

UPPER COLUMBIA RIVER

Quality Assurance Project Plan Methods Development for the White Sturgeon Sediment Toxicity Study

Prepared for
Teck American Incorporated
P.O. Box 3087
Spokane, WA 99220-3087

Prepared by

 **ENTRIX**
Down to Earth. Down to Business.™
University of Saskatchewan
44 Campus Drive
Saskatoon, SK S7N 3B5, Canada

 **Exponent®**
15375 SE 30th Place, Suite 250
Bellevue, WA 98007

 **HydroQual**
1200 MacArthur Boulevard
Mahwah, NJ 07430

Parametrix
411 108th Avenue NE, Suite 1800
Bellevue, WA 98004

Cardwell Consulting LLC
193 NW Kinderman Place
Corvallis, OR 97330-2253

April 2010

SECTION A: PROJECT MANAGEMENT

A1 TITLE AND APPROVAL SHEET

QUALITY ASSURANCE PROJECT PLAN METHODS DEVELOPMENT FOR WHITE STURGEON

Quality Assurance Project Plan Approvals

EPA Project Coordinator: Helen Bottcher Helen H Bottcher Date: 4/21/10

EPA Quality Assurance
(QA) Manager: Meja Titt Meja Titt for
Cinna Broo-Grove Date: 4/23/10

Teck Project Coordinator: Marko Adzic [Signature] Date: 04-21-10

Teck Technical
Coordinator: Dr. Anne Fairbrother Anne Fairbrother Date: 4/26/10

Principal Investigator: Dr. Markus Hecker [Signature] Date: 4/26/2010

Chemical Laboratory
Coordinator: Kris McCaig Kris McCaig Date: 04-21-10

Chemical Laboratory
Project Manager: Jeff Christian [Signature] Date: 4/26/10

Chemical Laboratory QA
Manager: Julie Gish Julie Gish Date: 4/26/10

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ACRONYMS AND ABBREVIATIONS

ASTM	American Society for Testing and Materials
AVS	acid volatile sulfide
CAS	Columbia Analytical Services
COC	chain-of-custody
DDT	dichlorodiphenyltrichloroethane
DO	dissolved oxygen
DOC	dissolved organic carbon
DQO	data quality objective
EDD	electronic data deliverable
ELS	early life stages
EPA	U.S. Environmental Protection Agency
LCS	laboratory control sample
MDL	method detection limit
MRL	method reporting limit
PCB	polychlorinated biphenyls
PEC	probable effects concentration
QA	quality assurance
QA/QC	quality assurance and quality control
QAPP	quality assurance project plan
QC	quality control
RI/FS	remedial investigation and feasibility study
SEM	simultaneously extracted metals
Site	Upper Columbia River site
SOP	standard operating procedure
TAL	target analyte list
TEC	threshold effects concentration
Teck	Teck American Incorporated
UCR	Upper Columbia River
U of S	University of Saskatchewan
UV	ultraviolet

UNITS OF MEASURE

cm	centimeter
cm/sec	centimeters per second
ft	feet
ft/sec	feet per second
mL	milliliter
sec	second

A3 DISTRIBUTION LIST

EPA Project Coordinator:	Helen Bottcher
EPA Quality Assurance Manager:	Maja Tritt
Teck Project Coordinator:	Marko Adzic
Teck Technical Team Coordinator:	Dr. Anne Fairbrother
Principal Investigator/Task Manager:	Dr. Markus Hecker
Chemical Laboratory Coordinator	Kris McCaig
Chemical Laboratory Project Manager:	Jeff Christian
Chemical Laboratory QA Manager:	Julie Gish

A4 INTRODUCTION AND TASK ORGANIZATION

A4.1 Introduction

This document presents the quality assurance project plan (QAPP) for methods development associated with future 2010 studies on sediment exposures to early life stages (ELS) of white sturgeon (*Acipenser transmontanus*) in the Upper Columbia River (UCR). The work described herein, and any associated observations and measurements recorded during this work, will not inform risk-management decisions for the remedial investigation/feasibility study (RI/FS). Rather, the primary objective of this work is to evaluate and inform critical design components and considerations of specifically designed flow-through fluvial simulation systems for future sediment toxicity tests using ELS of white sturgeon (the “test species”) at the University of Saskatchewan’s Aquatic Exposure Laboratory. An additional objective of this work is to identify and confirm appropriate reference and control sediments required for the aforementioned sediment toxicity tests.

Although the work described herein is intended only to inform the design of future sturgeon toxicity tests; and will not be used in risk assessment or site management decisions, the Data Quality Objective (DQO) process (USEPA 2006a) was used to guide the development of the requirements and rationale for activities presented herein.

A4.2 Task Organization

Roles and responsibilities of individuals including interfaces with the U.S. Environmental Protection Agency (EPA) and the Canadian Government are presented in Table A-1.

A5 PROBLEM DEFINITION AND OBJECTIVES

Based on experience and professional judgment of the principal investigator and researchers at the University of Saskatchewan (U of S) Aquatic Exposure Laboratory, flow-through fluvial simulation systems and associated exposure chambers have been developed and are commonly employed to evaluate surface water chronic exposures to a wide range of fish species including the test species. These systems, however, have not been specifically tested for the purpose of conducting flow-through sediment toxicity tests. As a result, and to ensure that future sediment toxicity tests using the test species (scheduled for 2010) are completed successfully, it is important that anticipated system design elements (e.g., depth of sediment) and operational conditions (e.g., flow rates) for

the fluvial flow-through system be tested and evaluated prior to the start of future sediment toxicity testing. In addition to specific exposure design considerations, it is also important to identify and confirm acceptable reference sediments. As such, objectives of this work are to:

- Evaluate and confirm the performance of flow-through fluvial simulation systems and associated exposure chambers for future ELS white sturgeon toxicity testing at the U of S Aquatic Exposure Laboratory
- Evaluate and confirm reference area sediments located upstream of Trail, British Columbia

A6 TASK DESCRIPTION

As noted within Section A5, primary objectives of this work are to evaluate and confirm the performance of flow-through fluvial simulation systems and associated exposure chambers for future sediment toxicity studies and confirmation of reference area sediments (including laboratory control sediments). Although the information and observations recorded during this work will not be used to inform risk-based management decisions, they will be used to inform and refine technical elements of future sediment toxicity tests using the test species. Tasks associated with this work can be divided into two general categories: 1) Fluvial-exposure system evaluation, and 2) Reference sediment confirmation (off-site tasks).

To help evaluate the flow-through fluvial simulation systems for future sediment toxicity testing, surface sediments (0 to 12 inches below ground surface) will be collected from the gravel bar at Deadman's Eddy, transported to the U of S Aquatic Exposure Laboratory, and employed in a number of experimental tests designed to evaluate and optimize system design elements in accordance with standard American Society for Testing and Materials (ASTM) guidelines for testing fish ELS (ASTM 2005; and see Table B-2 in Appendix B) as modified based on professional judgment for white sturgeon. Off-site tasks will include the collection of surface sediments and chemical analysis of potential reference sediments.

A7 DATA QUALITY OBJECTIVES, CRITERIA, AND DESIGN RATIONALE

The work described herein is intended to help inform study designs and will not be used for assessing risks or as input to site management decisions. However, the DQO process (USEPA 2006b) was used to guide the rationale for conduct of the methods development work.

A7.1 State the Problem

Flow-through fluvial simulation systems and associated exposure chambers have been developed and are routinely employed to evaluate surface water chronic exposures to fish including the test species. These systems, however, have not been specifically tested for the purpose of conducting flow-through sediment toxicity tests. As a result, and to ensure that future sediment toxicity tests using the test species (scheduled for 2010) are completed successfully, it is important that anticipated system design elements (e.g., depth of sediment) and operational conditions (e.g., flow rates) for the fluvial flow-through system be tested and evaluated prior to the start of future sediment toxicity testing. In addition identifying and confirming acceptable reference sediments is required for future sediment toxicity test using the test species.

A7.1.1 Resources and Schedule

Field mobilization for the collection of surface sediments from the gravel bar at Deadman's Eddy and off-site reference sediments is anticipated to take place in late April 2010. All on-site sediment sampling activities (i.e., from the gravel bar at Deadman's Eddy) would be completed within one day, and would precede any and all off-site sampling activities. On-site sediment samples would be immediately transported to the U of S Aquatic Exposure Laboratory so that laboratory evaluation of the flow-through fluvial exposure system (the "system") can be completed in May 2010. Upon obtaining all research and ground disturbance permits, and clearance from the Cultural Resources Working Group¹, a schedule of laboratory based activities to be completed at the U of S will be finalized. Laboratory testing and evaluation of the system and associated exposure chambers are not expected to extend beyond 45 calendar days. Evaluation and confirmation of reference area sediments will also be completed within the above-mentioned time frame.

A7.2 Identify the Goal of the Work

There are two primary overarching goals (objectives) associated with this work. They include: 1) Evaluating and confirming the performance of the flow-through fluvial exposure system and associated chambers for future sediment toxicity tests with ELS of white sturgeon; and 2) Evaluating and confirming suitable reference and control sediments for future sediment toxicity tests with ELS of white sturgeon.

¹ Research permit and ground disturbance permit applications have been submitted in parallel with this QAPP; while EPA has initiated the cultural review process at the time of writing.

Data generated during this work are for information purposes, and are intended to guide and support future sediment toxicity tests using ELS of white sturgeon. Information and/or observations recorded during this work will not be used, nor considered for risk-based evaluation or management decisions. A detailed description of the aforementioned objectives and associated measures are provided within Section A7.7.2 of this QAPP.

A7.3 Identify Information Inputs

Information inputs to support the objectives listed in Section A7.2 are presented below.

A7.3.1 Reference Sediments (Off-site)

Reference area sediments are essential for evaluating potential toxicological responses or ecological risks at a site as they are used to establish and gauge levels of responses in the absence of elevated chemical contaminant concentrations (ASTM 2000; USEPA 1994; 2000). A number of desirable characteristics (i.e., guidelines) for selecting reference area sediments have been identified (USEPA 2006b; RSET 2009). They include, but may not necessarily be limited to:

- Upstream from the Upper Columbia River study site (Site)
- Comparable physical setting as the study Site
- Un-impacted or minimally impaired (i.e., uncontaminated)
- Similar sediment grain size distribution and organic matter content as the study Site.

Data and observations collected as part of this work will be used to ascertain if the above-listed characteristics and recommendations are satisfied for potential reference area sediments. Therefore, at off-site reference area sediment locations, the following questions will be evaluated in regard to bulk surface sediments:

1. What is the grain size distribution?
2. What is the concentration of Target Analyte List (TAL) metals, and how do they compare to conservative toxicity benchmarks (e.g., threshold effects concentration [TEC] and probable effects concentration [PEC])?
3. What is the fraction of total organic carbon and pH?
4. What is the concentration of acid volatile sulfide (AVS) and simultaneously extracted metals (SEM)?
5. What is the concentration of organic chemicals (polychlorinated biphenyls [PCBs], and dichlorodiphenyltrichloroethane [DDT]); and how do they compare to conservative toxicity benchmarks (e.g., TECs)?

A7.3.2 White Sturgeon Flow-Through Fluvial Exposure Chamber System

A flow-through fluvial system and associated exposure chambers have been developed and are routinely employed to evaluate surface water chronic exposures to fish including the test species. These systems, however, have not been specifically tested for the purpose of conducting flow-through sediment toxicity tests. As a result, and to ensure that future sediment toxicity tests using the test species a number of questions need to be addressed to ensure that the fluvial exposure system and its operation is evaluated for the purpose of future sediment toxicity tests. These include the following, which are addressed in detail in Section A7.7.2:

- How can uniform flow conditions be established to minimize “dead spaces” at the inflow and outflow?
- Which hydrological operating condition (e.g., flow) will result in the smallest gradient between porewater and overlying water?
- What is the time to steady-state between porewater and overlying water under different hydrological operating conditions?
- What is the effect of different volumes and distributions of substrate on hydrological conditions in the chambers?
- What is the influence of sediment depth, depth of porewater sampling within the sediment layer, and sampled volume on exchange between overlying water and porewater?
- What are the optimum tank cleaning techniques?

A7.4 Geographic and Temporal Boundaries of the Work

Tasks associated with the work will be conducted in off- and on-site areas (Maps A-1 and A-2). Off-site tasks are those located upstream of the Trail facility in Canada (reference sites) and at the U of S Aquatic Exposure Laboratory; while on-site tasks are limited to the collection of surface sediments (0 to 12 inches) from the gravel bar at Deadman’s Eddy. Coordinates of specific sampling locations are described in Section A7.7.

To ensure that this work can be used to inform design elements of 2010 sturgeon exposure studies, field mobilization for on-site activities is anticipated to take place in April 2010; with off-site system design calibration tests extending into May and if necessary June 2010.

A7.5 Statistics and Types of Inferences

This step of the DQO process provides the data analysis approach for evaluating the data and drawing conclusions. Given that the primary goal of this work is for methods development and refinement of future toxicity testing designs, no risk-based management decisional data will be collected. Therefore, statistics are anticipated to be used only to describe the system components, e.g. average flow rates and measures of variability. Answers to the questions presented in Section A7.3 will provide the basis for selection of methods and procedures appropriate for use in future sediment toxicity tests using ELS of white sturgeon.

Evaluation of the flow-through fluvial system and associated exposure chambers within the laboratory will be evaluated with a series of dye (i.e., Fluorescein) experiments, visual observations, and measures of standard water quality parameters (e.g., dissolved oxygen, pH, conductivity, etc.). Use of sediments from the river, in addition to laboratory control sediments will facilitate the evaluation (i.e., attainment of steady-state conditions) and ensure appropriate linkage to sediments used in the future toxicity testing.

Evaluation of off-site reference area sediments will be informed by screening for metals as well as a limited number of organic chemicals (total PCBs and DDT) against conservative benchmarks.

A7.6 Performance or Acceptance Criteria

The purpose of this step of the DQO process is to specify acceptable limits on decision errors for the problem. Given that this work is only intended to inform future study designs (i.e., will not be used in risk assessment or site management decisions), this step is limited to generating information that is appropriate and applicable for use in evaluating the white sturgeon ELS flow-through fluvial exposure system and reference sediments. Evaluation of reference area sediments will consider chemical and biological characteristics. Test acceptability requirements recommended for this work are summarized within Table A-2. Measurement methods and associated calibration, precision and accuracy information for use in the design of the fluvial exposure system are shown in Table A-3.

A7.6.1 Sampling Completeness

External reference sediments will be located north of the Trail facility. Sediments for the white sturgeon ELS exposure system will be collected from an on-site location at the gravel bar at Deadman's Eddy. It is anticipated that a sufficient volume

(e.g., ~50 gallons for on-site sediments) and number of samples for methods development will be collected to complete the work.

A7.6.2 Contingencies

As noted within Section A7.1.1 laboratory evaluation of the flow-through fluvial exposure system is anticipated to be completed in late May/early June 2010. This timeline will provide sufficient opportunity to ensure that future sediment toxicity tests using ELS of white sturgeon can be initiated when eggs are available in mid-July 2010. To facilitate this outcome, components of the flow-through fluvial exposure system to be evaluated as outlined within Section A7.7.2 will be completed in order of relative importance and in parallel. For instance, the evaluation of cleaning techniques will be completed at the end, after the other parameters have been optimized. It is important to note that the flow-through fluvial exposure systems are modifications of systems commonly employed at the U of S Aquatic Exposure Laboratory to evaluate surface water chronic exposures to fish including the test species. It is important to note that although sturgeon eggs are not available until mid-July, it is anticipated that sediment samples for future toxicity tests using ELS of white sturgeon will be available by mid-June so the exposure system can be operational by the beginning of July 2010. Therefore, in the unlikely event that not all the design evaluation questions listed below (see Section A7.7.2) have been completely addressed, additional monitoring of system parameters may be instituted. This will ensure that the study outcome (survival, growth, reproduction endpoints) can be correlated with the appropriate exposure data that occur during the experimental period.

A7.7 Plan for Collecting Data

The plan for obtaining data is described herein and specific standard operating procedures (SOPs) for sediment sample collection methods are presented within Appendix A.

A7.7.1 Field Collection Methods

Sediment will be collected from both on-site and off-site locations. Because field sampling methods associated with these studies involve sediment collection or penetration and disturbance, Teck and its technical team will work with the potentially affected parties to assess the effects of the planned work and seek ways to avoid, minimize, or mitigate any adverse effects on historic properties. A cultural resources coordination plan (Appendix C) has been prepared to provide relevant background information about site-related cultural resources, define measures for protecting

resources, and define procedures for consulting with the appropriate state, federal, and tribal parties with interests in the cultural resources of the Site.

On-site Sampling Locations. During this work, one potential location between the trans-boundary reach and Kettle Falls will be sampled (Map A-1). Surface sediment samples will be collected using a steel shovel or scoop. The top 4 to 6 inches of sediment will be removed and samples collected from 4 to 12 inches depth will be collected in 5 gallon plastic buckets until approximately 50 gallons have been obtained. Sample collection will not exceed 12 inches in depth.

Off-site Sampling Locations. During this work, off-site sampling locations will be selected at putative reference sites (Map A-2). These include re-sampling areas previously used as reference locations. The objective is to evaluate and confirm off-site reference area sediments upstream of the Trail facility with sediment characteristics (grain size) similar to that of areas where white sturgeon spawn. Specifically, these locations include: Birchbank (riverine environment), Genelle (riverine environment), and Lower Arrow Lakes (reflective of the lacustrine conditions), with corresponding coordinates listed within Table A-4. Samples will be collected from the top 6 inches of sediment according to SOPs in Appendix A.

A7.7.2 Design of Fluvial White Sturgeon Exposure Systems

The white sturgeon ELS exposure systems to evaluate are a unique design, modified specifically for exposure of the sturgeon ELS to sediments in a manner that will meet all requirements of the ASTM fish early life stage test (ASTM 2005) under flow-through fluvial conditions. The study design and performance specifications (for biological and chemical measurements) are provided in the Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon (*Acipenser transmontanus*) prepared by Teck and submitted to EPA on April 5, 2010 and included in Appendix B for easy reference. In order to meet the specifications for the conduct of the sediment toxicity studies with white sturgeon ELS, exposure chambers will be designed to enable the adjustment of flow velocity, water replacement time, recirculation frequency, sediment thickness, porewater sampling volume and depth (Figure A-1). The following sections describe the parameters that will be evaluated during system development, which are summarized in Table A-5.

Flow Conditions

Objective. Evaluate and establish homogenous water flow conditions to ensure uniform distribution of influx and within the posterior chamber, and to minimize “dead spaces” at inflow and outflow.

Approach. A fluorescent dye (Fluorescein) will be used to measure water flow; such dyes are cost-effective and easily and accurately measured with a fluorometer or visualized with an ultraviolet (UV) lamp. After the dye is introduced into the test systems ($t=0$), it will be made visible by UV lighting, and dispersal of dye and associated water flows will be recorded by means of a digital video camera across the entire chamber. Additionally, water samples will be taken at $t=30$ sec, $t=120$ sec and $t=300$ sec at nine locations equally distributed over the diameter of the chamber. This will be repeated for diameters every 4 inches throughout the test chamber, resulting in a total of $9 \times 9 = 90$ water samples (Figure A-2). The first and last sampled diameters will be located at the inflow and outflow screens of the sediment exposure chamber, respectively, to identify potential dead-spaces or low-flow areas. Samples will be analyzed for dye concentrations using a microtiter plate fluorescent reader, and dye concentrations will be mapped throughout the chamber. Sampling will be conducted using 1 mL pipettes modified such that samples can be taken at different depths throughout the chambers.

Decision Criteria. This portion of the pilot studies aims to characterize possible dead-spaces with assumption that due to logistical and technical constraints small areas with low or altered flow, especially in the corners of the exposure systems or at the sediment-water interface cannot be completely avoided. If large areas with low-flow, as defined by a greater than 50 percent decrease in dye concentration (relative to the average dye concentration in all bottom samples taken across the chamber) in two of the three bottom samples taken per diameter sampled, occur, measures to minimize these dead-spaces have to be taken. These measures will include but are not limited to including additional jets to the spray-bar, re-arranging of jet-distribution, and rounding of corners.

Gradients

Objective. Evaluate potential gradients between porewater and overlying water under different hydrological conditions (e.g., flow velocity) using dyed sediments – monitor basic water quality parameters at different sediment depths, and at the sediment-water interface.

Approach. Gradients between porewater and overlying water will be measured by means of suction devices that will be installed at 4-inch intervals along the entire length of the centre of sediment exposure chamber. Suction devices will be buried at different depth to enable sampling of porewater in the top inch of sediment and in the sediment-surface water transitional zone (pseudo hyporheic area with and without gravel for habitat enrichment). Parameters that will be used to assess potential gradients between porewater and overlying water are dye concentration, dissolved oxygen concentration

(DOC), and conductivity. Flows to be tested range from high velocities that occur in the river in the vicinity of Deadman's Eddy to low velocities that are recorded in the flats areas of Marcus Flats; while maintaining conditions appropriate for white sturgeon ELS culture.

Decision Criteria. The goal of this experiment is to establish conditions under which the gradient between porewater and overlying water in the pseudo-hyporheic area is minimal while maintaining conditions appropriate for sturgeon ELS culture. Based on the achievable gradients, specific flow conditions for the definite test with white sturgeon ELS will be determined.

Time to Steady-state

Objective. Characterize time to steady-state between porewater and overlying water under different hydrological conditions (recirculation frequency, water replacement time, flow velocity) using dyed sediments – monitor basic water quality parameters at 0, 12, 24, 48 hours and every 48 hours thereafter until steady state is reached.

Approach. Basic water quality parameters will be monitored at 0, 12, 24, and 48 hours, and every 48 hours thereafter until steady state. Measurements will be made of conductivity, DOC, dissolved oxygen (DO), ammonia, nitrite, nitrate, hardness, alkalinity, and pH. Hydrological conditions to be assessed include:

- Recirculation frequency—2.7 to 7.8 re-circulations per hour.
- Water replacement time—1, 2, 3 and 4 complete water replacements per system per day.
- Flow velocity—see Section A7.7.2.1.

Decision Criteria. For the purposes herein, steady state is attained when changes over the entire exposure period are not projected to exceed 20 percent for conductivity while maintaining appropriate for white sturgeon ELS culture (e.g., sufficient DO concentrations).

Gravel Volume and Distributions

Objective. Identify the effect of different volumes and distribution of gravel on hydrological conditions in fluvial chambers.

Approach. Sediments will be layered at a thickness of 2, 3 and 4 inches into the exposure portion of the test chambers. Furthermore, different densities of 1 to 2 cm diameter gravel will be layered on top of the sediments to create a pseudo-hyporheic area. Densities of gravel to be tested will be 0, 3, 7, 10 and 13 stones per 100 cm².

Decision criteria. Influence of sediment volume and distribution will be assessed as described in Section A7.7.2.1.

Exchange between Overlying Water and Porewater

Objective. Evaluate the influence of sediment depth, depth of porewater sampling within sediment layer, and sampled volume on exchange between overlying water and porewater using dyed overlying water, and optimize: sampling depth, sample volume, number of sampling ports/devices per chamber, and suction strength and techniques.

Approach. Twelve porewater sampling ports will be equally distributed throughout the exposure chambers at a depth of 0.5 and 1 inch below the sediment surface. After introduction of dye into the test system, different volumes of porewater (0.5, 1 and 2.5 percent of total calculated porewater volume in sediment) will be sampled, and potential dilution of porewater with overlying water by fluorometric determination of dye concentration in samples will be determined. Furthermore, porewater will be sampled from different numbers of ports at the same time (4, 8 and 12).

Decision Criteria. Based on the data obtained during these experiments the sampling scenario (volume and number of ports to be sampled) under which minimum dilution of porewater from overlying water occurs will be selected for inclusion into the sampling protocol. Also, acceptable conditions (percent dilution of porewater) will be established by this work. It is anticipated that dilution will be minimal and not exceed 10 to 15 percent of total volume sampled.

Optimum Cleaning Techniques

Objective. Identify optimum cleaning techniques avoiding utilization of suction devices, and installment of large particle filters – with and without addition of diet (bloodworms, oligochaetes; semi-moist diet, other).

Approach. Food will be introduced simulating three feeding events per day using *Artemia*, worms and semi-moist diet. At days 2, 3, 4 and 5 chambers will be manually cleaned (daily) by carefully scraping surfaces with a spatula to remove as much biofilm as possible without significant resuspension of sediments. At 5, 10, 20 and 30 minutes after each cleaning event, bottom near water samples (approximately 1 cm above the sediment surface) will be taken as described in Section 7.7.2.1. Turbidity of samples as a measure of resuspended matter will be determined using the light scattering methods as described in EPA Method 180.1 or Standard Method 2130B (Standard Methods 1995).

Decision Criteria. Optimum cleaning techniques will be determined as a function of minimizing resuspension of sediment and efficiency of cleaning. It is acknowledged

that any type of physical removal of bio-growth will cause resuspension to a certain degree, and the final method to be established will be a compromise between efficiency of cleaning and amount of sediment resuspended during the cleaning event.

Artificial Laboratory Control Sediment

Objective. Selection of a laboratory control sediment that has physical characteristics appropriate for ELS of sturgeon and is comparable to sediments used historically in fish early life stage tests. The purpose of such a sediment is to benchmark this study with other fish early life stage studies reported in the literature to ensure comparability of results.

Approach. Several different materials will be considered, including silica sand and crushed or ground granite. It is important that the sediment be relatively course-grained (i.e., grain sizes between 0.5 and 2 mm diameter) to provide suitable substrate for sturgeon larvae. Materials will be obtained from commercial vendors (e.g., well drilling companies) who will be requested to provide certificates of analysis for contaminants (including metals) to verify their suitable for use as a control sediment. Sediments will be layered into the exposure chambers as described under A7.7.2.4, and water quality parameters and flow conditions will be assessed as described above (A7.7.2.1 and A7.7.2.3).

Decision Criteria. The purpose of this exercise as part of the pilot studies is to identify substrates with a range of comparable grain size distributions and color to those obtained from Columbia river, and to assess the potential influence these artificial sediment types may have on water quality. It is anticipated that acceptable criteria for key water quality parameters such as pH, DO, hardness, and alkalinity will not differ by more than 50 percent from average values determined for riverine sediments. It is acknowledged, however, that these artificial substrates do not contain significant amounts of organic materials, and thus, measurements of nutrients and organic carbon contents will not be used as decision criteria for these matrices.

A8 SPECIAL TRAINING/CERTIFICATES

All personnel working at the Site should have the appropriate health and safety training identified in the General Site Health and Safety Plan (Teck 2007) and U of S laboratory personnel will follow the laboratory safety guide (Appendix D).

A9 DOCUMENTATION AND RECORDS

Records will be maintained to document all activities and observations (data) associated with field sampling and with chemical analysis at the laboratories. Procedures for documentation of these activities are described in this section.

A9.1 Field Documentation

Field records that will be maintained for the pilot study include the following:

- Field logbooks
- Photo documentation
- Field data forms
- Sample tracking/chain-of-custody (COC) forms (when applicable).

Further details of the observations collected in the field are in the SOPs (Appendix A).

A9.2 Laboratory Documentation

All activities and results related to sample analysis (i.e., references) will be documented at the laboratory. Internal laboratory documentation procedures will be described in the laboratory quality assurance (QA) plans (refer to Appendix E).

Full laboratory data reports will be provided in both hard copy and electronic format to the analytical laboratory coordinator. Electronic data deliverables (EDDs) will be in spreadsheet format and will be compatible with the project database but will be qualified as “for information purposes only” within the database, as they will not be used to support risk assessment or site management decisions.

A9.2.1 Fluvial White Sturgeon Exposure Systems

All measurements conducted during the evaluation of the sturgeon exposure systems will be conducted by U of S Aquatic Exposure Laboratory. Specifications for all measuring equipment (fluorometer, conductivity meter, etc.) are provided in Table A-3. SOPs for instrument calibration and maintenance are in Appendix A. U of S will provide electronic data files to Teck for inclusion in the project database as “for information only.”

A9.2.2 Bulk Sediment – Off-site Locations

Sediment samples collected from the off-site reference locations will be submitted to Columbia Analytical Services (CAS) for measurement of selected analytes (Table A-2) in support of qualifying the off-site sediments as reference locations. CAS will provide a data package for each sample delivery group or analysis batch that is comparable in

content to a full Contract Laboratory Program package. It will contain all information required for a complete QA review, including the following:

- A cover letter discussing analytical procedures and any difficulties that were encountered
- A case narrative referencing or describing the procedures used and discussing any analytical problems and deviations from SOPs and this QAPP
- COC and cooler receipt forms
- A summary of analyte concentrations (to two significant figures for results <10, three significant figures for results >10), method reporting limits (MRLs), and method detection limits (MDLs)
- Laboratory data qualifier codes appended to analyte concentrations, as appropriate, and a summary of code definitions
- Sample preparation, digestion, extraction, dilution, and cleanup logs
- Instrument run logs
- Initial and continuing calibration data, including instrument printouts and quantification summaries, for all analytes
- Results for method and calibration blanks
- Results for all quality assurance and quality control (QA/QC) checks, including serial dilutions, laboratory control samples (LCSs), matrix spike samples, laboratory duplicate or triplicate samples, and any other quality control (QC) procedures required by applicable method protocols and laboratory SOPs
- Original data quantification reports and printouts of chromatograms and mass spectra for all analyses and samples as applicable
- All laboratory worksheets and standards preparation logs
- A page of example calculations for each analytical method included in the data package
- A documented data deliverable for each analytical method performed and reported.

Full laboratory data reports will be provided in electronic format to the chemical laboratory coordinator.

A9.3 Data Quality Documentation

Data verification (i.e., confirming the accuracy and completeness of field and laboratory data) will be completed by the Teck American Incorporated (Teck) technical team for data generated in the field, and by each laboratory for the data that it generates. Data

validation and data quality assessment for this task will be completed and provided to the Task Manager and the Technical Team Coordinator. Because these data will not be used in the RI/FS risk analysis or for risk management purposes, further external data validation will not be done.

SECTION B: ASSESSMENT AND OVERSIGHT

This task will rely on the knowledge and expertise of the Teck technical team. The field team and laboratories will stay in close verbal contact with Task Manager and the Technical Team Coordinator during all phases of this task. This level of communication will serve to keep the management team apprised of activities and events, and will allow for informal but continuous task oversight.

B1 ASSESSMENTS AND RESPONSE ACTIONS

Assessment activities will include readiness reviews prior to sampling as well as internal review while work is in progress.

Readiness reviews are conducted to ensure that all necessary preparations have been made for efficient and effective completion of each critical phase of work. The readiness review will be conducted prior to field sampling. The field supervisor will verify that all field equipment is ready for transfer. The field supervisor will also verify that the field team and subcontractor(s), as required, have been scheduled and briefed and that the contract for the subcontractor has been signed by both parties. Any deficiencies noted during this readiness review will be corrected prior to initiation of sampling activities.

The laboratories will be required to have implemented a review system that serves as a formal surveillance mechanism for all laboratory activities. Each phase of work is reviewed by a supervisor before it is approved for release. Details are provided in the laboratory QA plans.

B2 REPORTS TO MANAGEMENT

The laboratories will keep the appropriate Teck Laboratory Coordinator apprised of their progress on a weekly basis. The laboratories will provide the following information:

- Inventory and status of samples held at the laboratory in spreadsheet format by sample delivery group
- Summaries of out-of-control laboratory QC data that resulted in a requirement for corrective action and a description of the corrective actions implemented
- Descriptions and justification for any significant changes in methodology or QA/QC procedures.

The laboratories will be required to have implemented routine systems of reporting non-conformance issues and their resolution. These procedures are described in the

laboratory QA manual. Laboratory non-conformance issues will also be described in the field sampling report if they affect the quality of the data.

Data packages and EDDs will be prepared by the laboratory upon completion of analyses for each sample delivery group. The case narrative will include a description of any problems encountered, control limit exceedances (if applicable), and a description and rationale for any deviations from protocol. Copies of corrective action reports generated at the laboratory will also be included with the data package.

B3 REPORTING AND EPA OVERSIGHT

This section describes the process for reporting results to EPA, EPA oversight during the methods development, and how the most appropriate test methods for future testing will be determined from the method development evaluations performed under this QAPP.

B3.1 Reporting

Teck will provide EPA with a technical memorandum summarizing results of the reference sediment location selection and the studies conducted for evaluating the sturgeon exposure system. Appropriate appendices will be included with detailed data, and all data will be available electronically through the project database.

B3.2 EPA Oversight

EPA and Cultural Resources personnel may choose to observe sediment collection at Deadman's Eddy. This will ensure compliance with the Cultural Resources Plan and will allow immediate, real-time consultation on type of sediment to collect for use in exposure system design experiments. Teck will provide periodic updates to EPA's technical team through weekly telephone conferences during the development of the exposure system. This will allow consultation with EPA's experts to optimize system performance. In addition, EPA may choose to observe evaluation activities being completed at the U of S Aquatic Exposure Laboratory.

B3.3 System Design

Teck will consult with EPA on final design parameters for the sturgeon exposure system prior to setting up the chambers for the sediment toxicity study. Following the consultation, Teck will provide EPA with a written addendum to the Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon (*Acipenser transmontanus*) that describes the parameterization of the chambers along with the acceptable performance criteria (e.g., allowable excursions from preferred measures).

SECTION C: REFERENCES

- ASTM (American Society for Testing and Materials). 2000. Standard test methods for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates. E 1706-00. American Society for Testing and Materials, Philadelphia, PA.
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- USEPA. 2006b. Concepts and approaches for the bioassessment of non-wadeable streams and rivers. EPA/600/R-06/127. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.
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FIGURES

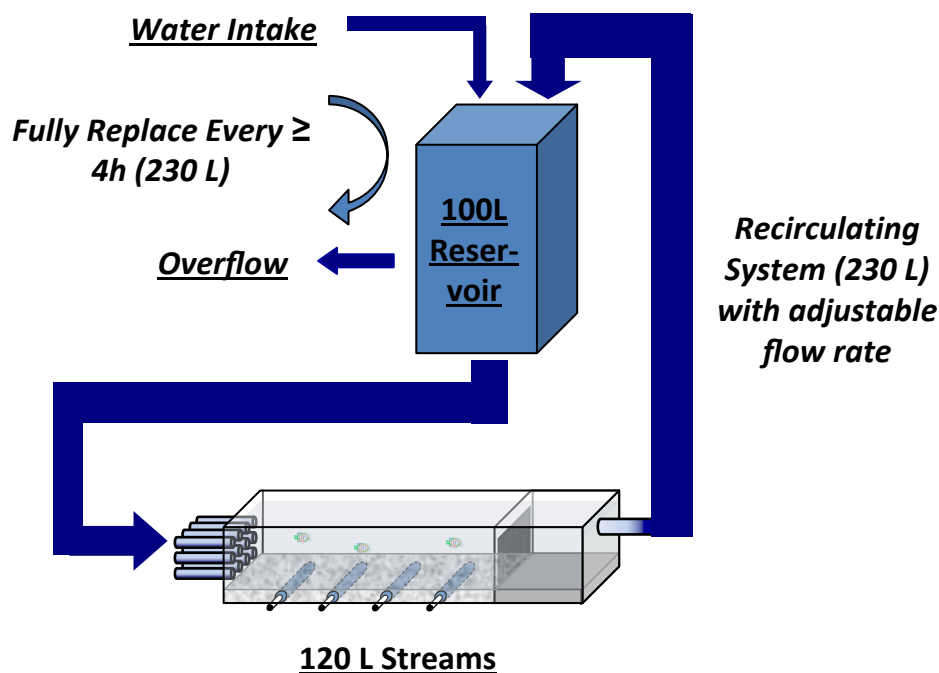
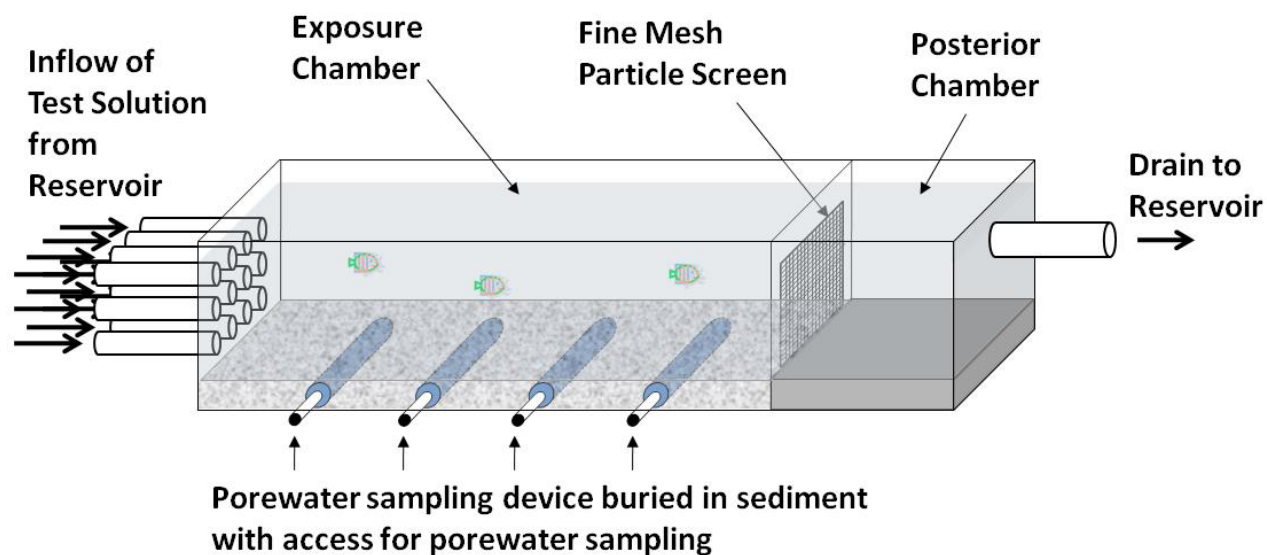
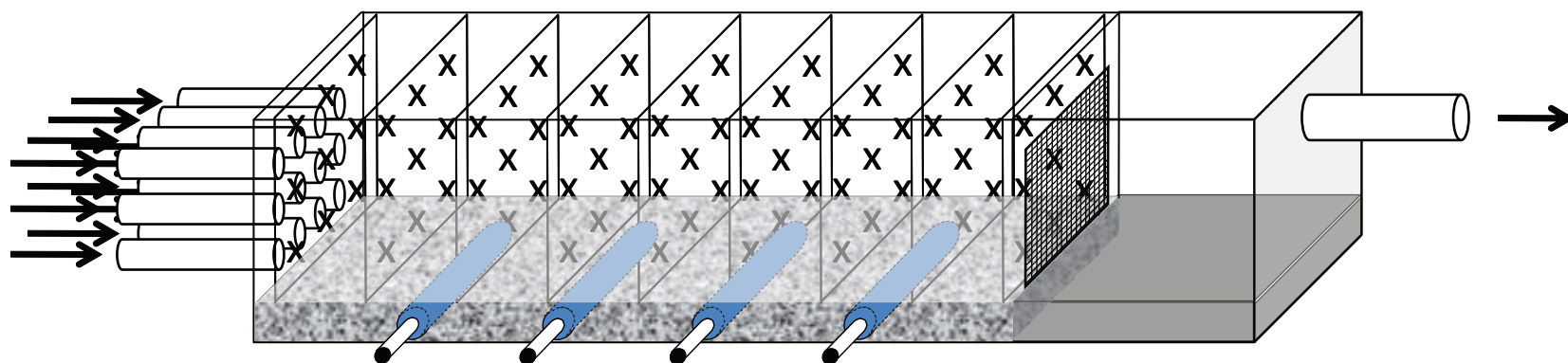


Figure A-1. Test Chamber Design for the Conduct of Chronic Sediment Exposure Studies with ELS White Sturgeon.

Note:

Inflow of water from reservoir is distributed over through a spraybar system (four to five evenly horizontally spaced perforated pipes) to insure constant flow distributed over the diameter of the test chamber. The posterior chamber enables consistent flow over sediments even at the posterior portion of the sediment layer so no dead spaces with standing or lower flow water can form. The effluent drains into the test solution reservoir, and water from the same reservoir is recirculated to the 12 inflow pipes by a high-flow pumping system allowing for adjusting flow rates through the exposure chamber.



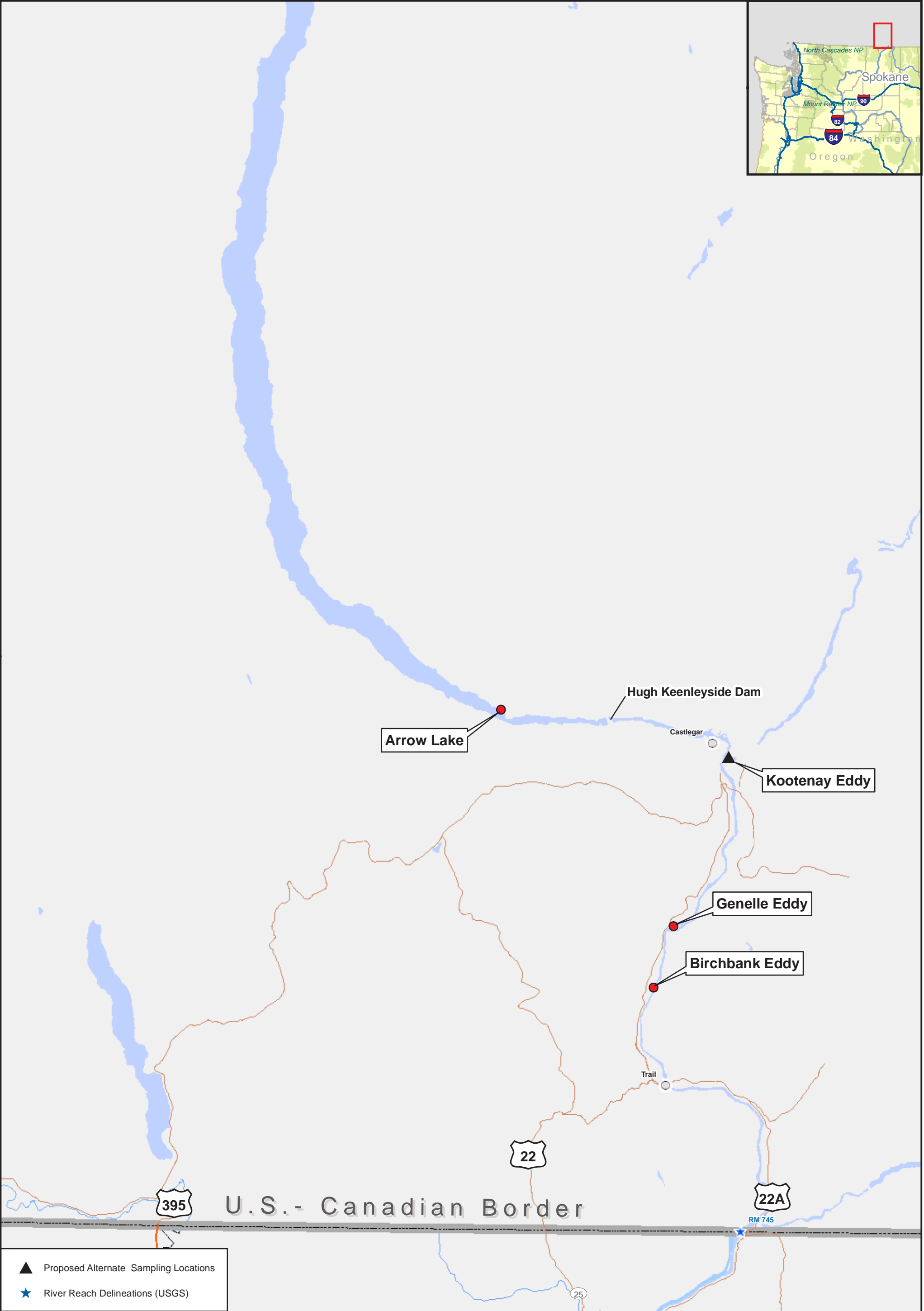
x = sampling point

Sampling volume = 1mL per sampling point

Figure A-2. Illustration of Sampling Point Distribution for Collecting Water Samples Throughout an Exposure Chamber During the Flow Condition Experiment.

MAPS





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Proposed Alternate Sampling Locations

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River Reach Delineations (USGS)

TABLES

Table A-1. Project Organization and Roles and Responsibilities of Individuals

Personnel	Role and Responsibility
Marko Adzic Project Manager	Serves as Teck's project coordinator and will have the primary responsibility for ensuring that Teck meets all requirements related to sample locations and sediment collections. He will also be responsible for coordinating with EPA, the Canadian Government, and managing the overall task schedule.
Dr. Anne Fairbrother Technical Coordinator	Is responsible for coordinating the tasks of all the team members to ensure that required activities are completed in sequence and on time. Dr. Fairbrother will work closely with the task manager to ensure that all requirements are met and study objectives achieved.
Dr. Markus Hecker Principal Investigator/Task Manager	Is responsible for conducting the methods development tests described for white sturgeon. He will work closely with Dr. Fairbrother to ensure that the objectives of the work are achieved. Coordinates and oversees field sampling and laboratory testing activities. Oversees day-to-day activities in the laboratory associated with white sturgeon exposure chamber evaluation.
Kris McCaig Chemical Laboratory Coordinator	Is responsible for ensuring that analytical chemistry analyses are completed satisfactorily prior to collection of samples for the definitive studies; coordinating receipt of samples by the laboratory and tracking the laboratory's progress; addressing QA issues related to the analytical chemistry; and addressing any scheduling issues. Ms. McCaig will report to the Technical Coordinator.

Table A-2. Measurement Performance Criteria for Reference Area Sediments (Whole Chemistry)

Parameter	Method	Target Detection Limit	Analytical Accuracy (% Recovery)	Analytical Precision (Relative % Deviation)	Overall Completeness (%)
TAL Metals	ICP/AES	See note a.	65-135	35	90
TOC	PSEP	0.05%	65-135	35	90
AVS	EPA 821-R-91-100	1 $\mu\text{mol/gm}$ dry sediment	53-143	30	90
SEM	EPA 821-R-91-100	0.01 $\mu\text{mol/gm}$ dry sediment	75-125	15	90
Grain Size	ASTM D422	NA	NA	NA	NA
pH	EPA 9045C	NA	NA	NA	NA
Total PCBs (congeners)	EPA 8082	0.053 - 1.0 $\mu\text{g/kg}$	53-143	50	90
Total DDT	EPA 8081A	1.0 $\mu\text{g/kg}$	53-143	50	90

Notes: a. Target detection limits are as follows:

Aluminum	20 mg/kg	Magnesium	500 mg/kg
Antimony	6 mg/kg	Manganese	1.5 mg/kg
Arsenic	1 mg/kg	Mercury	0.1 mg/kg
Barium	20 mg/kg	Nickel	4 mg/kg
Beryllium	0.5 mg/kg	Potassium	500 mg/kg
Cadmium	0.5 mg/kg	Selenium	3.5 mg/kg
Calcium	500 mg/kg	Silver	1 mg/kg
Chromium	1 mg/kg	Sodium	500 mg/kg
Cobalt	5 mg/kg	Thallium	2.5 mg/kg
Copper	2.5 mg/kg	Vanadium	5 mg/kg
Iron	10 mg/kg	Zinc	6 mg/kg
Lead	1 mg/kg		

Table A-3. Instrument Specifications and Calibration Methods

Parameter	Detection Range	Precision/ Reproducibility ^a	Method/Instrument	Calibration
RO/Lab Water Flow	8 to 114L/min	±1.0%	MIC Paddle Wheel Flow-Meter (Aquatic Eco-System item# ET13)	Filled graduated carboy to the 20L mark to ensure flow-meter measured volume accurately
Dye Fluorescence	125ppm to 15ppb	t.b.d. ^b	POLARstar OPTIMA	Ran a serial dilution of dye in lab water on POLARstar OPTIMA according to manufacturer's specifications
Temperature	-5 to 105 °C	±0.1°C	VWR Symphony Probe (Cat# 14002-860)	Calibrated by manufacturer
pH	-2.000 to 19.999	±0.002	VWR Symphony Probe (Cat# 14002-860)	Calibrated according to manufacturer's specifications
Conductivity	0 to 3000µS/cm ³	0.5% ± 1 digit or 0.01 µS/cm ³	VWR Symphony Probe (Cat# 11388-372)	Calibrated according to manufacturer's specifications
Dissolved Oxygen	0.00 to 90.00 mg/L	±0.2 mg/L	VWR Symphony Probe (Cat#11388-374)	Calibrated according to manufacturer's specifications
Ammonia-Nitrogen	0.0 to 2.0 mg/L	±0.05 – 1.0 mg/L (depending on concentration)	LaMotte Colorimetric Kit (Cat#3304)	Calibrated by manufacturer
Nitrite	0.02 to 0.30 mg/L	±0.02 – 0.1 mg/L (depending on concentration)	LaMotte Colorimetric Kit (Cat#7674)	Calibrated by manufacturer
Nitrate	0.25 to 10.0 mg/L	±0.25 – 2.0 mg/L (depending on concentration)	LaMotte Colorimetric Kit (Cat#3319)	Calibrated by manufacturer
Hardness	0 to 200 mg/L CaCO ₃ /L	±4 mg/L	LaMotte Colorimetric Kit (Cat#4824-DR-LT)	Calibrated by manufacturer
Alkalinity	0 to 200 mg/L CaCO ₃ /L	±4 mg/L	LaMotte Colorimetric Kit (Cat#4419-DR)	Calibrated by manufacturer
Total Chlorine	0 to 10 mg/L	±0.2 mg/L	LaMotte Colorimetric Kit (Cat#6905)	Calibrated by manufacturer

RO = reverse osmosis

^a Based on manufacturer's description.

^b t.b.d.: Will be determined as part of pilot experiments

Table A-4. Proposed Surface Sediment Sampling Locations

Sampling Area Description	UTM Coordinates	
	Easting	Northing
Deadman's Eddy Gravel Bar		
Northeast Corner	447158	5421097
Northwest Corner	447026	5421144
Southwest Corner	447023	5421127
Southeast Corner	447077	5421068
Off-Site Samples		
Genelle	448723.51	5450261.18
Birchbank	447251.03	5445720.5
Lower Arrow Lake	435940	5466319

Table A-5. Parameters, Methods, Measurements and Decision Criteria that will Inform the Design of the Exposure Systems and Test Conditions for the 2010 Studies with White Sturgeon ELS to Investigate Sediment Related Toxicity (listed here in order of operation)

Order	Parameter	Goal	Test Conditions	Measurement	Acceptance Criteria
1	Flow condition	Establish parameters and operational conditions that enable the maintenance of homogenous flow conditions in the test system.	Initial flow rate of 19 L/min, with incremental changes of +/- 2 L/min to achieve desired end state	Video record of fluorescein dye movement	Minimal dead spaces or low flow areas. Flow appropriate for ELS sturgeon, assumed to be: 1. Hatch through 14 dph: 8 to 12 L/min; 2. >14 dph: 16 to 24 L/min.
2	Gravel volume and distributions	Establish optimum density of gravel to create pseudo-hyporheic zone	Gravel: 0, 3, 7, 10 and 13 stones per 100 cm ²	Conductivity measurements	Minimum density of gravel that provides sufficient habitat for white sturgeon ELS without introducing excessive variation in water quality criteria as determined by conductivity (target value: < 30% variation among samples taken in the 1 cm water layer overlying the sediment surface across the exposure chamber).
3	Porewater sampling	Establish porewater sampling method	Airstone suction device in different depths of sediment using variable strength and duration of suction (via manual use of syringe). Initial volume to be collected 30 mL, with incremental changes of +/- 5 mL to obtain sufficient sample volume.	Only porewater is collected with no overlying water in the sample <ul style="list-style-type: none"> Dye concentration measurements. 	Extract only porewater and not overlying water in sufficient volume (target > 30 mL).
4	Sediment depth	Establish optimum depth of sediment for ELS sturgeon and to maximize porewater collection	Initial depth at 2 inches, with trials of 3 and 4 inches	Porewater sampling at 0.5 and 1 inch and overlying water sampling within the 1 cm of water overlying the sediment <ul style="list-style-type: none"> Dye concentration measurements. 	Optimal depth for maintaining flow conditions, surface-to-porewater gradients, and porewater collection.

Table A-5. Parameters, Methods, Measurements and Decision Criteria that will Inform the Design of the Exposure Systems and Test Conditions for the 2010 Studies with White Sturgeon ELS to Investigate Sediment Related Toxicity (listed here in order of operation) (continued)

Order	Parameter	Goal	Test Conditions	Measurement	Acceptance Criteria
5	Gradients between pore- and overlying water	Establish operational conditions that minimize gradients in water quality parameters between pore- and overlying water.	Each flow/sediment depth combination that is tested.	Time-resolved measurements of: <ul style="list-style-type: none"> • Dye concentration • Conductivity • DOC • pH 	Minimize gradient under optimal flow conditions.
6	Time to steady-state	Establish operational conditions that minimize time to steady-state.	Characterize time to steady-state between pore- and overlying water after establishing optimal flow and gravel conditions.	Time-resolved measurements of: <ul style="list-style-type: none"> • Alkalinity • Ammonia • Conductivity • DO • DOC • Hardness • pH 	Steady-state is attained when changes over the entire exposure period are not projected to exceed 20% for conductivity.
7	Cleaning methods	Establish most efficient method for cleaning	Introduce food 3X daily and scrape tanks at days 2, 3, 4 and 5.	Measure turbidity of samples using light scattering methods	Least turbidity per unit effort.
8	Laboratory control sediment	Define clean sediment with characteristics similar to UCR sediments	Research lab controls used in other bioassays Create sediment from clean silica sand and/or granite with grain size 0.5 to 2 mm and preference to dark color	Measure grain size and color	Grain size and color similar to UCR sediments.

APPENDIX A

STANDARD OPERATING PROCEDURES

LIST OF STANDARD OPERATING PROCEDURES

SOP-1	Positioning at Below-Water Stations
SOP-2	Below-Water Grab Sampling Procedures
SOP-3	Decontamination of Sampling Equipment
SOP-4	Sample Labeling
SOP-5	Field Documentation
SOP-6	Digital Camera Use and Documentation Procedures
SOP-7	Sample Custody
SOP-8	Standard Operating Procedure for Weighing Using Analytical Balances
SOP-9	Equipment Maintenance and Calibration
SOP-10	Experimental Exposure Systems for Field- and Laboratory-Based Studies of Aquatic Organisms Under Fluvial Conditions
SOP-11	Measurement of Water Quality and Processing of Water Samples During White Sturgeon (<i>Acipenser transmontanus</i>) Exposures
SOP-12	Data Package Review
SOP-13	Detection of Fluorescent Dye using the POLARstar OPTIMA

STANDARD OPERATING PROCEDURE SOP-1

POSITIONING AT BELOW-WATER STATIONS

Purpose

The purpose of this standard of practice (SOP) is to describe procedures used for locating sampling stations below water.

Scope and Applicability

This SOP is applicable for determining the horizontal and vertical location of below-water stations. The SOP applies to all below-water surface sediment groups regardless of collection device or whether it is a grab or core sample.

Equipment and Materials

The horizontal positioning equipment will consist of a global positioning system (GPS) instrument with Hypack or similar navigation software. The display will be capable of showing the present location of the vessel relative to the desired station location and will provide a bearing and distance to the station. The equipment will be capable of being pre-programmed with the National Oceanic and Atmospheric Administration (NOAA) nautical chart and sampling station locations. In the event normal GPS reception of four or more satellites is not available at a given location because of terrain blocking or other causes, alternative methods will be used to establish positions.

Vertical positioning will be done with the vessel fathometer, or, in shallow water, a lead line and tape measure or a surveyor's rod.

Typical Procedures/Guidelines

Horizontal Positioning

Horizontal positioning for below-water stations will be accomplished using differential GPS (DGPS) based on the U.S. Coast Guard (USCG) Maritime Differential GPS Service signal or GPS if the USCG differential signal cannot be received. USCG operates a GPS remote broadcast site from Spokane that broadcasts corrected GPS signals on marine radio beacon frequencies. Position errors with this system typically are within 1 to 3 meters (3.3 to 9.8 feet). The following

requirements apply to the GPS instrument and will be verified initially by the Field Team Leader (FTL) during the course of the work:

- The GPS unit will be configured such that satellites less than 8 degrees above the horizon will not be used in position computations.
- A minimum of four satellites will be used for computing all positions.

The GPS antenna will be mounted on the swing davit or top centerline of the A-frame. This will avoid the need for computing distance offsets for the antenna location.

If adequate GPS signals are not received, alternative methods for sample positioning will be used based on radar and/or laser rangefinder equipment. These methods will measure distances to at least two known points on the nautical chart or to two marker buoys equipped with radar reflectors and light reflective surfaces. These marker buoys will be deployed based on GPS positioning so that their locations can be plotted on a special navigation grid sheet along with the station locations to be sampled. The vessel will be navigated until it is within a circle of 40 meters (131 feet) or less around the station location. A buoy will be deployed to mark the position. This buoy will have a short mooring line slope of about 1.1 to 1 so as to minimize the lateral excursion of the buoy on the surface of the water. The vessel will be maneuvered so that the buoy is no more than 10 meters (33 feet) from the vessel at the time the sampler or corer contacts the bottom. A radar and/or laser rangefinder fix will be taken at the time of the grab or core.

The sampling vessel must locate and remain fixed on the general sampling location before sampling can begin. The vessel operator (VO) will be responsible for navigating the boat to each sample location. For submerged sediment sampling, the boat will be positioned so that it is within a circle of 20 meters (66 feet) or less around the planned position at the time that the sediment sampler or corer contacts the bottom. The FTL will verify that the sample location is within the allowable position circle. If the actual sample position is outside the 20-meter radius, the vessel will be re-positioned. If conditions are such that the vessel's position cannot be maintained within the 20-meter radius, the sample will not be taken.

All submerged sediment samples will be taken without anchoring the vessel except when current or wind conditions preclude holding the vessel on station within the 20-meter position circle. The VO and FTL will determine whether anchoring is required for taking the sediment sample.

When the vessel is not under power, it will typically swing perpendicular to the wind or current. If the sampling equipment is deployed over the side of the vessel instead of the stern, this will be done on the upwind or upcurrent side to prevent the vessel drifting onto the hydrowire (winch wire) once the sampler has contacted the bottom. In shallow water (less than about 10 meters

deep) with drift rates exceeding about 0.5 meter per second (m/sec) (1.6 feet per second [ft/sec]), the sampler may not perform correctly because of its lateral speed when it hits the bottom. Under high drift rates, it will be necessary to either anchor or hold the vessel in position using engine power. The adverse effects of drift rate decrease as the water depth increases because the vessel must drift a longer distance on the surface to pull the sampler out of alignment on the bottom.

The angle of the hydrowire to the vertical will be kept to approximately 5 degrees or less for all sampling activities, if possible, and will be measured using a wire angle indicator. Once wire angles exceed 5 degrees, the offset error between the vessel and the sampler increases significantly. If this condition occurs, the vessel will be repositioned and another sample attempt made.

The VO is responsible at all times for the safe and prudent operation of the boat and the conditions under which any operation will be performed. This is particularly important for operations under stormy conditions, in shallow water, in strong currents, or any situation that affects the maneuverability and stability of the boat. Although the FTL is responsible for all scientific operations, it is the VO who will be responsible for any actions concerning operation of the boat.

Vertical Positioning

The vertical position of below-water stations will be determined using a fathometer. In areas where the depth is too shallow, a lead line or survey rod will be used to measure the distance from the water surface to the riverbed. The depth to the station from the water surface will be converted to elevation based on the pool elevation established at the beginning of the day.

STANDARD OPERATING PROCEDURE SOP-2¹

BELOW-WATER GRAB SAMPLING PROCEDURES

Purpose

The purpose of this SOP is to describe sample collection and processing procedures for sediment samples collected using below-water grab sampling equipment.

Scope and Applicability

This sediment sampling SOP is divided into two procedures: the first procedure is applicable to sample locations that can be accessed by boat without disruption of bottom sediment or damage to the vessel, and the second procedure is applicable to below-water sample locations that are in shallow water or in areas that cannot be accessed by boat. Below-water sample locations include transect samples, focus area samples, tributary mouth samples, and bioassay and reference area samples (including samples from which porewater will be isolated). Under certain conditions (e.g., high water), beach samples may also be obtained from sediment that is submerged at the time of sampling. Vessel and sample positioning required prior to below-water sediment sampling is described in SOP-1, Positioning at Below-Water Stations.

Below-Water Sampling From Vessel

This procedure is applicable to below-water samples collected using a van Veen grab sampler operated from a vessel.

Equipment and Materials

The equipment and materials required for below-water grab sampling from a vessel are listed in Table 1.

Sample Collection Procedures

Vessels equipped with a van Veen grab sampler will be used to collect below-water sediment samples. A backup van Veen grab will also be available in the event that the primary grab is damaged, malfunctions, or is lost. Sampling locations will be approached at slow boat speeds with minimal wake to minimize disturbance of bottom sediments prior to sampling,

¹ This SOP is also SOP-4 in the April 2010 *Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon (Acipenser transmontanus)*.

particularly in shallow sampling locations. Sediment samples will be handled carefully to minimize disturbance during collection and transportation to the analytical laboratory.

Preparation for Sampling

After all sampling equipment and supplies have been transported to the work vessel, the van Veen grab can be assembled on deck. Because of the depth of water and the weight of the sampler, a hydraulic winching system will be used to control the rate of the sampler ascent and descent. When not in use, the sampler will always be secured when the vessel is underway.

The hydrowire will be attached to the sampler using a ball-bearing swivel (or similar hardware) to minimize any twisting forces during deployment and recovery. The hydrowire, swivel, and shackles will all have a working load capacity at least three times the weight of a full sampler (e.g., approximately 250 kilograms [kg] or 550 pounds [lb]).

At the direction of the field team leader (FTL), the vessel will move to the sampling station and, depending on surface wind and wave conditions, will be held or anchored on station using the differential global positioning system (DGPS) (or GPS if the DGPS cannot be received) and navigation system (see SOP-1, Positioning at Below-Water Stations). At this time, relevant information on sampling date, time, station location and coordinates, local water depth, and weather observations will be recorded in the field notebook in accordance with sample documentation requirements identified in the main body of the FSP.

Sampling Procedure

A decontaminated van Veen sampler (see SOP-3) will be used to collect the sediment samples. Once the sampler is ready for deployment, the sampler will be locked open with the safety pin. The sampler will be deployed using the hydraulic winch and an overhead davit or boom. While the winch operator slowly picks up the sampler with the hydrowire, another team member will be responsible for safely guiding the sampler over the side of the boat and into position over the intended sampling location. The safety pin will be removed while keeping hands and fingers outside the sampler. The deckhand will indicate to the winch operator when the sampler is at the surface and ready for sampling. At this time, the FTL will notify the vessel operator (VO) that sampling will commence and that the VO should prepare to collect a DGPS/GPS fix.

The position of the sampler relative to the riverbed can be shown by configuring the display of the vessel's depth sounder. Alternatively, the sampler may be lowered at a controlled rate of speed approximately equal to 30 centimeters per second (cm/sec) (1 foot per second [ft/sec]). The location of the sampler as it is lowered through the water column would then be determined either by rigging the hydrowire to a meter wheel or using pre-marked meter lengths on the cable itself.

Under no circumstances should the sampler be allowed to “free fall” to the bottom. Doing so may result in premature triggering of the sampler, or an excessive bow wake around the sampler, or improper orientation of the sampler. When the sampler reaches the mudline, the cable will be drawn taut and DGPS/GPS coordinates recorded. **Note: to ensure that the position fix is representative of the actual location sampled, the antenna for the GPS unit must be located as close as practical to the sampler (e.g., within 1 to 2 meters).** After the sample is collected, the sampling device will be lifted slowly off the bottom, then steadily raised to the surface at a speed of about 30 cm/sec (1 ft/sec). Care will be used to avoid swinging or tipping the sampler during retrieval.

Once aboard the vessel, the sampler will be gently lowered onto a sampling table or stand. This waist-high stand may be constructed of wood and should be large enough to hold the sampler and allow for at least two team members to retrieve the sample. Ideally, the stand will be approximately 1 meter wide by 1.5 meters long (3 feet by 4 feet), one end of which will extend over the transom or gunwale of the work vessel.

The access doors on the top of the sampler, which consist of wire mesh screens and rubber flaps, allow visual characterization of the sediment surface and aid in assessing sample acceptability. Each surface grab sample will be retrieved aboard the vessel and evaluated for the following acceptance criteria, which are illustrated generally in Figure 1:

- Overlying water is present and has low turbidity.
- Adequate penetration depth is achieved.
- The sampler is not overfilled (no contact with doors).
- The sediment surface is undisturbed.
- There are no signs of winnowing or leaking from the sampling device.

Grab samples not meeting these criteria will be rejected near the location of sample collection and steps repeated until the criteria have been met or until three attempts at a location have failed. Deployments will be repeated within a 20-meter (66-foot) radius of the proposed sample location, or other suitable location based on observed conditions, as determined by FTL. If adequate penetration is not achieved after multiple attempts, less volume will be accepted and noted in the field notebook. Chemical replicate samples will be a split sample. The following are minimum penetration depths based on sample grain size:

- Coarse to medium sand: 4 to 5 cm
- Fine sand: 6 to 7 cm
- Silt and clay: 10 cm

Before sample processing commences, the material in the sampler will be photographed and the photograph labeled with station location, date, and time of sample. The overlying water will then be siphoned off near one corner of the sampler. Next, a decontaminated stainless steel trowel or spoon may be used to collect only the upper 10 to 15 cm (4.5 to 6 inches) of sediment from inside the sampler, without touching the sidewalls. The sampler and sampling table will be decontaminated between stations and rinsed with site water between grabs in accordance with the equipment decontamination procedures described in SOP-3.

Sample Processing Procedures

After an acceptable grab sample is retrieved, the following processing steps will be completed on the vessel for all discrete grab samples (the procedures for processing of composite samples are in SOP-3):

1. Physical characterization will be performed in general accordance with the American Society for Testing and Materials (ASTM) D2488-00 visual-manual description procedure. The following information will be recorded on the Sediment Sample Data Sheet and signed by the FTL:
 - Date, type, and name of person logging the sample
 - Weather conditions
 - Sample location number and coordinates
 - Project designation
 - Depth of water at the location and surface elevation
 - Equipment used
 - Whether an examination was made by the cultural resources observer and whether the observer established the presence of any historical or cultural resources requiring this target sampling station to be abandoned, in accordance with the process described in the main body of the FSP
 - Sediment texture
 - Sediment color
 - Presence, type, and strength of odors
 - Grab penetration depth (nearest centimeter)
 - Degree of leakage or sediment surface disturbance
 - Any obvious abnormalities such as wood/shell fragments or large organisms

- Estimation of sample recovery volume (e.g., percent full)
- 2 The sediment will be transferred to an aluminum-foil-lined stainless steel bowl for homogenization with disposable hand tools. Sediment that is in direct contact with the sides of the grab sampler should not be included with the sediment placed in the bowl to avoid potential contamination from the device. Team members conducting the sample processing will don a clean pair of disposable nitrile gloves and collect the upper 10 to 15 centimeters of sediment using disposable hand tools.
- 3 Any obvious abnormalities present (e.g., wood/shell fragments or large organisms) and coarser grained sediment (e.g., pebbles and gravel) will be removed from the sample by hand or using disposable hand tools.
- 4 In the event that one grab sample does not have an adequate volume for the sample aliquot, repeat the sampling procedure at the same station and combine and thoroughly homogenize the samples.
- 5 The homogenized sample will be distributed into the appropriate pre-labeled sample containers, filled to capacity, and stored in a cooler with a maximum temperature of 4 degrees Celsius (°C).
- 6 The sampling equipment will be decontaminated according to the procedures described in SOP-3.
- 7 Any excess sediment remaining after processing will be returned to the water, and the deck will be rinsed clean after all grab samples are collected and before moving to the next station.
- 8 The sampler will be secured and moved to the next sample location.

Below-Water Sampling in Shallow Water

This procedure is applicable to below-water samples collected in shallow water using hand-operated equipment.

Equipment and Materials

The equipment and materials required for below-water grab sampling by hand in shallow water are listed in Table 2.

Sample Collection Procedures

Shallow-water sampling locations may be accessed by vessel or by land. The sampling team will be equipped with a petite ponar grab sampler and disposable hand tools, with backups

available in the event of equipment failure. Sediment samples will be handled carefully to minimize disturbance during collection and transportation to the analytical laboratory.

Preparation for Sampling

All sampling equipment and supplies will be transported to the sampling station by water vessel or land vehicle. For approach by water, the vessel will maneuver as close to shore as possible, to water depths of 2 feet or less, near the sampling station at the direction of the FTL; the field team will disembark with the necessary field equipment. Approach via land vehicle will use the best available path.

At the direction of the FTL, personnel will identify the sampling station using the DGPS (or GPS if the DGPS cannot be received) and navigation system (see SOP -1, Positioning at Below-Water Stations). The sampler will observe the general hydrologic and geologic conditions present at the proposed location and determine whether the location is suitable for sampling of fine-grained sediment. If the materials present are primarily gravel, cobbles, or rocks, an alternative sample location in the vicinity will be identified and the DGPS or GPS coordinates will be determined and recorded in the field notebook. All relevant information on sampling date, time, station location and coordinates, local water depth, and weather observations will be recorded in the field notebook in accordance with sample documentation requirements identified in the main body of the FSP.

Sampling Procedure

The petite ponar grab sampler is the preferred equipment for collection of sediment samples in shallow water areas. The petite ponar will be operated by hand. Under no circumstances should the sampler be allowed to “free fall” to the bottom. Doing so may result in premature triggering of the sampler, or an excessive bow wake around the sampler, or improper orientation of the sampler. After the sample is collected, the sampling device will be lifted slowly off the bottom, then steadily raised to the surface.

The access doors on the top of the sampler, which consist of wire mesh screens and rubber flaps, allow visual characterization of the sediment surface and aid in assessing sample acceptability. Each surface grab sample will be evaluated for the following acceptance criteria, which are illustrated generally in Figure 1:

- Overlying water is present and has low turbidity.
- Adequate penetration depth is achieved.
- The sampler is not overfilled (no contact with doors).
- The sediment surface is undisturbed.
- There are no signs of winnowing or leaking from the sampling device.

Grab samples not meeting these criteria will be rejected near the location of sample collection and steps repeated until the criteria have been met or until three attempts at a location have failed. If adequate penetration is not achieved after multiple attempts or the sample is too coarsely grained, multiple grabs of lesser volume will be accepted and noted in the field notebook. If all attempts at use of the petite ponar fail, disposable hand tools may be used to gather the sediment sample. At such locations, the reasons for using hand tools will be thoroughly detailed in the field notebook.

Before sample processing commences, the material in the sampler will be photographed and the photograph labeled with station location, date, and time of sample. The overlying water will then be siphoned off near one corner of the sampler. Next, a decontaminated stainless steel trowel or spoon may be used to collect only the upper 10 to 15 cm (4.5 to 6 inches) of sediment from inside the sampler, without touching the sidewalls. The sampler will be decontaminated between stations and rinsed with site water between grabs in accordance with the equipment decontamination procedures described in SOP-3.

Sample Processing Procedures

After an acceptable grab sample is retrieved, the following processing steps will be completed for all discrete grab samples (the procedures for processing of composite samples are in SOP-3):

1. Physical characterization will be performed in general accordance with the American Society for Testing and Materials (ASTM) D2488-00 visual-manual description procedure. The following information will be recorded on the Sediment Sample Data Sheet and signed by the FTL:
 - Date, type, and name of person logging the sample
 - Weather conditions
 - Sample location number and coordinates
 - Project designation
 - Depth of water at the location and surface elevation
 - Equipment used
 - Whether an examination was made by the cultural resources observer and whether the observer established the presence of any historical or cultural resources requiring this target sampling station to be abandoned, in accordance with the process described in the main body of the FSP
 - Sediment texture
 - Sediment color

- Presence, type, and strength of odors
 - Grab penetration depth (nearest centimeter)
 - Degree of leakage or sediment surface disturbance
 - Any obvious abnormalities such as wood/shell fragments or large organisms
 - Estimation of sample recovery volume (e.g., percent full)
- 2 The sediment will be transferred to an aluminum-foil-lined stainless steel bowl for homogenization with disposable hand tools. Sediment that is in direct contact with the sides of the grab sampler should not be included with the sediment placed in the bowl to avoid potential contamination from the device. Team members conducting the sample processing will don a clean pair of disposable nitrile gloves and collect the upper 10 to 15 centimeters of sediment using disposable hand tools.
 - 3 Any obvious abnormalities present (e.g., wood/shell fragments or large organisms) and coarser-grained sediment (e.g., pebbles and gravel) will be removed from the sample by gloved hand or by disposable hand tools.
 - 4 In the event that one grab sample does not have an adequate volume for the sample aliquot, repeat the sampling procedure at the same station and combine and thoroughly homogenize the samples.
 - 5 The homogenized sample will be distributed into the appropriate pre-labeled sample containers, filled to capacity, and stored in a cooler with a maximum temperature of 4°C.
 - 6 Any reusable sampling equipment will be decontaminated according to the procedures described in SOP-3.
 - 7 Any excess sediment remaining after processing will be returned to the water, and the deck will be rinsed clean after all grab samples are collected and before moving to the next station.
 - 8 The sampler will be secured and moved to the next sample location.

Table 1. Equipment List for van Veen Grab Sampling Operations

Description	Quantity
van Veen grab—0.11 m ² area (20-L capacity), stainless steel	1 ea
Lead weights for van Veen grab—3.4 kg (7.5 lb)	2 ea
Lead weights for van Veen grab—5.0 kg (11 lb)	2 ea
Plastic floats for van Veen Grab	2 ea
Spare parts and tool kit for van Veen grab	1 ea
Field Sampling Plan	1 copy
Daily sampling schedule	2 copies
Health and Safety Plan	1 copy
Personal protection equipment as required by the Health and Safety Plan	as needed for day ^a
Field notebook	1 ea
Camera—35 mm with flash unit (or digital with flash unit)	1 ea
For 35 mm camera—Film, 400/36, color print	2 rolls
For digital camera— memory cards	2 ea
Camera kit (batteries, charger, instructions, lens cleaner, and wipes)	1 ea
Pens—ballpoint, black ink	4 ea
Duct tape	2 rolls
Measuring tape	1 ea
Stainless steel ruler	1 ea
Decontamination equipment as listed in SOP-3	as needed for day ^a
Disposable tubing to siphon off water in sampler	as needed for day ^a
Disposable stainless steel spoons, scoops, or spatula for sediment sample transfer	as needed for day ^a
Aluminum-foil-lined stainless steel bowls for sediment homogenization	as needed for day ^a
Sample bottles as specified in the sample matrix table	as needed for day ^a
Coolers with ice for sample storage	as needed for day ^a
Field data sheets, chain-of-custody forms, sample labels, custody seals, and related materials	as needed for day ^a
Nitrile gloves	as needed for day ^a
Paper towels or Kimwipes	as needed for day ^a

^a To be determined for each day's sampling and stocked before vessel departs.

ea = each; kg = kilogram; L = liter; lb = pound; m² = square meter; mm = millimeter

Table 2. Equipment List for Petite Ponar Grab Sampling Operations

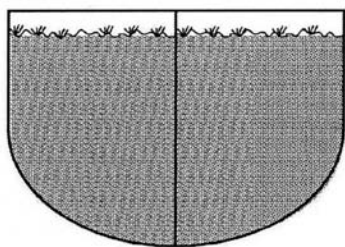
Description	Quantity
Petite ponar grab sampler (2.5-L capacity), stainless steel	1 ea
Spare parts and tool kit for petite ponar grab	1 ea
Field Sampling Plan	1 copy
Daily sampling schedule	2 copies
Health and Safety Plan	1 copy
Personal protection equipment as required by the Health and Safety Plan	as needed for day ^a
Field notebook	1 ea
Camera—35 mm with flash unit (or digital with flash unit)	1 ea
For 35 mm camera—Film, 400/36, color print	2 rolls
For digital camera— memory cards	2 ea
Camera kit (batteries, charger, instructions, lens cleaner, and wipes)	1 ea
Pens—ballpoint, black ink	4 ea
Duct tape	2 rolls
Measuring tape	1 ea
Stainless steel ruler	1 ea
Decontamination equipment as listed in SOP-3	as needed for day ^a
Disposable tubing to siphon off water in sampler	as needed for day ^a
Disposable stainless steel spoons, scoops, or spatula for sediment sample transfer (and for last resort sample collection)	as needed for day ^a
Aluminum-foil-lined stainless steel bowls for sediment homogenization	as needed for day ^a
Sample bottles as specified in the sample matrix table	as needed for day ^a
Coolers with ice for sample storage	as needed for day ^a
Field data sheets, chain-of-custody forms, sample labels, custody seals, and related materials	as needed for day ^a
Nitrile gloves	as needed for day ^a
Paper towels or Kimwipes	as needed for day ^a

^a To be determined for each day's sampling and stocked before vessel departs.

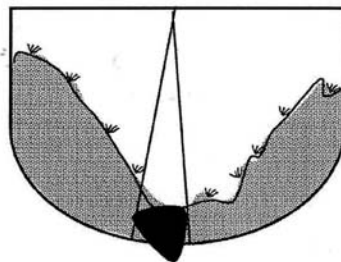
ea = each

L = liter

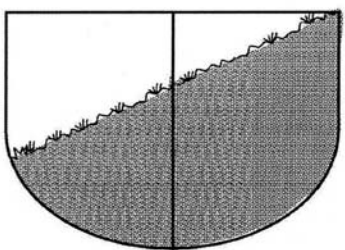
mm = millimeter



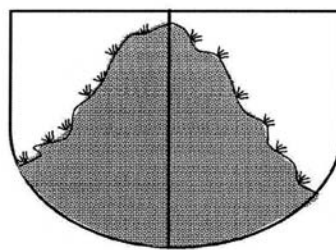
Acceptable if Minimum
Penetration Requirement Met
and Overlying Water is Present



Unacceptable
(Washed, Rock Caught in Jaws)



Unacceptable (Canted with
Partial Sample)



Unacceptable
(Washed)

Figure 1. Examples of Acceptable and Unacceptable Grab Samples

STANDARD OPERATING PROCEDURE SOP-3

DECONTAMINATION OF SAMPLING EQUIPMENT

Purpose

The purpose of this standard of practice (SOP) is to describe decontamination of sampling equipment prior to, during, and after sampling.

Scope and Applicability

This SOP for decontamination of sampling equipment is applicable to nondisposable sampling equipment that comes into contact with sediment. All nondisposable equipment will be decontaminated between uses. This means that decontamination will occur at the following times:

- Prior to commencement of the sampling event
- Between sample locations
- Between sampling intervals
- Upon completion of the sampling event

It is not necessary to decontaminate sampling equipment between multiple grabs from the same location, or between grabs from different locations that will be composited together to form a single composite sample.

The rigor of decontamination depends on the nature of the equipment, as follows:

- Rigorous decontamination will be performed on all equipment used to directly handle and process sediment samples for analysis. This equipment includes sample spoons, knives, bowls, trowels, and any other nondisposable equipment that comes into contact with sediment that will be submitted for analysis.
- Limited decontamination will be performed on equipment used to access and gather submerged sediment. This equipment includes the van Veen grab sampler, the Eckman box dredge, and other similar devices. The possibility of cross-contamination using this equipment is limited because sediment gathered by them will be removed for sample processing without touching the sidewalls of the sampler.

Equipment and Materials

- Distilled water rinse
- Potable water rinse
- Alconox (or other nonphosphate detergent) and potable water solution
- 10 percent nitric acid solution (HNO_3)
- Hexane
- Large plastic tubs and buckets for detergent solution and water rinse
- Spray bottles
- Nylon bristle brushes
- Plastic sheeting
- Sealable tubs or drums to contain rinsate fluids
- Aluminum foil
- Personal protective equipment (PPE), including nitrile gloves and goggles
- Paper towels or Kimwipes

Equipment Decontamination Procedures

Preparation for Decontamination

Safety goggles and nitrile gloves will be worn by all personnel who are assisting in the decontamination of equipment. Sealable containers, such as tubs, will be used to collect all rinsate fluids.

Decontamination Procedure for Sediment Samplers

The field sampling team will decontaminate large sampling equipment using the following steps:

1. Rinse excess sediment from equipment using site water.
2. Wash all equipment surfaces that contacted the potentially contaminated sediment with detergent solution (Alconox or other laboratory-grade detergent), using a brush as needed to remove particulate matter and surface films.
3. Rinse with potable water.
4. Maintain decontaminated equipment in a position and location where it will not come into contact with contaminants.
5. Transfer decontamination fluids to a sealable container.

Decontamination Procedure for Nondisposable Sample-Handling and Processing Equipment

The field sampling team will decontaminate nondisposable sampling-handling and processing equipment using the following steps:

1. Rinse excess sediment from equipment using site water.
2. Transfer equipment to decontamination station tubs.
3. Wash all equipment surfaces that contacted the potentially contaminated sediment with detergent solution (Alconox or other laboratory-grade detergent), using a brush as needed to remove particulate matter and surface films.
4. Rinse with potable water.
5. Rinse with 10 percent nitric acid (HNO₃).
6. Rinse with distilled water in spray bottle.
7. Spray with hexane in spray bottle.
8. Air dry.
9. Rinse with distilled water in spray bottle.
10. Air dry.
11. Wrap equipment with aluminum foil, if appropriate, to reduce the need for subsequent cleaning if equipment is to be stored or transported.
12. Transfer decontamination fluids to a sealable container.

Key Checks/Items

The need for rigorous decontamination procedures is expected to be limited because all sample handling and processing equipment that comes into direct contact with the sediment submitted for analysis will be disposable. Under these circumstances, field blanks demonstrating the adequacy of decontamination are not required and are not included as part of the field sampling program.

STANDARD OPERATING PROCEDURE SOP-4

SAMPLE LABELING

Scope and Applicability

This standard operating procedure (SOP) describes the general procedures for sample labeling and specifically, the labels that will be used for sediment collection.

Sample Identifier Labels

Sample identifiers will be established before field sampling begins and assigned to each sample as it is collected. Preprinted, barcode labels should be used, when possible. Labels must be printed on waterproof paper with waterproof adhesive backing.

Sample identifiers consist of codes designed to fulfill three purposes: 1) to identify related samples (i.e., composites) to ensure proper data analysis and interpretation; 2) to obscure the relationships between samples so that laboratory analysis will be unbiased by presumptive similarities between samples; and 3) to track individual sample containers to ensure that the laboratory receives all of the material associated with a single sample. To accomplish these purposes, each container may have two different codes associated with it: the individual sediment sample number(s), and the composite numbering. These codes and their uses are described below.

Individual Sample Numbering

Each distinct sampling location will be assigned a unique identifier. Sample IDs will be numbered sequentially beginning with the letters "TAI" (Teck American, Inc.), country, location, site, sampler type, and grab (or porewater suction) number (e.g., TAI-US-DME-1-VV-1). The codes will include the following information:

Country:

CAN = Canada

US = United States

Location:

DME = Deadman's Eddy

BBE = Birchbank Eddy

GE = Genelle Eddy

KE = Kootenay Eddy

LALL = Lower Arrow Lake Lower

Site:

1, 2, 3, ... N

Sample type:

VV = van Veen sampler

EBC = Eckman box corer

PD = Ponar dredge

STANDARD OPERATING PROCEDURE SOP-5

FIELD DOCUMENTATION

Scope and Applicability

The integrity of each sample from the time of collection to the point of data reporting must be maintained throughout the study. Proper record keeping will be implemented in the field to allow samples to be traced from collection to final disposition.

All information pertaining to field operations during sample collection must be properly documented to ensure transparency (and reproducibility) of methods and procedures. Several types of field documents will be used for this purpose by field personnel.

Field Logbooks

During field sampling events, field logbooks are used to record all daily field activities on each vessel used for sediment collection. The purpose of the field logbook is to document events that occur during field activities and to record data measured in the field to ensure transparency and reproducibility.

The field logbook is the responsibility of, and maintained by the Field Team Leader (FTL) for each vessel. The site logbook will be placed in the project files at the conclusion of field activities.

The field logbook will be bound and waterproof with consecutively numbered pages. All entries will be made using indelible ink and no erasures will be made. Any necessary corrections in the logbook should consist of a single line-out deletion, followed by the author's initials and the date. The author will initial and date each page of the field logbook, sign and date the last page at the end of each day, and draw a line through the remainder (unused portion) of that page.

- The project name, dates of the field work, site name, and location (city and state) should be written on the cover of the field logbook. If more than one logbook is used during a single sampling event, then the upper right hand corner of the logbook will be annotated (e.g., Volume 1 of 2, 2 of 2) to indicate the number of logbooks used during the field event. Field logbooks will be stored in a secure manner when not in use in the field. At a minimum, the following information will be recorded in the field logbook:
- Project name and location.

- Purpose and description of the field task.
- Project start date and end date.
- Date and time of entry (24-hour clock).
- Time and duration of daily sampling activities.
- Weather conditions at the beginning of the field work and any changes that occur throughout the day, including the approximate time of the change (e.g., wind speed and direction, rain, thunder, wave action, vessel traffic, temperature of both the air and water).
- Name and affiliation of person making entries and other field personnel and their duties, including the times that they are present.
- The location and description of the work area, including sketches, map references, and photograph log, if appropriate.
- Level of personal protection being used.
- Onsite visitors (names and affiliations), if any, including the times that they are present (e.g., cultural resource personnel, agency observers, etc.).
- The name, affiliation, and telephone number(s) of any key field contacts.
- Notation of the coordinate system used to determine the station location information.
- The sample identifier and analysis code for each sample to be submitted for laboratory analysis, if not included on separate field data sheets.
- All field measurements made (or reference to specific field data sheets used for this purpose), including the time that the measurement was collected and the date of calibration, if appropriate.
- The sampling location name, date, gear, water depth (if applicable), and sampling location coordinates, if not included on separate field data sheets.
- The type of vessel used (e.g., size, power, type of engine) (for aquatic sampling only).
- Specific information on each type of sampling activity.
- The sample type (e.g., surface sediment), sample number, sample tag number, and preservatives used (if any), if not included on separate field data sheets.
- Sample storage methods.
- Cross-references of numbers for duplicate samples.
- A description of the sample.
- Photographs (uniquely identified) taken at the sampling location, if any.
- Details of the work performed.
- Variations, if any, from the project-specific Quality Assurance Project Plan (QAPP) or standard operating protocols and reasons for deviation.

- Details pertaining to unusual events which might have occurred during sample collection (e.g., possible sources of sample contamination, equipment failure, unusual appearance of sample integrity).
- References to other logbooks or field forms used to record information (e.g., field data sheets, health and safety log).
- Sample shipment information (e.g., shipping manifests, COC form numbers, carrier, air bill numbers, time addresses).
- A record of quantity of investigation derived wastes (if any) and storage and handling procedures.

During the field day, as listed above, a summary of all site activities should be recorded in the logbook. The information need not duplicate anything recorded in other field logbooks or field forms (e.g., Site Health and Safety Officer's logbook, calibration logbook, field data sheets), but should summarize the contents of the other logbooks and refer to the page locations in these logbooks for detailed information.

If measurements are made at any location, the measurements and equipment used must either be recorded in the field logbook or reference must be made to the logbook and page number(s) on which they are recorded. All maintenance and calibration records for equipment should be traceable through field records to the person using the instrument and to the specific piece of instrumentation itself.

Upon completion of the field sampling event, the FTL will be responsible for submitting all field logbooks to be copied. A discussion of copy distribution is provided below.

Sample Processing and Field Data Forms

Sample processing and field data forms will be generated during this field sampling event to record the relevant sample information collected during a sampling event. Upon completion of the field sampling event, the FTL will be responsible for submitting all field data forms to be copied. A discussion of copy distribution is provided below.

Equipment Calibration Records

Equipment calibration records, including instrument type and serial number, calibration supplies used, calibration methods and calibration results, date, time, and personnel performing the calibration, should be recorded in the field logbook. At a minimum, equipment used during the investigation should be calibrated daily in accordance with the manufacturers' recommendations.

Distribution of Copies

Two copies of all field logbooks and additional field data forms will be made at completion of the field event. The first copy will be stamped with a “COPY” stamp. This copy will be placed in the project file and will be available for general staff use. The second copy will be stamped with a “FILE” stamp. This copy will be placed in the data management file with the laboratory data packages and will be used by the data management and quality assurance staff only. The original field logbooks and forms will be placed in a locked file cabinet at the Project Manager’s location.

STANDARD OPERATING PROCEDURE SOP-6¹

DIGITAL CAMERA USE AND DOCUMENTATION PROCEDURES

Purpose

The purpose of this standard of practice (SOP) is to describe the use of digital cameras and procedures for digital camera data management.

Scope and Applicability

This SOP is applicable to taking digital photographs and placing the digital data in a database. Digital photographs may be taken to document field activities, site conditions and features, and sampling locations.

Equipment and Materials

- Digital camera
- Spare batteries
- 12-volt charger
- Digital camera carrying case and manual
- Digital Photograph Data Form
- Permanent marker
- Personal computer

Typical Camera Features

- Save pictures (in standard mode) directly to a memory stick or comparable
- Auto focus; manual focus available if required
- Zoom
- Brightness control
- Playback of photos on camera screen
- Display of photograph number, date, and time

¹ This SOP is also SOP-3 in the April 2010 *Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon (Acipenser transmontanus)*.

- Flash
- Timer
- Display showing time remaining on battery and remaining disk capacity
- Ability to protect and delete images that have been taken

Camera Use

Digital cameras will be used by the field team to document field activities. Each field team will be directly responsible for the camera and ensure that it is not exposed to excessive heat/cold or moisture. The field team leader (FTL) will be responsible for digital photograph documentation or for assigning documentation duties to a team member.

Digital photographs will be taken to document field activities and locations. Examples of field activities for which photo documentation will be useful include: (1) sample collected in bowls, van Veen, or other collection device along with station and sample identifiers, (2) station vicinity with associated river mile and compass directions noted, and (3) field sampling techniques utilized, such as equipment use/operation.

Digital photographs will be collected at a high-pixel setting such that enlargements can be made with minimal degradation in picture quality.

Photograph Documentation

Field Team Responsibilities

Each field team will keep a daily hard copy log of all photographs. The following digital photograph data will be collected:

- Camera identifier (type, model, equipment number)
- Project and Event ID—this information is obtained from the FTL.
- Team members—list each team member.
- Photograph number—record the number of the photograph and the photograph file name (as coded below).
- Date and time—as provided by the camera display .
- Description—the target of the photograph.
- Station—identify a station ID (such as sample location ID), if applicable.
- Northing and easting—list the coordinates of the subject of the photograph and the compass direction in which the picture is being taken. Coordinates can be acquired from a global positioning system (GPS) or from the GIS system if the location has been surveyed. Coordinates do not need to be recorded for a station if the location was surveyed prior to photograph collection.

Notes—record any other pertinent information (including coordinates of location where picture was taken [see above]).

Digital Photograph File Name

At the end of each field day, the member of the field team who is responsible for the camera will transfer the electronic data from the camera to the field operations computer. The folder structure will be as follows:

\\DATA\PHOTOS\VESSEL_xx\YYYYMMDD\LOCATION\file [1, 2, 3,N]

The third structure level shown above (e.g., VESSEL xx, VESSEL yy) continues for all sampling teams. The notation YYYYMMDD represents the year, month, day. The LOCATION is general area in the river (e.g., Marcus Flats, China Bend). The individual files for the day (e.g., file1, file 2, file N) will be placed within this folder using the default file identifier provided by the camera.

Transfer of Information and Archive

After the photograph disks have been uploaded, the original hard copy of the photograph log will be initialed and dated by the team member who downloaded the photographs, then archived by the FTL.

Sample Processing Coordinator Responsibilities

The FTL will be responsible for: (1) reviewing electronic photographs and the logs as they are made available to ensure consistency and completeness of annotations; (2) collecting and archiving the hard copies of the photograph logs; (3) reviewing electronic photographs and the logs as they are made available to ensure consistency and completeness of annotations; and (4) notifying the sampling team leader of apparent inconsistencies and making recommendations for corrective action.

Key Checks/Items

- Make sure the camera's battery is fully charged on a daily basis
- Keep extra memory sticks available
- To save battery life, use flash only when necessary
- Make sure the camera quality level is set at "best" or equivalent (High pixel)
- Review photograph records periodically to ensure that the electronic photographs and the data log agree
- Leave enough time at the end of the field day to transfer the data

STANDARD OPERATING PROCEDURE SOP-7

SAMPLE CUSTODY

Scope and Applicability

This SOP describes procedures for custody management of environmental samples. Chain-of-custody (COC) forms ensure that samples are traceable from the time of collection through processing and analysis until final disposition. A sample is considered to be in a person's custody if any of the following criteria are met:

- 1 The sample is in the person's possession
- 2 The sample is in the person's view after being in possession
- 3 The sample is in the person's possession and is being transferred to a designated secure area
- 4 The sample has been locked up to prevent tampering after it was in the person's possession.

At no time is it acceptable for samples to be outside of project personnel's custody unless the samples have been transferred to a secure area (i.e., locked up and custody sealed). If the samples cannot be placed in a secure area, then a field team member must physically remain with the samples (e.g., at lunch time one team member must remain with the samples).

Chain-of-Custody Forms

The chain-of-custody (COC) form is critical because it documents sample possession from the time of collection through the final disposition of the sample. The form also provides information to the laboratory regarding what analyses are to be performed on the samples that are shipped.

The COC form will be completed after each field collection activity and before the samples are shipped to the laboratory. Sampling personnel are responsible for the care and custody of the samples until they are shipped. When transferring possession of the samples, the individuals relinquishing and receiving the samples must sign the COC form(s), indicating the time and date that the transfer occurs. The COC forms each consist of 3-part carbonless paper with white, yellow, and pink copies. The white sheet and the yellow sheet will be placed into a plastic sealable bag and secured to the inside top of each transfer container (e.g., cooler). The pink sheet will be retained by the field staff for filing at the Project Manager's location. Each COC form has a unique number. This number and the samples on the form shall be recorded in the field

logbook. Computer-generated COC forms may be used instead of the hardcopy forms. If computer-generated forms are used, then the forms will be printed in triplicate, sequentially numbered, and all three sheets signed so that two sheets can accompany the shipment to the laboratory and one sheet can be retained on file at the Project Manager's location.

The individual sample labels will be recorded on the COC form. The COC form will also identify the sample collection date and time, the type of sample, the project, and the sampling personnel. In addition, the COC form provides information on the preservative or other sample pretreatment applied in the field and the analyses to be conducted by referencing a list of specific analyses or the statement of work for the laboratory. The COC form will be sent to the laboratory along with the sample(s).

Procedures

The following guidelines will be followed to ensure the integrity of the samples:

1. At the end of each sampling day and prior to shipping or storage, COC entries will be made for all samples and COCs will be filled out for all samples. Information on the COCs will be checked against field logbook entries.
2. At the bottom of each COC form is a space for the signatures of the persons relinquishing and receiving the samples and the time and date that the transfer occurred. Usually the FTL will relinquish the samples. The time that the samples were relinquished should match. Each COC form must be appropriately signed and dated by the sampling personnel. The person who relinquishes custody of the samples must also sign this form.
3. The COC form should not be signed until the information has been checked for inaccuracies by the FTL. All changes should be made by drawing a single line through the incorrect entry and initialing and dating it. Revised entries should be made in the space below the entries. Any blank lines remaining on the COC form after corrections are made should be marked out with single lines that are initialed and dated. This procedure will preclude any unauthorized additions.
4. At the bottom of each COC form is a space for the signatures of the persons relinquishing and receiving the samples and the time and date that the transfer occurred. The time that the samples were relinquished should match exactly the time they were received by another party. Under no circumstances should there be any time when custody of the samples is undocumented.
5. If samples are sent by a commercial carrier not affiliated with the laboratory, such as Federal Express (FedEx) or United Parcel Service (UPS), the name of the carrier should be recorded on the COC form. Any tracking numbers supplied by the carrier should be also entered on the COC form. The time of transfer should be as close to the actual drop-off time

as possible. After the COC forms are signed and the “pink” copy has been removed, they should be sealed inside the transfer container.

6. If errors are found after the shipment has left the custody of sampling personnel, a corrected version of the forms must be made and sent to all relevant parties. Minor errors can be rectified by making the change on a copy of the original with a brief explanation and signature. Errors in the signature block may require a letter of explanation.
7. Samples that are archived internally at Parametrix or a Parametrix authorized laboratory must be accompanied by a COC form and an Archive Record form.
8. Upon completion of the field sampling event, the FS will be responsible for submitting all COC forms to be copied.

Custody Seal

As security against unauthorized handling of the samples during shipping, two custody seals will be affixed to each sample cooler. The custody seals will be placed across the opening of the cooler (front right and back left) prior to shipping. Be sure the seals are properly affixed to the cooler so they cannot be removed during shipping. Additional tape across the seal may be prudent.

Shipping Air Bills

When samples are shipped from the field to the testing laboratory via a commercial carrier (e.g., Federal Express, UPS), an air bill or receipt is provided by the shipper. Upon completion of the field sampling event, the FS will be responsible for submitting the sender’s copy of all shipping air bills to be copied. The air bill number (or tracking number) should be noted on the applicable COC forms or alternatively the applicable COC form number should be noted on the air bill to enable the tracking of samples if a cooler becomes lost.

Acknowledgement of Sample Receipt Forms

In most cases, when samples are sent to a testing laboratory, an Acknowledgment of Sample Receipt form is faxed to the project QA/QC coordinator the day the samples are received by the laboratory. It is the responsibility of the person receiving this form (designated by Project Manager) to review the form and make sure that all the samples that were sent to the laboratory were received by the laboratory and that the correct analyses were requested. If an error is found, the laboratory must be called immediately. Decisions made during the telephone conversation should be documented in writing on the Acknowledgment of Sample Receipt Form. In addition, corrections should be made to the COC form and the corrected version of the COC form should be faxed to the laboratory.

The Acknowledgment of Sample Receipt form (and any modified COC forms) will then be submitted to be copied.

Archive Record Forms

On occasion, samples are archived at an authorized laboratory. If samples are to be archived, it is the responsibility of the project manager or analytical laboratory manager to complete an Archive Record form. This form is to be accompanied by a copy of the COC form for the samples, and will be placed in a locked file cabinet. The original COC form will remain with the samples in a resealable plastic bag.

STANDARD OPERATING PROCEDURE SOP-8¹

STANDARD OPERATING PROCEDURE FOR WEIGHING USING ANALYTICAL BALANCES

Purpose

This SOP specifies sample weighing procedures for the members of the Environmental Toxicology Laboratory (ETL).

Scope and Applicability

This SOP applies to the ETL for samples supplied from the Upper Columbia River white sturgeon studies

Procedures for Weighing Samples

When weighing samples or chemicals the following procedures are to be followed:

1. Check level and cleanliness of balance.
2. Balances must not be affected by room drafts; close balance doors.
3. Always weigh into containers or use weigh papers. Under no circumstances should excess standard or reagent be returned to its original container.
4. Weigh quickly. It is better to accurately weigh close to a desired weight in one or two steps than to repeatedly manipulate a sample to obtain a round number. For example 10.02 and 9.81 are just as good weights as 10.00.
5. Objects weighed must be at room temperature.
6. Choose a balance appropriate for the mass to be weighed. For example, a 4 decimal place balance is need for a 0.0100 g standard whereas a 40.00 g soil sample requires only a 2 place balance.
7. At the beginning of each phase of the study the balance must be checked using standard reference weights and the results recorded on the Analytical Balance Maintenance Calibration Record form.
8. Routine and preventative maintenance is purchased from the balance manufacturer at recommended intervals, nominally once in 2 years.

¹ This SOP is also SOP-11 in the April 2010 *Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon (Acipenser transmontanus)*.

STANDARD OPERATING PROCEDURE SOP-9¹

EQUIPMENT MAINTENANCE AND CALIBRATION

Purpose

The purpose of this SOP is to provide guidelines for the maintenance and calibration of equipment used in the Upper Columbia River (UCR) white sturgeon studies conducted by the University of Saskatchewan (U of S) Environmental Toxicology Laboratory (ETL).

Scope and Application

This SOP will be applied to all equipment used in the UCR white sturgeon studies including multi-parameter meters, pipettors, and balances.

Safety Considerations

There are no safety issues for this SOP.

Equipment, Materials, and Reagents

The equipment used in UCR white sturgeon studies will be:

- Sartorius balance (Mettler Toledo, Inc, serial number 37030129)
- Ohaus Adventurer Pro balances (VWR, Cat. #11379-144)
- Certified balance weights (Troemner Inc., serial number 13637)
- VWR sympHony Multiparameter Research meters (VWR, Cat #11388-328)
- Pipetman pipettors (P100, P200, P1000, P5000).

Method, Procedures, and Requirements

This SOP will provide details on the methods used to calibrate and maintain equipment used in the UCR white sturgeon experiments. In cases where the calibration procedures are identical to the manufacturer's descriptions, it will refer to these and the according document will be included with this SOP as an attachment.

¹ This SOP is also SOP-12 in the April 2010 *Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon (Acipenser transmontanus)*.

Equipment Calibration

Balances (Sartorius, Mettler, and Ohaus)

The balances will be calibrated before each use. They will be calibrated using check weights provided by the manufacturer. The weight of the check weights will be recorded in a balance log along with the date of use, user's name, and whether the balance recorded the check weights within 10 percent (Attachment 1a through 1c).

VWR symphony Multiparameter Research Meters

pH probe

The pH meter will undergo a two point calibration before each use. Two standard buffer solutions are recommended for precise calibration. The first, near the electrode isopotential point (pH 7.0) and the second near the expected sample pH (pH 4.0 or 10.0). Calibration will be recorded on a data sheet once it is performed (Attachment 2a).

Conductivity probe

The conductivity meter will undergo a two point calibration before each use. Standard buffer solutions will be used. Calibration will be recorded on a data sheet once it is performed (Attachment 2b).

Dissolved oxygen (DO) probe

No user calibration is required (Attachment 2c).

Temperature probe

No user calibration is required.

Note that a similar research meter of another brand or model may be selected instead of the VWR symphony Multiparameter Research Meter. A single research meter will be used from the beginning to the end of each study to ensure consistent results.

Pipetman pipettors

Pipettors will be calibrated on a weekly basis. Each pipettor will be used to draw up a volume of liquid that will then be weighed to confirm that the volume is correct. The serial number of each pipettor will be recorded along with the date, presumed volume, weight of the liquid, calibrator's name and whether the pipettor was adjusted will be recorded on a log sheet (Attachments 3a and 3b).

Equipment Maintenance

Balances

The balances will be brushed off after each use, and kept covered between uses.

VWR symphony Multiparameter Research Meters

Between measurements, the probes will be rinsed with distilled water. The filling hole cover will be removed when taking measurements, but will be put back in place during storage. The

pH probe will be kept in buffer solution of 3M KCl in between uses. All other probes will be stored according to manufacturer's protocol (Attachments 2a through 2c).

Records, Documentation, and Quality Control Requirements

All maintenance and calibration procedures shall be entered in the appropriate notebooks and instrument logs. Instrument logs are kept with each piece of equipment, and a copy of all files is kept with Shanda Sedgwick in Room 125 at the Toxicology Building.

Responsibilities

Project Manager. Dr. Markus Hecker will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principal investigator and other U of S personnel, Teck American personnel, and the sponsor Project Manager.

Principal Investigator. Prof. John P. Giesy will advise the Project Manager in overseeing and approving all project activities, authorize necessary actions and adjustments related to U of S activities to accomplish program quality assurance (QA) objectives; and act as liaison between agencies, staff, and the sponsor Project Manager.

Study Team Leaders (STLs). David Vardy and Jonathon Doering, under the supervision of Markus Hecker, will oversee all research activities and supervise all personnel involved with the assemblage of the experimental exposure systems. The STLs will ensure that proper sample collection, preservation, storage, transport, and COC quality control (QC) procedures are followed and will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

Quality Assurance (QA) Manager. Dr. Shaun Roark will initiate audits on work completed by project personnel. The manager will review program QA activities, quality problems, and quality-related requests. In response to experimental findings, the QA manager will approve corrective actions. The QA manager will report quality non-conformances to the Project Manager.

Questions or Comments

Please feel free to contact the following persons with any questions, comments, etc., you may regarding the procedures outlined in this SOP.

Markus Hecker
mhecker@entrix.com
(306) 966-5233

Paul D. Jones, Ph.D.
paul.jones@usask.ca
(306) 966-5062

John P. Giesy, Ph.D.
john.giesy@usask.ca
(306) 966-2096

Recommendation for your weighing system

GWP® Recommendation:	MTCDN-101-20091130-11
Company:	University of Saskatchewan
Contact:	Howard Zhang
Department / Position:	Toxicology Centre
Street:	44 Campus Dr
Zip Code / City:	Saskatoon, S7N 5B3
State / Country:	Saskatchewan
Phone:	(306) 966-1204
Email:	xiao.wei.zhang@usask.ca

Characterization of your weighing process

- | | |
|--|-------|
| • Accuracy of weighing process | 2 % |
| • Smallest net weight | 10 mg |
| • Maximum weighing load | 200 g |
| • Safety factor | 1 |
| • Impact of wrong measuring results on the business process | low |
| • Impact of wrong measuring results on humans, animals and the environment | low |
| • Wrong measuring results can be discovered very easily | yes |
| • Applicable standards and regulations | none |

GWP® Recommendation: MTCDN-101-20091130-11

Balance

AB265-S

**Weighing Parameters**

Minimum weight according to the specifications for 2 % accuracy
(Expansion factor $k = 2$, without safety factor)

Specified: 3 mg¹⁾ Required: 10 mg

Safety factor

Achieved: 3.3 Required: 1

For the determination of the actual minimum weight the issuing of a minimum weight certificate is recommended.

Installation, qualification and training

Starter Pac

**Service contract**

Maintenance

Tests²⁾

Calibration (by service)	yearly
Repeatability (by user)	monthly
Sensitivity (by user)	quarterly

**Weights**

Weight 1:	200 g	Class:	F2, F1 or E2
Weight 2:	10 g	Class:	F1 or E2



For correct handling of weights we recommend the use of appropriate tweezers and/or gloves.

Recalibration interval of weights:³⁾ every three years

Test tolerances⁴⁾

Sensitivity	
Weight 1:	Control limit: 2 g
Weight 2:	Control limit: 100 mg
Repeatability	
Weight 2:	Control limit: 0.1 mg



GWP® Recommendation: MTCDN-101-20091130-11

Remarks

- 1) The minimum weight depends on the settings of the balance, the tare weight as well as the balance's environment. The minimum weight of your instrument in situ can be smaller than the specified value given in the Operating Instructions. A precise determination of the minimum weight on site is documented in the METTLER TOLEDO Minimum Weight Certificate.
- 2) Original recommendation for Repeatability (by user): none
Reason for the change: Required for the verification of the pipettes.
- 3) If country specific regulations apply, they have to be considered.
- 4) Various weighing parameters can contribute to the measurement uncertainty of a weighing result. While repeatability is the dominant uncertainty factor when weighing small net amounts, parameters such as sensitivity have a greater influence with larger net weights. With the exception of the repeatability test, all control limits are set to 1/2 of the weighing accuracy in order to have a security reserve accounting for any other influences on the result (control limit = test weight * weighing accuracy / 2).
For repeatability tests however, the influence of the other parameters are negligible for small test weights, thus a security reserve is not necessary for the control limit (control limit = weighing accuracy * smallest net weight / k).
If a safety factor >1 is defined, then the warning limits of the appropriate parameters are calculated by the control limits divided by the safety factor.

General remarks

Used in the toxicology environment and for the verification of pipettes.

GWP® Recommendation: MTCDN-101-20091130-11

Disclaimer

These general recommendations are for information only and are not binding in any way. To reach utmost weighing accuracy, and to allow an optimal use of the weighing instruments, it is necessary to conduct calibration and test procedures in a regular and timely manner. This document is offered as a recommendation to improve weighing accuracy and to optimize the use of the weighing instruments. It is based on specifically selected parameters like risk and process tolerance. Other factors, which might also have an influence, like installation location, history of the instrument, experience of laboratory staff, safety management system of user etc., have not been taken into account. Therefore these intervals are to be considered as recommendations. Depending on the circumstances the use of modified / other time schedules might be appropriate. The final responsibility for the test procedures is with the user of the equipment. This recommendation does not extend our warranty in any way.

GWP® Recommendation V 2.4, Tables V 2.44

Created on Monday, November 30, 2009 by

Michael Birtzu

Email michael.birtzu@mt.com

www.mt.com/GWP

For more information

**Mettler-Toledo Inc.
Laboratory Division**

2915 Argenta Road, Uuni 6
Mississauga, Ontario L5N 8G6
Phone (416) 888-8537
Fax (614) 781-3906

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SOP for Periodic Repeatability Tests (Routine Tests)

Title:	SOP for Periodic Repeatability Tests
Document No.:	
Author:	METTLER TOLEDO Laboratory & Weighing Technologies
Number of Pages:	4
Controls	
Replacement Document:	N/A
Reason for Revision:	N/A
Release Date:	17. Oct. 2007
Release for Routine Operation	
Reviewed by:	
Date:	
Signature:	
Approved by:	
Date:	
Signature:	

General

Basic Rules for Handling Balances

- Before using a balance, make sure the balance was left on power for a sufficient period of time (mentioned in the balance operating instructions).
- Make sure the balance is leveled.
- Minimize environmental influences, e.g. open windows, direct sunlight or strong drafts.
- Do not enter the draft shield with hands. Use gloves or long tweezers.
- Place objects gently and in the center of the pan.

Basic Rules for Handling Weights

Test Weights

- Only an external test weight with calibration certificate can make a balance a “traceable” piece of equipment.
- Test weights should always be placed gently on a clean weighing pan and put back immediately in their storage place after use.
- Test weights (since they are also part of measuring equipment) need to be re-calibrated at specified intervals (ISO 9001).
- Any incident, which might have affected the value of the test weight, should trigger an immediate re-calibration. METTLER TOLEDO’s calibration services will give advice on this.

How to Store Test Weights

- Test weights should be stored in their original box.
- Test weights should be stored in the same room as the balance they are used with, since temperature differences between test weights and their surrounding lead to measurement errors.
- Test weights that have not been stored at the same temperature need acclimatization, which can take several hours.

How to Move Weights

- Test weights should only be handled with appropriate tools such as tweezers, forks, handles or gloves (see METTLER TOLEDO’s accessories for weights).
- These tools should be exclusively used for transferring test weights, to avoid possible contamination.

Repeatability Test

Preparation

- Before performing the test, the test weight must be acclimatized to the ambient temperature of the balance.
- Prepare a sheet where you can note the readings of the repeatability test points. Prepare 3 columns titled "w/o Test Wght.", "With Test Wght." and "Differences".

Test Procedure

- Empty the pan
- If required, place tare load on the weighing pan
- Tare the balance (if required press zero)
- Read the stable value from the display and note it in the column "w/o Test Wght."
- Place the test weight in the center of the weighing pan
- Read the stable value from the display and note it in the column "With Test Wght."
- Remove the test weight
- Repeat the measurements from "tare the balance" to "remove test weight" until the customer defined number of "w/o Test Wght." and "With Test Wght." readings is performed

Evaluation

- Subtract each "w/o Test Wght." from the corresponding "With Test Wght." to calculate the difference.
- Calculate the standard deviation of the differences.
- Evaluate whether standard deviation exceeds the defined "Control Limit" ¹⁾.

Deviation

Control Limit ¹⁾

- If the control limit is exceeded, report the problem to the laboratory supervisor or the person responsible of the balance.
- Mark the balance as "out of control limits".
- Contact METTLER TOLEDO service organization for advice.

¹⁾ – Values within the control limit: no action is necessary.

- Values beyond the control limit show that weighing process is no longer under control and immediate action is therefore required.

Mettler-Toledo AG

Laboratory & Weighing Technologies
Im Langacher
P.O. Box LabTec
CH-8606 Greifensee
Switzerland

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Printed in Switzerland 11793057

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For more information

SOP for Periodic Sensitivity Tests (Routine Tests)

Title:	SOP for Periodic Sensitivity Tests
Document No.:	
Author:	METTLER TOLEDO Laboratory & Weighing Technologies
Number of Pages:	4
Controls	
Replacement Document:	N/A
Reason for Revision:	N/A
Release Date:	17. Oct. 2007
Release for Routine Operation	
Reviewed by:	
Date:	
Signature:	
Approved by:	
Date:	
Signature:	

General

To test the sensitivity of a balance, a large test weight is more suitable than a small one.

Basic Rules for Handling Balances

- Before using a balance, make sure the balance was left on power for a sufficient period of time (mentioned in the balance operating instructions).
- Make sure the balance is leveled.
- Minimize environmental influences, e.g. open windows, direct sunlight or strong drafts.
- Do not enter the draft shield with hands. Use gloves or long tweezers.
- Place objects gently and in the center of the pan.

Basic Rules for Handling Weights

Test Weights

- Only an external test weight with calibration certificate can make a balance a “traceable” piece of equipment.
- Test weights should always be placed gently on a clean weighing pan and put back immediately in their storage place after use.
- Test weights (since they are also part of measuring equipment) need to be re-calibrated at specified intervals (ISO 9001).
- Any incident, which might have affected the value of the test weight, should trigger an immediate re-calibration. METTLER TOLEDO’s calibration services will give advice on this.

How to Store Test Weights

- Test weights should be stored in their original box.
- Test weights should be stored in the same room as the balance they are used with, since temperature differences between test weights and their surrounding lead to measurement errors.
- Test weights that have not been stored at the same temperature need acclimatization, which can take several hours.

How to Move Weights

- Test weights should only be handled with appropriate tools such as tweezers, forks, handles or gloves (see METTLER TOLEDO’s accessories for weights).
- These tools should be exclusively used for transferring test weights, to avoid possible contamination.

Sensitivity Test

Preparation

- Before the test is performed, the test weight must be acclimatized to the ambient temperature of the balance.
- Prepare a sheet where you can note the readings of the sensitivity test.

Test Procedure

- Empty the pan
- If required, place the tare load on the weighing pan
- Tare the balance (if required press zero)
- Place the test weight on the weighing pan
- Read the stable value from the display and note it

Evaluation

- Evaluate whether the noted value exceeds the defined "Warning Limit" ¹⁾.
- Evaluate whether the noted value exceeds the defined "Control Limit" ²⁾.

Deviation

Warning Limit ¹⁾ (where defined)

- If the warning limit is exceeded, repeat the test.
- If the warning limit is exceeded again, report that the warning limit was not met to the laboratory supervisor or the person responsible for the balance.
- Level the balance, perform adjustment with built-in or external adjustment weight and repeat the test.
- If the warning limit is still exceeded, report the problem to the laboratory supervisor or the person responsible of the balance. Optionally, contact METTLER TOLEDO's service organization for advice.

Control Limit ²⁾

- If the control limit is exceeded, report the problem to the laboratory supervisor or the person responsible of the balance.
- Mark the balance as "out of control limits".
- Contact METTLER TOLEDO service organization for advice.

¹⁾ – Values within the warning limit: no action is necessary.
– Values between the warning and control limit are within the tolerance but must be kept under surveillance. Corrective action may be appropriate, depending on the direction in which the values are changing.

²⁾ – Values within the control limit, see ¹⁾
– Values beyond the control limit show that weighing process is no longer under control and immediate action is therefore required.

Mettler-Toledo AG

Laboratory & Weighing Technologies
Im Langacher
P.O. Box LabTec
CH-8606 Greifensee
Switzerland

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pH Meter

pH Autocalibration with Two Buffers

1. Press the **power** key to turn on the meter. **See 1.**
2. Select two buffers that bracket the expected sample pH and are one to four pH units apart. **See 2.** See **Autocalibration Buffer Selection** section.

3. Press the **calibrate** key. **See 3.**

4. Rinse the electrode with deionized water and blot dry with a lint-free tissue. **See 4.**

NOTE: Do not wipe or rub the electrode, as static buildup will occur.

5. Insert the electrode into the first buffer and gently stir – if a stir probe is being used press the **stir** key to start and stop stirring (benchtop models only). **See 5.**
6. Wait for the **pH** icon to stop flashing and the **►** icon to start flashing. The meter should display the temperature corrected pH buffer value. **See 6.**

If the displayed buffer value is incorrect or a customer buffer is being used manually enter the value by pressing the **up/down arrow** keys to adjust each digit and the **digits** key to move to the next digit.

7. Press the **calibrate** key. **See 7.**
8. Rinse the electrode with deionized water and blot dry with a lint-free tissue. **See 8.**

NOTE: Do not wipe or rub the electrode, as static buildup will occur.

9. Insert the electrode into the second buffer and gently stir – if a stir probe is being used press the **stir** key to start and stop stirring (benchtop models only). **See 9.**
10. Wait for the **pH** icon to stop flashing and the **►** icon to start flashing. The meter should display the temperature corrected pH buffer value. **See 10.**

11. Press the **calibrate** key and then press the **measure** key to save and end the calibration. The slope will be displayed and then the meter will proceed to the measurement mode. **See 11a & 11b.**

Autocalibration Buffer Selection

1. Press the **setup** key.
2. Press the **up arrow** key until PH is displayed on the top line.
3. Press the **line select** key to move the **►** icon to the middle line. Press the **up arrow** key until bUF is displayed.

4. Press the **line select** key to move the **►** icon to the bottom line. Press the **up arrow** key to select the buffer set. USA is default setting.

USA = 1.68, 4.01, 7.00, 10.01, and 12.46
EUR0 = 1.68, 4.01, 6.86, and 9.18

5. Press the **line select** key to move the **►** icon to the top line. Press the **measure** key to return to the measurement mode.

Measurement Mode Selection

1. Press the **setup** key.
2. Press the **up arrow** key until rEAd is displayed on the top line.
3. Press the **line select** key to move the **►** icon to the middle line. Press the **up arrow** key until tYPE is displayed.

4. Press the **line select** key to move the **►** icon to the bottom line. Press the **up arrow** key to select the measurement mode. AUtO is default setting.

COnT = Continuous measurement mode
AUtO = AUTO-READ™ measurement mode
tImE = Timed measurement mode

5. Press the **line select** key to move the **►** icon to the top line. Press the **measure** key to return to the measurement mode.

pH Measurements

1. Rinse the electrode with deionized water, blot dry with a lint-free tissue, and insert the electrode into the sample.
2. If the meter is in AUTO-READ mode, press the **measure** key to take a measurement. Once the reading stabilizes the **AR** icon will stop flashing and the display will freeze. Press the **measure** key again to take a new measurement.

If a stir probe is connected it will start stirring when the **measure** key is pressed and stop stirring when the **AR** icon stops flashing (benchtop meters only).

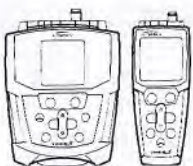
3. If the meter is in the continuous or timed measurement modes, the meter will continuously take measurements and update the display. Once the reading stabilizes the **pH** icon will stop flashing.

If a stir probe is connected press the **stir** key to start stirring and press the **stir** key again to stop stirring (benchtop meters only).

Printing Data

In the AUTO-READ and continuous measurement modes, every time the **measure** key is pressed the meter logs the data on the display. In the timed measurement mode, the meter logs the data at the predetermined time interval.

1. Press the **view log** key.
2. Use the **up arrow** key to scroll between CAL0 (print the calibration log) and SEnd (print the measurement log).
3. Press the **line select** key to print the data.



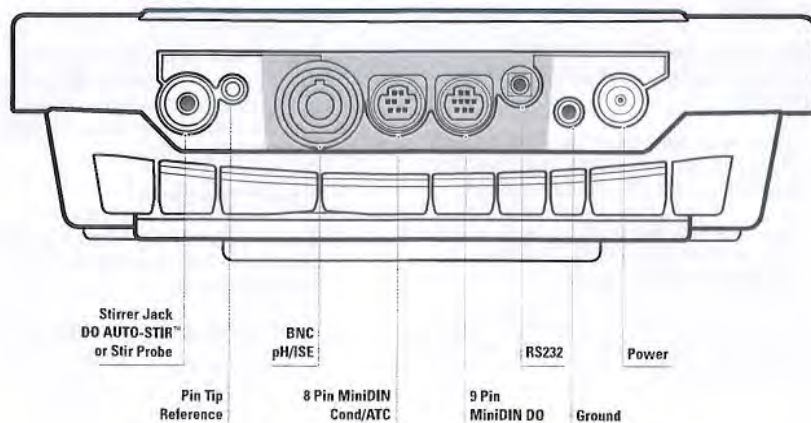
Key Description

	power		up arrow		line select		view log
	calibrate		down arrow		setup		stir
	measure		digits				

All connectors on the symphony™ SB90M5 benchtop meter are depicted below. All connectors on the SP90M5 portable meter are highlighted in gray.

The SB70P, SP70P, SB80PI, SP80PI, SB80PD, SP80PD, SB80PC, and SP80PC meters will have fewer connectors. For example, the SP70P portable pH meter will only have BNC, 8 pin miniDIN (ATC), and RS232 connectors.

Meter Connections



For the most current contact information, visit www.vwr.com

EN

pH Meter

Preparation

1. Power adapter (benchtop models only) - Select the appropriate wall socket plug and slide the plug plate into the groove on the back of the adapter. **See A.**

Batteries - Select four AA Alkaline batteries. Confirm that the meter is off and remove the battery cover. Orient and insert the batteries as depicted in the battery compartment housing. Replace the cover. **See B & C.**

2. Prepare the electrode according to the directions in the electrode instruction manual. In general, these steps include:

- Gently remove the protective cap/sleeve from the sensing element and save for storage.
- If the electrode is refillable, remove the fill hole cover and fill the electrode with the appropriate fill solution.
- Soak the electrode in pH electrode storage solution, VWR® Cat. No. 14002-828. **See D.**

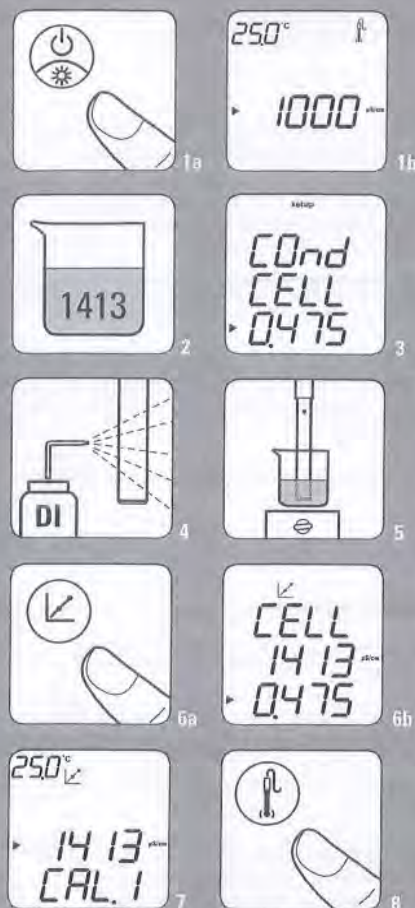
3. Meter connections - Connect the power adapter to the meter and then to the wall socket (benchtop meters only). **See E.** Connect the pH electrode to the BNC input on the meter. **See F.** Connect the ATC probe to the 8 pin miniDIN input on the meter. **See G.** Connect the stir probe to the stir jack input on the meter (benchtop meters only). **See H.**

NOTE: Make sure that all unused inputs on the meter are covered with the black caps. ▲

Meter Overview

1. To power on the meter press the **power** key.
2. Press the **line select** key to choose the top, middle, or bottom display line. The ► icon will point to the selected line.
3. In the measurement screen press the **up arrow** key to change the value on the top display line to pH, RmV, mV, or a blank line.
4. To escape out of any meter function, press and hold the **measure** key until the meter returns to the measurement mode.
5. The symphony™ pH meters can be calibrated using up to five pH buffers.

Conductivity Meter



Conductivity Autocalibration with One Standard

1. Press the **power** key to turn on the meter. **See 1a.** Use the **line select** key to move the ► icon to the conductivity measurement line. **See 1b.**
2. Select the VWR® conductivity standard (100 µS/cm, 1413 µS/cm, or 12.9 mS/cm) that has the closest conductivity to your expected sample value. **See 2.**
3. In the setup mode enter the nominal cell constant value for the conductivity probe. **See 3.** The meter will use the nominal cell constant value to recognize the conductivity standard during autocalibration. **See Nominal Cell Constant Selection** section.
4. Rinse the conductivity probe with deionized water and blot dry with a lint-free tissue. **See 4.**
5. Insert the conductivity probe into the conductivity standard and gently stir – if a stir probe is being used press the **stir** key to start and stop stirring (benchtop models only). **See 5.**
6. Press the **calibrate** key. **See 6a.** The meter will show the manual calibration display for about five seconds. **See 6b.** Do not press any keys.
7. After about five seconds the meter will proceed to the direct and autocalibration display. Wait for the **µS/cm** or **mS/cm** icon to stop flashing and the ► icon to start flashing. The meter should display the conductivity standard value at 25 °C. **See 7.**
8. Press the **measure** key to save and end the calibration. **See 8.** The calculated cell constant will be displayed and then the meter will proceed to the measurement mode.

Nominal Cell Constant Selection

1. Press the **setup** key.
2. Press the **up arrow** key until COnd is displayed on the top line.
3. Press the **line select** key to move the ► icon to the middle line. Press the **up arrow** key until CELL is displayed.
4. Press the **line select** key to move the ► icon to the bottom line. Enter the nominal cell constant by pressing the **up/down arrow** keys to adjust each digit and the **digits** key to move to the next digit. 0.475 cm⁻¹ is default setting.
5. Press the **line select** key to move the ► icon to the top line. Press the **measure** key to return to the measurement mode.

Reference Temperature Selection

1. Press the **setup** key.
2. Press the **up arrow** key until COnd is displayed on the top line.
3. Press the **line select** key to move the ► icon to the middle line. Press the **up arrow** key until trEF is displayed.
4. Press the **line select** key to move the ► icon to the bottom line. Press the **up arrow** key to select 15 °C, 20 °C, or 25 °C as the reference temperature. 25 °C is default setting.
5. Press the **line select** key to move the ► icon to the top line. Press the **measure** key to return to the measurement mode.

Conductivity Measurements

1. Rinse the conductivity probe with deionized water, blot dry with a lint-free tissue, and insert the probe into the sample.
2. If the meter is in AUTO-READ™ mode, press the **measure** key to take a measurement. Once the reading stabilizes the **AR** icon will stop flashing and the display will freeze. Press the **measure** key again to take a new measurement.

If a stir probe is connected it will start stirring when the **measure** key is pressed and stop stirring when the **AR** icon stops flashing (benchtop meters only).

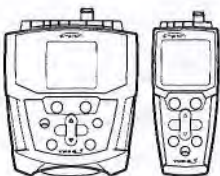
3. If the meter is in the continuous or timed measurement modes, the meter will continuously take measurements and update the display. Once the reading stabilizes the **µS/cm** or **mS/cm** icon will stop flashing.

If a stir probe is connected press the **stir** key to start stirring and press the **stir** key again to stop stirring (benchtop meters only).

Printing Data

In the AUTO-READ and continuous measurement modes, every time the **measure** key is pressed the meter logs the data on the display. In the timed measurement mode, the meter logs the data at the predetermined time interval.

1. Press the **view log** key.
2. Use the **up arrow** key to scroll between CAL0 (print the calibration log) and SEnd (print the measurement log).
3. Press the **line select** key to print the data.



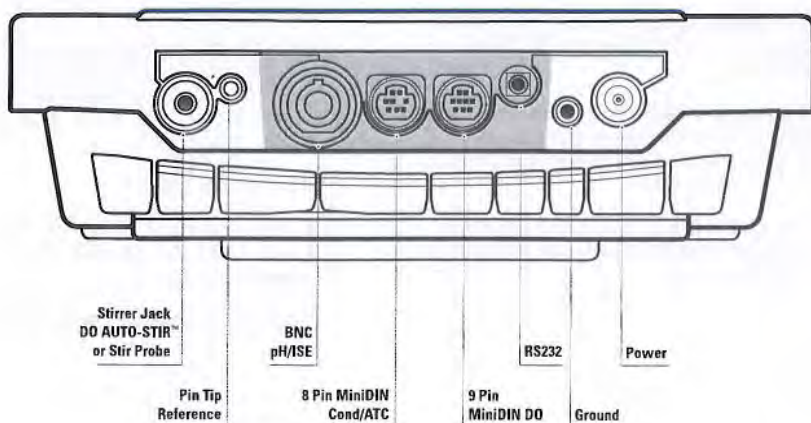
Key Description

	power		up arrow		line select		view log
	calibrate		down arrow		setup		stir
	measure		digits				

All connectors on the sympHony™ SB90M5 meter are depicted below. All connectors on the SP90M5 portable meter are highlighted in gray.

The SB70C, SP70C, SB80PC, and SP80PC meters will have fewer connectors. For example, the SP70C portable conductivity meter will only have 8 pin miniDIN and RS232 connectors.

Meter Connections



For the most current contact information, visit www.vwr.com

EN

Conductivity Meter

Preparation

1. Power adapter (benchtop models only) - Select the appropriate wall socket plug and slide the plug plate into the groove on the back of the adapter. **See A.**

Batteries - Select four AA Alkaline batteries. Confirm that the meter is off and remove the battery cover. Orient and insert the batteries as depicted in the battery compartment housing. Replace the cover. **See B & C.**

2. Prepare the conductivity probe according to the directions in the probe instruction manual. In general, this includes rinsing the probe with deionized water. **See D.**

3. Meter connections - Connect the power adapter to the meter and then to the wall socket (benchtop meters only). **See E.** Connect the conductivity probe to the 8 pin miniDIN input on the meter. **See F.** Connect the stir probe to the stir jack input on the meter (benchtop meters only). **See G.**

NOTE: Make sure that all unused inputs on the meter are covered with the black caps. ▲

Meter Overview

1. To power on the meter press the **power** key.
2. Press the **line select** key to choose the top, middle, or bottom display line. The ► icon will point to the selected line.
3. In the measurement screen press the **up arrow** key to change the value on the middle display line to $\mu\text{S}/\text{cm}$ or mS/cm , mg/L , ppt, $\text{M}\Omega\text{-cm}$, or a blank line.
4. To escape out of any meter function, press and hold the **measure** key until the meter returns to the measurement mode.
5. The sympHony™ conductivity meters can perform a one point manual calibration, up to a three point autocalibration, or up to a five point direct calibration.

DO Meter

Quick Start Guide

Meter Overview

1. To power on the meter press the **power** key.
2. Press the **line select** key to choose the top, middle, or bottom display line. The ► icon will point to the selected line.
3. In the measurement screen press the **up arrow** key to change the value on the bottom display line to % saturation, mg/L, barometric pressure (no icon), or a blank line.
4. To escape out of any meter function, press and hold the **measure** key until the meter returns to the measurement mode.
5. The symphony™ DO meters can perform an air calibration using water saturated air, water calibration using air saturated water, manual calibration (Winkler titration), or zero point calibration.

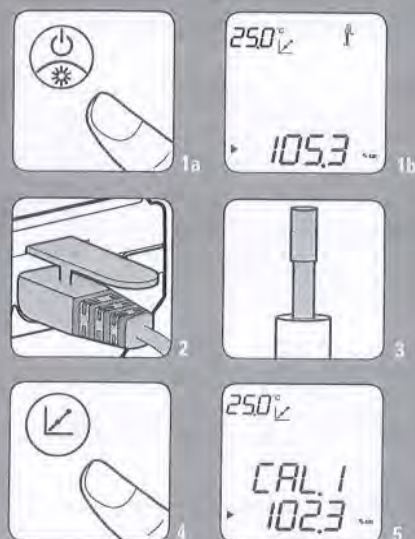
Air Calibration

1. Press the **power** key to turn on the meter. **See 1a.** Use the **line select** key to move the ► icon to the DO measurement line. **See 1b.**

2. Make sure the DO probe is connected to the meter and fully polarized. **See 2.**

Allow the probe to polarize for 30 to 60 minutes when the probe is first connected to the meter, the membrane cap is changed, or the electrolyte solution is changed.

3. Insert the probe into the prepared calibration sleeve or BOD bottle and allow five minutes for the probe to equilibrate. **See 3.**
4. Press the **calibrate** key. **See 4.**
5. When the reading stabilizes the meter will display 102.3% saturation and then proceed to the measurement mode. **See 5.**



DO Calibration Type Selection

1. Press the **setup** key.
2. Press the **up arrow** key until dO is displayed on the top line.
3. Press the **line select** key to move the ► icon to the middle line. Press the **up arrow** key until CALt is displayed.
4. Press the **line select** key to move the ► icon to the bottom line. Press the **up arrow** key to select the calibration type. Alr is default setting.
Alr = Water Saturated Air Calibration
H2O = Air Saturated Water Calibration
mAn = Manual Calibration (Winkler titration)
SEtO = Zero Point Calibration
5. Press the **line select** key to move the ► icon to the top line.
6. Press the **measurement** key to return to the measurement mode.

Measurement Mode Selection

1. Press the **setup** key.
2. Press the **up arrow** key until rEAd is displayed on the top line.
3. Press the **line select** key to move the ► icon to the middle line. Press the **up arrow** key until tYPE is displayed.
4. Press the **line select** key to move the ► icon to the bottom line. Press the **up arrow** key to select the measurement mode. AUtO is default setting.
COnt = Continuous measurement mode
AUtO = AUTO-READ™ measurement mode
tImE = Timed measurement mode
5. Press the **line select** key to move the ► icon to the top line.
6. Press the **measure** key to return to the measurement mode

DO Measurements

1. Rinse the DO probe with deionized water, blot dry with a lint-free tissue, and insert the probe into the sample.
2. If the meter is in AUTO-READ mode, press the **measure** key to take a measurement. Once the reading stabilizes the **AR** icon will stop flashing and the display will freeze. Press the **measure** key again to take a new measurement.

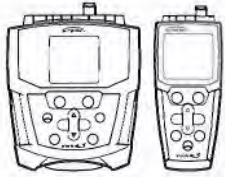
If a stir probe is connected it will start stirring when the **measure** key is pressed and stop stirring when the **AR** icon stops flashing. If an AUTO-STIR™ BOD probe is connected, the stir button on the probe can be pressed to take a new measurement and begin stirring. (Benchtop meters only)
3. If the meter is in the continuous or timed measurement modes the meter will continuously take measurements and update the display. Once the reading stabilizes the **mg/L** or **%sat** icon will stop flashing.

If a stir probe or AUTO-STIR BOD probe is connected, press the **stir** key on the meter to start stirring and press the **stir** key again to stop stirring (Benchtop meters only).

Printing Data

In the AUTO-READ and continuous measurement modes, every time the **measure** key is pressed the meter logs the data on the display. In the timed measurement mode the meter logs the data at the predetermined time interval.

1. Press the **view log** key.
2. Use the **up arrow** key to scroll between CALO (print the calibration log) and SEnd (print the measurement log).
3. Press the **line select** key to print the data.



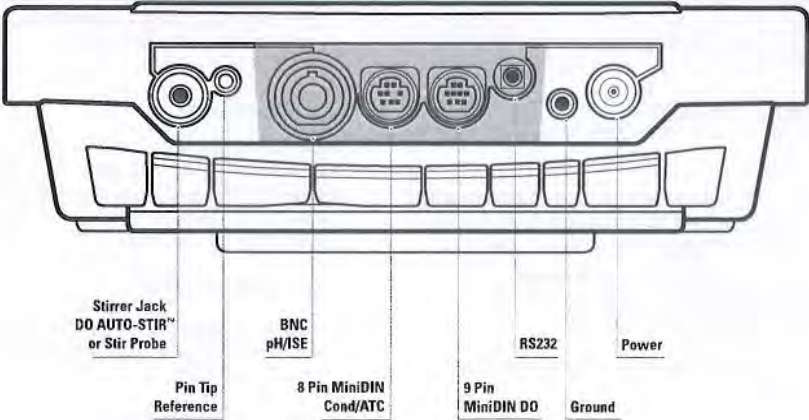
Key Description

	power		up arrow		line select		view log
	calibrate		down arrow		setup		stir
	measure		digits				

All connectors on the symphony™ SB90M5 meter are depicted below. All connectors on the SP90M5 portable meter are highlighted in gray.

The SB70D, SP70D, SB80PD, and SP80PD meters will have fewer connectors. For example, the SP70D portable DO meter will only have 9 pin miniDIN, 8 pin miniDIN (ATC), and RS232 connectors.

Meter Connections



For the most current contact information, visit www.vwr.com

EN

DO Meter

Preparation

1. Power adapter (benchtop models only) - Select the appropriate wall socket plug and slide the plug plate into the groove on the back of the adapter. **See A.**

Batteries - Select four AA Alkaline batteries. Confirm that the meter is off and remove the battery cover. Orient and insert the batteries as depicted in the battery compartment housing. Replace the cover. **See B & C.**

2. Prepare the DO probe according to the directions in the probe instruction manual. In general, these steps include:

- Unscrew the membrane cap from the DO probe, fill the membrane cap about $\frac{3}{4}$ full with DO probe electrolyte solution, and screw the membrane cap onto the DO probe.
- Connect the DO probe to the meter and allow the probe to polarize for about 30-60 minutes.

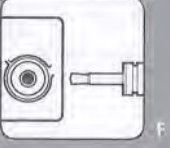
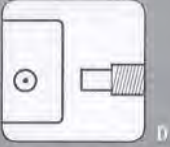
NOTE: A DO reading of zero and no change in temperature indicates that the DO probe is not connected. Firmly reconnect the DO probe to the meter. ▲

3. Calibration sleeve or BOD bottle.

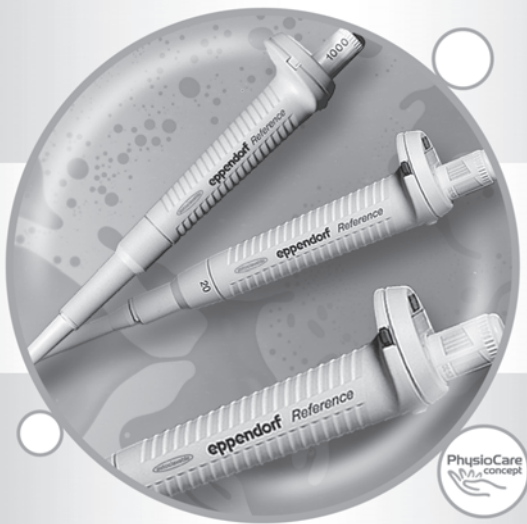
- To prepare the calibration sleeve remove the cap from the sleeve and remove the sponge from the cap. Saturate the sponge with distilled water and then squeeze all the excess water out of the sponge. Reassemble the calibration sleeve and insert the DO probe.
- To prepare a BOD bottle fill the bottle with about 50 mL of distilled water. Insert the probe into the BOD bottle (Use a BOD bottle adapter if the DO probe does not fit directly into the bottle). Make sure the probe is suspended about half an inch above the distilled water and there is no water on the surface of the DO probe.

4. Meter connections - Connect the power adapter to the meter and then to the wall socket (benchtop meters only). **See D.** Connect the DO probe to the 9 pin miniDIN input on the meter. **See E.** Connect the stir probe or the stir connector on the AUTO-STIR™ BOD probe to the stir jack input on the meter (benchtop meters only). **See F.**

NOTE: Make sure that all unused inputs on the meter are covered with the black caps. ▲



Attachment 3a



Eppendorf Reference®

Operating Manual

eppendorf

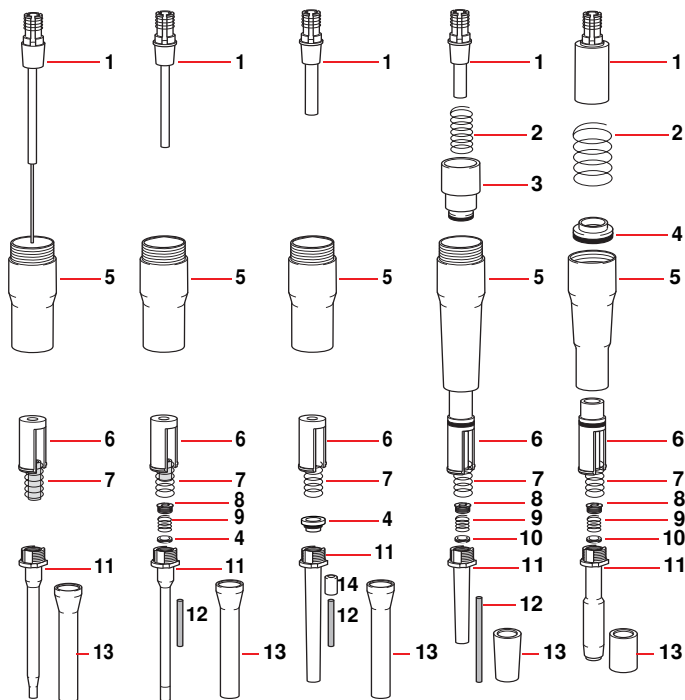
1 Fig. / Abb. 1

Reference variabel / Reference adjustable

0.1 – 2.5 μL 10 – 100 μL 20 – 200 μL 100 – 1000 μL 500 – 2500 μL
 0.5 – 10 μL
 2 – 20 μL

Reference fix

1 – 50 μL 100 μL 200 – 250 μL 500 – 2500 μL



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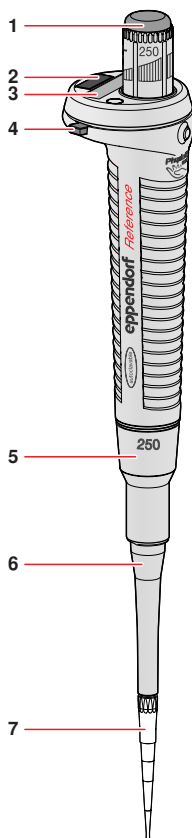
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1 Main Illustration



1 Control button

The control button is for setting the volume, dispensing and ejecting the pipette tip.

2 Volume display

Displays the preset volume.

3 Labeling area

The provided autoclavable, blank labels can be labeled with a waterproof pen and affixed at this location.

4 Catch

Hold down the catch when you set the volume using the control button.






5 Lower part

6 Ejector sleeve

7 Pipette tip

2 Warning signs and hazard icons

2.1 Hazard icons

	Biological hazard		Health hazard
	General hazard		Material damage
	Notice		

2.2 Danger levels

The danger level is part of a safety note and defines the potential consequences if the information contained therein is not observed.

DANGER	<i>Will</i> lead to serious injuries or death.
WARNING	<i>May</i> lead to serious injuries or death.
CAUTION	May lead to minor or moderate injuries.
NOTICE	May lead to material damage.
Notice	Provides useful information.

3 General safety notes



WARNING! Damage to health due to handling infectious liquids and pathogenic germs.

- ▶ Observe the national regulations for handling these substances, the biological security level of your laboratory, the material safety data sheets and the manufacturer's application notes.
- ▶ Wear your personal protective equipment (PPE).
- ▶ Follow the instructions regarding hygiene, cleaning and decontamination.
- ▶ For complete instructions regarding the handling of germs or biological material of risk group II or higher, please refer to the "Laboratory Biosafety Manual" (Source: World Health Organization, current edition of the Laboratory Biosafety Manual).



WARNING! Damage to health due to toxic, radioactive or aggressive chemicals.

- ▶ Observe the national regulations for handling these substances as well as the material safety data sheets and manufacturer's application notes.
- ▶ Wear your personal protective equipment (PPE).



CAUTION! Danger to persons from improper handling.

- ▶ Never aim the opening of a Reference equipped with pipette tip at yourself or other persons.
- ▶ Only initiate dispensing if it is safe to do so.
- ▶ For all dispensing tasks, make sure that you are not endangering yourself or anyone else.



CAUTION! Poor safety due to incorrect accessories and spare parts.

The use of accessories and spare parts other than those recommended by Eppendorf may impair the safety, functioning and precision of the device. Eppendorf cannot be held liable or accept any liability for damage resulting from the use of incorrect or non-recommended accessories and spare parts or from the improper use of such equipment.

- ▶ Only use accessories and original spare parts recommended by Eppendorf.
-

**NOTICE! Damage to device from missing pipette tips.**

- ▶ Only use the Reference with attached pipette tips.

**NOTICE! Carry-over, contamination and incorrect dispensing results due to the repeated use of pipette tips.**

The pipette tips are for single use only. Prolonged use can have a negative impact on dispensing tasks.

- ▶ Use the pipette tips only once.
- ▶ Do not autoclave the epDualfilter T.I.P.S.

**NOTICE! Incorrect dispensing volume with special liquids and due to temperature differences.**

Solutions which differ greatly from water in terms of their physical data, or temperature differences between the pipette, pipette tip and liquid can result in incorrect dispensing volumes.

- ▶ Avoid temperature differences between pipette, pipette tip and liquid.
- ▶ Ensure that the temperature remains constant between 20 and 25 °C, $\pm 0,5$ °C.
- ▶ Check the dispensing volume and readjust the pipette in case of deviations.

**NOTICE! Damage to device due to penetration of liquids.**

- ▶ Do not deposit the Reference with filled pipette tip.
- ▶ Only use pipettes in the Reference-Serie when a pipette tip has been attached.
- ▶ Do not allow any liquids to penetrate the inside of the housing.

4 Intended use

The Reference is a lab device intended for dispensing liquids in the volume range from 0.1 μ L to 2.5 mL, in combination with matching pipette tips. In vivo applications (applications in or on the human body) are not permitted.

The Reference may only be operated by trained specialist staff. All users must have read the operating manual carefully and familiarized themselves with the device's mode of operation.

5 Technical data

5.1 Reference fix

Model / Volume μL	Color code	epT.I.P.S. μL	Systematic error		Random error	
			%	μL	%	μL
1	light gray	0.5– 20	± 2.5	± 0.025	± 1.8	± 0.018
2	light gray	0.5– 20	± 2.0	± 0.04	± 1.2	± 0.024
5	light gray	0.5– 20	± 1.5	± 0.075	± 0.8	± 0.04
10	light gray	0.5– 20	± 1.0	± 0.1	± 0.5	± 0.05
10	yellow	2– 200	± 1.0	± 0.1	± 0.5	± 0.05
20	yellow	2– 200	± 0.8	± 0.16	± 0.3	± 0.06
25	yellow	2– 200	± 0.8	± 0.2	± 0.3	± 0.075
50	yellow	2– 200	± 0.7	± 0.35	± 0.3	± 0.15
100	yellow	2– 200	± 0.6	± 0.6	± 0.2	± 0.2
200	blue	50–1,000	± 0.6	± 1.2	± 0.2	± 0.4
250	blue	50–1,000	± 0.6	± 1.5	± 0.2	± 0.5
500	blue	50–1,000	± 0.6	± 3	± 0.2	± 1
1,000	blue	50–1,000	± 0.6	± 6	± 0.2	± 2
1,500	red	500–2,500	± 0.6	± 9	± 0.2	± 3
2,000	red	500–2,500	± 0.6	± 12	± 0.2	± 4
2,500	red	500–2,500	± 0.6	± 15	± 0.2	± 5

5.2 Reference variable

Model μL	Color code	epT.I.P.S. μL	Volume μL	Systematic error		Random error	
				%	μL	%	μL
0.1– 2.5	dark gray	0.1– 10	0.25	± 12.0	± 0.030	± 6.0	± 0.015
			1.25	± 2.5	± 0.031	± 1.5	± 0.019
			2.5	± 1.4	± 0.035	± 0.7	± 0.018
0.5– 10	light gray	0.5– 20	1	± 2.5	± 0.025	± 1.8	± 0.018
			5	± 1.5	± 0.075	± 0.8	± 0.04
			10	± 1.0	± 0.1	± 0.4	± 0.04
2– 20	light gray	0.5– 20	2	± 3.0	± 0.06	± 2.0	± 0.04
			10	± 1.0	± 0.1	± 0.5	± 0.05
			20	± 0.8	± 0.16	± 0.3	± 0.06
2– 20	yellow	2– 200	2	± 5.0	± 0.1	± 1.5	± 0.03
			10	± 1.2	± 0.12	± 0.6	± 0.06
			20	± 1.0	± 0.2	± 0.3	± 0.06
10– 100	yellow	2– 200	10	± 3.0	± 0.3	± 0.7	± 0.07
			50	± 1.0	± 0.5	± 0.3	± 0.15
			100	± 0.8	± 0.8	± 0.15	± 0.15
50– 200	yellow	2– 200	50	± 1.0	± 0.5	± 0.3	± 0.15
			100	± 0.9	± 0.9	± 0.3	± 0.3
			200	± 0.6	± 1.2	± 0.2	± 0.4
50– 250	blue	50–1,000	50	± 1.4	± 0.7	± 0.3	± 0.15
			100	± 1.1	± 1.1	± 0.3	± 0.3
			250	± 0.6	± 1.5	± 0.2	± 0.5
100–1,000	blue	50–1,000	100	± 3	± 3	± 0.3	± 0.3
			500	± 1	± 5	± 0.2	± 1
			1,000	± 0.6	± 6	± 0.2	± 2
500–2,500	red	500–2,500	500	± 1.5	± 7.5	± 0.3	± 1.5
			1,250	± 0.8	± 8	± 0.2	± 2
			2,500	± 0.6	± 15	± 0.2	± 5

The technical data is only valid when the quoted **ependorf** pipette tips are used. Tests carried out in accordance with ISO 8655 for piston-stroke pipettes with an air cushion using a precision balance with evaporation trap approved by the standardization authorities.

Number of determinations:

10; degassed, bidistilled water, 20 °C – 25 °C, constant to ± 0.5 °C; with pre-wetted pipette tip; dispensing carried out on inner wall of vessel. If the place where the pipette is used is at extremely high altitude, an adjustment must be made in line with the ambient air pressure.

6 Function principle

The pipettes in the Reference-Serie are piston-stroke pipettes that operate according to the air-cushion principle.

The Reference-Serie consists of fixed-volume pipettes and pipettes with an adjustable volume setting.

The control button is multi-functional. The function executed by the pipette depends on how far its control button is pressed down.

Ex
20 °C

Three steps are necessary to operate the pipette:

1. Measuring stroke

Press the control button down until the first stop. The desired volume of liquid is dispensed or, when the button is released, aspirated.

2. Blow-out

Press the button down a little more until the next stop.
Any liquid remaining in the pipette tip is emptied.

3. Ejection

Press the button all the way down.
The pipette tip is ejected.

7 Operation

The pipette can be individually labelled. The autoclavable blank adhesive label provided can be marked with a permanent marker and fits onto the identification area on the top of the housing.

7.1 Volume setting

The volume is adjusted by pressing down the lateral catch and turning the control button at the same time.

It is advisable to carry out volume setting from the higher down to the lower value. i.e. first go above the desired volume and then return to the lower value.

7.2 Pipette tips

The pipette can function only when a pipette tip is attached into which the liquid is aspirated.

To facilitate the search for a suitable tip, the color of the control buttons corresponds to the color of the **ependorf** tip racks.

When pipetting liquids with wetting properties different to those of water, please observe the recommendations contained in Chapter 7.5.

7.3 Aspirating liquid

- The liquid which is to be aspirated is taken from a suitable vessel.
- Attach suitable pipette tip to the pipette firmly (observe the color coding).
- Press down the control button to the first stop (measuring stroke).
- Immerse the pipette tip vertically approx. 3 mm into the liquid.
- Allow the control button to slide back **slowly**.
- Pull the tip out of the liquid **slowly**.
- To remove any remaining droplets, dab with non-fibrous cellulose material. When doing so, ensure that no liquid comes out of the tip.

7.4 Dispensing liquid

- Hold the tip at an angle against the inside wall of the tube.
- Press down the control button slowly to the first stop (measuring stroke) and wait until the liquid stops flowing.
- Press down the control button to the second stop (blow-out) until the tip is completely empty.
- Hold down the control button and pull the tip up the inner wall of the tube.
- Tip is ejected by pressing the control button to the final stop.

7.5 Special notes

To guarantee the highest degree of precision and accuracy, we recommend pre-wetting all new tips by aspirating and dispensing liquid two or three times before pipetting.

Finally, with the tip not in contact with the liquid, empty it completely on the inner wall of the tube (via blow-out).

Explanation: Why does the pipette tip have to be **pre-wetted**?

To compensate for the properties of the liquid.

Wetting liquids (serum, detergent) form a thin film on the inner wall of the pipette tip. When the first pipetting is carried out, the volume dispensed would thus be too low.

When pipetting serum or high-viscosity solutions, wait a few seconds when aspirating and dispensing liquid.

8 Testing / Alignment

The serial number of the pipette is located on its control button.

8.1 Testing

Distilled water is dispensed from a pre-wetted tip into a tube and is then weighed.

Volumes 0.1 – 10 μL :

The test is performed with a highly sensitive analytic balance (resolution balance: 0.001 mg) by releasing the volume.



The bidistilled water, weighing vessel, pipette and pipette tip must all be the same temperature!

To calculate the volume, divide the weight by the density of the water (at 20 °C: 0.9982 mg/ μL ; mg/ μL = g/mL).

Volumes > 10 – 2.500 μL :

For a volume of more than 10 μL , a balance with a resolution of 0.01 mg is sufficient.

8.2 Alignment

8.2.1 When should alignment be carried out?

The pipettes in the Reference-Serie were tested during production in accordance with the measurement conditions for water listed in Chapter 2.

In the case of doubts arising about the accuracy of the pipetted volume, the following points should first be checked:

- Is the pipette leaking? (This is one possible reason for dispensed volumes being too low; troubleshooting and solutions are contained in Chapter 7)
- What is the temperature of the sample? (In open tubes, water at room temperature cools down due to evaporation.)
- What is the temperature of the pipette?
- What is the temperature of the air?
- Has mg been converted into μL ?
- Does the sample have a different density to that of water?
- Is the pipetting speed too high?

If the place where the pipette is used is at extremely high altitude, an adjustment must be made in line with the ambient air pressure. At 1,000 m above sea level, there is a volume error of approx. -0.4% .

Assistance with these questions is contained in **eppendorf's** SOP (Standard Operating Procedure). An SOP can be called up from our home page www.eppendorf.com

If these checks prove to be unsuccessful, it is safe to assume that the alignment of the pipette has altered (e.g. due to several components having been replaced).

8.2.2 Follow-up alignment in the case of error

From a technical point of view, this is a zero-point shift. The value by which the setting of the pipette is shifted remains constant across the entire measuring range. If, for example, in the case of a $10 - 100\ \mu\text{L}$ pipette, follow-up alignment of $1\ \mu\text{L}$ is carried out at $100\ \mu\text{L}$ ($=1\%$), the pipette is also adjusted by $1\ \mu\text{L}$ at $10\ \mu\text{L}$ ($=10\%$!)

Alignment Reference fix:

To assist you in finding the basic setting again, round adhesive labels with an arrow are provided as an alignment aid.



– Stick the alignment aid onto the control button.

Determine the volume by weighing and calculation
(see point a – c "Alignment Reference variable").

d



Pierce the calibration seal attached to the opening for adjustments with Side B of the key provided. This destroys the seal which should be removed. Undo the screw inside a little until the control button can be turned.

- e Adjust control button by the volume determined.
One revolution of the control button corresponds to the following values for water:

Reference fix	Vol./revol.
1, 2, 5, 10 µL	approx. 0.5 µL
10, 20 µL	approx. 1 µL
25, 50 µL	approx. 2.4 µL
100 µL	approx. 5 µL
200, 250 µL	approx. 12 µL
500, 1000 µL	approx. 46 µL
1500, 2000, 2500 µL	approx. 118 µL

Clockwise rotation: decrease in volume.

Counterclockwise rotation: increase in volume.

- f Tighten the screw until the control button can no longer be turned.
Then continue as described in steps f and g of the Alignment Reference variable.
If the nominal volume does not correspond with the measuring result, repeat steps d – g.
Then reseal the adjustment opening using one of the calibration seals supplied.

Alignment Reference variable:

- The pipette, tip and water must all be the same temperature (20 – 25 °C, constant to ± 0.5 °C).
- Set the Reference variable to the desired nominal volume.
- With a pipette tip attached to the pipette, the desired volume is pipetted and weighed 10 times. The average of this weighing is converted into µL using the following formula:

$$\text{Volume} = \frac{\text{Weight}}{\text{Density of liquid}} \\ \text{(at the temperature specified)}$$

The value obtained is the actual setting
(density of water at 20 °C: 0.9982 mg/µL; mg/µL = g/mL).

d



Pierce the calibration seal attached to the opening for adjustments with Side B of the key provided. This destroys the seal which should be removed. Carefully attach the key to the adjusting sleeve inside.

- e Turn the wrench to adjust the volume display of the pipette (with piston stroke unchanged) to the actual volume (measurement under step c).
- f Remove the wrench.
- g Repeat step c). The readings must be within the tolerances specified in the technical data.

If the nominal value still does not agree with the measuring result, repeat steps d – g.

Since this adjustment affects the entire measuring range, it is imperative to check the other volumes of this pipette specified in the technical data.

Then reseal the adjustment opening using one of the calibration seals supplied.

8.2.3 Adjustment for liquids with a density different to that of water

It is possible to adjust the pipette for **one specific volume of liquid** with a density different to that of water in such a way that the volume displayed corresponds to the volume pipetted.

All other values for the adjustable pipettes are now out of alignment, i.e. an adjustable-volume pipette has been converted into a fixed-volume pipette!

Proceed as described in Chapter 8.2.2.



A pipette set in this way delivers a pipetting value that correlates with that in the display **only for the liquid used and for the volume tested!** For this reason, we very strongly recommend labeling the converted pipette **as a fixed-volume pipette** for "Solution y"!

The error for liquids with a higher vapor pressure (e.g. organic solvents) cannot be aligned in this way. In this case, we recommend using an **eppendorf** positive-displacement pipette.

9 Care / Sterilization

9.1 Care

Depending on the frequency of use, all parts of the pipette should be cleaned from time to time in a soap solution or should be carefully wiped clean using isopropanol. They should then be rinsed in distilled water and dried.

The seals are maintenance-free and the pistons should be lubricated lightly (using the grease for pipettes provided) when cleaned or replaced.

Severe contamination caused by the liquid entering the pipette can be removed after the pipette has been disassembled (see Part B, Maintenance).

For information about replacing defective parts, please see Part B, Maintenance.

9.2 Sterilization

The Reference-Serie including the blank label provided (marked with a permanent marker) is fully autoclavable at 121 °C for 20 minutes.

Before autoclaving, unscrew the pipette at the central junction by rotating about one revolution. This enables steam to penetrate more easily into the pipette during autoclaving.

After autoclaving, the pipette may have to be dried at room temperature. Retighten the central junction only after the pipette has completely cooled.

The tip cone may have to be tightened again with the wrench (see part B, Maintenance).

The Reference pipettes may be temporarily subjected to the UV light of a sterile bench (≥ 254 nm).

9.3 Decontamination prior to dispatch



If the Reference pipette is to be checked, repaired or calibrated by Eppendorf AG or another service partner, it must be free of hazardous substances and clean!

A form called "Decontamination certificate for return of goods" and general notes about decontamination are available on our home page:

www.eppendorf.com

A signed decontamination certificate must be enclosed with the pipette when it is returned. The serial number of the Reference must be entered on the decontamination certificate.

The Reference can be decontaminated of potentially infectious substances by being steam-autoclaved (see Sec. 9.2).

The surfaces of the pipette can be disinfected with alcohol (ethanol, propanol) or with a disinfectant containing alcohol.

10 Troubleshooting

Error	Cause	Solution
Droplets on the inner wall of the pipette tip.	<ul style="list-style-type: none"> – Uneven wetting of the plastic wall. – A pipette tip with poor wetting properties has been used. 	<ul style="list-style-type: none"> – Attach a new pipette tip. – Use an original eppendorf tip.
Pipette is dripping and/or the volume pipetted is incorrect.	<ul style="list-style-type: none"> – The tip is loose. – A poorly fitting pipette tip has been used. – Liquid with a high vapor pressure has been pipetted. – Tip has been taken out of the liquid too quickly. <p>The pipette is dripping because:</p> <ul style="list-style-type: none"> – Piston is contaminated. – Piston is damaged. – Seals are damaged. – Tip cone loose. 	<ul style="list-style-type: none"> – Press the tip firmly in place. – Use an original eppendorf tip. – In this case, we recommend pipetting using a positive-displacement pipette. – Remove the tip slowly from the liquid. – Clean and lightly lubricate the piston. – Replace the piston and seal (see Part B, Maintenance). – Replace all seals (see Part B, Maintenance). – Lightly tighten tip cone with wrench (see Part B, Maintenance). Exchange, where necessary.

Error	Cause	Solution
Control button jams, moves erratically.	<ul style="list-style-type: none"> – Piston contaminated. – Seals contaminated. – Penetration of solvent vapors. 	<ul style="list-style-type: none"> – Clean piston and lubricate lightly. – Disassemble pipette. Clean all seals and exchange where necessary (see Part B, Maintenance). – Unscrew pipette at the central junction and ventilate. Clean piston and lubricate lightly.
Pipette blocked, too little liquid is aspirated.	<ul style="list-style-type: none"> – Liquid has penetrated the tip cone and dried. <p>For 25 to 500 μL pipettes:</p> <ul style="list-style-type: none"> – The filling tube in the nose cone is blocked. 	<ul style="list-style-type: none"> – Unscrew pipette at the central junction, rinse lower part first with warm water, then with distilled water and allow to dry. <p>Or:</p> <ul style="list-style-type: none"> – Disassemble pipette. Replace ejector seal in the tip cone (see Part B, Maintenance). <p>For 25 to 50 μL:</p> <ul style="list-style-type: none"> – Replace tip cone. <p>For 100 to 500 μL:</p> <ul style="list-style-type: none"> – Replace the filling tube in the tip cone (see Part B, Maintenance).

10.1 If there is doubt that dispensing data are correct

To avoid dispensing errors, the precision and accuracy of the Reference pipettes need to be checked regularly. The PICASO II software program (see ordering information) is available to determine permitted systematic and random measuring deviation (see Chapter 2 "Technical data"). An SOP (**S**tandard **O**perating **P**rocedure) for checking pipettes can be called up from our home page www.eppendorf.com.

For liquids whose physical data deviate significantly from those of water, calibration needs to be changed in accordance with Chapter 8.2.3. Calibration will also need to be changed if the pipette is being used at a very high altitude.

Maintenance

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Ordering information

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Please only use the accessories recommended by eppendorf.

Using spare parts and disposables which we have not recommended can reduce the precision, accuracy and life of the pipette. We do not honor any warranty or accept any responsibility for damage resulting from such action.

Guarantee and warranty

In the case of warranty claims please contact your responsible Eppendorf contractual partner.

Wear-and-tear parts are excluded from the warranty. When returning the pipette to the Eppendorf contractual partner the necessary data for utilization and the performance of decontamination (see Part A section 6.3) should be included.

Servicing must be carried out by the Service of the Eppendorf contractual partner. No warranty shall apply with misuse or opening of the device by unauthorized persons.

For information on replacing pistons and seals as well as on disassembling and assembling the different models in the Reference-Serie please see page 1 at the front of this manual.

The fix and variable pipettes are of identical construction. You should therefore refer to the figure corresponding to the volume of your pipette or the volume range it falls in.

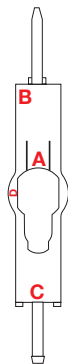
I Exchanging the piston

- Unscrew pipette at the central junction.
- Press control button and hold down. Hold piston at the top of the piston mounting and pull off. If the piston fits too tightly, the spring at the piston mounting can be pressed down slightly with side B of the wrench and the piston then pulled off.
- Attach new piston down to the stop and lubricate lightly.

II Exchanging the seals

The lower parts of the Reference-Serie can be completely disassembled for cleaning and maintenance using the wrench provided.

The wrench has the following functions:



A = Narrow opening: for loosening and tightening the tip cone.
Wide opening (D): For tightening the tip cone (with the lettering facing the pipette tip).

The wrench is designed in such a way that the tip cone cannot be tightened too much.

B = For loosening the screw in the tip cone.
During assembly, for mounting the screw, spring and seal and tightening the screw.
During alignment, for adjusting the volume display.

C = For removing the seal in the tip cone.

Removing the seals

Fig. 2 – 6 on the following pages show you how to remove the seals. The numbers shown are identical with the numbers in the Ordering information (page 94) and the numbers of the parts on page 1 of this manual.

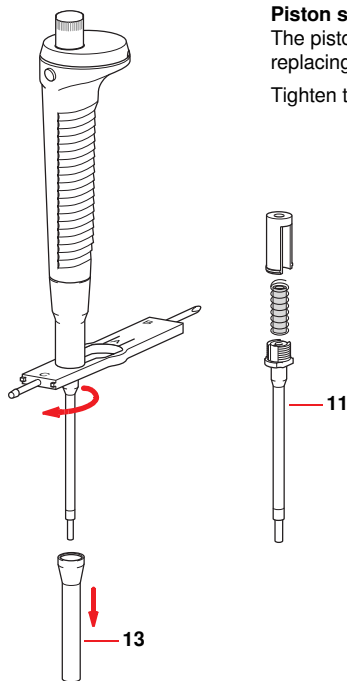
0.1 – 2.5 μL , 0.5 – 10 μL and 2 – 20 μL (Fig. 2)

Push the control button all the way down and pull off the ejector sleeve (13).

Piston seal

The piston seal in the tip cone is exchanged by replacing the entire tip cone (11).

Tighten the tip cone (see IV of this part).



10 – 100 μL (Fig. 3)

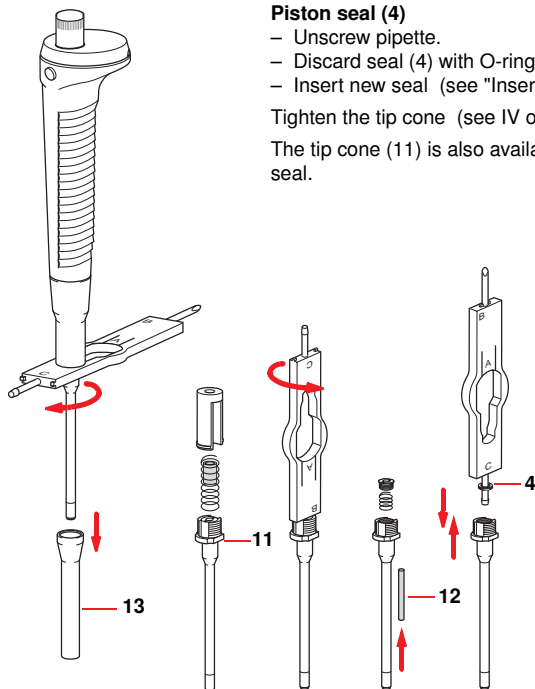
Push the control button all the way down and pull off the ejector sleeve (13).

Piston seal (4)

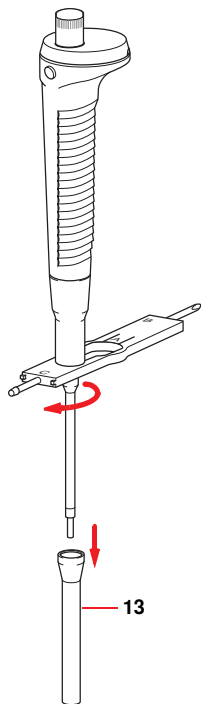
- Unscrew pipette.
- Discard seal (4) with O-ring.
- Insert new seal (see "Inserting the seals").

Tighten the tip cone (see IV of this part).

The tip cone (11) is also available complete with seal.



50 – 200 μL (Fig. 4)

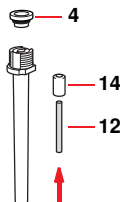
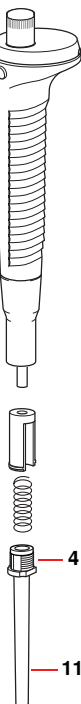


Push the control button all the way down and pull off the ejector sleeve (13).

Piston seal (4)

- Unscrew pipette.
- Remove piston seal (4) from tip cone (with the piston in the grip of the pipette) and discard.
- Place new piston seal onto tip cone.

Tighten the tip cone (see IV of this part).



50 – 250 μ L (Fig. 5)

Push the control button all the way down and pull off the ejector sleeve (13).

Piston seal (4)

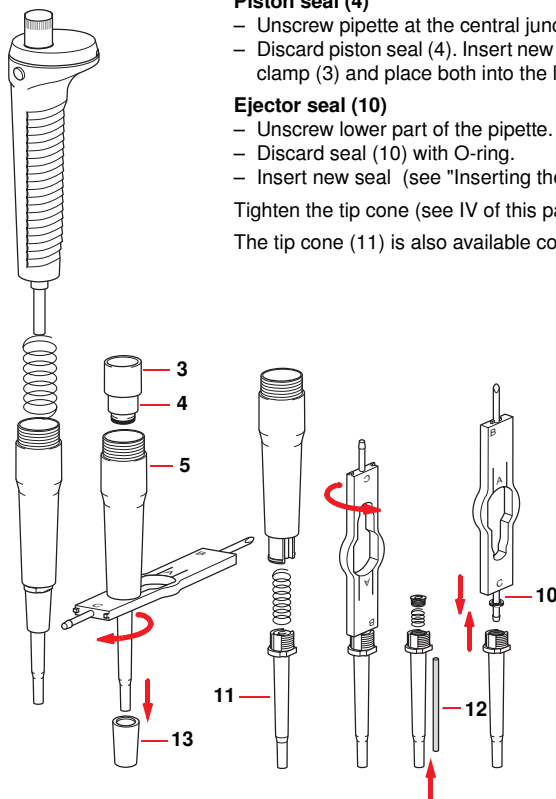
- Unscrew pipette at the central junction.
- Discard piston seal (4). Insert new piston seal into the clamp (3) and place both into the lower part (5).

Ejector seal (10)

- Unscrew lower part of the pipette.
- Discard seal (10) with O-ring.
- Insert new seal (see "Inserting the seals").

Tighten the tip cone (see IV of this part).

The tip cone (11) is also available complete with seal.



100 – 1000 and 500 – 2500 μL (Fig. 6)

Push the control button all the way down and pull off the ejector sleeve (13).

Piston seal (4)

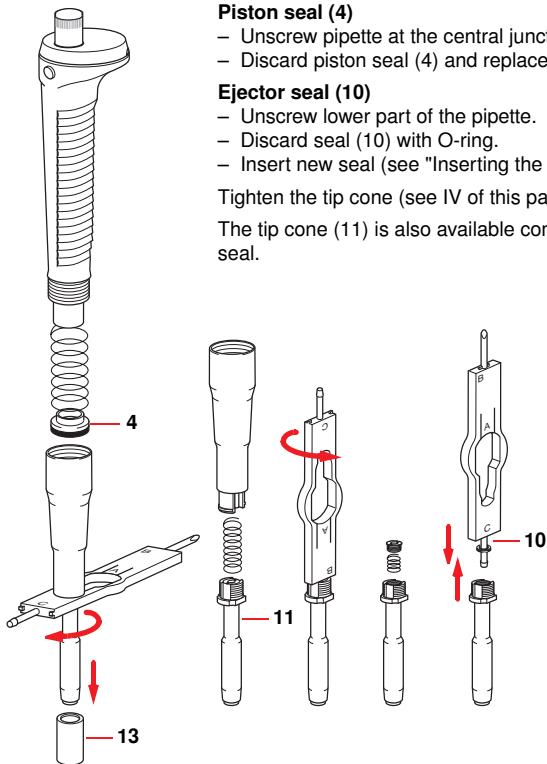
- Unscrew pipette at the central junction.
- Discard piston seal (4) and replace.

Ejector seal (10)

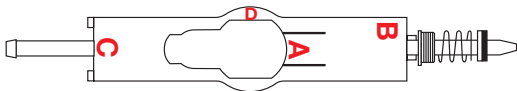
- Unscrew lower part of the pipette.
- Discard seal (10) with O-ring.
- Insert new seal (see "Inserting the seals").

Tighten the tip cone (see IV of this part).

The tip cone (11) is also available complete with ejector seal.



Inserting the seals



Pull new seal off the pin and push screw, spring, seal (with plastic part first) in that order onto side B of the wrench as shown in the figure and screw lightly into tip cone. Do not tighten too much. Assemble pipette again.

III Exchanging the filling tube

10 – 100 μL , 50 – 200 μL and 50 – 250 μL (Fig. 3, 4, 5)

Filling tube (12)

- Unscrew lower part of the pipette.
- Push filling tube (12) (and damping tube (14) for 50 – 250 μL) out of the tip cone from below with the wire punch. Push in new tube from above.

IV Exchanging the tip cone

After the ejector sleeve has been pulled off, the tip cone together with the seal (11) can be loosened with the wrench (A, narrow opening) and exchanged (together with the seal. See Fig. 2 – 6).

Tighten the tip cone:

Place wrench with the wide opening (A, lettering facing the pipette tip) onto the tip cone and tighten until it locks into position by turning half a revolution. The wrench is designed in such a way that the tip cone cannot be tightened too much.

Caution

After exchanging parts or completing other maintenance, always check that the pipette functions correctly (see Part A, Section 5 and 7 of this Manual).

If a problem cannot be solved with the aid of the recommendations above, please return the **eppendorf** Reference to your **eppendorf** appointed dealer.

I Reference fixed-volume

(Please see page 1 (Fig. 1) of this manual).

Only parts with order numbers are available separately.

Pipettes / spare parts

Models	Order number International	Order number North America
Gray control button (for 10 µL pipette tips)		
1 µL	4900 000.010	022470400
2 µL	4900 000.028	022470451
5 µL	4900 000.036	022470507
10 µL	4900 000.044	022470558
Yellow control button (for 200 µL pipette tips)		
10 µL	4900 000.109	022470604
20 µL	4900 000.117	022470752
25 µL	4900 000.150	022470809
30 µL		022470850
50 µL	4900 000.125	022470957
75 µL		022471007
80 µL		022471058
90 µL		022471104
100 µL	4900 000.133	022471155
150 µL		022471201
200 µL		022471252
Blue control button (for 1000 µL pipette tips)		
200 µL	4900 000.508	
250 µL	4900 000.540	022471309
300 µL		022471350
500 µL	4900 000.516	022471457
800 µL		022471554
900 µL		022471601
1000 µL	4900 000.524	022471651
Red control button (for 2500 µL pipette tips)		
1500 µL	4900 000.923	022471708
2000 µL	4900 000.907	022471759
2500 µL	4900 000.915	022471805

Reference® – Part B – Ordering information

	Order number International	Order number North America
1 Piston		
1 – 10 µL (gray control button)	4910 810.001	022475088
20 µL	4910 811.008	
10 – 20 µL (yellow control button)		022475100
25 – 50 µL	4900 810.003	022475118
100 µL, incl. piston seal (4)	4910 812.004	
75 – 100 µL, incl. piston seal (4)		022475126
150 – 200 µL		022475142
250 µL		022475169
200 – 250 µL, incl. piston seal (4)	4910 813.000	
300 – 1000 µL, incl. piston seal (4)		022475185
500 – 1000 µL, incl. piston seal (4)	4910 814.007	
500 – 2500 µL, incl. piston seal (4)	4910 815.003	022475207
2 Piston spring		
1 – 250 µL	4910 825.009	
250 µL		022475223
300 – 1000 µL		022475240
500 – 1000 µL	4910 826.005	
1500 – 2500 µL	4910 827.001	022475266
3 Clamp	not sold separately	
4 Piston seal		
75 – 100 µL, incl. screw (8), spring (9)		022475282
100 µL, incl. screw (8), spring (9)	4910 820.007	
150 – 200 µL, incl. screw (8), spring (9)		022475304
200 – 250 µL, incl. screw (8), spring (9), ejector seal (10)	4910 821.003	
250 µL, incl. screw (8), spring (9), ejector seal (10)		022475321
300 – 1000 µL, incl. screw (8), spring (9), ejector seal (10)		022475347
500 – 1000 µL, incl. screw (8), spring (9), ejector seal (10)	4910 822.000	
500 – 2500 µL, incl. screw (8), spring (9), ejector seal (10)	4910 823.006	022475363

5 Lower housing

6 Ejector, includes ejector spring (7)

- 1 – 20 µL, incl. ejector tube
- 25 – 100 µL, incl. ejector tube
- 150 – 200 µL
- 200 – 250 µL
- 250 µL
- 300 – 1000 µL
- 500 – 1000 µL
- 1500 – 2500 µL

Order number
International
not sold separately

Order number
North America

4910 839.000	022475380
4910 840.008	022475401
	022475428
4910 841.004	
	022475461
	022475487
4910 842.000	
4910 843.007	022475509

7 Ejector spring

(1 – 100 µL includes ejector tube)

not sold separately

8 Screw for tip cone

not sold separately

9 Tip cone spring

not sold separately

10 Ejector seal

not sold separately

11 Tip cone, complete

- 1 – 10 µL gray, incl. seal
- 10 – 20 µL yellow, incl. seal
- 25 – 50 µL, incl. (8), (9), (4), (12)
- 75 – 100 µL, incl. (8), (9), (4), (12)
- 100 µL, incl. (8), (9), (4), (12)
- 150 – 200 µL, incl. (12), (14)
- 200 – 250 µL, incl. (8), (9), (10), (12)
- 250 µL, incl. (8), (9), (10), (12)
- 300 – 500 µL, incl. (8), (9), (10), (12)
- 500 µL, incl. (8), (9), (10), (12)
- 800 – 1000 µL, incl. (8), (9), (10)
- 1000 µL, incl. (8), (9), (10)
- 500 – 2500 µL, incl. (8), (9), (10)

4910 830.002	022475541
4910 832.005	022475584
4900 830.004	022475606
	022475622
4910 833.001	
	022475649
4910 834.008	
	022475665
	022475681
4900 831.000	
	022475703
4910 835.004	
4910 836.000	022475720

12 Filling tube (5 pieces, 1 wire punch)

- 75 – 100 µL
- 100 µL
- 150 – 200 µL
- 200 – 250 µL
- 250 µL
- 300 – 500 µL
- 500 µL

	022475746
4910 837.007	
	022475762
4910 838.003	
	022475789
	022475801
4900 837.009	

13 Ejector sleeve

1	–	100 µL
150	–	200 µL
200	–	1000 µL
250	–	1000 µL
500	–	2500 µL

Order number International Order number North America

4910 845.000	022475827
	022475843
4910 846.006	
	022475860
4910 847.002	022475886

14 Damping tube

not sold separately

Lower part complete, incl. piston and (3) – (13)

1	–	10 µL
		10 µL (gray button)
10	–	20 µL (yellow button)
25	–	50 µL
75	–	100 µL
		100 µL
150	–	200 µL
200	–	250 µL
		250 µL
300	–	500 µL
		500 µL
800	–	1000 µL
		1000 µL
500	–	2500 µL

4910 890.005	
	022475924
4910 892.008	022475967
4900 890.007	022475983
	022476009
4910 893.004	
	022476025
4910 894.000	
	022476041
	022476068
4900 891.003	
	022476084
4910 895.007	
4910 896.003	022476106

Reference repair set

4910 805.008	022475045
--------------	-----------

(tube of grease for pipettes, 1 wrench, 6 blank labels, 1 wire punch, 1 filling tube each for 75 – 100 µL, 150 – 200 µL, 250 µL and 300 – 500 µL, 1 damping tube for 50 – 200 µL)

Calibration aid labels (5 pieces)

4900 805.000	022475002
--------------	-----------

Grease for pipettes

0013 063.010	022458507
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Wrench

4910 092.001	022475029
--------------	-----------

Calibration seal, red (5 pieces)

4910 601.003	
--------------	--

II Reference adjustable-volume

(Please see page 1 (Fig. 1) of this manual).

Only parts with order numbers are available separately.

Pipettes / spare parts

Models	Order number International	Order number North America
Dark gray control button (for 10 µL pipette tips) 0.1 – 2.5 µL	4910 000.085	022470001
Light gray control button (for 20 µL pipette tips) 0.5 – 10 µL 2 – 20 µL	4910 000.018 4910 000.026	022470051 022470108
Yellow control button (for 200 µL pipette tips) 2 – 20 µL 10 – 100 µL 50 – 200 µL	4910 000.034 4910 000.042 4910 000.093	022470159 022470205 022470256
Blue control button (for 1000 µL pipette tips) 50 – 250 µL 100 – 1000 µL	4910 000.050 4910 000.069	022470302
Red control button (for 2500 µL pipette tips) 500 – 2500 µL	4910 000.077	022470353
1 Piston 0.1 – 2.5 µL 0.5 – 10 µL 2 – 20 µL 10 – 100 µL, incl. piston seal (4) 50 – 200 µL, incl. piston seal (4) 50 – 250 µL, incl. piston seal (4) 100 – 1000 µL, incl. piston seal (4) 500 – 2500 µL, incl. piston seal (4)	4910 809.003 4910 810.001 4910 811.008 4910 812.004 4910 816.000 4910 813.000 4910 814.007 4910 815.003	022475061 022475088 022475100 022475126 022475142 022475185 022475207
2 Piston spring 50 – 250 µL 100 – 1000 µL 500 – 2500 µL	4910 825.009 4910 826.005 4910 827.001	022475240 022475266
3 Clamp	not sold separately	

	Order number International	Order number North America
4 Piston seal		
10 – 100 µL, incl. screw (8), spring (9)	4910 820.007	022475282
50 – 200 µL, with 2 O-rings	4910 824.002	022475304
50 – 250 µL, incl. screw (8), spring (9), ejector seal (10)	4910 821.003	
100 – 1000 µL, incl. screw (8), spring (9), ejector seal (10)	4910 822.000	022475347
500 – 2500 µL, incl. screw (8), spring (9), ejector seal (10)	4910 823.006	022475363
5 Lower housing	not sold separately	
6 Ejector , includes ejector spring (7)		
0.1 – 2.5 µL, incl. ejector tube	4910 839.000	022475380
0.5 – 10 µL, incl. ejector tube	4910 839.000	022475380
2 – 20 µL	4910 839.000	022475380
10 – 100 µL	4910 840.008	022475401
50 – 200 µL	4910 844.003	022475444
50 – 250 µL	4910 841.004	
100 – 1000 µL	4910 842.000	022475487
500 – 2500 µL	4910 843.007	022475509
7 Ejector spring (0.1–2.5, 0.5–10, 2–20, 10–100 µL incl. ejector tube)	not sold separately	
8 Screw for tip cone	not sold separately	
9 Tip cone spring	not sold separately	
10 Ejector seal	not sold separately	
11 Tip cone, complete		
0.1 – 2.5 µL, incl. seal	4910 829.004	022475525
0.5 – 10 µL, incl. seal	4910 830.002	022475541
2 – 20 µL, light gray, incl. seal	4910 831.009	022475568
2 – 20 µL, yellow, incl. seal	4910 832.005	022475584
10 – 100 µL, incl. (8), (9), (4), (12)	4910 833.001	022475622
50 – 200 µL, incl. (12), (14)	4910 851.000	022475649
50 – 250 µL, incl. (8), (9), (10), (12)	4910 834.008	
100 – 1000 µL, incl. (8), (9), (10)	4910 835.004	022475703
500 – 2500 µL, incl. (8), (9), (10)	4910 836.000	022475720

	Order number International	Order number North America
12 Filling tube , (5 pieces, 1 wire punch)		
10 – 100 µL	4910 837.007	022475746
50 – 200 µL, incl. 2 pieces of (14)	4910 853.002	022475762
50 – 250 µL	4910 838.003	
13 Ejector sleeve		
0.1 – 2.5 µL	4910 845.000	022475827
0.5 – 10 µL	4910 845.000	022475827
2 – 20 µL	4910 845.000	022475827
10 – 100 µL	4910 845.000	022475827
50 – 200 µL	4910 852.006	022475843
50 – 250 µL	4910 846.006	
100 – 1000 µL	4910 846.006	022475860
500 – 2500 µL	4910 847.002	022475886
14 Damping tube		
Lower part , complete, incl. piston and (3) – (14)	not sold separately	
0.1 – 2.5 µL	4910 889.007	022475908
0.5 – 10 µL	4910 890.005	022475924
2 – 20 µL, light gray	4910 891.001	022475941
2 – 20 µL, yellow	4910 892.008	022475967
10 – 100 µL	4910 893.004	022476009
50 – 200 µL	4910 897.000	022476025
50 – 250 µL	4910 894.000	
100 – 1000 µL	4910 895.007	022476084
500 – 2500 µL	4910 896.003	022476108
Reference repair set	4910 805.008	022475045
(tube of grease for pipettes, 1 wrench, 6 blank labels, 1 wire punch, 1 filling tube each for 75 – 100 µL, 150 – 200 µL, 250 µL and 300 – 500 µL, 1 damping tube for 50 – 200 µL)		
Calibration aid labels (5 pieces)	4900 805.000	022475002
Grease for pipettes	0013 063.010	022458507
Calibration seal, red (5 pieces)	4910 601.003	
Wrench	4910 092.001	022475029

III Additional accessories

	Order number International	Order number North America
Pipette carousel, incl. 6 pipette supports	3115 000.003	022444905
Pipette holder (replacement for stand)	3115 600.019	022260588
Pipette wall mount (with adhesive surface for attachment to lab benches and wall)	3115 000.020	022444913
PICASO II (Pipette Calibration Software)	3113 004.001	
PICASO accessories	see eppendorf catalog	
Operating manual	4910 900.132	

IV Pipette tips

epT.I.P.S.

(The packaging units stated represent the minimum ordering quantity).

	Length	Color code	Order number International	Order number North America
Standard , in bags, 2x 500 = 1000 tips				
0.1 – 10 µL	34 mm	dark gray	0030 000.811	022492004
0.1 – 20 µL	40 mm	medium gray	0030 000.838	022492012
0.5 – 20 µL L	46 mm	light gray	0030 000.854	022492021
2 – 200 µL	53 mm	yellow	0030 000.870	022492039
50 – 1000 µL	71 mm	blue	0030 000.919	022492055
500 tips				
500 – 2500 µL	115 mm	red	0030 000.951	022492071
Set , 1 Box, incl. 5x 96 tips				
0.1 – 10 µL	34 mm	dark gray	0030 073.207	022491407
0.1 – 20 µL	40 mm	medium gray	0030 073.223	022491415
0.5 – 20 µL L	46 mm	light gray	0030 073.240	022491423
2 – 200 µL	53 mm	yellow	0030 073.266	022491431
50 – 1000 µL	71 mm	blue	0030 073.304	022491458
5x 48 tips				
500 – 2500 µL	115 mm	red	0030 073.347	022491474
Reloads , 10x 96 = 960 tips				
0.1 – 10 µL (in stacks)	34 mm	dark gray	0030 073.363	022491504
0.1 – 20 µL	40 mm	medium gray	0030 073.380	022491512
0.5 – 20 µL L	46 mm	light gray	0030 073.401	022491521
2 – 200 µL (in stacks)	53 mm	yellow	0030 073.428	022491539
50 – 1000 µL	71 mm	blue	0030 073.460	022491555
10x 48 = 480 tips				
500 – 2500 µL	115 mm	red	0030 073.509	022491571
Reloads PCR-clean , 10x 96 = 960 tips				
0.1 – 10 µL (in stacks)	34 mm	dark gray	0030 073.746	022491709
0.1 – 20 µL	40 mm	medium gray	0030 073.762	022491717
0.5 – 20 µL L	46 mm	light gray	0030 073.789	022491725
2 – 200 µL (in stacks)	53 mm	yellow	0030 073.800	022491733
50 – 1000 µL	71 mm	blue	0030 073.843	022491750
10x 48 = 480 tips				
500 – 2500 µL	115 mm	red	0030 073.886	022491776

Reference – Part B – Ordering information

	Length	Color code	Order number International	Order number North America
Box, 1 box plus 96 tips				
0.1 – 10 µL	34 mm	dark gray	0030 073.002	022491300
0.1 – 20 µL	40 mm	medium gray	0030 073.029	022491318
0.5 – 20 µL L	46 mm	light gray	0030 073.045	022491326
2 – 200 µL	53 mm	yellow	0030 073.061	022491334
50 – 1000 µL	71 mm	blue	0030 073.100	022491351
48 tips				
500 – 2500 µL	115 mm	red	0030 073.142	022491377
Racks, plus 10x96=960 tips				
0.1 – 10 µL	34 mm	dark gray		022491903
0.1 – 20 µL	40 mm	medium gray		022491911
0.5 – 20 µL L	46 mm	light gray		022491920
2 – 200 µL	53 mm	yellow		022491938
50 – 1000 µL	71 mm	blue		022491954
480 tips				
500 – 2500 µL	115 mm	red		022491971
Racks PCR-clean, plus 10x96=960 tips				
0.1 – 10 µL	34 mm	dark gray		022491806
0.1 – 20 µL	40 mm	medium gray		022491814
0.5 – 20 µL L	46 mm	light gray		022491822
2 – 200 µL	53 mm	yellow		022491831
50 – 1000 µL	71 mm	blue		022491857
240 tips				
500 – 2500 µL	115 mm	red		022491873
Racks, sterile, plus 10x96=960 tips				
0.1 – 20 µL	40 mm	medium gray		022492250
2 – 200 µL	53 mm	yellow		022492276
50 – 1000 µL	71 mm	blue		022492292
Racks Eppendorf Biopur, colorless, pyrogen-free, DNA-free, RNase-free, ATP-free				
5x 96 = 480 tips				
0.1 – 20 µL	40 mm	medium gray	0030 075.005	022491067
2 – 200 µL	53 mm	yellow	0030 075.021	022491083
50 – 1000 µL	71 mm	blue	0030 075.064	022491105
240 tips				
500 – 2500 µL	115 mm	red	0030 075.102	022491121

Reference – Part B – Ordering informations

			Length	Color code	Order number International	Order number North America
Singles (Eppendorf Biopur) , colorless, pyrogen-free, DNA-free, RNase-free, ATP-free, individually wrapped, 100 tips						
0.1	– 20 µL		40 mm	medium gray	0030 010.019	022491130
2	– 200 µL		53 mm	yellow	0030 010.035	022491148
50	– 1000 µL		71 mm	blue	0030 010.051	022491156
ep Dualfilter T.I.P.S. , PCR-clean, sterile, in racks, 10x 96 = 960 tips						
0.1	– 10 µL	S	34 mm	dark gray	0030 077.504	022491202
0.1	– 10 µL	M	40 mm	medium gray	0030 077.512	022491211
0.5	– 20 µL	L	46 mm	light gray	0030 077.520	022491229
2	– 20 µL		53 mm	yellow	0030 077.539	022491270
2	– 100 µL		53 mm	yellow	0030 077.547	022491237
2	– 200 µL		55 mm	yellow	0030 077.555	022491296
20	– 300 µL		55 mm	orange	0030 077.563	022491245
50	– 1000 µL		76 mm	blue	0030 077.571	022491253
GELoader® tips (f. 0.5 – 10 µL)						
1 set	= 200 tips		62 mm	light gray	0030 001.222	022351656

Reference – Part B – Ordering informations

	Length	Color code	Order number International	Order number North America
Reloads, LoRetention PCR clean,				
10 x 96 = 960 tips				
0.1 – 10 µL	34 mm	dark gray	0030 072.006	022493010
0.5 – 20 µL L	46 mm	light gray	0030 072.014	022493012
2 – 200 µL	53 mm	yellow	0030 072.022	022493014
50 – 1000 µL	71 mm	blue	0030 072.030	022493016
Reloads, LoRetention, autoclavable,				
10 x 96 = 960 tips				
0.1 – 10 µL	34 mm	dark gray	0030 072.049	022493018
0.5 – 20 µL L	46 mm	light gray	0030 072.057	022493020
2 – 200 µL	53 mm	yellow	0030 072.065	022493022
50 – 1000 µL	71 mm	blue	0030 072.073	022493024
Racks, LoRetention PCR clean,				
10 x 96 = 960 tips				
0.1 – 10 µL	34 mm	dark gray		022493026
0.5 – 20 µL L	46 mm	light gray		022493028
2 – 200 µL	53 mm	yellow		022493030
50 – 1000 µL	71 mm	blue		022493032
Racks, LoRetention Dualfilter, PCR clean, sterile and pyrogen free				
10 x 96 = 960 tips				
0.1 – 10 µL S	34 mm	dark gray	0030 077.610	022493000
0.5 – 20 µL L	46 mm	light gray	0030 077.628	022493002
2 – 100 µL	53 mm	yellow	0030 077.644	022493006
20 – 300 µL	55 mm	orange	0030 077.636	022493004
50 – 1000 µL	76 mm	blue	0030 077.652	022493008

Eppendorf Offices

AUSTRALIA / NEW ZEALAND

Eppendorf South Pacific Pty. Ltd.
Tel. +61 2 98 89 50 00
Fax +61 2 98 89 51 11
E-Mail: Info@eppendorf.com.au
Internet: www.eppendorf.com.au

AUSTRIA

Eppendorf Austria GmbH
Tel. +43 (0) 1 890 13 64 - 0
Fax +43 (0) 1 890 13 64 - 20
E-Mail: office@eppendorf.at
Internet: www.eppendorf.at

BRAZIL

Eppendorf do Brasil Ltda.
Tel. +55 11 3095 9344
Fax +55 11 3095 9340
E-Mail: eppendorf@eppendorf.com.br
Internet: www.eppendorf.com.br

CANADA

Eppendorf Canada Ltd.
Tel. +1 905 826 5525
Fax +1 905 826 5424
E-Mail: canada@eppendorf.com
Internet: www.eppendorfn.com

CHINA

Eppendorf China Ltd.
Tel. +86 21 68760880
Fax +86 21 50815371
E-Mail: market.info@eppendorf.cn
Internet: www.eppendorf.cn

FRANCE

EPPENDORF FRANCE S.A.R.L.
Tel. +33 1 30 15 67 40
Fax +33 1 30 15 67 45
E-Mail: eppendorf@eppendorf.fr
Internet: www.eppendorf.fr

GERMANY

Eppendorf Vertrieb
Deutschland GmbH
Tel. +49 2232 418-0
Fax +49 2232 418-155
E-Mail: vertrieb@eppendorf.de
Internet: www.eppendorf.de

INDIA

Eppendorf India Limited
Tel. +91 44 42 11 13 14
Fax +91 44 42 18 74 05
E-Mail: info@eppendorf.co.in
Internet: www.eppendorf.co.in

ITALY

Eppendorf s.r.l.
Tel. +390 2 55 404 1
Fax +390 2 58 013 438
E-Mail: eppendorf@eppendorf.it
Internet: www.eppendorf.it

JAPAN

Eppendorf Co. Ltd.
Tel. +81 3 5825 2363
Fax +81 3 5825 2365
E-Mail: info@eppendorf.jp
Internet: www.eppendorf.jp

NORDIC

Eppendorf Nordic Aps
Tel. +45 70 22 29 70
Fax +45 45 76 73 70
E-Mail: nordic@eppendorf.dk
Internet: www.eppendorf.dk

SOUTH & SOUTHEAST ASIA

Eppendorf Asia Pacific Sdn. Bhd.
Tel. +60 3 8023 2769
Fax +60 3 8023 3720
E-Mail: asiapacific@eppendorf.com.my
Internet: www.eppendorf.com.my

SPAIN

Eppendorf Ibérica S.L.U.
Tel. +34 91 651 76 94
Fax +34 91 651 81 44
E-Mail: iberica@eppendorf.es
Internet: www.eppendorf.es

SWITZERLAND

Vaudaux-Eppendorf AG
Tel. +41 61 482 1414
Fax +41 61 482 1419
E-Mail: vaudaux@vaudaux.ch
Internet: www.eppendorf.ch

UNITED KINGDOM

Eppendorf UK Limited
Tel. +44 1223 200 440
Fax +44 1223 200 441
E-Mail: sales@eppendorf.co.uk
Internet: www.eppendorf.co.uk

USA

Eppendorf North America
Tel. +1 516 334 7500
Fax +1 516 334 7506
E-Mail: info@eppendorf.com
Internet: www.eppendorfn.com

OTHER COUNTRIES

Internet:
www.eppendorf.com/worldwide



Your local distributor: www.eppendorf.com/worldwide

Eppendorf AG · 22331 Hamburg · Germany · Tel: +49 40 538 01-0 · Fax: +49 40 538 01-556
E-Mail: eppendorf@eppendorf.com

Eppendorf North America, Inc. · 102 Motor Parkway, Suite 410 · Hauppauge, NY 11788-5178 · USA
Tel: +1 516 334 7500 · Toll free phone: +1 800 645 3050 · Fax: +1 516 334 7506
E-Mail: info@eppendorf.com

Application Support


Europe, International: Tel: +49 1803 666 789 · E-Mail: support@eppendorf.com
North America: Tel: +1 800 645 3050 ext. 2258 · E-Mail: support_na@eppendorf.com
Asia, Pacific: Tel: +60 3 8023 2769 · E-Mail: support_asiapacific@eppendorf.com

Attachment 3b

Eppendorf SOP

Standard Operating Procedure for Pipettes

eppendorf



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1 Test and pipette conditions

- i** These test and pipetting conditions are valid for all Eppendorf pipettes and dispensers. Follow EN ISO 8655 "Volume-measuring equipment with pistons".

For the calibration or adjustment of pipettes, the scales and measuring station should fulfill the following requirements:

1.1 Balances

1.1.1 Balance type

Use semi-microbalances and microbalances to calibrate pipettes.

Some companies offer balances that are specially designed to meet the requirements of pipette calibration, e.g. Sartorius and Mettler.

1.1.2 Accuracy

When selecting the balances, ensure that they are suitable for the accuracy of the pipette. This helps to ensure that deviations within a measuring series are recorded exactly for the assessment of the systematic and random measurement deviations in accordance with EN ISO 8655.

For a pipette volume of less than or equal to 10 μL , balances with a 6-digit display must be used. With larger volumes, balances with a 5-digit display are sufficient.

1.1.3 Minimum requirements for balances

Selected volume* of the pipette to be tested	Display resolution (mg)
1 μL to 10 μL	0.001
>10 μL to 100 μL	0.01
>100 μL to 1 000 μL	0.1
>1 mL to 10 mL	0.1
>10 mL to 200 mL	1

* For practical reasons, the nominal volume can be used for the selection of the balances.

1 Test and pipette conditions

1.2 Measuring station

1.2.1 Evaporation

Take evaporation protection into account while carrying out the measurement.

With volumes < 50 µL in particular, errors caused by the evaporation of the test liquid must be taken into consideration.

This can be ensured by using a liquid trap or other equipment to prevent evaporation.

1.2.2 Measuring station

For electronic measurement data processing, we recommend calibration software and accessories for your balance (PICASO, order no. 3113 004.001).

(see also EN ISO 8655, Part 6)

1.2.3 Test room

The tests should be carried out in a draft-free room under constant climatic conditions.

The test room should have a constant temperature between 15 °C and 30 °C and a constant relative humidity above 50 %.

1.2.4 Temperature differences

Before the test, the device to be tested and test liquid must have stood in the test room for a sufficient amount of time, at least 2 hours, in order to reach equilibrium with the storage conditions.

Direct sunlight and other influences which could affect the temperature should be avoided at all costs.

1.2.5 Test liquid

Distilled or deionized water of "Quality 3 in accordance with ISO 3696", degassed or at equilibrium with air. The water must be at room temperature.

1.2.6 Operating Manual

Observe the operating instructions for your pipette.

2 Calibration

2.1 Scope of testing

Note concerning the nominal volume:

The nominal volume of an adjustable-volume pipette is the largest volume to be set by the user and specified by the manufacturer.

When using the Combitip with the Multipette, the nominal volume is the largest possible dispensing volume of the Combitip. For Multipette plus, the nominal volume is 1/5 of the Combitip filling volume. For the Multipette stream / Xstream, the nominal volume is the filling volume of the Combitip.

2.1.1 Adjustable-volume pipettes

For variable pipettes, check **3 different volumes** with 10 measured values each:

- the nominal volume,
- approx. 50 % of the nominal volume,
- 10 % of the nominal volume.

2.1.2 Multi-channel pipettes

For Multi-channel pipettes check **each channel separately** with 3 different volumes, each with 10 measured values:

- the nominal volume,
- approx. 50 % of the nominal volume,
- 10 % of the nominal volume.

2.1.3 Multipette

For

- Multipette,
- Multipette plus,
- Multipette stream,
- Multipette Xstream

check the nominal volume with 10 measured values with the Eppendorf Combitip used.

2.1.4 Bottle-top dispenser and Top Buret

For bottle-top dispensers and the Top Buret, check the nominal volume with 10 measured values.

2 Calibration

2.2 Measurement

2.2.1 Work method

1. Place the selected pipette tip on the tip cone of the pipette or on the corresponding Combitip in the Multipipette.
2. Implement the following settings:
 - Adjustable-volume piston-stroke pipettes: the smallest volume to be tested
 - Multipettes: the nominal volume
 - Bottle-top dispenser and Top Buret: the nominal volume
3. For all Multipettes with fully drawn up Combitip plus, first discard the first dispensing step.
4. Fill test liquid up to a height of min. 3 mm in the weighing vessel.
5. For piston-stroke pipettes fill the pipette tip 5x with test liquid and empty it (pre-wet), in order to create a moisture balance in the dead air volume.
6. Replace the single-use tip.
7. Pre-wet the tip 1x.

2.2.2 Removing the test volume from the reservoir

1. Hold the pipette vertically.
2. Dip the pipette tip into the test liquid by a few millimeters.
3. Draw in the volume to be tested slowly and evenly. The waiting time of 1 to 3 seconds, for Research 1 to 10 mL 5 seconds must be observed.
(the waiting time corresponds to the size of the tip, see operating instructions.)
4. Pull the pipette tip slowly out of the liquid, wiping it on the vessel wall.

2.2.3 Dispensing the test volume into the weighing vessel

1. Rest the filled tip up against the wall of the weighing vessel at an angle.
2. Dispense the test liquid slowly until the first stop (measuring stroke).
3. Press the control button to the second stop (blow-out) and dispense the remaining liquid in the tip (does not apply for dispensers and burets).
4. Hold down the control button and pull the tip up the vessel wall.
5. Let the control button slide back into position.
6. Determine the weight.
7. Complete all measurements of a measuring series as described and calculate the systematic and random error (see p. 9).
8. In case of adjustable-volume pipettes, determine the measurement with the nominal volume, 50 % and 10 % of the nominal volume. Always begin the test with 10 % of the nominal volume.

3 Evaluation

3.1 Calculating the systematic error

Mean value of the dispensed volume:

$$\bar{x} = \frac{\sum \text{All measured values}}{n} \cdot Z$$

n = Number of measured values

To convert the measured values into volume values, use correction factor Z for the dependency of the test liquid on temperature and air pressure for each individual value (see *Factor Z for distilled water* on p. 29).

Systematic error e_s in micro liter:

$$e_s = \bar{x} - x_{\text{nominal}}$$

Systematic error e_s in percent:

$$e_s = 100 \frac{(\bar{x} - x_{\text{nominal}})}{x_{\text{nominal}}}$$

3.2 Calculating the random error

Random error as repeat standard s:

$$s = \sqrt{\frac{\sum (x_i \cdot Z - \bar{x})^2}{n - 1}}$$

Random error as coefficient of variation CV:

$$CV (\%) = \frac{s}{\bar{x}} \cdot 100$$

The specifications of the tested pipette can be found in the relevant operating instructions or technical specifications (see *Technical specifications* on p. 30).

4 Sterilization and cleaning

- i** Further notes on cleaning and sterilization can found in the relevant operating manual.

4.1 Sterilization

Parameter for autoclaving

- 121 °C
- 20 minutes
- 1 bar overpressure

4.1.1 Reference and Biomaster

The Reference pipettes and the Biomaster are fully autoclavable.

1. Prior to autoclaving, unscrew the upper and lower part of the pipette apart by approx. one turn to enable the vapor to enter more easily.
2. After autoclaving, allow the pipette to cool down to room temperature and to dry completely and then screw together.

4.1.2 Research plus

The Research plus pipettes are completely autoclavable.

1. You can put the Research plus into the autoclave as a whole unit or with the lower part removed. Do not disassemble the lower part.
2. For 5 mL and 10 mL pipettes: remove the old protection filter. Add a new protection filter and install it after autoclaving. Autoclave the protection filter only once.
After autoclaving:
3. Cool the pipette down to room temperature and leave to dry.
4. For 5 mL / 10 mL pipettes: the protection filter swells during autoclaving. Slightly squeeze the protection filter when installing it into the cone tip.

4.1.3 Research and Research pro

With the Research and Research pro pipettes, the lower section is autoclavable.

With single-channel models prior to autoclaving

1. Pull off the ejector sleeve with depressed ejector.
2. Unscrew the lower part of the pipette.

With the multi-channel versions, you can autoclave the entire lower section.

After autoclaving, assemble the parts only after room temperature has been reached and the parts have been dried.

4.1.4 Top Buret

The Top Buret can not be autoclaved.

4 Sterilization and cleaning

4.1.5 Varipette and Multipette

The

- Varipette,
- Multipette,
- Multipette plus,
- Multipette stream and
- Multipette Xstream

are not autoclavable.

4.1.6 Varispenser

The Varispenser and Varispenser plus are only autoclavable when fully assembled.

With the Varispenser plus

1. Set the discharge valve toggle to the dispensing position (→).
2. Release the volume setting knob, move to the middle position and leave released.
3. Place the dispenser on a cloth and autoclave, avoiding contact with hot surfaces.
4. Only use the dispenser again when it has cooled down to room temperature.

4.1.7 Xplorer

The lower part on the Xplorer pipettes can be autoclaved.

1. Pull off the ejector sleeve with depressed ejector.
2. On the lower part of the pipette, push the ring labeled **PUSH UP TO RELEASE** upward until the lower part is released.

After autoclaving, assemble the parts only after room temperature has been reached and the parts have been dried.

4.2 Cleaning



All areas of the underside of the pipette can be cleaned in a soap solution or isopropanol 60 % provided that no differing instructions are given in the operating manual.

1. Clean the parts in soap solution or isopropanol.
2. Rinse the parts in distilled water.
3. Allow the parts to dry completely and then assemble.
4. Lightly lubricate the piston of the pipette (Eppendorf special grease

5 Leakage check

5.1 Pipette leakage check

In order to carry out a leakage check on a pipette, follow the procedure outlined below:

1. For adjustable-volume pipettes: set the nominal volume.
2. For volumes $<20\ \mu\text{L}$ pre-wet the tip several times.
3. Hold the pipette vertically with a full tip for approx. 30 sec. Do not touch the pipette tip.
4. Observe the meniscus of the liquid on the tip opening. If there is a leak in the pipette, a drip will form on the tip opening.

6 Troubleshooting

6.1 Possible error causes and elimination of errors

- i** Observe the error descriptions in the operating instructions.

Error	Cause	Elimination
Droplets on the inner wall of the pipette tip.	Uneven wetting of the plastic wall.	<ul style="list-style-type: none"> ▶ Attach a new pipette tip.
Pipette is dripping, pipetted volume is incorrect.	<ul style="list-style-type: none"> • Tip loose. • Incorrect pipette tip. 	<ul style="list-style-type: none"> ▶ Press the tip on firmly. ▶ Use original Eppendorf tip.
	Pipette is leaking because: <ul style="list-style-type: none"> • The piston is contaminated. • The piston is damaged. • The seal is damaged. 	<ul style="list-style-type: none"> ▶ Clean the piston and lubricate slightly. ▶ Replace the piston and piston sealing and lubricate slightly. ▶ Replace the seal.
Liquid cannot be aspirated correctly or dripping occurs.	<ul style="list-style-type: none"> • Leakage in the Combitip plus. • Combitip plus has heated up. 	<ul style="list-style-type: none"> ▶ Replace the Combitip plus with a new Combitip plus. ▶ Ensure that the temperature is uniform as the liquid expands when heated.
The control button is jammed, runs jerky.	<ul style="list-style-type: none"> • The piston is contaminated. • The seal is contaminated. • The piston is damaged. • Penetration of solvent vapors 	<ul style="list-style-type: none"> ▶ Clean the piston and lubricate slightly. ▶ Disassemble the pipette, clean all seals, replace if necessary. ▶ Replace the piston and piston seal and lubricate slightly. ▶ Unscrew the lower part of the pipette and aerate the lower part. ▶ Clean the piston and lubricate slightly.

7 Adjustment

7.1 General information

Prior to delivery, all pipettes are adjusted with distilled or de-ionized, degassed water under the conditions described in Chapters 1 to 3 and according to ISO 3696 (see *Calibration* on p. 7). If you have doubts regarding the accuracy of the volume entered, please check the following points first:

- Is there a leak in the device (see *Leakage check* on p. 12)?
Exception: Biomaster
- Does the temperature of the pipetted liquid correspond to:
 - the temperature of the device?
 - the ambient air temperature?
- Is the set volume correct?
- Has the liquid density and air pressure been taken into consideration?
- Does the density of the pipetted liquid deviate from the double-distilled, degassed water?
- Was the work completed correctly, as described in the "Calibration" chapter (see p. 7) and "Evaluation" chapter (see p. 9)?
- Were original Eppendorf pipette tips used?

Volume errors can also occur when pipetting liquids with high vapor pressure, where the density or viscosity deviates considerably from the values of water.

Adjustments should only be carried out on the device after these conditions have been taken into consideration.

7.2 Adjusting adjustable-volume pipettes

-
- i** Observe the operating instructions for your pipette. There you will find detailed descriptions on adjustment.
-

The adjustment of the adjustable-volume pipettes:

- Research
- Reference
- Biomaster

refers to a zero point shift.

7 Adjustment

EN

Standard Operating Procedure for Pipettes

7.2.1 Procedure

Requirements

Device, original Eppendorf tip, test liquid and ambient air must have the same temperature (15 – 30 °C) at a constant temperature of $\pm 0.5^{\circ}\text{C}$ during the test (according to EN ISO 8655, Part 6).

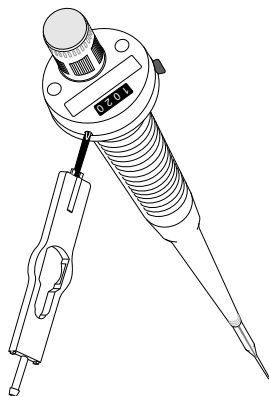
- i** If your Research or Reference has an adjustment seal, removing it prior to adjusting. After adjusting, lock the adjustment opening with a new adjustment seal. Adjustment seals can be reordered (see operating manual).

1. Set the pipette to the smallest volume to be tested.
2. Use a matching original Eppendorf tip for single-channel pipettes. For Multi-channel pipettes, use any channel.
3. Pipette the set volume 10 times.
4. Conduct a weighing after every volume dispensing.
5. Calculate the mean value after 10 measurements (see *Evaluation* on p. 9).
The calculated mean value of these weighings (observe conversion factor $Z = \text{mg to } \mu\text{L}$) yields the actual volume.
6. For adjusting, introduce the suitable tool into the opening intended for this purpose, or place it on the designated location, and adjust the actual volume (see the following figures or operating manual).
7. Check the set volume by measuring again. If the target volume does not match the result of the measurement, repeat steps 2 to 6.
8. After the adjustment, check the measured values for accuracy at 50% of the nominal volume and check the nominal volume for accuracy.

7.2.2 Biomaster

Auxiliary equipment

- Supplied pipette wrench (order no. 4910 092.001)



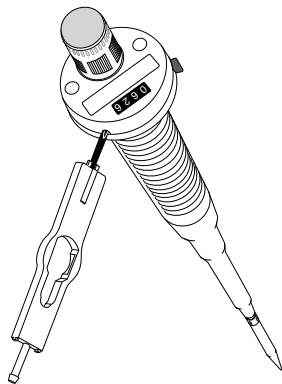
1. Using the pipette wrench, set the digital display of the pipette, with unaltered piston stroke, to the actual volume value of the measurement.
2. Pull off the pipette wrench.
3. Set the pipette to the target volume in the usual manner.
4. Mark the adjustment on the pipette.

7 Adjustment

7.2.3 Reference adjustable-volume

Auxiliary equipment

- Supplied pipette wrench (order no. 4910 092.001)
- Supplied red ADJ seal



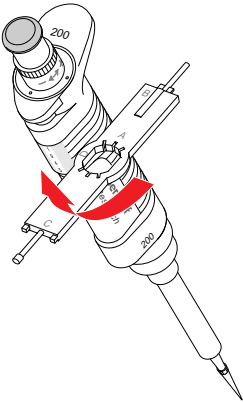
1. Insert side B of the pipette wrench into the adjustment opening in the lid.
2. Using the pipette wrench, set the digital display of the pipette, with unaltered piston stroke, to the actual volume value of the measurement.
3. Pull off the pipette wrench.
4. Set the pipette to the target volume in the usual manner.
5. After the adjustment has been successfully completed, lock the adjustment opening with a red ADJ seal.

7 Adjustment

7.2.4 Research adjustable-volume

Auxiliary equipment

- Supplied pipette wrench (order no. 3111 501.016)
- Supplied red ADJ seal



1. Insert side D of the pipette wrench vertically into the lateral adjustment opening of the pipette grip.
2. Tilt the pipette wrench to the vertical position.
3. Turn the volume setting ring toward – or +. This adjusts the piston stroke of the pipette. The digital display does not change in the process. A revolution corresponds to:

Volume range	Vol. / revolution
0.1 – 2.5 µL	approx. 0.1 µL
0.5 - 10 µL	approx. 0.5 µL
2 - 20 µL	approx. 1 µL
10 - 100 µL	approx. 5 µL
20 - 200 µL	approx. 10 µL
100 - 1 000 µL	approx. 50 µL
500 - 5 000 µL	approx. 250 µL
1 -10 mL	approx. 510 µL

4. Pull off the pipette wrench.
5. Move the volume setting ring back and forth a bit until the digital indicator and stroke system engage with each other again.
6. After the adjustment has been successfully completed, lock the adjustment opening with a red ADJ seal.

7 Adjustment

EN

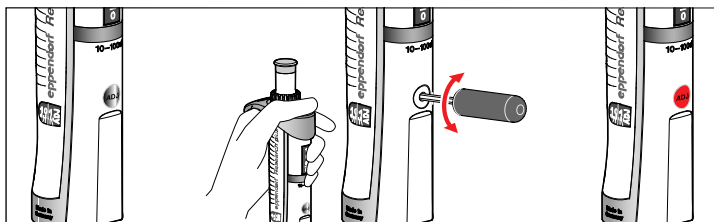
7.2.5 Research plus adjustable-volume - adjustment to environmental parameters

Adjustment change for specific liquid densities, changed altitudes or pipette tips that are not used to calculate the random and systematic error.

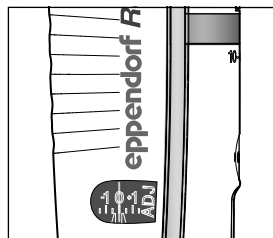
The Research plus was adjusted, tested and fitted with a gray adjustment seal with the abbreviation "ADJ" before delivery. The adjustment display on the side reads "0". If the adjustment is changed, the volume changes by a certain value. Strictly speaking, the change only applies to the testing volume.

Auxiliary equipment

- Supplied adjustment tool (order no. 3120 633.006)
- Supplied red adjustment seal (ADJ)



1. Remove the gray adjustment seal.
2. Keep the ejector pressed.
3. Insert the adjustment tool (from the delivery package).
4. Turn the adjustment tool until the adjustment display shows the desired value.
5. Place the Research plus on a horizontal surface (table). When completing the adjustment, look absolutely vertically at the window and read the set value via the backsight in the viewing window.



6. Carry out weighings to verify accuracy and precision.
7. After the tests, close the opening with the red adjustment seal (from the delivery package).

If the adjustment is meant for a specific liquid, mark the pipette accordingly. Use the labeling area on the pipette for this purpose and write down the liquid and the volume.

7 Adjustment

EN

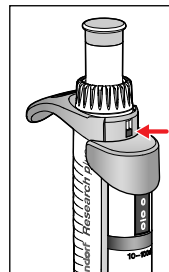
Standard Operating Procedure for Pipettes

7.2.6 Research plus adjustable-volume - change to the factory adjustment

Auxiliary equipment

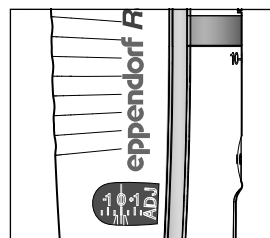
- Supplied safety plug tool
- Supplied pin to loosen the safety plug

It is possible to change the factory adjustment with the corresponding accessories for a Research plus with adjustable volume setting. If the factory adjustment of the Research plus has been changed by a user, this can be recognized by a red safety plug behind the ejector. If the Research plus has been adjusted and calibrated by Eppendorf AG, this is indicated by a gray safety plug.

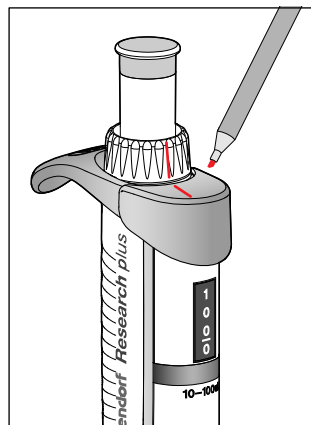


1. Check whether the adjustment display on the side is set to "0".

If the adjustment display is not set to "0", you will first need to set it to "0" with the adjustment tool. In this case, instead of continuing with the factory adjustment modification, carry out a gravimetric test of the Research plus with the adjustment display set to "0".

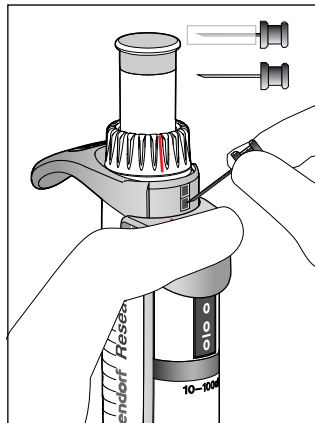


2. Provide the volume setting ring and the ejector with a common mark with a pen. This mark serves as an orientation for factory adjustment changes. When changing the factory adjustment, you can turn the volume setting ring, without the volume display changing. The mark on the volume setting ring and the ejector informs you how far you have moved from the factory setting.

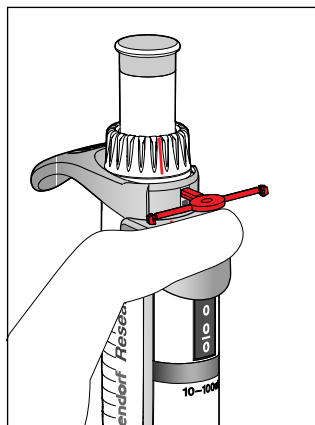


7 Adjustment

3. Keep the ejector pressed and remove the safety plug with the pin.

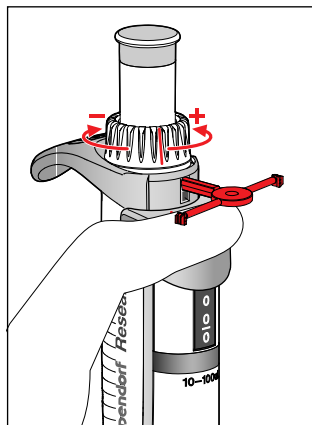


4. Continue to keep the ejector pressed. Insert the safety plug tool such that the counter locking mechanism is pushed down.



7 Adjustment

5. Turn the volume setting ring slightly to change the volume. Proceed as shown in the figure.



EN

Standard Operating Procedure for Pipettes

7 Adjustment

This results in the following approximate volume changes:

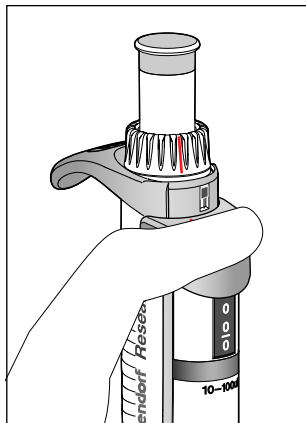
Single-channel				
Nominal volume Color code	+½ revolution	+¼ revolution	–¼ revolution	–½ revolution
2.5 µL dark gray	0.106 µL	0.053 µL	–0.053 µL	–0.106 µL
10 µL medium gray	0.53 µL	0.27 µL	–0.27 µL	–0.53 µL
20 µL light gray	1.06 µL	0.53 µL	–0.53 µL	–1.06 µL
20 µL yellow	1.07 µL	0.54 µL	–0.54 µL	–1.07 µL
100 µL yellow	5.4 µL	2.7 µL	–2.7 µL	–5.4 µL
200 µL yellow	10.8 µL	5.4 µL	–5.4 µL	–10.8 µL
300 µL orange	10.7 µL	5.4 µL	–5.4 µL	–10.7 µL
1 000 µL blue	54 µL	27 µL	–27 µL	–54 µL
5 mL purple	271 µL	135 µL	–135 µL	–271 µL
10 mL turquoise	542 µL	271 µL	–271 µL	–542 µL

Multi-channel				
Nominal volume Color code	+½ revolution	+¼ revolution	–¼ revolution	–½ revolution
10 µL medium gray	0.53 µL	0.27 µL	–0.27 µL	–0.53 µL
100 µL yellow	5.4 µL	2.7 µL	–2.7 µL	–5.4 µL
300 µL orange	10.7 µL	5.4 µL	–5.4 µL	–10.7 µL

The values mentioned are theoretical values and are for orientation purposes only. The volume changes mentioned apply to each volume setting.

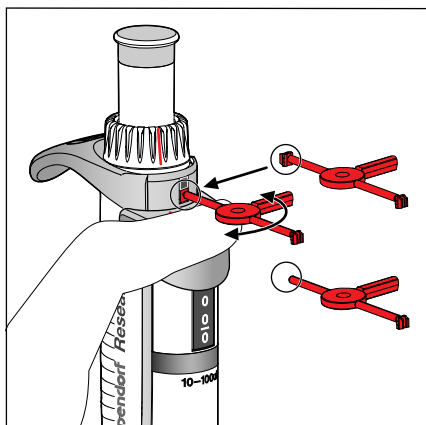
7 Adjustment

6. Slide the locking mechanism up and carry out a gravimetric test of the changes which have been made.



7. If the measured gravimetric values meet your requirements: insert the red safety plug on the tool into the opening of the Research plus and break it off from the tool.

The red safety plug on the pipette indicates that the Research plus has been adjusted by the user. If the adjustment display had also been set to "0" before, you must close the opening with a new, red adjustment seal at the position for the adjustment seal.



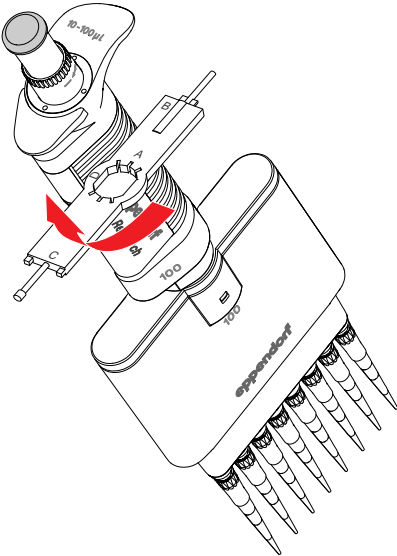
8. Document the changes made and the measurements conducted. Remove the mark on the volume setting ring and the ejector. The red safety plug on the pipette indicates that the Research plus has been adjusted and calibrated by the user.

7 Adjustment

7.2.7 Research multi-channel

Auxiliary equipment

- Supplied pipette wrench (order no. 3111 501.016)
- Supplied red ADJ seal



1. Insert side D of the pipette wrench vertically into the lateral adjustment opening of the pipette grip.
2. Tilt the pipette wrench to the vertical position.
3. Turn the volume setting ring toward – or +. This adjusts the piston stroke of the pipette. The digital display does not change in the process. A revolution corresponds to:

Volume range	Vol. / revolution
0.5 - 10 µL	approx. 0.5 µL
10 - 300 µL	approx. 5 µL
30 µL	approx. 10 µL

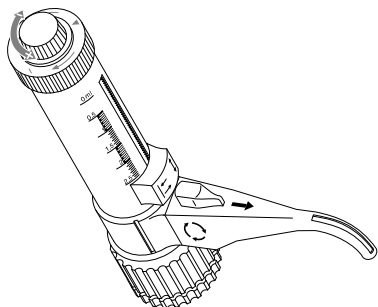
4. Pull off the pipette wrench.
5. Move the volume setting ring back and forth a bit until the digital indicator and stroke system engage with each other again.
6. After the adjustment has been successfully completed, lock the adjustment opening with a red ADJ seal.

7 Adjustment

7.2.8 Research plus multi-channel

Conduct the adjustment as described for the Research plus single-channel pipette (see p. 18).

7.2.9 Varispenser plus



- ▶ Turn the fine adjustment toward + or -. A revolution corresponds to the smallest dispensing step:

Reduce volume:

- ▶ Turn toward -.

Increase volume:

- ▶ Turn toward +.

The factory adjustment is carried out at 20 °C with double distilled, vented water.

7.2.10 Xplorer

- i** The adjustment of the Xplorer pipette is described on the CD enclosed with the pipette.

If a different adjustment was selected in the options for the Xplorer pipette, a wrench symbol will appear in the display header.



Another symbol showing the selected adjustment is displayed to the right of the wrench symbol. If you switch back to the factory adjustment later, the previously selected adjustment is deleted, and both symbols disappear from the header.

1P ADJ

3P ADJ

Gly



2P ADJ

Eth



7 Adjustment

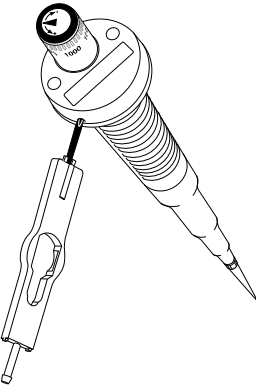
7.3 Adjusting fixed-volume pipettes

The adjustment process for fixed-volume pipettes is the same as for adjustable-volume pipettes (see *Adjusting adjustable-volume pipettes* on p. 14). For fixed-volume pipettes, 10 measured values of the nominal volume are tested.

7.3.1 Reference fixed

Auxiliary equipment

- Adhesive label as an adjusting aid for the basic setting
- Supplied pipette wrench (order no. 4910 092.001)



1. In order to simplify relocating the basic setting, attach the adhesive label supplied as an adjusting aid to the control button.
2. Using the B side of the pipette wrench, loosen the inside screw until the control button can be turned.
3. Set the control button to the calculated actual volume value of the measurement (see *Adjusting adjustable-volume pipettes* on p. 14). One revolution of the control button corresponds to the following (based on water):

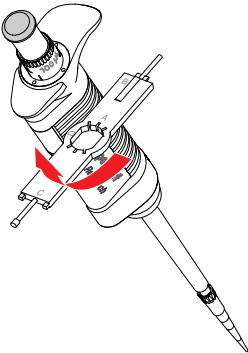
Reference fixed-volume	Vol. / revolution
1, 2, 5, 10 μL	approx. 0.5 μL
10, 20 μL	approx. 1 μL
25, 50 μL	approx. 2.4 μL
100 μL	approx. 5 μL
200, 250 μL	approx. 12 μL
500, 1 000 μL	approx. 46 μL
1 500, 2 000, 2 500 μL	approx. 118 μL

7 Adjustment

7.3.2 Research fixed

Auxiliary equipment

- Supplied pipette wrench (order no. 3111 501.016)



1. Insert side D of the pipette wrench vertically into the lateral adjustment opening of the pipette grip.
2. Move the tool into the vertical position.
3. Turn the volume setting ring toward – or +. This adjusts the piston stroke of the pipette. With reference to water, one revolution of the volume setting ring corresponds to:

Volume range	Vol. / revolution
10	approx. 0.8 µL
20	approx. 0.8 µL
25	approx. 0.8 µL
50	approx. 0.8 µL
100	approx. 0.8 µL
200	approx. 38 µL
250	approx. 38 µL
500	approx. 38 µL
1 000	approx. 38 µL

7.3.3 Research plus fixed

Adjustment is carried out as described for the Research plus adjustable-volume (see p. 18).

7 Adjustment

7.4 Physical influences of liquids

It is possible to adjust the previously described devices for a volume of a liquid with a different density than water so that the displayed volume value corresponds to the pipetted volume.

-
- i** With adjustable-volume pipettes, all other values are then readjusted, as the adjustable-volume pipette then becomes a fix-volume pipette.
-

The adjustment process is comparable to the described procedure (see p. 15). The difference is that the mean value of the weighings is converted to microliters according to the formula:

$$\text{Pipetting volume} = \frac{\text{Mean value of weighings}}{\text{Density of liquid weighed}}$$

1. Determine the mean value and convert it to microliters.
The calculated value is the nominal value.
 2. The digital display of variable pipettes, or the volume of fix-volume pipettes, must be set to the calculated actual value.
 3. Check the value set for the liquid gravimetrically. The accordingly set device only delivers a dispensing value, which corresponds to the digital display, for the used liquid and set volume.
 4. If necessary correct and check the setting.
 5. Label the measuring device following adjustment with the measured value and the name of the liquid which was used for the adjustment.
-

- i** Following adjustment, the certificate accompanying the pipette becomes invalid.
-

8 Factor Z for distilled water

8.1 Factor Z overview table

Factor Z ($\mu\text{L}/\text{mg}$) in accordance with EN ISO 8655 for distilled water depending on test temperature and air pressure:

Temperature (°C)	Air pressure (kPa)						
	80	85	90	95	100	101.3	105
15	1.0017	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020
15.5	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020	1.0021
16	1.0019	1.0020	1.0020	1.0021	1.0021	1.0021	1.0022
16.5	1.0020	1.0020	1.0021	1.0021	1.0022	1.0022	1.0022
17	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024	1.0024
18	1.0022	1.0023	1.0023	1.0024	1.0025	1.0025	1.0025
18.5	1.0023	1.0024	1.0024	1.0025	1.0025	1.0026	1.0026
19	1.0024	1.0025	1.0025	1.0026	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0027	1.0028	1.0028
20	1.0026	1.0027	1.0027	1.0028	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0029	1.0030	1.0030
21	1.0028	1.0029	1.0029	1.0030	1.0031	1.0031	1.0031
21.5	1.0030	1.0030	1.0031	1.0031	1.0032	1.0032	1.0032
22	1.0031	1.0031	1.0032	1.0032	1.0033	1.0033	1.0033
22.5	1.0032	1.0032	1.0033	1.0033	1.0034	1.0034	1.0034
23	1.0033	1.0033	1.0034	1.0034	1.0035	1.0035	1.0036
23.5	1.0034	1.0035	1.0035	1.0036	1.0036	1.0036	1.0037
24	1.0035	1.0036	1.0036	1.0037	1.0037	1.0038	1.0038
24.5	1.0037	1.0037	1.0038	1.0038	1.0039	1.0039	1.0039
25	1.0038	1.0038	1.0039	1.0039	1.0040	1.0040	1.0040
25.5	1.0039	1.0040	1.0040	1.0041	1.0041	1.0041	1.0042
26	1.0040	1.0041	1.0041	1.0042	1.0042	1.0043	1.0043
26.5	1.0042	1.0042	1.0043	1.0043	1.0044	1.0044	1.0044
27	1.0043	1.0044	1.0044	1.0045	1.0045	1.0045	1.0046
27.5	1.0045	1.0045	1.0046	1.0046	1.0047	1.0047	1.0047
28	1.0046	1.0046	1.0047	1.0047	1.0048	1.0048	1.0048
28.5	1.0047	1.0048	1.0048	1.0049	1.0049	1.0050	1.0050
29	1.0049	1.0049	1.0050	1.0050	1.0051	1.0051	1.0051
29.5	1.0050	1.0051	1.0051	1.0052	1.0052	1.0052	1.0053
30	1.0052	1.0052	1.0053	1.0053	1.0054	1.0054	1.0054

9 Technical specifications

EN

Standard Operating Procedure for Pipettes

The following specifications are valid for the following conditions:

Liquid: Distilled or deionized water

Reference temperature: 20 °C to 25 °C ± 0.5 °C

Number of determinations: 10, in accordance with EN ISO 8655 with original Eppendorf pipette tips

i Technical specifications subject to change!

9.1 Fix-volume pipettes

9.1.1 Reference fix

Model	Test tip epT.I.P.S. Color code Volume range Length	Error limits			
		Error			
		Systematic		Random	
		\pm %	\pm μ L	\pm %	\pm μ L
1 μ L	light gray 0.5 - 20 μ L L 46 mm	± 2.5	± 0.025	± 1.8	± 0.018
2 μ L		± 2.0	± 0.04	± 1.2	± 0.024
5 μ L		± 1.5	± 0.075	± 0.8	± 0.04
10 μ L		± 1.0	± 0.1	± 0.5	± 0.05
10 μ L	yellow 2 - 200 μ L 53 mm	± 1.0	± 0.1	± 0.5	± 0.05
20 μ L		± 0.8	± 0.16	± 0.3	± 0.06
25 μ L		± 0.8	± 0.2	± 0.3	± 0.075
50 μ L		± 0.7	± 0.35	± 0.3	± 0.15
100 μ L		± 0.6	± 0.6	± 0.2	± 0.2
200 μ L	blue 50 - 1 000 μ L 71 mm	± 0.6	± 1.2	± 0.2	± 0.4
250 μ L		± 0.6	± 1.5	± 0.2	± 0.5
500 μ L		± 0.6	± 3	± 0.2	± 1
1 000 μ L		± 0.6	± 6	± 0.2	± 2
1 500 μ L	red 500 - 2 500 μ L 115 mm	± 0.6	± 9	± 0.2	± 3
2 000 μ L		± 0.6	± 12	± 0.2	± 4
2 500 μ L		± 0.6	± 15	± 0.2	± 5

9 Technical specifications

9.1.2 Research fix

Model	Test tip epT.I.P.S. Color code Volume range Length	Error limits			
		Error			
		Systematic		Random	
		± %	± µL	± %	± µL
10 µL	yellow 2 - 200 µL 53 mm	± 1.2	± 0.12	± 0.6	± 0.06
20 µL		± 1.0	± 0.2	± 0.3	± 0.06
25 µL		± 1.0	± 0.25	± 0.3	± 0.075
50 µL		± 0.7	± 0.35	± 0.3	± 0.15
100 µL		± 0.6	± 0.6	± 0.2	± 0.2
200 µL	blue 0.05 - 1 mL 71 mm	± 0.6	± 1.2	± 0.2	± 0.4
250 µL		± 0.6	± 1.5	± 0.2	± 0.5
500 µL		± 0.6	± 3	± 0.2	± 1
1 000 µL		± 0.6	± 6	± 0.2	± 2

9 Technical specifications

9.1.3 Research plus fix

Research plus fixed volume					
Model	Test tip epT.I.P.S. Color code Volume range Length	Error limits			
		Error			
		Systematic		Random	
		± %	± µL	± %	± µL
10 µL	medium gray 0.1 - 20 µL 40 mm	± 1.2	± 0.12	± 0.6	± 0.06
20 µL	light gray 0.5 - 20 µL L 46 mm	± 0.8	± 0.16	± 0.3	± 0.06
10 µL	yellow 2 - 200 µL 53 mm	± 1.2	± 0.12	± 0.6	± 0.06
20 µL		± 1.0	± 0.2	± 0.3	± 0.06
25 µL		± 1.0	± 0.25	± 0.3	± 0.08
50 µL		± 0.7	± 0.35	± 0.3	± 0.15
100 µL		± 0.6	± 0.6	± 0.2	± 0.2
200 µL		± 0.6	± 1.2	± 0.2	± 0.4
200 µL	blue 50 - 1 000 µL 71 mm	± 0.6	± 1.2	± 0.2	± 0.4
250 µL		± 0.6	± 1.5	± 0.2	± 0.5
500 µL		± 0.6	± 3	± 0.2	± 1
1 000 µL		± 0.6	± 6	± 0.2	± 2

9 Technical specifications

9.2 Adjustable volume pipettes

9.2.1 Reference variable

Model	Test tip epT.I.P.S. Color code Volume range Length	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
0.1 - 2.5 µL	dark gray 0.1 - 10 µL 34 mm	0.25 µL	± 12.0	± 0.03	± 6.0	± 0.015
		1.25 µL	± 2.5	± 0.031	± 1.5	± 0.019
		2.5 µL	± 1.4	± 0.035	± 0.7	± 0.018
0.5 - 10 µL	light gray 0.5 - 20 µL 46 mm	1 µL	± 2.5	± 0.025	± 1.8	± 0.018
		5 µL	± 1.5	± 0.075	± 0.8	± 0.04
		10 µL	± 1.0	± 0.1	± 0.4	± 0.04
2 - 20 µL	light gray 0.5 - 20 µL 46 mm	2 µL	± 3.0	± 0.06	± 2.0	± 0.04
		10 µL	± 1.0	± 0.1	± 0.5	± 0.05
		20 µL	± 0.8	± 0.16	± 0.3	± 0.06
2 - 20 µL	yellow 2 - 200 µL 53 mm	2 µL	± 5.0	± 0.1	± 1.5	± 0.03
		10 µL	± 1.2	± 0.12	± 0.6	± 0.06
		20 µL	± 1.0	± 0.2	± 0.3	± 0.06
10 - 100 µL	yellow 2 - 200 µL 53 mm	10 µL	± 3.0	± 0.3	± 0.7	± 0.07
		50 µL	± 1.0	± 0.5	± 0.3	± 0.15
		100 µL	± 0.8	± 0.8	± 0.15	± 0.15
50 - 200 µL	yellow 2 - 200 µL 53 mm	50 µL	± 1.0	± 0.5	± 0.3	± 0.15
		100 µL	± 0.9	± 0.9	± 0.3	± 0.3
		200 µL	± 0.6	± 1.2	± 0.2	± 0.4
50 - 250 µL	blue 50 - 1 000 µL 71 mm	50 µL	± 1.4	± 0.7	± 0.3	± 0.15
		100 µL	± 1.1	± 1.1	± 0.3	± 0.3
		250 µL	± 0.6	± 1.5	± 0.2	± 0.5
100 - 1 000 µL	blue 50 - 1 000 µL 71 mm	100 µL	± 3.0	± 3	± 0.3	± 0.3
		500 µL	± 1.0	± 5	± 0.2	± 1
		1 000 µL	± 0.6	± 6	± 0.2	± 2
500 - 2 500 µL	red 500 - 2 500 µL 115 mm	0.5 mL	± 1.5	± 7.5	± 0.3	± 1.5
		1.0 mL	± 0.8	± 8	± 0.2	± 2
		2.5 mL	± 0.6	± 15	± 0.2	± 5

9 Technical specifications

9.2.2 Research variable

Model	Test tip epT.I.P.S. Color code Volume range Length	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
0.1 - 2.5 µL	dark gray 0.1 - 10 µL 34 mm	0.25 µL	± 12.0	± 0.03	± 6.0	± 0.015
		1.25 µL	± 2.5	± 0.031	± 1.5	± 0.019
		2.5 µL	± 1.4	± 0.035	± 0.7	± 0.018
0.5 - 10 µL	light gray 0.5 - 20 µL L 46 mm	1 µL	± 2.5	± 0.025	± 1.8	± 0.018
		5 µL	± 1.5	± 0.075	± 0.8	± 0.04
		10 µL	± 1.0	± 0.1	± 0.4	± 0.04
2 - 20 µL	yellow 2 - 200 µL 53 mm	2 µL	± 5.0	± 0.1	± 1.5	± 0.03
		10 µL	± 1.2	± 0.12	± 0.6	± 0.06
		20 µL	± 1.0	± 0.2	± 0.3	± 0.06
10 - 100 µL	yellow 2 - 200 µL 53 mm	10 µL	± 3.0	± 0.3	± 1.0	± 0.1
		50 µL	± 1.0	± 0.5	± 0.3	± 0.15
		100 µL	± 0.8	± 0.8	± 0.2	± 0.20
20 - 200 µL	yellow 2 - 200 µL 53 mm	20 µL	± 2.5	± 0.5	± 0.7	± 0.14
		100 µL	± 1.0	± 1	± 0.3	± 0.3
		200 µL	± 0.6	± 1.2	± 0.2	± 0.4
100 - 1 000 µL	blue 0.05 - 1 mL 71 mm	100 µL	± 3.0	± 3	± 0.6	± 0.6
		500 µL	± 1.0	± 5	± 0.2	± 1
		1 000 µL	± 0.6	± 6	± 0.2	± 2
0.5 - 5 mL	purple 0.1 - 5 mL 120 mm	0.5 mL	± 2.4	± 12	± 0.6	± 3
		2.5 mL	± 1.2	± 30	± 0.25	± 6.25
		5.0 mL	± 0.6	± 30	± 0.15	± 7.5
1 - 10 mL	turquoise 1 - 10 mL 165 mm	1.0 mL	± 3.0	± 30	± 0.6	± 6
		5.0 mL	± 0.8	± 40	± 0.2	± 10
		10.0 mL	± 0.6	± 60	± 0.15	± 15

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9.2.3 Research pro

Model	Test tip epT.I.P.S. Color code Volume range Length	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
0.5 - 10 µL	light gray 0.5 - 20 µL L 46 mm	1 µL	± 2.5	± 0.025	± 1.8	± 0.018
		5 µL	± 1.5	± 0.075	± 0.8	± 0.04
		10 µL	± 1.0	± 0.1	± 0.4	± 0.04
5 - 100 µL	yellow 2 - 200 µL 53 mm	10 µL	± 2.0	± 0.2	± 1.0	± 0.1
		50 µL	± 1.0	± 0.5	± 0.3	± 0.15
		100 µL	± 0.8	± 0.8	± 0.2	± 0.2
20 - 300 µL	orange 20 - 300 µL 55 mm	30 µL	± 2.5	± 0.75	± 0.7	± 0.21
		150 µL	± 1.0	± 1.5	± 0.3	± 0.45
		300 µL	± 0.6	± 1.8	± 0.2	± 0.6
50 - 1 000 µL	blue 50 - 1 000 µL 71 mm	100 µL	± 3.0	± 3	± 0.6	± 0.6
		500 µL	± 1.0	± 5	± 0.2	± 1
		1 000 µL	± 0.6	± 6	± 0.2	± 2
500 - 5 000 µL	purple 0.1 - 5 mL 120 mm	0.5 mL	± 3.0	± 15	± 0.6	± 3
		2.5 mL	± 1.2	± 30	± 0.25	± 6.25
		5.0 mL	± 0.6	± 30	± 0.15	± 7.5

9 Technical specifications

9.2.4 Research plus variable

Research plus adjustable single-channel						
Model	Test tip epT.I.P.S. Color code Volume range Length	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
0.1 - 2.5 µL Increment: 0.002 µL	dark gray 0.1 - 10 µL 34 mm	0.1 µL	± 48	± 0.048	± 12	± 0.012
		0.25 µL	± 12	± 0.03	± 6	± 0.015
		1.25 µL	± 2.5	± 0.031	± 1.5	± 0.019
		2.5 µL	± 1.4	± 0.035	± 0.7	± 0.018
0.5 - 10 µL Increment: 0.01 µL	medium gray 0.1 - 20 µL 40 mm	0.5 µL	± 8	± 0.04	± 5	± 0.025
		1 µL	± 2.5	± 0.025	± 1.8	± 0.018
		5 µL	± 1.5	± 0.075	± 0.8	± 0.04
		10 µL	± 1.0	± 0.1	± 0.4	± 0.04
2 - 20 µL Increment: 0.02 µL	light gray 0.5 - 20 µL L 46 mm	2 µL	± 5	± 0.1	± 1.5	± 0.03
		10 µL	± 1.2	± 0.12	± 0.6	± 0.06
		20 µL	± 1.0	± 0.2	± 0.3	± 0.06
2 - 20 µL Increment: 0.02 µL	yellow 2 - 200 µL 53 mm	2 µL	± 5	± 0.1	± 1.5	± 0.03
		10 µL	± 1.2	± 0.12	± 0.6	± 0.06
		20 µL	± 1.0	± 0.2	± 0.3	± 0.06
10 - 100 µL Increment: 0.1 µL	yellow 2 - 200 µL 53 mm	10 µL	± 3	± 0.3	± 1	± 0.1
		50 µL	± 1	± 0.5	± 0.3	± 0.15
		100 µL	± 0.8	± 0.8	± 0.2	± 0.2
20 - 200 µL Increment: 0.2 µL	yellow 2 - 200 µL 53 mm	20 µL	± 2.5	± 0.5	± 0.7	± 0.14
		100 µL	± 1	± 1	± 0.3	± 0.3
		200 µL	± 0.6	± 1.2	± 0.2	± 0.4
30 - 300 µL Increment: 0.2 µL	orange 20 - 300 µL 55 mm	30 µL	± 2.5	± 0.75	± 0.7	± 0.21
		150 µL	± 1	± 1.5	± 0.3	± 0.45
		300 µL	± 0.6	± 1.8	± 0.2	± 0.6
100 - 1 000 µL Increment: 1 µL	blue 50 - 1 000 µL 71 mm	100 µL	± 3	± 3	± 0.6	± 0.6
		500 µL	± 1	± 5	± 0.2	± 1
		1 000 µL	± 0.6	± 6	± 0.2	± 2

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Research plus adjustable single-channel						
Model	Test tip epT.I.P.S. Color code Volume range Length	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
0.5 - 5 mL Increment: 0.005 mL	purple 0.1 - 5 mL 120 mm	0.5 mL	± 2.4	± 12	± 0.6	± 3
		2.5 mL	± 1.2	± 30	± 0.25	± 6
		5.0 mL	± 0.6	± 30	± 0.15	± 8
1 - 10 mL Increment: 0.01 mL	turquoise 1 – 10 mL 165 mm	1.0 mL	± 3	± 30	± 0.6	± 6
		5.0 mL	± 0.8	± 40	± 0.2	± 10
		10.0 mL	± 0.6	± 60	± 0.15	± 15

9.2.5 Biomaster

Model	Pipette tip	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
Biomaster 4830	Mastertip	2 µL	± 6.0	± 0.12	± 4.0	± 0.08
		3 µL	± 5.0	± 0.15	± 3.0	± 0.09
		5 µL	± 4.0	± 0.2	± 2.0	± 0.1
		10 µL	± 3.0	± 0.3	± 1.5	± 0.15
		20 µL	± 2.5	± 0.5	± 0.8	± 0.16

9.2.6 Varipette

Model	Pipette tip	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
Varipette 4720	Varitip S	2.5 µL	± 1.0	± 0.025	± 0.2	± 0.005
		5 µL	± 0.4	± 0.02	± 0.2	± 0.01
		10 µL	± 0.3	± 0.03	± 0.2	± 0.02
Varipette 4720	Varitip P	1 µL	± 0.6	± 0.006	± 0.2	± 0.002
		5 µL	± 0.5	± 0.025	± 0.1	± 0.005
		10 µL	± 0.3	± 0.03	± 0.1	± 0.01

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EN

9.2.7 Xplorer

Model Increment	Test tip epT.I.P.S. Color code Volume range Length	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
0.5 - 10 µL Increment: 0.01 µL	medium gray 0.1 - 20 µL 40 mm	1 µL	± 2.5	± 0.025	± 1.8	± 0.018
		5 µL	± 1.5	± 0.075	± 0.8	± 0.04
		10 µL	± 1.0	± 0.1	± 0.4	± 0.04
5 - 100 µL Increment: 0.1 µL	yellow 2 - 200 µL 53 mm	10 µL	± 2.0	± 0.2	± 1.0	± 0.1
		50 µL	± 1.0	± 0.5	± 0.3	± 0.15
		100 µL	± 0.8	± 0.8	± 0.2	± 0.2
15 - 300 µL Increment: 0.1 µL	orange 15 - 300 µL 55 mm	30 µL	± 2.5	± 0.75	± 0.7	± 0.21
		150 µL	± 1.0	± 1.5	± 0.3	± 0.45
		300 µL	± 0.6	± 1.8	± 0.2	± 0.6
50 - 1 000 µL Increment: 1 µL	blue 50 - 1 000 µL 71 mm	100 µL	± 3.0	± 3.0	± 0.6	± 0.6
		500 µL	± 1.0	± 5.0	± 0.2	± 1
		1 000 µL	± 0.6	± 6.0	± 0.2	± 2
0.25 - 5 mL Increment: 0.005 mL	purple 0.1 - 5 mL 120 mm	0.5 mL	± 3.0	± 15.0	± 0.6	± 3
		2.5 mL	± 1.2	± 30.0	± 0.25	± 6.25
		5 mL	± 0.6	± 30.0	± 0.15	± 7.5
0.5 - 10 mL Increment: 0.01 mL	turquoise 1 – 10 mL 165 mm	1 mL	± 3.0	± 30.0	± 0.60	± 6.0
		5 mL	± 0.8	± 40.0	± 0.20	± 10.0
		10 mL	± 0.6	± 60.0	± 0.15	± 15.0

9 Technical specifications

9.3 Multi-channel pipettes

9.3.1 Research

Model	Test tip epT.I.P.S. Color code Volume range Length	Volume in μL	Error limits			
			Error			
			Systematic		Random	
			$\pm \%$	$\pm \mu\text{L}$	$\pm \%$	$\pm \mu\text{L}$
Research 8-channel 0.5 – 10 μL	light gray 0.5 - 20 μL L 46 mm	1	± 8.0	± 0.08	± 5.0	± 0.05
		5	± 4.0	± 0.2	± 2.0	± 0.1
		10	± 2.0	± 0.2	± 1.0	± 0.1
Research 12-channel 0.5 – 10 μL		see 8-channel				
Research 8-channel 10 – 100 μL	yellow 2 - 200 μL 53 mm	10	± 3.0	± 0.3	± 2.0	± 0.2
		50	± 1.0	± 0.5	± 0.8	± 0.4
		100	± 0.8	± 0.8	± 0.3	± 0.3
Research 12-channel 10 – 100 μL		see 8-channel				
Research 8-channel 30 – 300 μL	orange 20 - 300 μL 55 mm	30	± 3.0	± 0.9	± 1.0	± 0.3
		150	± 1.0	± 1.5	± 0.5	± 0.75
		300	± 0.6	± 1.8	± 0.3	± 0.9
Research 12-channel 30 – 300 μL		see 8-channel				

9 Technical specifications

9.3.2 Research pro

Model	Test tip epT.I.P.S. Color code Volume range Length	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
Research pro 8-channel / 12-channel 0.5 – 10 µL	light gray 0.5 - 20 µL L 46 mm	1 µL	± 5.0	± 0.05	± 3.0	± 0.03
		5 µL	± 3.0	± 0.15	± 1.5	± 0.075
		10 µL	± 2.0	± 0.2	± 0.8	± 0.08
Research pro 8-channel / 12-channel 5 – 100 µL	yellow 2 - 200 µL 53 mm	10 µL	± 2.0	± 0.2	± 2.0	± 0.2
		50 µL	± 1.0	± 0.5	± 0.8	± 0.4
		100 µL	± 0.8	± 0.8	± 0.25	± 0.25
Research pro 8-channel / 12-channel 20 – 300 µL	orange 20 - 300 µL 55 mm	30 µL	± 2.5	± 0.75	± 1.0	± 0.3
		150 µL	± 1.0	± 1.5	± 0.5	± 0.75
		300 µL	± 0.6	± 1.8	± 0.25	± 0.75
Research pro 8-channel / 12-channel 50 - 1 250 µL	green 50 - 1 250 µL 76 mm	120 µL	± 6.0	± 7.2	± 0.9	± 1.08
		600 µL	± 2.7	± 16.2	± 0.4	± 2.4
		1 200 µL	± 1.2	± 14.4	± 0.3	± 3.6

9.3.3 Research plus

Research plus adjustable multi-channel						
Model	Test tip epT.I.P.S. Color code Volume range Length	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
0.5 – 10 µL Increment: 0.01 µL	medium gray 0.1 - 20 µL 40 mm	0.5 µL	± 12	± 0.06	± 8.0	± 0.04
		1 µL	± 8.0	± 0.08	± 5.0	± 0.05
		5 µL	± 4.0	± 0.2	± 2.0	± 0.1
		10 µL	± 2.0	± 0.2	± 1.0	± 0.1
10 – 100 µL Increment: 0.1 µL	yellow 2 - 200 µL 53 mm	10 µL	± 3.0	± 0.3	± 2.0	± 0.2
		50 µL	± 1.0	± 0.5	± 0.8	± 0.4
		100 µL	± 0.8	± 0.8	± 0.3	± 0.3
30 – 300 µL Increment: 0.2 µL	orange 20 - 300 µL 55 mm	30 µL	± 3.0	± 0.9	± 1.0	± 0.3
		150 µL	± 1.0	± 1.5	± 0.5	± 0.75
		300 µL	± 0.6	± 1.8	± 0.3	± 0.9

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9.3.4 Xplorer

Model Increment	Test tip epT.I.P.S. Color code Volume range Length	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
0.5 - 10 µL Increment: 0.01 µL	medium gray 0.1 - 20 µL 40 mm	1 µL	± 5.0	± 0.05	± 3.0	± 0.03
		5 µL	± 3.0	± 0.15	± 1.5	± 0.075
		10 µL	± 2.0	± 0.2	± 0.8	± 0.08
5 - 100 µL Increment: 0.1 µL	yellow 2 - 200 µL 53 mm	10 µL	± 2.0	± 0.2	± 2.0	± 0.2
		50 µL	± 1.0	± 0.5	± 0.8	± 0.4
		100 µL	± 0.8	± 0.8	± 0.25	± 0.25
15 - 300 µL Increment: 0.1 µL	orange 15 - 300 µL 55 mm	30 µL	± 2.5	± 0.75	± 1.0	± 0.3
		150 µL	± 1.0	± 1.5	± 0.5	± 0.75
		300 µL	± 0.6	± 1.8	± 0.25	± 0.75
50 - 1 200 µL (only 8-channel) Increment: 1 µL	green 50 - 1 250 µL 76 mm	120 µL	± 6.0	± 7.2	± 0.9	± 1.08
		600 µL	± 2.7	± 16.2	± 0.4	± 2.4
		1 200 µL	± 1.2	± 14.2	± 0.3	± 3.6

9 Technical specifications

9.4 Multipette

The following specifications for the Multipette plus, Multipette stream / Multipette Xstream apply for the following conditions:

- Using the Combitips plus
- Liquid: Distilled or deionized water
- Reference temperature: 20 to 25 °C, ± 0.5 °C
- Number of determinations: 10 according to EN ISO 8655, with original Eppendorf Combitip plus

Multipette stream / Multipette Xstream:

- Volume test in "DIS" mode
- Set speed level: 7

9.4.1 Multipette plus

Combitip plus	Testing volume	Error limits			
		Error			
		Systematic		Random	
		\pm %	\pm μ L	\pm %	\pm μ L
0.1 mL	2 μ L	± 1.6	± 0.032	± 3.0	± 0.06
	20 μ L	± 1.0	± 0.2	± 2.0	± 0.4
0.2 mL	4 μ L	± 1.3	± 0.052	± 2.0	± 0.08
	40 μ L	± 0.8	± 0.32	± 1.5	± 0.6
0.5 mL	10 μ L	± 0.9	± 0.09	± 1.5	± 0.15
	100 μ L	± 0.8	± 0.8	± 0.6	± 0.6
1 mL	20 μ L	± 0.9	± 0.18	± 0.9	± 0.18
	200 μ L	± 0.6	± 1.2	± 0.4	± 0.8
2.5 mL	50 μ L	± 0.8	± 0.4	± 0.8	± 0.4
	500 μ L	± 0.5	± 2.5	± 0.3	± 1.5
5 mL	100 μ L	± 0.6	± 0.6	± 0.6	± 0.6
	1 000 μ L	± 0.5	± 5	± 0.25	± 2.5
10 mL	200 μ L	± 0.5	± 1	± 0.6	± 1.2
	2 000 μ L	± 0.5	± 10	± 0.25	± 5
25 mL	500 μ L	± 0.4	± 2	± 0.6	± 3
	5 000 μ L	± 0.3	± 15	± 0.25	± 12.5
50 mL	1 000 μ L	± 0.3	± 3	± 0.5	± 5
	10 000 μ L	± 0.3	± 30	± 0.3	± 30

9 Technical specifications

9.4.2 Multipette stream / Multipette Xstream

Combitip plus	Dispensing range (min./max.)	Testing volume	Error limits			
			Error			
			Systematic		Random	
			± %	± µL	± %	± µL
0.1 mL (white piston) Increment: 0.1 µL	1 - 100 µL	10 µL	± 1.6	± 0.16	± 2.5	± 0.25
		50 µL	± 1.0	± 0.5	± 1.5	± 0.75
		100 µL	± 1.0	± 1	± 0.5	± 0.5
0.2 mL (blue piston) Increment: 0.2 µL	2 - 200 µL	20 µL	± 1.3	± 0.26	± 1.5	± 0.3
		100 µL	± 1.0	± 1	± 1.0	± 1
		200 µL	± 1.0	± 2	± 0.5	± 1
0.5 mL Increment: 0.5 µL	5 - 500 µL	50 µL	± 0.9	± 0.45	± 0.8	± 0.4
		250 µL	± 0.9	± 2.25	± 0.5	± 1.25
		500 µL	± 0.9	± 4.5	± 0.3	± 1.5
1 mL Increment: 1 µL	10 - 1 000 µL	100 µL	± 0.9	± 0.9	± 0.55	± 0.55
		500 µL	± 0.6	± 3	± 0.3	± 1.5
		1 000 µL	± 0.6	± 6	± 0.2	± 2
2.5 mL Increment: 2.5 µL	25 - 2 500 µL	250 µL	± 0.8	± 2	± 0.45	± 1.125
		1 250 µL	± 0.5	± 6.25	± 0.3	± 3.75
		2 500 µL	± 0.5	± 12.5	± 0.15	± 3.75
5 mL Increment: 5 µL	50 - 5 000 µL	0.5 mL	± 0.8	± 4	± 0.35	± 1.75
		2.5 mL	± 0.5	± 12.5	± 0.25	± 6.25
		5.0 mL	± 0.5	± 25	± 0.15	± 7.5
10 mL Increment: 10 µL	0.1 - 10 mL	1.0 mL	± 0.5	± 5	± 0.25	± 3
		5.0 mL	± 0.4	± 20	± 0.25	± 13
		10.0 mL	± 0.4	± 40	± 0.15	± 15
25 mL (blue adapter) Increment: 25 µL	0.25 - 25 mL	2.5 mL	± 0.3	± 8	± 0.35	± 8.8
		12.5 mL	± 0.3	± 38	± 0.25	± 31
		25.0 mL	± 0.3	± 75	± 0.15	± 38
50 mL (dark gray adapter) Increment: 50 µL	0.5 - 50 mL	5.0 mL	± 0.3	± 15	± 0.50	± 25
		25.0 mL	± 0.3	± 75	± 0.20	± 50
		50.0 mL	± 0.3	± 150	± 0.15	± 75

9 Technical specifications

9.5 Varispenser / Top Buret

The following specifications for Varispenser and Top Buret are valid for the following conditions:

Liquid:	Distilled or deionized water
Reference temperature:	20 to 25 °C, ± 0.5 °C unchanging
Number of determinations:	10, in accordance with EN ISO 8655

9.5.1 Varispenser and Varispenser plus

Setting range	Tested volume	Systematic error (inaccuracy)	Random error (imprecision)
0.5 – 2.50 mL	2.5 mL	± 0.6 %	≤ 0.1 %
1.00 – 5.00 mL	5.0 mL	± 0.5 %	≤ 0.1 %
2.00 – 10.0 mL	10.0 mL	± 0.5 %	≤ 0.1 %
5.00 – 25.0 mL	25.0 mL	± 0.5 %	≤ 0.1 %
10.0 – 50.0 mL	50.0 mL	± 0.5 %	≤ 0.1 %
20.0 – 100.0 mL	100.0 mL	± 0.5 %	≤ 0.1 %

9.5.2 Top Buret

Size	Setting range	Systematic error (inaccuracy)	Random error (imprecision)
C	25 mL	± 0.2 %	≤ 0.1 %
H	50 mL	± 0.2 %	≤ 0.1 %

9 Technical specifications

9.6 Error limits in accordance with EN ISO 8655

The error limits always refer to the overall system: pipette and tip. If the nominal volume of the pipette lies between two entries in the table, the absolute error values for the next largest nominal volume apply.

The absolute error limits in µL referring to the nominal volume apply to each volume which can be set on the piston stroke pipette. The list below includes the absolute and relative error limits in relation to the volume.

9.6.1 Air-cushion pipettes, fixed and adjustable volume

Nominal volume	Error limits			
	Measurement errors			
	Systematic		Random	
	± %	± µL	± %	± µL
1 µL	±5.0 %	±0.05 µL	±5.0 %	±0.05 µL
2 µL	±4.0 %	±0.08 µL	±2.0 %	±0.04 µL
5 µL	±2.5 %	±0.125 µL	±1.5 %	±0.075 µL
10 µL	±1.2 %	±0.12 µL	± 0.8 %	±0.08 µL
20 µL	±1.0 %	±0.2 µL	±0.5 %	±0.1 µL
50 µL	±1.0 %	±0.5 µL	±0.4 %	±0.2 µL
100 µL	± 0.8 %	±0.8 µL	±0.3 %	±0.3 µL
200 µL	± 0.8 %	±1.6 µL	±0.3 %	±0.6 µL
500 µL	± 0.8 %	±4.0 µL	±0.3 %	±1.5 µL
1 000 µL	± 0.8 %	±8.0 µL	±0.3 %	±3.0 µL
2 000 µL	± 0.8 %	±16.0 µL	±0.3 %	±6.0 µL
5 000 µL	± 0.8 %	±40.0 µL	±0.3 %	±15.0 µL
10 000 µL	±0.6 %	±60.0 µL	±0.3 %	±30.0 µL

i For Multi-channel pipettes, the error values are two times the values specified for single-channel pipettes.

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Standard Operating Procedure for Pipettes

9.6.2 Positive displacement pipettes (Biomaster)

Nominal volume	Error limits			
	Measurement errors			
	Systematic		Random	
	± %	± µL	± %	± µL
5 µL	±2.5 %	±0,13 µL	±1.5 %	±0.08 µL
10 µL	±2.0 %	±0.2 µL	±1.0 %	±0.1 µL
20 µL	±2.0 %	±0.4 µL	± 0.8 %	±0,16 µL
50 µL	±1.4 %	±0,7 µL	±0.6 %	±0.3 µL
100 µL	±1.5 %	±1.5 µL	±0.6 %	±0.6 µL
200 µL	±1.5 %	±3.0 µL	±0.4 %	±0.8 µL
500 µL	±1.2 %	±6.0 µL	±0.4 %	±2.0 µL
1 000 µL	±1.2 %	±12.0 µL	±0.4 %	±4.0 µL

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9.6.3 Dispenser (Multipette)

Nominal volume	Error limits			
	Measurement errors			
	Systematic		Random	
	± %	± µL	± %	± µL
0.001 mL	±5.0 %	±0.05 µL	±5.0 %	±0.05 µL
0.002 mL	±5.0 %	±0.1 µL	±5.0 %	±0.1 µL
0.003 mL	±2.5 %	±0.075 µL	±3.5 %	±0.11 µL
0.01 mL	±2.0 %	±0.2 µL	±2.5 %	±0.25 µL
0.02 mL	±1.5 %	±0.3 µL	±2.0 %	±0.4 µL
0.05 mL	±1.0 %	±0.5 µL	±1.5 %	±0.75 µL
0.1 mL	±1.0 %	±1.0 µL	±1.0 %	±1.0 µL
0.2 mL	±1.0 %	±2.0 µL	±1.0 %	±2.0 µL
0.5 mL	±1.0 %	±5.0 µL	±0.6 %	±3.0 µL
1 mL	±1.0 %	±10.0 µL	±0.4 %	±4.0 µL
2 mL	± 0.8 %	±16.0 µL	±0.4 %	±8.0 µL
5 mL	±0.6 %	±30.0 µL	±0.3 %	±15.0 µL
10 mL	±0.5 %	±50.0 µL	±0.3 %	±30.0 µL
25 mL	±0.5 %	±125.0 µL	±0.3 %	±75.0 µL
50 mL	±0.5 %	±250 µL	±0.25 %	±125.0 µL
100 mL	±0.5 %	±500 µL	±0.25 %	±250.0 µL
200 mL	±0.5 %	±1 000 µL	±0.25 %	±500.0 µL

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9.6.4 Single-stroke dispenser (Varispenser)

Nominal volume	Error limits			
	Measurement errors			
	Systematic		Random	
	± %	± µL	± %	± µL
0.01 mL	±2.0 %	±0.2 µL	±1.0 %	±0.1 µL
0.02 mL	±2.0 %	±0.4 µL	±0.5 %	±0.1 µL
0.05 mL	±1.5 %	±0.75 µL	±0.4 %	±0.2 µL
0.1 mL	±1.5 %	±1.5 µL	±0.3 %	±0.3 µL
0.2 mL	±1.0 %	±2.0 µL	±0.3 %	±0.6 µL
0.5 mL	±1.0 %	±5.0 µL	±0.2 %	±1.0 µL
1 mL	±0.6 %	±6.0 µL	±0.2 %	±2.0 µL
2 mL	±0.6 %	±12.0 µL	±0.2 %	±4.0 µL
5 mL	±0.6 %	±30.0 µL	±0.2 %	±10.0 µL
10 mL	±0.6 %	±60.0 µL	±0.2 %	±20.0 µL
25 mL	±0.6 %	±150.0 µL	±0.2 %	±50.0 µL
50 mL	±0.6 %	±300.0 µL	±0.2 %	±100.0 µL
100 mL	±0.6 %	±600.0 µL	±0.2 %	±200.0 µL
200 mL	±0.6 %	±1 200 µL	±0.2 %	±400,0 µL

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9.6.5 Piston stroke burets

Nominal volume	Error limits			
	Measurement errors			
	Systematic		Random	
	± %	± µL	± %	± µL
<1 mL	±0.6 %	±6,0 µL	±0.1 %	±1,0 µL
2 mL	±0.5 %	±10,0 µL	±0.1 %	±2,0 µL
5 mL	±0.3 %	±15,0 µL	±0.1 %	±5,0 µL
10 mL	±0.3 %	±30,0 µL	±0.1 %	±10,0 µL
20 mL	±0.2 %	±40,0 µL	±0.1 %	±20,0 µL
25 mL	±0.2 %	±50,0 µL	±0.1 %	±25,0 µL
50 mL	±0.2 %	±100,0 µL	±0.1 %	±50,0 µL
100 mL	±0.2 %	±200 µL	±0.1 %	±100,0 µL

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Eppendorf offices

AUSTRALIA & NEW ZEALAND

Eppendorf South Pacific Pty. Ltd.
Phone: +61 2 9889 5000
Fax: +61 2 9889 5111
E-mail: Info@eppendorf.com.au
Internet: www.eppendorf.com.au

AUSTRIA

Eppendorf Austria GmbH
Phone: +43 (0) 1 890 13 64 - 0
Fax: +43 (0) 1 890 13 64 - 20
E-mail: office@eppendorf.at
Internet: www.eppendorf.at

BRAZIL

Eppendorf do Brasil Ltda.
Phone: +55 11 30 95 93 44
Fax: +55 11 30 95 93 40
E-mail: eppendorf@eppendorf.com.br
Internet: www.eppendorf.com.br

CANADA

Eppendorf Canada Ltd.
Phone: +1 905 826 5525
Fax: +1 905 826 5424
E-mail: canada@eppendorf.com
Internet: www.eppendorfn.com

CHINA

Eppendorf China Ltd.
Phone: +86 21 38560500
Fax: +86 21 38560555
E-mail: market.info@eppendorf.cn
Internet: www.eppendorf.cn

CZECH REP. & SLOVAKIA

Eppendorf Czech & Slovakia s.r.o.
Phone: +420 323 605 454
Fax: +420 323 605 454
E-mail: eppendorf@eppendorf.cz
Internet: www.eppendorf.cz /
www.eppendorf.sk

FRANCE

Eppendorf France S.A.R.L.
Phone: +33 1 30 15 67 40
Fax: +33 1 30 15 67 45
E-mail: eppendorf@eppendorf.fr
Internet: www.eppendorf.fr

GERMANY

Eppendorf Vertrieb
Deutschland GmbH
Phone: +49 2232 418-0
Fax: +49 2232 418-155
E-mail: vertrieb@eppendorf.de
Internet: www.eppendorf.de

INDIA

Eppendorf India Limited
Phone: +91 44 42 11 13 14
Fax: +91 44 42 18 74 05
E-mail: info@eppendorf.co.in
Internet: www.eppendorf.co.in

ITALY

Eppendorf s.r.l.
Phone: +390 2 55 404 1
Fax: +390 2 58 013 438
E-mail: eppendorf@eppendorf.it
Internet: www.eppendorf.it

JAPAN

Eppendorf Co. Ltd.
Phone: +81 3 5825 2363
Fax: +81 3 5825 2365
E-mail: info@eppendorf.jp
Internet: www.eppendorf.jp

NORDIC

Eppendorf Nordic Aps
Phone: +45 70 22 2970
Fax: +45 45 76 7370
E-mail: nordic@eppendorf.dk
Internet: www.eppendorf.dk

SOUTH & SOUTHEAST ASIA

Eppendorf Asia Pacific Sdn. Bhd.
Phone: +60 3 8023 2769
Fax: +60 3 8023 3720
E-mail: eppendorf@eppendorf.com.my
Internet: www.eppendorf.com.my

SPAIN

Eppendorf Ibérica S.L.U.
Phone: +34 91 651 76 94
Fax: +34 91 651 81 44
E-mail: iberica@eppendorf.es
Internet: www.eppendorf.es

SWITZERLAND

Vaudaux-Eppendorf AG
Phone: +41 61 482 1414
Fax: +41 61 482 1419
E-mail: vaudaux@vaudaux.ch
Internet: www.eppendorf.ch

UNITED KINGDOM

Eppendorf UK Limited
Phone: +44 1223 200 440
Fax: +44 1223 200 441
E-mail: sales@eppendorf.co.uk
Internet: www.eppendorf.co.uk

USA

Eppendorf North America, Inc.
Phone: +1 516 334 7500
Fax: +1 516 334 7506
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Eppendorf North America, Inc. · 102 Motor Parkway, Suite 410 · Hauppauge, NY 11788-5178 · USA

Tel: +1 516 334 7500 · Toll free phone: +1 800 645 3050 · Fax: +1 516 334 7506 · E-Mail: info@eppendorf.com

Application Support

Europe, International: Tel: +49 1803 666 789 · E-Mail: support@eppendorf.com

North America: Tel: +1 800 645 3050 ext. 2258 · E-Mail: techserv@eppendorf.com

Asia Pacific: Tel: +60 3 8023 6869 · E-Mail: support_asiapacific@eppendorf.com

STANDARD OPERATING PROCEDURE SOP-10¹

EXPERIMENTAL EXPOSURE SYSTEMS FOR LABORATORY STUDIES OF AQUATIC ORGANISMS UNDER FLUVIAL CONDITIONS

Purpose

The purpose of this SOP is to describe the methods to assemble exposure system structures, where to obtain the parts, and how to arrange the experimental setup for laboratory studies under simulated fluvial conditions.

Scope and Applicability

This SOP describes the design, construction, maintenance and utilization of artificial flow-through exposure systems. The flow regime in the recirculating exposure units can be adjusted such that it simulates fluvial conditions of interest (e.g., the flow regime can be adjusted such that it accommodates the specific requirements for different life-stages or riverine fish such as sturgeon).

Summary of Method

The method described herein covers the initial construction and assemblage of all materials required for a fully functioning exposure system as well as considerations to be taken when choosing an experimental layout. Details of all building materials and suppliers are provided. A list of supplier contact information is included at the end of the SOP.

Safety Considerations

All safety considerations will be in accordance with University of Saskatchewan (U of S) Department of Health, Safety and Environment (DHSE) procedures and with the requirements of the U of S Environmental Toxicology Laboratory (ETL) Safety Manual. These requirements include:

1. All persons involved in research at the ETL shall complete basic laboratory safety, new employee orientation, bio-safety, and radiation safety courses offered by the DHSE (summer student orientation will suffice for summer students);

¹ This SOP is also SOP-13 in the April 2010 *Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon (Acipenser transmontanus)*.

2. All persons involved in research using vertebrate subjects must have taken proper training through the Animal Use and Care Committee.

Other job-specific safety training will be provided by qualified members of the ETL.

Specific safety concerns are:

1. Personnel will be working in close proximity to water and electricity, such that electrocution is an imminent danger. For this reason, it is important to assure that all equipment is certified for this use and to always wire equipment and use ground fault interruption (GFI) circuits at all times. Do not stand in water while touching wiring, extension chords, or electrically powered equipment.
2. The exposure system frames and wet tables are heavy structures and care should be taken when lifting and assembling.
3. All chemicals and flammable liquids that are to be used should be disposed of in properly labeled waste containers and stored in appropriate storage bins.

Equipment and Materials

Flow-through Exposure Chambers

Artificial flow-through exposure chambers and screen dividers are constructed from ¼-inch plexi-glass or ¼-inch high density polyethylene (HDPE). The material and structures are supplied and fabricated by WD Plastics (Saskatoon, SK) and QMP plastics (Saskatoon, SK), respectively. Each exposure chamber contains removable screen dividers for controlling flow regime.

¼-inch puck board reservoir baffles and exposure chamber dividers are also supplied and fabricated by WD Plastics.

Incubation/hatching Jars

6L McDonald-type egg hatching jars are supplied by Aquatic Ecosystems (Apopka, FL; Cat. #J30).

Wet Table

The fiberglass wet tables are open 4-foot by 4-foot boxes that are 8 inches deep. A 2-inch female thread by female thread bulkhead fitting is installed at the bottom of one end for a stand pipe. The wet table, with installed bulkhead, is fabricated by Progressive Yard Works Ltd (Saskatoon, SK).

Exposure System Table Frame

The steel table frame is supplied and fabricated by Elance, steel fabricating Co. Ltd (Saskatoon, SK). Each exposure system table frame consist of a 4-foot ¼-inch by x 4-foot ¼-inch welded L-bracket top frame, four 30-inch removable steel legs, four 4-inch steel bolts and four steel nuts.

Reservoir and Mixing Tank

100L polyethylene reservoir tanks (part # 70394-0) and 1000L polyethylene oval vertical mixing tanks with 8-inch lids and 1-inch female thread by female thread bulkheads (part # 60014-1) are supplied by Quality Molded Plastics Ltd (Saskatoon, SK).

Pumps

Pulsafeeder pulsatron diaphragm metering pumps (model # LEH7SAPHC3-XXX) are supplied by Viking Pump of Canada Inc. and magnetic drive march pumps (part # W30HD) are supplied by Aquatic Eco-systems Inc, Saskatoon, SK.

Chillers

Water chillers are supplied by Aqua Logic Inc (Apopka, FL).

Plumbing Equipment

Bulkhead fittings are supplied by Western/Westlund. Ball valves, adapters, bushings, tees, PVC piping, and clamps are supplied by Aquifer Distribution Ltd (Saskatoon, SK). Some plumbing equipment can also be found at Home Depot. Quick coupler cam locks are supplied by Green Line Hose and Fittings (Saskatoon, SK), and hose/tubing is supplied by Goodall Rubber Company of Canada (Saskatoon, SK).

Tools and Miscellaneous Equipment

Tools such as drills, drill bits, Teflon tape, and saws can be found at Home Depot. Miscellaneous equipment such as PVC/ABS cement, silicon, fiberglass mosquito netting, etc., can also be found at Home Depot or Canadian Tire.

Methods and Procedures

Overview

A holding tank contains the reverse osmosis and dechlorinated lab water mixture supplied to the experimental exposure systems. The water is delivered from the tank to the 100L exposure system reservoir via a metering pump. The water then travels from the reservoir to the exposure system treatment chambers via a re-circulating march pump. The water flows from one end of the treatment chamber to the other and exits through a drain hole, which in turn is

connected back to the 100L reservoir. There is an overflow drain out the back of each reservoir to discard wastewater and a baffle to prevent short-circuiting of the inflow to the overflow drain (Figure 1). The water may be cooled to the desired temperature by either placing a chiller unit inside the 100L reservoir or by re-circulating chilled water through the wet table.

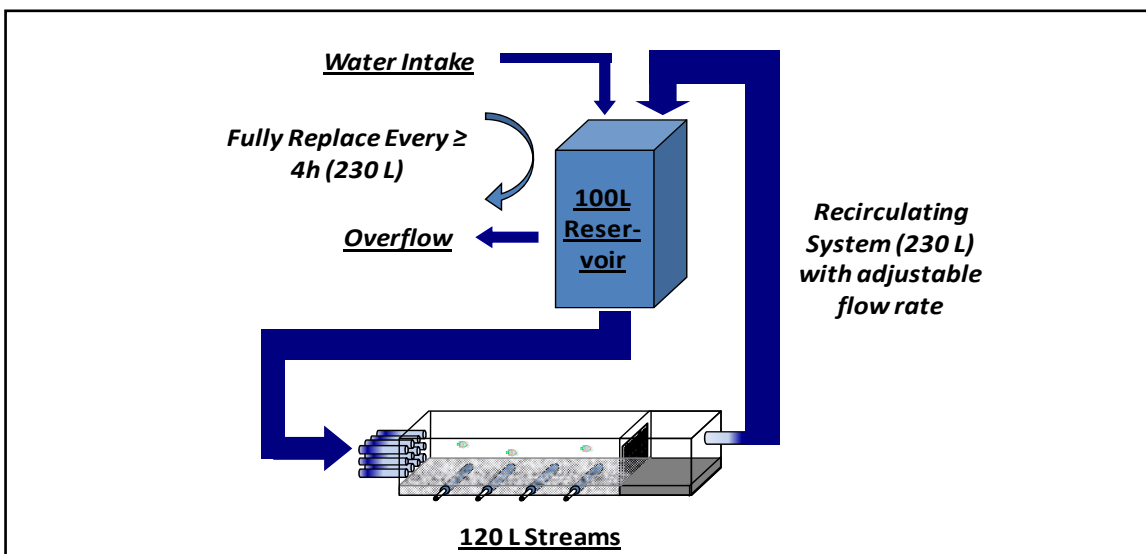


Figure 1. Experimental exposure system. Each true replicate exposure system consists of one 100L reservoir and one 120L chambers. Each chamber can hold up to 24,000 cm³ of sediment.

Experimental Setup

- All piping threads should be wrapped in Teflon tape prior to attachment.
- All PVC/ABS piping joints should be glued with the appropriate cement.

Exposure system table frames

- Arrange the experimental exposure systems in such a fashion that enables easy access, preferably from all sides.
- Assemble the steel table frame. Attach four steel legs to the steel top frame by sliding each leg into a sleeve on the frame and secure with a bold and nut.

Wet table

- A fiberglass wet table is placed inside the table frame.
- If using a water bath to cool the treatment chambers
 - Fabricate a 6-inch standpipe from 2-inch diameter PVC piping
 - Attach the wet table standpipes into the bulkhead fitting of the wet table using a 2-inch socket by male adapter.

- Attach a drain hose to the bulkhead fitting on the underside of the wet table using a 2-inch PVC male adapter and run the drain hose back to the chilled water supply.
- Attach a delivery hose from the chilled water supply to the wet table frame using PVC piping and a clamp. Attach a ball valve at the end so that the water flow can be adjusted.

Treatment chambers and incubation/hatching jars

- Each wet table can hold up to six 40L plexiglass treatment chambers, or three 120L HDPE treatment chambers, side by side.
- Attach a 1-inch female thread by female thread bulkhead to the 2-inch hole at the end part of each chamber.
- Each treatment chamber has removable screen inserts to control the flow regime. Silicon fiberglass mosquito netting is placed over the rectangular holes on the inserts in order to reduce water flow and to prevent the fish from passing through.
- Egg hatching jars are placed at the end of the exposure chambers. The test solution is pumped through the top of the standpipe of the hatching jar (from the exposure chamber) when the experiment is initiated and flows into the reservoir and is re-circulated (Figure 2.).¹

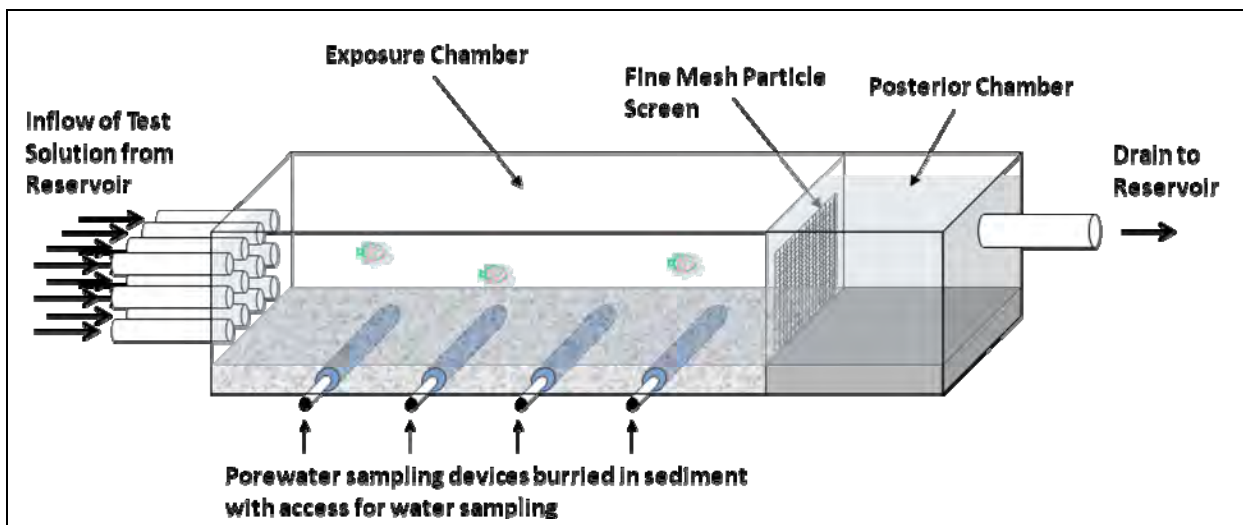


Figure 2. Exposure Chamber. This schematic represents one exposure chamber.¹

- A right angle 1-inch male PVC adapter is attached to the exterior side of the bulkhead fitting at the end of the exposure chambers.

¹ Final exposure chamber design may vary, and will be adjusted in accordance with the methods established during the 2010 pilot studies.

- Tubing is attached to the adapters.
- Depending on the number of replicates per table, combine the drain pipe tubing from common doses into one hose with an appropriate splitter.
- Run one drain hose (per true replicate) from each splitter back into the appropriate reservoir.
- Place a 48-inch by 24-inch by ¼-inch puck board divider between each true replicate on the table.
- Each chamber is capable of holding up to 24,000 cubic cm of sediment given a depth of 7.6 cm.
- Cylindrical, ceramic airstones are placed horizontally 1.5 inches below sediment surface at 3-inch intervals. Airstones are inserted into a grommet built into the side of the exposure chamber to allow water to be pulled through the airstone into a 2.5 mL syringe for collection. A valve is placed onto the grommet to regulate flow¹.

Reservoirs

- Reservoirs and lids must be cut to the appropriate size to fit under the table frame.
- Drill a 2-inch output hole in the center of one face of the reservoir near the bottom so that it will align with the march pumps. Attach a 1-inch female thread by female thread bulkhead. Attach a 1-inch ball valve and a male cam lock to the bulkhead.
- Drill a 2-inch intake hole in the center of the reservoir on the same face as the previous hole near the top (about 3 inches below the lid). Attach a 1-inch female thread by female thread bulkhead. Attach a male cam lock to the bulkhead.
- Drill a 2-inch overflow hole to the far side, near the top, in the reservoir face directly opposite the face in which the two previous holes were drilled. Make sure the center of this hole is at least 1-inch lower than the previously drilled intake hole. This hole is the overflow hole and must be lower than the previously drilled intake hole. Attach a 1-inch female thread by female thread bulkhead.
- Drill a 4-inch hole in the center of the bottom of the 23-inch by 8-inch by ¼-inch puck board baffle. Silicon this baffle, with the hole on the bottom, to the inside corner nearest the overflow hole of the reservoir. Make sure the baffle will separate the intake water from the overflow water.
- Drill a 2-inch hole in the corner of the reservoir lid opposite the reservoir overflow hole. Attach a 1-inch female thread by female thread bulkhead and a 1-inch male PVC adapter.

- Place the reservoirs under the exposure system frame and wet table.
- Attach black flexible sump-pump hose to the overflow drain at the back of the reservoir to discard of the wastewater.
- Attach the drain hose from each splitter of the exposure chambers to the 1-inch male PVC adapter in the lid of the appropriate reservoir.

March pump and manifold¹

- Attach a female cam lock to the intake of the march pump. Connect the march pump intake to the male cam lock at the bottom output of the reservoir.
- Attach a 4-way or 3-way splitter, depending on the number of pseudo-replicate exposure chambers per true replicate being used, to the output of the march pump. This is the initial piece of the manifold that will allow for water flow regulation to each exposure chamber.
- Attach two ball valves in series to each output arm of the splitter (this will allow the water flow to be shut off without disrupting the flow settings).
- Attach male PVC adapters and tubing to the second ball valve of each of the manifold arms and run the tubing to the right angle ½-inch PVC nozzle that was previously attached to the front of each treatment chamber.
- The PVC nozzle will be attached to a spray-bar assembly. The spray-bar assembly will consist of four vertical spray bars, each regulated by a ball valve. Water will enter from either end of the spray-bar assembly to deliver an even flow².

Metering pumps

- Place the metering pumps next to their corresponding reservoirs.
- Attach a 3/8-inch inner diameter hose barb to a female cam lock and attach to the male intake cam lock at the top of the reservoir.
- Attach 3/8-inch inner diameter tubing from the hose barb to the output of the corresponding metering pump.

Mixing/holding tanks and receiving tanks

- Position the mixing/holding tanks or the receiving tank (used in continuous delivery of test solution from river or lake source) in such a way that they are easily accessible and so that they are as close as possible to their corresponding exposure systems.
- Attach a 3-way splitter to the 1-inch bulkhead of the tank.

¹ Final manifold design may vary depending on number of chambers per table.

² Final spray-bar assembly design may vary.

- Attach ball valves to each of the output arms of the splitter.
- Attach a 3/8-inch inner diameter hose barb to the ball valves on the splitter.
- Attach 3/8-inch inner diameter tubing from the intake of the metering pumps to the 3/8-inch inner diameter hose barb on the splitter on the corresponding tank.

Pump Settings and Flow Rate

Metering pump

Using the current pumping system the turnover rate of the water/mixture in the 205L recirculating system can be varied from one to four times per 24-hour period. This is controlled by the settings on the metering pump.

Metering pump settings and adjustments:

- Disconnect the cam lock on the reservoir intake at the top of the reservoir.
- Position the hose so that it is discharging into a graduated cylinder.
- Time the discharge for 60 seconds using a stopwatch and a measuring cylinder (or for 15 seconds and multiply by 4).
- Regulate the discharge rate (ml/min) by adjusting the frequency and stroke knobs on the metering pump and repeat timing of discharge until the desired flow is achieved:
 - Set stroke rate to nearest value of flow (Table 1.). Use stroke length to calibrate fine adjustment to achieve desired rate. Stroke length should be kept as close to 100 percent as possible for maximum efficiency.

Table 1. Stroke rate adjustments for metering pump.

Stroke rate	10	15	20	30	40	50	60	70	80	90	100
mL/min	110	140	210	330	400	480	580	695	755	800	900
L/day	160	200	300	475	575	690	835	1000	1090	1150	1300

- Ensure that the hose is reconnected once the flow rate is achieved.

March pump and manifold

The recirculation rate of the water/mixture from the reservoir to the treatment chambers can be varied from approximately 2L/min to 15L/min by adjusting the ball valves on the manifold attached to the march pump. Never completely shut off the ball valves on the manifold as the back pressure may damage the pump.

Manifold settings:

- Place a beaker under the right angle ½-inch PVC nozzle in the front of each treatment chamber corresponding to the manifold of interest.
- Fully open the ball valves on the manifold.
- Start the march pump and measure the flow rate into the beakers. Adjust the second ball valves on the manifold arms until the flow into each exposure chamber is at the desired rate.
- If the flow ever needs to be shut off for an exposure chamber, adjust the first ball valves on the manifold so that the set flow rate remains the same once normal flow is restored. This will prevent having to re-calibrate the manifold.

Take-Down of Exposure System

Ideally, all cleaning and acid washing should be done as soon as possible once the experiment is terminated. All tanks, streams and wet tables must be scrubbed clean before algae hardens and dries. Aquarium/ wet lab appropriate soap should be used for cleaning.

- Mixing tanks should be scrubbed using soap and water to remove any algal/fungal buildup and then acid washed (5percent hydrochloric acid [HCl]) for a minimum of 8 hours.
- Exposure chambers can be cleaned first by scrubbing with water and soap and then with an acid bath (5 percent HCl). The plexiglass should be subjected to the acid bath for a minimum of 8 hours, and then well rinsed with clean water.
- Reservoirs can be cleaned first by scrubbing with water and soap and then with an acid bath (5 percent HCl). The plastic should be subjected to the acid bath for a minimum of 8 hours, and then well rinsed with clean water.
- To clean the March pump, run clean water through it to dilute any contaminants. Note: Do not use acid as it will corrode the metal parts of the pump.
- To clean the metering pump, run clean water through it to dilute any contaminants. If needed, remove the four screws to disassemble the head assembly. Check the injection valve assembly, the discharge valve cartridge and the suction valve cartridge - these may have lime or other substances blocking them or preventing the ball from functioning properly. Note: Do not use acid as it will corrode the metal parts of the pump.

- Clean the area of concern with a small brush, being careful not to scratch or damage the apparatus.

Manufacturers' Contact Information

WD Plastics

826- 56th St. East

Saskatoon, SK, S7K 5Y8

(PH) 306-934-6844 (FAX) 306-934-6842

Elance Steel Fabricating Company Ltd.

40 Unger St. North Corman Park

Site 404, Box 3, RR #4

Saskatoon, SK, S7K 3J7

(PH) 306-931-4412 (FAX) 306-931-7683

Progressive Yard Works Ltd.

3423 Millar Ave.

Saskatoon, SK, S7K 6J4

(PH) 306-244-6911 (FAX) 306-244-6913

Aquatic Eco-Systems, Inc.

2395 Apopka Blvd.

Apopka, FL, 32703

Orders and General Inquiries: 877-347-4788

Free Tech Support: 407-598-1401

Fax: 407-886-6787

Aquifer Distribution Ltd.

227A Venture Crescent

Saskatoon, SK, S7K 6N8

(PH) 306-242-1567 (FAX) 306-665-2115

Quality Molded Plastics Ltd. (QMP)

Site 412, Box 280, RR#4

71st. St. Rd. & Highway 16W

Saskatoon, SK, S7K 3J7

(PH) 306-242-4494 (FAX) 306-242-4122

Goodall Rubber Co. of Canada
2902 Miners Ave
Saskatoon, SK, S7K 4Z7
(PH) 306-652-3791 (FAX) 306-652-5848

Green Line Hose and Fittings
2520 Millar Ave.
Saskatoon, SK, S7K 4K2
(PH) 306-653-5001 (FAX) 306-653-5008

Marine Aquaria
2854 Calgary Trail
Edmonton, AB
(PH) 780-761-1101 (FAX) 780-761-1102

Viking Pump of Canada.
8912-60 Avenue
Edmonton, AB T6E 6A6
1-888-VIK-PUMP (845-7867)
(FAX) 780-466-9131
For parts ordering 519-256-5438

Kootenay Trout Hatchery
4522 Fenwick Road
Fort Steel, BC, V0B 1N0
(PH) 250-429-3214 (FAX) 250-429-3202

Records, Documentation and Quality Control Requirements

All procedures, activities, anomalies and/or deviation from the specified method shall be recorded in a bound, serially numbered, laboratory notebook. In case of routine procedures that are identical to those conducted at an earlier time, the earlier description can be referred to without writing the complete procedures down again by indicating procedure, notebook page, date and initial.

Responsibilities

Project Manager. Dr. Markus Hecker will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principal investigator and other U of S personnel, Teck American personnel, and the sponsor Project Manager.

Principal Investigator. Prof. John P. Giesy will advise the Project Manager in overseeing and approving all project activities, authorize necessary actions and adjustments related to U of S activities to accomplish program quality assurance (QA) objectives; and act as liaison between agencies, staff, and the sponsor Project Manager.

Study Team Leaders (STL). David Vardy and Jonathon Doering, under the supervision of Markus Hecker, will oversee all research activities and supervise all personnel involved with the assemblage of the experimental exposure systems. The STLs will ensure that proper sample collection, preservation, storage, transport, and chain-of-custody quality control procedures are followed and will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

Quality Assurance (QA) Manager. Dr. Shaun Roark will initiate audits on work completed by project personnel. The manager will review program QA activities, quality problems, and quality-related requests. In response to experimental findings, the QA manager will approve corrective actions. The QA manager will report quality non-conformances to the Project Manager.

Questions or Comments

Please feel free to contact the following persons with any questions, comments, etc., you may regarding the procedures outlined in this SOP.

Markus Hecker
mhecker@entrix.com
(306) 966-5233

Paul D. Jones, Ph.D.
paul.jones@usask.ca
(306) 966-5062

John P. Giesy, Ph.D.
john.giesy@usask.ca
(306) 966-2096

STANDARD OPERATING PROCEDURE SOP-11¹

MEASUREMENT OF WATER QUALITY AND PROCESSING OF WATER SAMPLES DURING WHITE STURGEON (*ACIPENSER TRANSMONTANUS*) EXPOSURES

Purpose

The purpose of this SOP is to describe methods necessary to make water quality (WQ) measurements on exposure chambers and process water samples during white sturgeon exposures in the University of Saskatchewan (U of S) laboratory.

Scope and Application

This SOP applies to the ETL for samples supplied from the Upper Columbia River white sturgeon studies.

Summary of Method

The method described herein describes the appropriate methods for taking daily WQ measurements from exposure chambers during white sturgeon exposures. In addition, methods for processing periodic water samples for extended analysis are described.

Safety Considerations

All safety considerations will be in accordance with U of S Department of Health, Safety and Environment (DHSE) procedures and with the requirements of the U of S Environmental Toxicology Laboratory (ETL) Safety Manual. These requirements include: all persons involved in research at the ETL shall complete basic laboratory safety, new employee orientation, bio-safety, and radiation safety courses offered by the DHSE (summer student orientation will suffice for summer students); all persons involved in research using vertebrate subjects must have taken proper training through the Animal Use and Care Committee. Other job-specific safety training will be provided by qualified members of the ETL.

Specific safety concerns are:

¹ This SOP is also SOP-16 in the April 2010 *Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon (Acipenser transmontanus)*.

Personnel will be working in close proximity to water and electricity, such that electrocution is an imminent danger. For this reason, it is important to assure that all equipment is certified for this use and to always wire equipment and use ground fault interruption (GFI) circuits at all times. Do not stand in water while touching wiring, extension chords, or electrically powered equipment.

Equipment, Materials, and Reagents

Daily WQ measurements

- Ammonia nitrogen kit (LaMotte, Cat #7674)
- Nitrate nitrogen kit (LaMotte, Cat #7418-01)
- Hardness kit (LaMotte, Cat #4482-DR-LI)
- Multi-function meter for pH, temperature, dissolved oxygen, and conductivity (VWR, Cat #11388-328)

Periodic WQ measurements

- Chlorine kit (VWR, Cat #66170-136)
- Sulfate kit (VWR, Cat #66121-530)

Preparation of water samples for metal analysis and detailed WQ analysis

- Water filtration apparatus (VWR, Cat #28199-406)
- 0.45 µm water filters (VWR, Cat #28157-960)
- Plastic forceps (VWR, Cat #83009-010)
- Ultrapure nitric acid (VWR, Cat #CANX0408-7)
- Water sample bottles (VWR, Cat #EP156-125WMN)
- 1 mL pipetter
- 1 mL pipette tips
- Water bath (plastic tote will work)
- Ultra-pure water
- Hydrochloric acid (HCl)
- Small Petri dishes (VWR, Cat #25384-332)
- -20°C freezer

Preparation of water samples for total organic carbon (TOC) and dissolved organic carbon (DOC) analysis

- Water filtration apparatus (VWR, Cat #28199-406)

- 0.45 µm water filters (VWR, Cat #28157-960)
- Water sample bottles (VWR, Cat #EP156-125WMN)
- HCl
- Small Petri dishes (VWR, Cat #25384-332)
- -20°C freezer

Methods, Procedures, and Requirements

Daily water quality measurements¹

1. Selected WQ measurements (temperature, oxygen, pH, conductivity) should be made from one exposure chamber in each recirculating system every day. Chambers should be alternated so that WQ measurements are made on each different chamber over 2 to 3 days.
2. Using the multi-parameter WQ monitor, make measurements for temperature, pH, dissolved oxygen and conductivity.
3. Record all WQ measurements on the appropriate data sheet(s) following quality assurance/quality control (QA/QC) requirements.
4. Any abnormal WQ measurements, especially high temperatures should be immediately reported to a team leader.

Periodic WQ measurements¹

1. WQ kits to make measurements for nitrite, nitrate, ammonia, chlorine, phosphate, and sulfate are available to be used at important time points or when needed (at least once per week per exposure system).
2. Make kit measurements for those parameters that are not performed by the Liber lab (see subsequent Section). Copies of kit instructions are available in SOP notebooks and as electronic copies from Upper Columbia River (UCR) team members.
3. These measurements should be made in one exposure chamber per recirculating system the first day water is supplied to the mesocosms, before eggs/larvae are added to chambers, after eggs/larvae are added to chambers, once per week during the exposures in the field, at exposure termination, and whenever UCR team members deem necessary.
4. Record all WQ measurements on the appropriate data sheet(s) following QA/QC requirements.

¹ Any samples sent to CAS laboratory will be collected, stored and shipped according to CAS protocols.

5. Any abnormal WQ measurements, especially high ammonia levels, should be immediately reported to a team leader.

Preparation of water samples for metal analysis and detailed water quality analysis¹

1. Water samples should be prepared as outlined in the sampling plan provided by the UCR team leaders.
2. Place water filtration units, water filters, water sample transfer bottles and plastic tweezers into a 5 percent HCl bath 12 to 24 hours before the samples will be taken.
3. Rinse the water filtration units, filters, bottles, and tweezers in an Ultrapure water bath.
4. Remove a 150 mL water sample from one exposure chamber in each exposure treatment (i.e., each river water treatment, each metal dose, controls) with a water sample transfer bottle.
5. Assemble the water filtration units with water filters in place.
6. Place the water samples into the filtration units.
7. Allow samples an appropriate amount of time to pass through filters.
8. Disassemble filtration units and decant filtered water samples into properly labeled 60 or 125 mL sample bottles. Discard excess filtered sample.
9. Acidify filtered samples with 1 percent of total sample volume of Ultrapure nitric acid.
10. Cap bottles.
11. Place filters into properly labeled individual Petri dishes.
12. Freeze filtered water and filter samples at -20°C until analysis by Columbia Analytical Services.
13. Record samples taken on the proper data sheet(s) and in the laboratory notebook.
14. All sample transfers, both between laboratories and from the field, should be performed with proper chain-of-custody (COC) documentation (see SOP-ETL4006; SOP-9 in *Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon [Acipenser transmontanus]*).

Preparation of water samples for TOC and DOC analysis¹

1. Water samples should be prepared as outlined in the sampling plan provided by the UCR team leaders.
2. Place water filtration units, water filters, water sample transfer bottles and plastic tweezers into a 5 percent hydrochloric acid bath 12 to -24 hours before the samples will be taken.
3. Rinse the water filtration units, filters, bottles, and tweezers in an Ultrapure water bath.

4. Remove a 150 mL water sample from one exposure chamber in each exposure treatment (i.e., each river water treatment, each metal dose, controls) with a water sample transfer bottle.
5. Assemble the water filtration units with water filters in place.
6. Place the water samples into the filtration units.
7. Allow samples an appropriate amount of time to pass through filters.
8. Disassemble filtration units and decant filtered water samples into properly labeled 125 mL sample bottles. Fill bottles to approximately 1 inch from top and cap. Discard excess filtered sample. The filtered sample contains the DOC content.
9. Place the filter into a properly labeled Petri dish. The filter contains the undissolved portion of the carbon content.
10. Preserve the water samples for analysis by acidifying the samples with 1 mL of HCl.
11. Freeze water samples and filters at -20°C.
12. The water sample represents DOC content; the filter plus the water sample represents TOC content. Carbon analysis will be performed using a TOC analyzer (TOC-5050A, Shimadzu, Mandel Scientific, Guelph, Ontario).
13. Record samples taken on the proper data sheet(s) and in the laboratory notebook.
14. All sample transfers, both between laboratories and from the field, should be performed with proper COC documentation (see SOP-ETL4006; SOP-9 *Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon [Acipenser transmontanus]*) for COC details.

Preparation of Porewater Samples for Metal Analysis¹

1. Porewater samples should be prepared as outlined in the sampling plan provided by the UCR team leaders.
2. Place water filtration units, water filters, water sample bottles, and plastic tweezers into a 5 percent hydrochloric acid bath 12 to 24 hours before the samples will be taken.
3. Attach 2.5 mL syringe to the grommet of the exposure chamber using airline tubing.
4. Open valve and pull 2.5 mL of water into syringe and properly discard. Pull another 2.5 mL of porewater into the syringe and transfer into clean, sample bottle.
5. Repeat steps 1 through 4 for the remaining airstones using one sample bottle per exposure chamber.
6. Process porewater samples following procedure detailed in the "Preparation of water samples for metal analysis and detailed water quality analysis" section above.

Records, Documentation and QC Requirements

All procedures, activities, anomalies and/or deviation from the specified method shall be recorded in a bound, serially numbered, laboratory notebook. In case of routine procedures that are identical to those conducted at an earlier time, the earlier description can be referred to without writing the complete procedures down again by indicating procedure, notebook page, date and initial.

Responsibilities

Project Manager. Dr. Markus Hecker will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principal investigator and other U of S personnel, Teck American personnel, and the sponsor Project Manager.

Principal Investigator. Prof. John P. Giesy will advise the Project Manager in overseeing and approving all project activities, authorize necessary actions and adjustments related to U of S activities to accomplish program QA objectives; and act as liaison between agencies, staff, and the sponsor Project Manager.

Study Team Leaders (STLs). David Vardy and Jonathon Doering under the supervision of Markus Hecker will oversee all research activities and supervise all personnel involved with the culture and maintenance of early white sturgeon life-stages. The STLs will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

Quality Assurance (QA) Manager. Dr. Shaun Roark will initiate audits on work completed by project personnel. The manager will review program QA activities, quality problems, and quality-related requests. In response to experimental findings, the QA manager will approve corrective actions. The QA manager will report quality non-conformances to the Project Manager.

Questions or Comments

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Markus Hecker
mhecker@entrix.com
(306) 966-5233

Paul D. Jones, Ph.D.
paul.jones@usask.ca
(306) 966-5062

John P. Giesy, Ph.D.
john.giesy@usask.ca
(306) 966-2096

STANDARD OPERATING PROCEDURE SOP-12¹

DATA PACKAGE REVIEW

Purpose

The purpose for data package review is to ensure that final results reported are an accurate representation of the raw data generated during analysis. Data packages must function as stand-alone units. They must contain all information necessary to verify the reported results and to completely document the quality control (QC) procedures utilized during the analysis. Any deviations from the written protocol and/or quality control procedures which do not meet the documented limits must be clearly noted in the data package.

Scope and Applicability

Data package review is applicable to all data packages generated by the Environmental Toxicology Laboratory (ETL) at University of Saskatchewan (U of S). Two levels of review will be performed on each data package prior to submission of the data package to the Quality Assurance (QA) Coordinator. The first level of review will be performed by the primary analyst (analyst who performed the analysis or his/her designee). The second level of review is to be performed by the lead supervisor or their designee. Both levels of data package review must be documented utilizing the appropriate checklist. A QC review will be performed at a frequency of 20 percent of samples.

Safety Considerations

All personnel shall adhere to prudent safety practices as specified in the project Health and Safety Plan (HASP) (TCAI 2007).

Equipment, Materials, and Reagents

The data package reviewers will require basic office equipment including, at a minimum, pens, a calculator and a computer. The computer must be loaded with Reflections for access to the LIMS software and should have the ability to review raw analytical data when required.

¹ This SOP is also SOP-17 in the April 2010 *Draft Quality Assurance Project Plan for the Assessment of Sediment Toxicity to White Sturgeon (Acipenser transmontanus)*.

Method, Procedures, and Requirements

Three levels of data review will routinely be performed:

- Analyst review
- Technical review
- QA review.

Primary Analyst Review

Once the data package has been generated, in accordance with ETL standard operating procedures (SOPs), the analyst, or their designee, will perform the first level of review. This review, detailed below, will verify the completeness of the data package prior to submission for technical review.

- A. Review the Lot Folder Tracking Form. Verify that the header information is correct. Verify that the collection and analysis dates for samples are correct.
- B. Each package will contain a data package checklist appropriate for the method performed. Verify that the data package includes all required forms as listed on the appropriate data package checklist. Verify that the data package is assembled in the order detailed on the appropriate data package checklist. Verify that the review of the contents have been performed by checking off each specific item on the appropriate data package checklist.
- C. The primary analyst must verify each item on the appropriate Review Checklist. The review of each item must be noted by checking the appropriate item on the checklist.
- D. Review the LIMS Worklist printout and the client and internal chains-of-custody (COCs). Verify that all samples on the client and internal COCs match the samples on the worklist and that there are no transcription errors or omissions.
- E. Verify that there are no transcription errors on the LIMS worklist printout by checking the results against the raw data.
- F. Check all QC sample results. Ensure that all quality control samples met acceptance criteria specified in the appropriate method SOP. If any QC samples do not meet criteria, verify that this is noted in the Case Narrative and also in the Analyst Comments section of the data package checklist. Verify that there is sufficient explanation regarding data acceptability.
- G. Verify that all unused lines and entries on all forms have been lined out, dated, and initialed.

- H. Verify that the correct calibration standards were used for quantitation.
- I. Verify that all raw data for the analyses are included.
- J. Verify correct calculation of results by recalculating the reported result on the Example Calculation Form.
- K. Verify that all pages in the data package that require analyst signatures have been signed and dated by the analyst.
- L. Document all variations from the SOP or problems with the analytical run in the Case Narrative.
- M. If applicable, verify that the data package is consecutively paginated and that an electronic data deliverable (EDD) has been generated.

Technical Review

The technical review is the second level review and is performed by the Lead Supervisor or his/her designee. The technical review is performed to confirm the completeness of the package as submitted by the analyst and to verify the technical validity of the reported results, and is detailed below.

- A. Review the Lot Folder Tracking Form. Verify that all header information is included and is accurate. Review the items listed in the Case Narrative Form and add pertinent information, as appropriate. Sign and date the Lot Folder Tracking Form for Technical Review.
- B. Each package will contain a data package checklist appropriate for the method performed. Verify that the data package includes all required forms as listed on the data package checklist. Verify that the data package is assembled in the order detailed on the appropriate data package checklist. Verify that the primary analyst has checked off all applicable items on the appropriate data package checklist.
- C. Each data package will contain the appropriate Method Review Checklists. Verify that the primary analyst has completely and correctly filled out the Review Checklists. Verify that the primary analyst has signed and dated the checklist. Verify that items listed on the form have been included in the data package and that all information is accurate. Sign and date the Review Checklists as the Reviewer.
- D. Review the LIMS worklist printout. Verify that there were no transcription errors for the reported results by reviewing the raw data. Verify that all QC sample recoveries are reported correctly. Sign and date the LIMS report as the Reviewer.

- E. Review the internal COC to ensure that custody was maintained within the laboratory. Signatures and dates must be present for all exchanges of samples between personnel. Verify that all client COCs are included for all samples contained on the internal COC.
- F. Review the Sample Preparation Form, if applicable. This form must include preparation information for all samples present in the analytical run(s). Verify that all header information, sample identification, sample initial and final weights or volumes, units, solvent used with manufacturer's name and lot number, spike volumes and solution identifications have been completed. Ensure that sample preparation steps and holding time requirements have been met. Verify that the spiking solutions are traceable to certified reference materials and the traceability has been clearly documented in the data package. Verify that none of the stock or working standards used for spiking have expired. Verify that the source used for the laboratory control spike (LCS) is different from the source used for the initial calibration standards. The source for the matrix spike (MS) may be from the same source as the LCS or the initial calibration standards. All unused lines must be crossed out, initialed, and dated.
- G. Review the Additional Sample Preparation Information Form, if applicable. The steps involved in the preparation process must be clearly defined with all initial and final volumes clearly stated. All unused lines must be crossed out, dated and initialed.
- H. Review the Sample Preparation Comments Form, if applicable. Verify that the header information is complete and accurate. Verify that the analyst included any comments regarding the sample preparation process which may affect the results and which deviate from the specified method. If there were no reportable instances which affect the data, verify that the analyst indicates this. All unused lines must be crossed out, dated and initialed by the analyst.
- I. Review the Sample Extract Dilution Form, if applicable. Verify that the header information is complete and accurate. Verify that the sample IDs and extract and solvent volumes and units are correct. Verify that the resulting dilution factors are correctly calculated. All unused lines must be crossed out, dated and initialed by the analyst.
- J. Review the Solvent Purity Form, if applicable. Verify that the header information is complete and accurate. Verify that any solvents used for the method have had the solvent purity verified. As laboratory deionized water is continuously monitored, documentation using this form is not required.
- K. Verify the calibration of the instrument. Documentation of the calibration may consist of the Calibration Form, instrument calibration reports, or the use of a spreadsheet or other

documentation specified in the method SOP. Raw responses must be checked for accurate data transcription and acceptable calibration results. Verify that all calibration information presented on the raw data has been accurately transcribed onto the calibration forms. Verify that all instrument calibration criteria (initial calibration verification [ICV], continuing calibration verification [CCV], instrument drift checks, etc.) meet the requirements detailed in the laboratory quality control plan (LQCP) and/or method SOP. For daily calibration checks, verify that the daily standard is checked against the correct initial calibration. Any manual integrations of calibration standards require clear identification, and an explanation for the use of manual integration.

- L. Verify that all calibration and QC standard sources meet the requirements of the LQCP and associated method SOP. Verify that the calibration solutions are traceable to certified reference materials and the traceability has been clearly documented in the data package.
- M. Verify that all associated raw data are included in the data package. Review the responses for all sample and QC analyses. Raw responses must be checked for accurate data transcription, if applicable. If a spreadsheet is used for interpretation of raw data, check for accurate data transcription. Confirm analysis holding time is within requirements. Verify that final concentrations have been calculated correctly by checking 10 percent of reported results. Check raw data for obvious problems, this may include elevated baselines, peak tailing, retention-time shifts, interfering ions, etc.
- N. Verify that the analysis was in control by evaluating the recoveries observed for the QC samples. The method blank result should be below the method specific limits (method detection limits, practical quantification limit, method reporting limit, etc). If there are concentrations present in the method blank at levels above the method specific limits, then professional judgment must be used to determine if the data are acceptable. This must be noted on the Case Narrative.
- O. Verify that a matrix spike was performed and is included in the Data Package. Verify that the matrix spike recoveries are acceptable.
- P. Verify that all standards used for the sample preparation and analysis have not expired. Verify that all required stock and working standards logbook pages are included. Verify that all standards can be traced back to a certified standard reference material and that all Certificates of Analysis are included.
- Q. Verify that the report is consecutively paginated and that the EDD has been generated. If the report is not paginated and/or the EDD is not generated, perform these functions.

- R. Although each data package is a stand-alone entity assessment of results particularly for laboratory control samples and certified reference materials should be compared to those of preceding data packages to detect possible trending of data with time. Should any trending be detected the data in all packages should be examined closely to determine the cause of any trends observed.

QC Review

- A. A QC review will be performed at a frequency of 20 percent of samples. The QC review will be performed utilizing the QC Lot Folder Review Checklist. For each portion of the data package to be reviewed, check each item listed on the QC Lot Folder Review Form to ensure completeness and accuracy.
- B. If QC or technical discrepancies are identified in the 20 percent data package review, the QC reviewer should use their professional judgment in determining whether more than 20 percent of the data packages should be reviewed.
- C. Verify that anomalies, variations, or problems are stated in the Case Narrative Section.
- D. Verify that all necessary forms are included in the data package.
- E. Verify that the field COC, Lab COC, LIMS worklist and COC information is complete and accurate.
- F. Verify that all sample preparation information is complete and accurate, and that sample prep and analytical holding time requirements have been met.
- G. Verify that the calibration information is complete and accurate.
- H. Verify that the sample response information is complete and accurate.
- I. Verify that method blank, LCS, and MS samples were performed and accurately reported. Verify that the QC samples meet the method specific criteria.
- J. Sample integrity and traceability will be assessed in two samples per data package by performing a full audit trail analysis. The audit trail will track the sample documentation from field collection through final reporting and will include verification of sample documentation, sample container labeling and all COC and analytical procedures.
- K. Sign and date the QC Review Checklist.
- L. Upon completion of the QC review, any discrepancies in the data package should be brought to the attention of the Lead Supervisor for resolution.

Records, Documentation, and QC Requirements

- A. The primary analyst shall document any anomalies and/or deviations from the specified method in the appropriate sections of the data package and list them in the Case Narrative Form. The primary analyst will sign and date any forms as the analyst.
- B. The technical reviewer will record any problems noted during the technical review. The technical reviewer will return the data package to the analyst for corrections prior to submission of the data package. The technical reviewer must sign and date all forms as the reviewer.
- C. The technical reviewer, or his/her designee, will paginate the report.
- D. Generation of EDDs will be performed by the technical reviewer or by a designee of the technical reviewer or lead supervisor. As the nature of the EDD will vary considerably for each sample type and analytical procedure it is not possible to provide a definitive description of specific EDDs. EDDs will take the format of summary tables which may be directly extracted from the data package but may also consist of scanned documents in electronic format suitable for electronic storage, transmission and retrieval. QC procedures for summary tables will be determined based on the method of generation. EDDs will be provided in a format that will allow them to be suitably protected from electronic manipulation of the data.
- E. The QC reviewer will document any findings on the QC Lot Folder Review Checklist and notify the Lead Supervisor(s) and primary analyst.

Responsibilities

Individuals and their project responsibilities are identified in a work plan or quality assurance project plan (QAPP) for each project. Any changes in personnel or their responsibilities will be noted in a protocol amendment and placed on file with other project records.

References

Field and Laboratory Policies and Procedures Manual, 1996, Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824.

Data Quality Objectives Process for Superfund, Office of Emergency and Remedial Response, EPA 540-R-93-071, September 1993, United States Environmental Protection Agency, Washington DC 20460.

TCAI (Teck Cominco American, Inc.). 2007. Upper Columbia River: Draft general health and safety plan for the remedial investigation and feasibility study. Prepared for Teck Cominco American Incorporated, December 27, 2007. Integral Consulting Inc., Mercer Island, Washington, and Parametrix, Bellevue, Washington.

STANDARD OPERATING PROCEDURE SOP-13

DETECTION OF FLUORESCENT DYE USING THE POLARstar OPTIMA

Purpose

The purpose of this SOP is to describe the detection of fluorescein dye in water using the POLARstar OPTIMA in the University of Saskatchewan (U of S) laboratory.

Scope and Applicability

The detection of fluorescein dye allows the measurement of flow regimes through experimental exposure systems, as well as the passage of surface water into porewater within experimental sediment exposure systems.

Summary of Method

Once dyed samples are collected from either the surface or porewater, the POLARstar OPTIMA fluorometric function is used according to the manufacturer's instructions to quantify fluorescence intensity of the sample. A list of supplier contact information is included in section 6.0 of this SOP.

Safety Considerations

All safety considerations will be in accordance with University of Saskatchewan (U of S) Department of Health, Safety and Environment (DHSE) procedures and with the requirements of the U of S Environmental Toxicology Laboratory (ETL) Safety Manual. These requirements include:

1. All persons involved in research at the ETL shall complete basic laboratory safety, new employee orientation, bio-safety, and radiation safety courses offered by the DHSE (summer student orientation will suffice for summer students)
2. All persons involved in research using vertebrate subjects must have taken proper training through the Animal Use and Care Committee
3. All chemicals and flammable liquids that are to be used should be disposed of in properly labeled waste containers and stored in appropriate storage bins.

Equipment, Materials, and Reagents

Necessary materials include:

- 96 well plate
- Pipettor capable of dispensing 250µL
- Fluorescein sodium salt (Sigma-Aldrich CAS: 518-47-8)
- POLARstar OPTIMA (BMG LABTECH, San Diego, CA, USA).

Method Performance

Surface or porewater samples are collected according to the sampling procedures outlined in SOP-2. 200µL of each sample are pipetted into one well per desired replicate on a 96 well plate. The 96 well plate is then inserted into the POLARstar OPTIMA reader and analyzed according to manufacturer's manual using the fluorometric function of the instrument. Fluorescein must be analyzed using the POLARstar OPTIMA filters $\lambda_{ex}485$ and $\lambda_{em}520$. For the manufacturer's manual see Attachments 1 and 2 of this document.

Dye concentration is to be determined relative to quantified concentrations measured in a standard dilution series of fluorescein dye that is to be run in parallel on the same plate (Table 1; Figure 1). The range of dye concentrations for calculation of the standard curve is 0.5 to 125 µg/mL but may be adjusted in accordance with the specific requirements of an experiment. Laboratory water used for the experiments with the fluvial exposure systems that has no dye added serves as a blank. Concentration of dye in individual samples is calculated by solving the equation obtained for the standard curve (4th degree polynomial function) for X. Alternatively, points from the lower and upper end of the curve are to be dropped until a linear regression can be fitted through the all remaining data point with an R^2 of greater 0.90. If necessary, samples are to be diluted to fall within the linear range of the standard curve if the linear standard curve option is selected. All samples, standards and blank are to be measure in triplicates or quadruplicates.

Table 1. Plate layout for the determination of Fluorescein concentrations in water samples using a 96 well microtiter plate and the POLARstar OPTIMA fluorometric function.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	SA1	SA2
B	BL	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	SA1	SA2
C	BL	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	SA1	SA2
D	SA3	SA3	SA3	SA8	...		SA13	...		SA18	...	
E	SA4	SA4	SA4	SA9	...		SA14	...		SA19	...	
F	SA5	SA5	SA5	SA10	...		SA15	...		SA20	...	
G	SA6	SA6	SA6	SA11	...		SA16	...		SA21	...	
H	SA7	SA7	SA7	SA12	...		SA17	...		SA22	...	

BL = Blank (Lab Water)

ST = Fluorescein Standard: ST1: 0.5 µg/mL; ST2: 1.0 µg/mL; ST3: 2.0 µg/mL; ST4: 3.9 µg/mL; ST5: 7.8 µg/mL; ST6: 16 µg/mL; ST7: 31 µg/mL; ST8: 62 µg/mL; ST9: 125 µg/mL

SA1 – SA22 = Samples 1 - 22

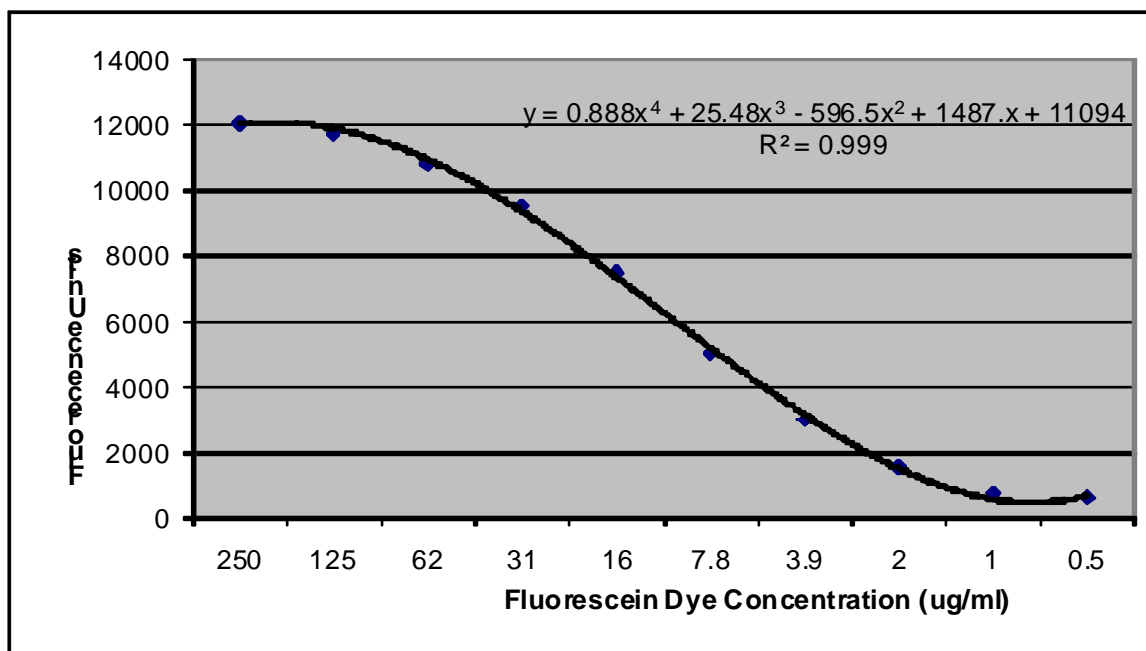


Figure 1. Example of a fluorescein standard curve for the calculation of dye concentrations in water samples.

Manufacturers' Contact Information

Sigma-Aldrich
2149 Winston Park Dr., Oakville, Ontario L6H 6J8
Ph: 800-565-1400 e-mail: canada@sial.com

BMG LABTECH (Canadian supplier: Fisher Scientific)
112 Colonnade Road, Nepean, Ontario K2E 7L6
Ph: 613-228-6275 Web: www.fishersci.ca

Records, Documentation and QC Requirements

All procedures, activities, anomalies and/or deviation from the specified method shall be recorded in a bound, serially numbered, laboratory notebook. In case of routine procedures that are identical to those conducted at an earlier time, the earlier description can be referred to without writing the complete procedures down again by indicating procedure, notebook page, date and initial.

Responsibilities

Project Manager. Dr. Markus Hecker will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principal investigator and other U of S personnel, Teck Cominco personnel, and the sponsor Project Manager.

Principal Investigator. Professor John P. Giesy will advise the Project Manager in overseeing and approving all project activities, authorize necessary actions and adjustments related to U of S activities to accomplish program QA objectives; and act as liaison between agencies, staff, and the sponsor Project Manager.

Study Team Leaders (STL). David Vardy and Jonathon Doering, under the supervision of Markus Hecker, will oversee all research activities and supervise all personnel involved with the assemblage of the experimental exposure systems. The STLs will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed and will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

Quality Assurance (QA) Manager. Dr. Shaun Roark will initiate audits on work completed by project personnel. The manager will review program QA activities, quality problems, and quality-related requests. In response to experimental findings, the QA manager will approve

corrective actions. The QA manager will report quality non-conformances to the Project Manager.

Questions or Comments

Please feel free to contact the following persons with any questions, comments, etc., you may regarding the procedures outlined in this SOP.

Markus Hecker
mhecker@entrix.com
(306) 966-5233

John P. Giesy, Ph.D.
john.giesy@usask.ca
(306) 966-2096

**FLUOstar OPTIMA
POLARstar OPTIMA
LUMIstar OPTIMA**

Operating Manual

Revision G

This manual was designed to guide FLUOstar OPTIMA, POLARstar OPTIMA and LUMIstar OPTIMA users through the basic hardware features of the instrument.

Headquarters**Germany**

BMG LABTECH GmbH
Hanns-Martin-Schleyer Str. 10
77656 Offenburg
Phone: +49 781 96968-0
Fax: +49 781 96968-67
germany@bmglabtech.com

Subsidiaries**Australia**

BMG LABTECH Pty. Ltd.
2/24 Carbine Way
Mornington 3931
Victoria
Phone: +61 3 5973 4744
Fax: +61 3 5973 4711
australia@bmglabtech.com

China

BMG LABTECH Co. Ltd.
RM2-201, Hua Fu Jing Yuan
NO11. An Ding Men Wai Avenue
Dongcheng District
Beijing, P.R. China 100011
Phone: +86 10 84110632
Fax: +86 10 84129679
china@bmglabtech.com

France

BMG LABTECH SARL
7, Rue Roland Martin
94500 Champigny s/ Marne
Phone: +33 1 48 86 20 20
Fax: +33 1 48 86 47 07
france@bmglabtech.com

Japan

BMG LABTECH JAPAN Ltd.
2 FT.S.-1 Building
1-6-2, Shimo-cho, Omiya-ku
Saitama-city 330-0844
Phone: +81 48 6477217
Fax: +81 48 6477218
japan@bmglabtech.com

United Kingdom

BMG LABTECH Ltd.
P.O. Box 73
Aylesbury Bucks, HP 20 2 QJ
United Kingdom
Phone: +44 1296 33 66 50
Fax: +44 1296 33 66 51
uksales@bmglabtech.com

USA

BMG LABTECH Inc.
2415 Presidential Dr.
Bldg. 204, Suite 118
Durham, NC 27703
Phone: +1 919 806 1735
Fax: +1 919 806 8526
usa@bmglabtech.com

FLUOstar OPTIMA, POLARstar OPTIMA & LUMIstar OPTIMA

The FLUOstar OPTIMA is a multifunctional microplate reader that can perform a wide variety of applications for fluorescence intensity, time-resolved fluorescence, absorbance and luminescence.

The POLARstar OPTIMA can measure in the above-mentioned modes and additionally in fluorescence polarization mode and simultaneous dual emission. The LUMIstar OPTIMA is a luminescence microplate reader that can be upgraded to include all above mentioned modes.

The versatile optical system allows switching from top to bottom optics.

All instruments achieve high-performance measurement data in a wide wavelength range. The instruments have a built-in incubator and can be configured with up to two reagent injectors.

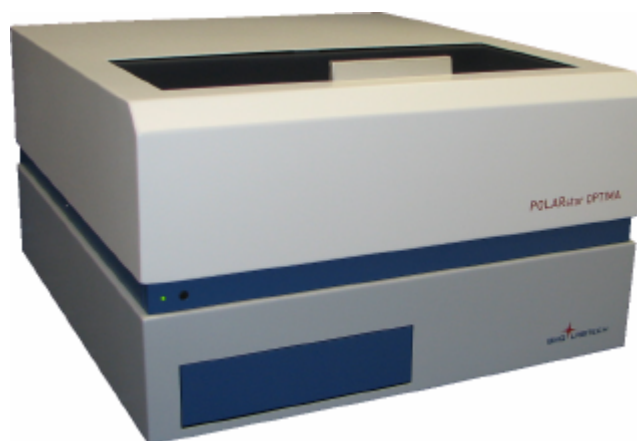


Figure 1: POLARstar OPTIMA

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1 Technical Specifications

Measurement principles:

FLUOstar OPTIMA:

- Fluorescence intensity
- Time-resolved fluorescence
- Luminescence (opt.)
- Absorbance (opt.)
- Upgradeable to POLARstar OPTIMA

POLARstar OPTIMA:

- Fluorescence polarization
- Simultaneous dual emission fluorescence
- Fluorescence intensity
- Time-resolved fluorescence
- Luminescence (opt.)
- Simultaneous dual emission luminescence (opt.)
- Absorbance (opt.)

LUMIstar OPTIMA:

- Luminescence
- Simultaneous dual emission luminescence (opt.)
- Upgradeable to FLUOstar / POLARstar OPTIMA

Light source:

FLUOstar OPTIMA & POLARstar OPTIMA:

- High-energy xenon flash-lamp

Detector:

Side window, current type photomultiplier tube

Filters:

FLUOstar OPTIMA & POLARstar OPTIMA:

- 2 filter wheels:
with 8 excitation and 8 emission filter positions

LUMIstar OPTIMA:

- Emission filter wheel with 8 filter positions

Gain control:Software selectable gain
Automatic gain adjustment**Plate Carrier:**Auto lock microplate carrier.
All microplate formats up to 1536-well in all detection modes.
Microplates should fulfil the SBS specification and non-SBS formats should fit: (lxwxh) (mm) max: 128x86x20; min: length 124.**Reagent injectors:**Up to two built-in reagent injectors
Individual injection volumes for each well
Injection volumes definable down to 3 µl
Up to 4 independent injection actions per well
Variable injection speed (100 µl/s to 420 µl/s)**Shaking:**Linear, orbital and figure eight shaking
Programmable shake time and diameter**Incubation:**Incubation range from ambient +8°C to 45°C, in 0.1°C steps
Extended incubation up to 60°C (optional)
Temperature monitoring (without incubation)
Temperature stability 0.2°C**Shortest reading time:**

Fluorescence intensity:	-	15 s for 96-wells
	-	30 s for 384-wells
	-	57 s for 1536-wells
Luminescence:	-	20 s for 96-wells
	-	55 s for 384-wells

Fluorescence intensity:Limit of detection 0.9 fmol / well fluorescein
Spectral range (ex. and em.): 240 ... 740 nm
Dynamic range 8 decades

Time-resolved fluorescence:	Limit of detection 70 amol / well europium Spectral range (ex. and em.): 240 ... 740 nm Dynamic range: 6 decades
Luminescence:	Limit of detection <50 amol / well ATP Spectral range 240 ... 740 nm Dynamic range: 9 decades
Fluorescence polarization:	Limit of detection: <5 mP SD at 1 nM fluorescein Spectral range: (ex. and em.): 380 ... 740 nm Dynamic range: 4 decades
Absorbance:	Spectral range 240 ... 740 nm Dynamic range 0.000-3.000 OD Reproducibility 0.010 OD for 0-2 OD range, 0.030 OD for 2-3 OD range
Computer interface:	RS232, 38400 baud, binary communication protocol
Power requirements:	100/115/230 V, 50/60 Hz Consumption: max. 205 VA Fuses: <ul style="list-style-type: none">- T2.5A/250V for main power 230 V- T3.15A/250V for main power 115 V- T3.15A/250V for main power 100 V (use original type Wickmann only)
Dimensions:	Height: 27 cm, width: 44 cm and length 48 cm
Weight:	28 kg
Ambient conditions:	Operating temperature: 15°C to 35°C Storage temperature: -10°C to 50°C Humidity of atmosphere: 20% to 80% Non-condensing
Instrument conformity:	Over voltage category II; contamination class II; protection class I
Robotic capabilities:	Stacker for 50 microplates (optional)

Specifications are subject to change without notice.

2 Installation

When unpacking the instrument, please check to ensure that all parts are included.

The shipping box should contain:

FLUOstar OPTIMA or POLARstar OPTIMA or LUMIstar OPTIMA

- Control and evaluation software (CD ROM in a cover inside this manual)
- Manual
- Power cord
- RS232 cable
- Service box containing:
 - Allen key (1.5mm)
 - 2 extra fuses-Wickmann original:
 - T2.5A/250V for main power 230 V
 - T3.15A/250V for main power 115 V
 - T3.15A/250V for main power 100 V
 - injector needle cleaner (if with reagent injectors)

Call BMG LABTECH immediately if any of these items are missing.

The area designated for the instrument should be free of dust, liquids and acidic vapours. The table's surface should be flat and even. Avoid areas subject to vibrations and direct sunlight.



Upon unpacking and positioning the reader make sure to unlock the transport pin (section 2.1 Transport Pin) before any power connection (section 2.2 Power and Communication Connections).

2.1 Transport Pin

When the instrument is shipped or moved to a different location, the transport pin should be in the locked position.

The transport pin is located in the back left corner of the reagent box (figure 2). Once the instrument is in its permanent location, the transport pin should be unlocked to free the plate carrier. To do this lift up the transport pin and turn it counter-clockwise and leave the pin in the groove.

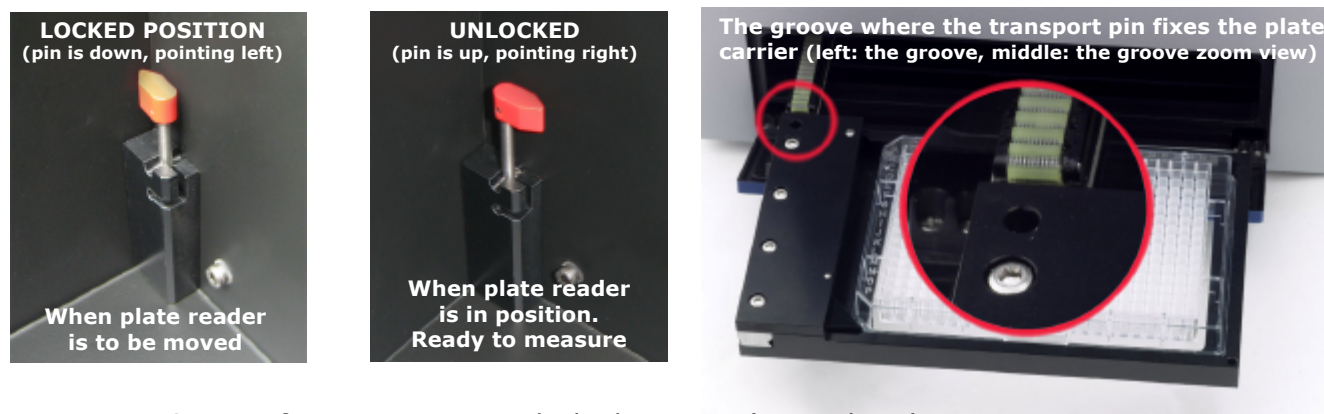


Figure 2: Left: transport pin in locked position (pin is down).
Middle: transport pin in unlocked position (pin is up).
Right: the groove where the transport pin can lock the plate carrier

If the instrument needs to be moved to a new location, the plate carrier should be in the locked position otherwise the transport system could be damaged.

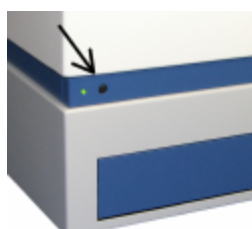


Figure 3: Plate In / Plate Out button.

Press and hold the plate in / plate out button for 3 seconds, hereafter the plate carrier will automatically move to its lock position. Once the reader is switched off, the transport pin can be turned (counter clockwise) and moved down. Also upon shutting down the control software the plate carrier will be moved to its transport lock position.

Check that the transport system is locked:

Open the plate carrier door with a fingernail and check if the plate carrier is fixed by gently trying to move the plate carrier, left to right and front to back. If it is not locked, lift the transport pin and try to position the plate carrier manually until the transport pin falls in position and locks the plate carrier. You may have to move the plate carrier slightly until the pin locks the plate carrier.

2.2 Power and Communication Connections



Before connecting the instrument's power cable and turning the power switch on, make sure the transport pin is unlocked (section 2.1)

- **Power Connection**

First check that the power switch on the back of the instrument is in the "Off" position. Inspect the voltage information on the label next to the power switch to ensure that it corresponds to the local main power specifications. Also make sure the power cable is grounded. Hereafter the power cable can be connected to the instrument.

- **Connection Check**

Locate the RS232 cable (9-pin type) in the shipping box. Connect it to the FLUOstar OPTIMA (or POLARstar OPTIMA or LUMIstar OPTIMA) and to the RS232 port on the PC.



Only connect a computer that corresponds to EN 60950 and UL 1950 for data processing instruments

In order to make a 'Connection check', the software needs to be installed. Please refer to the software manual part of this binder to install the software. You can perform a connection check within the setup menu of the OPTIMA software (go to 'Setup | Connection' and click 'Connection check'). If the instrument and PC are communicating, a 'Connection OK' message will appear. If there is no connection: first check that the reader is turned on, then try another COM port. If the 'Connection Ok' still does not appear, make sure that it is correctly configured, e.g. not a virtual port.

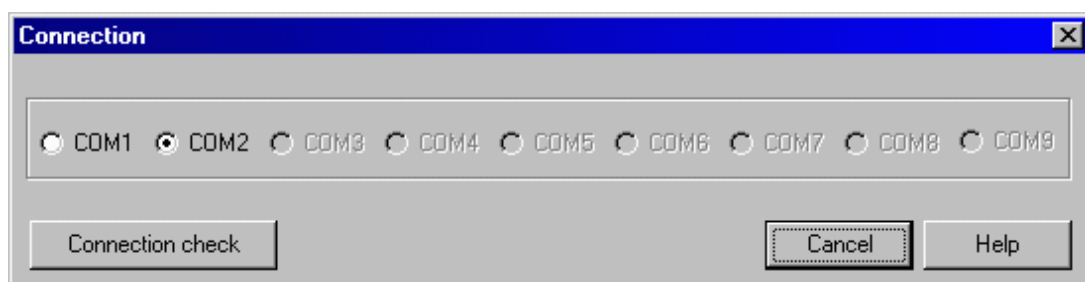


Figure 4: Connection check window ('Setup / Connection')

3 Instrument Overview

Front View

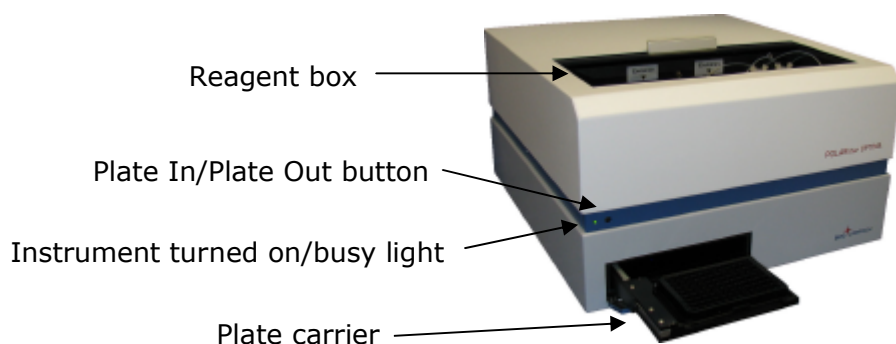


Figure 6: POLARstar OPTIMA

A constant green light means the instrument is turned on. A flashing green light means the instrument is busy (e.g. performing a measurement, plate in/out, priming, etc.). A faster flashing (5 flashes per second) means an error has occurred.

Back View

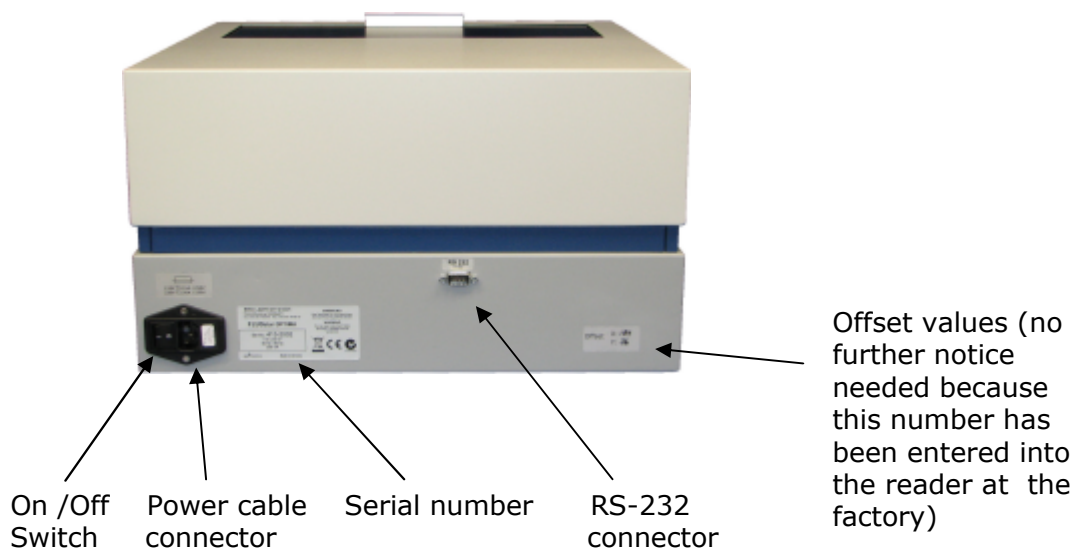


Figure 7: Back of FLUOstar OPTIMA, POLARstar OPTIMA and LUMIstar OPTIMA

Top View, Reagent Box

FLUOstar OPTIMA & POLARstar OPTIMA

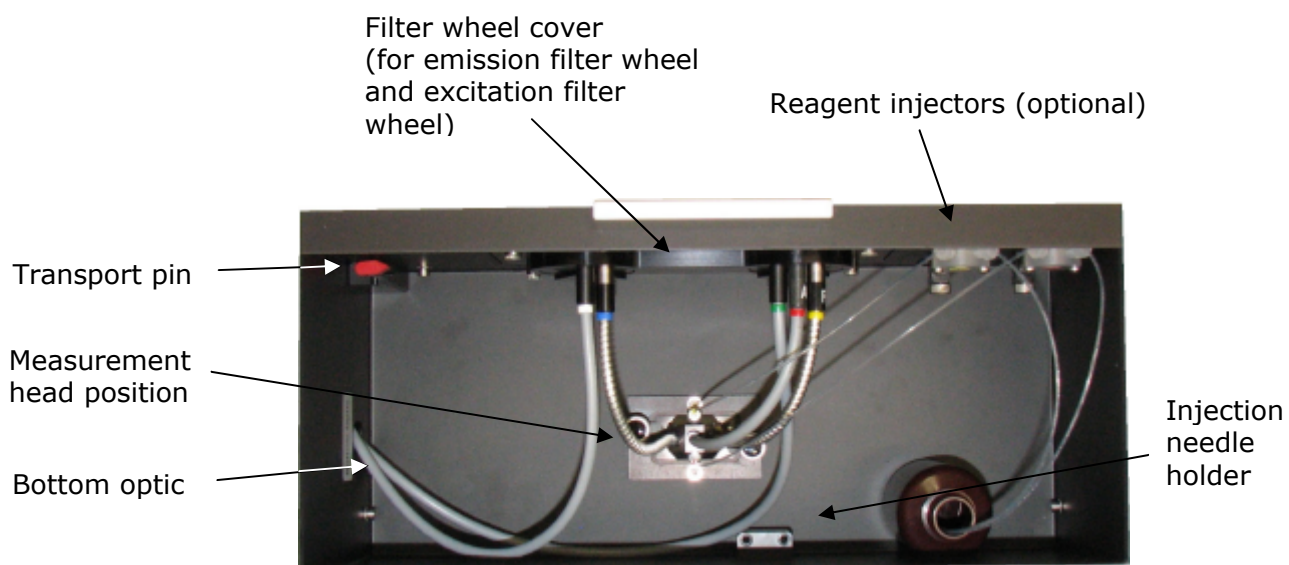


Figure 8: FLUOstar OPTIMA & POLARstar OPTIMA top view of reagent box with combi-optic and 2 reagent injectors

Top View, Reagent Box

LUMIstar OPTIMA

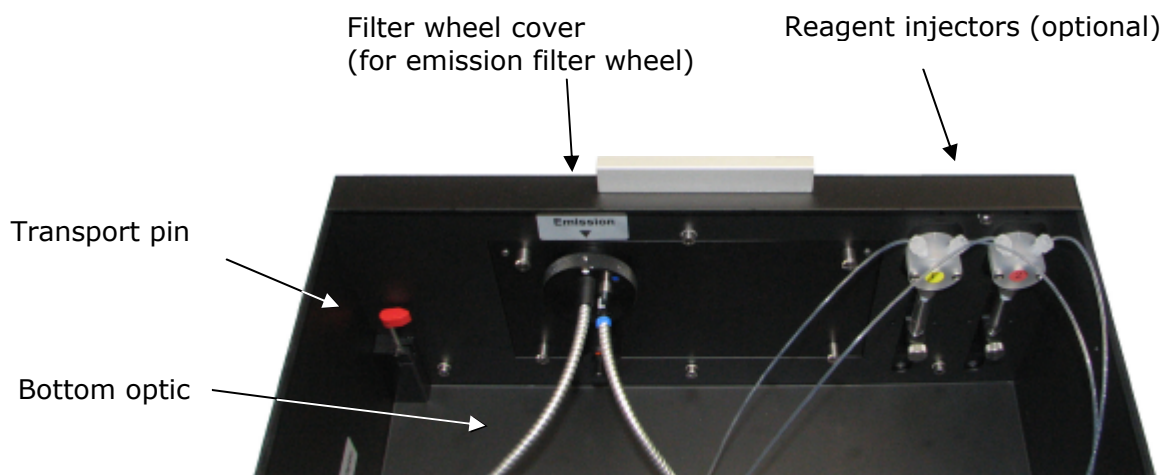


Figure 9: LUMIstar OPTIMA top view of reagent box with 2 reagent injectors

4 Description of components

4.1 Light Guides (optics)

All standard equipped readers have UV/Vis light guides for top reading. For optimal performance, there are different top reading optics available for fluorescence intensity, fluorescence polarization, luminescence, absorbance and time-resolved fluorescence.

4.2 Installation and changing of light guides

The readers are equipped with quick-fix mountings for easy exchange of light guides and easy to position spacers (for more about spacers see 4.5). No tools are required for changing the light guides. Remove the light guides from the positioning wheel by hand and turn the holders on the measurement head frame to release the black mounting piece of the light guides.

Quick-fix: in released position

Quick-fix: in fixed position

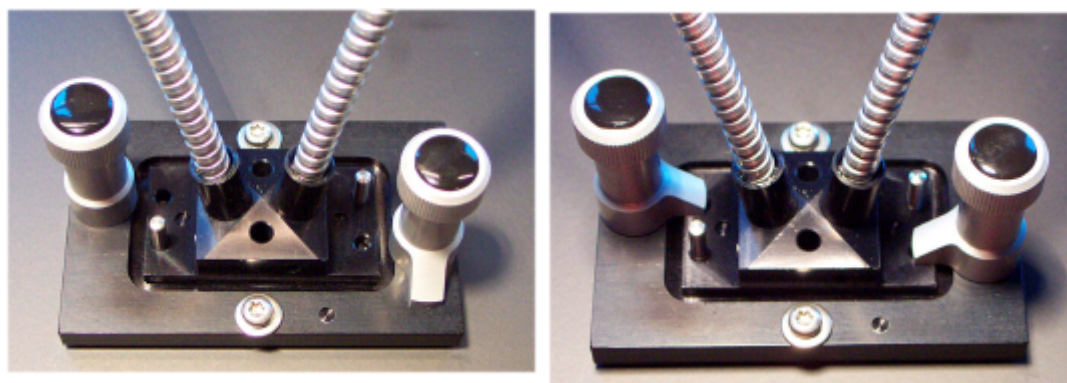


Figure 10: Quick-fix - pull up and turn to change optic

Note: Always hold the light optics by the black mounting piece.

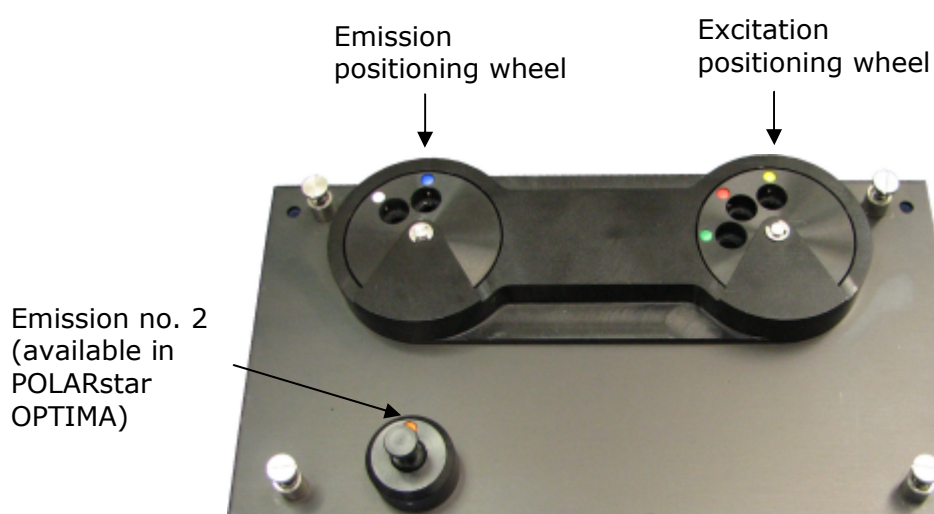


Figure 11: Filter wheel cover with positioning wheels

4.2.1 Combination Optics (fluorescence intensity and absorbance)

The combination measurement head is made up of two liquid-filled light guides for fluorescence intensity and a quartz fiber for absorbance measurement (figure 12).

To position the measurement head with the quick-fix holders, see section 4.2 Installation and changing of light guides.

For fluorescence measurements: excitation enters through the yellow marked light guide and emission is measured through the blue marked light guide.

For absorbance measurements: The grey, red marked, absorbance light guide excites from above and the absorbance is measured through the bottom optic.



Figure 12: Combination optics

Regarding reagent injection: be careful when you position the needles in the measurement head (see figure 13) to avoid damage to the reagent needles as well as the optics. The optional reagent injectors need to be available.

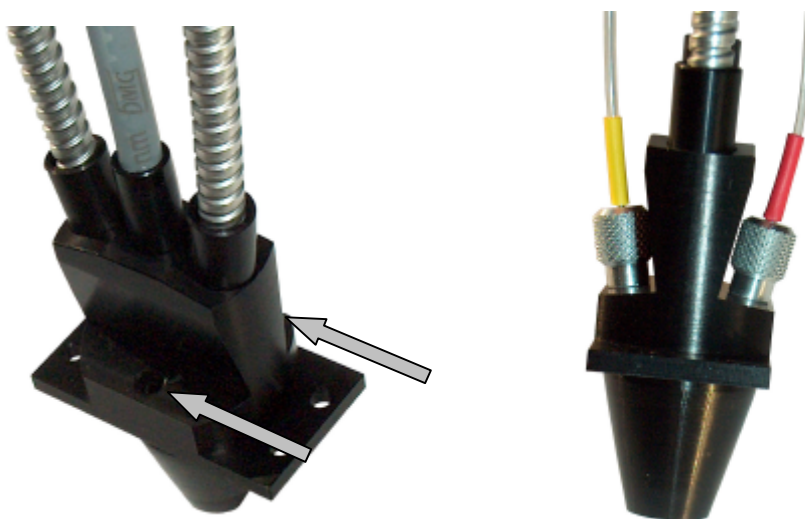


Figure 13: Holes to position reagent needles

4.2.2 Fluorescence Intensity Optics

The fluorescence intensity and time-resolved fluorescence light guides are liquid-filled and should be connected to the excitation and emission positioning wheels.

To position the measurement head with the holders, see section 4.2 Installation and changing of light guides.

Regarding reagent injection: be careful when you position the needles in the measurement head (see figure 15), to avoid damage to the reagent needles as well as the optics. The optional reagent injectors need to be available.



Figure 14: Fluorescence intensity light guide

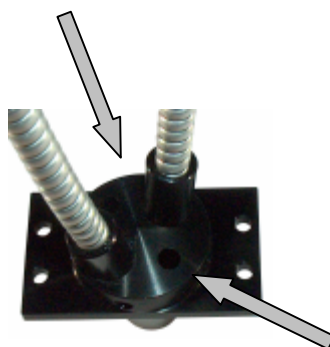


Figure 15: Holes to position reagent needles

4.2.3 Absorbance Optics

The light guide for absorbance mode is available with one light guide in the 'optimized absorbance optic' (figure 16) or in combination with fluorescence intensity in the 'combination optics' (see 4.2.1).

The 'optimized absorbance optic' connects to the red-marked excitation-positioning wheel.

For injection in absorbance mode see the combination optic.



Figure 16: optimized absorbance optic

4.2.4 Luminescence Optics

The luminescence optic has one light guide, which is silver in colour. The light guide connects to the emission side. To position the measurement head with the holders, see section 4.2 Installation and changing of light guides.

There are two dedicated luminescence versions: one covering up to 96-well formats, with a light guide that is 3 mm in diameter and one that covers plate formats up to 384-well and this is 2 mm in diameter.



Figure 17: Luminescence optics

Regarding reagent injection: be careful when you position the needles in the measurement head (see figure 18), to avoid damage to the reagent needles as well as the optics. The optional reagent injectors need to be available

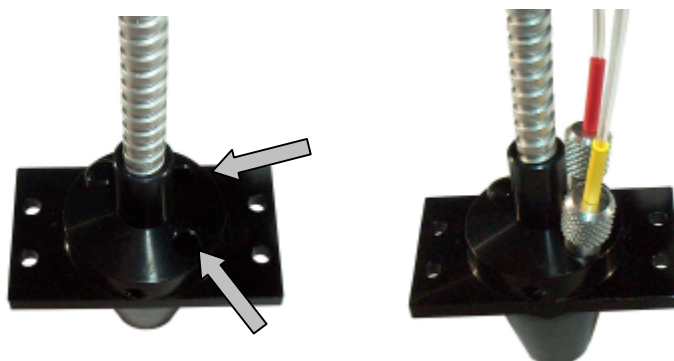


Figure 18: Holes to position reagent needles

For reading the luminescence, the luminescence option needs to be present.

4.2.5 Fluorescence Polarization Optics

The light guides for the fluorescence polarization form a triangle at the base of the optic. When positioning the measurement head, make sure the base of the optics is oriented so that it points towards the front of the instrument (figure 19).

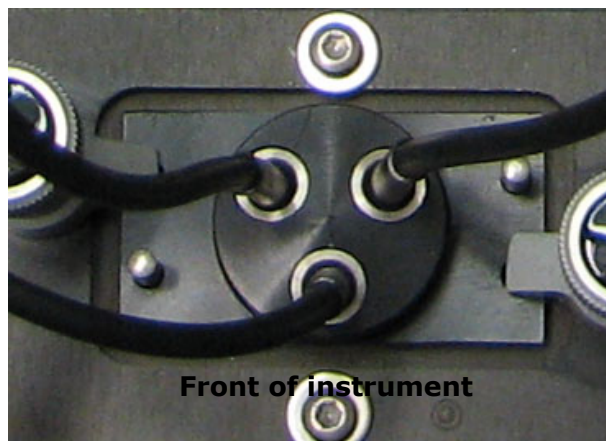


Figure 19: Top view: the fluorescence polarization optics "points" towards the front of instrument.

Position the light guides as follows: right light guide into excitation position, left light guide into the upper emission position (PMT 1) and the center light guide connects to the lower emission position (PMT 2).

Only the POLARstar OPTIMA can measure fluorescence polarization. It is possible to upgrade the FLUOstar OPTIMA to a POLARstar OPTIMA.

Regarding filters for fluorescence polarization, see section 4.4.3.



Figure 20: fluorescence polarization measurement head

4.2.6 Dual Emission Optics

The dual emission optic is designed for assays in which you excite at one wavelength and measure two emission wavelengths simultaneously. This is only possible in the POLARstar OPTIMA because this action takes two PMT's. The dual emission optic looks like the polarization optics, but are not capable of polarization. The optic is installed in the same way as the polarization optic (figure 20).

4.2.7 High Density Optics

The high-density optic has 1 excitation light guide surrounded by 6 emission light guides. This optic is designed to minimize cross talk in plate formats such as 384- and 1536-well plates.

Insert the black mounting piece into the measurement head socket as described in section 4.2 Installation and changing of light guides.

The single black light guide connects to the yellow-marked position of the excitation wheel. The six-bundled optic is inserted into the blue-marked position of the emission wheel.

Compared to 96-well plates, the 1536-well plates have a low profile. To be able to read this format the "1536 fluorescence option" is needed.

Injection is not possible in 1536 well plate formats.



Figure 21: High density optics

4.2.8 Bottom Optics

The bottom optics are used to measure fluorescence, luminescence and absorbance. The bottom optics enter the reagent box on the left side and are connected to the left position of the excitation and emission wheels.

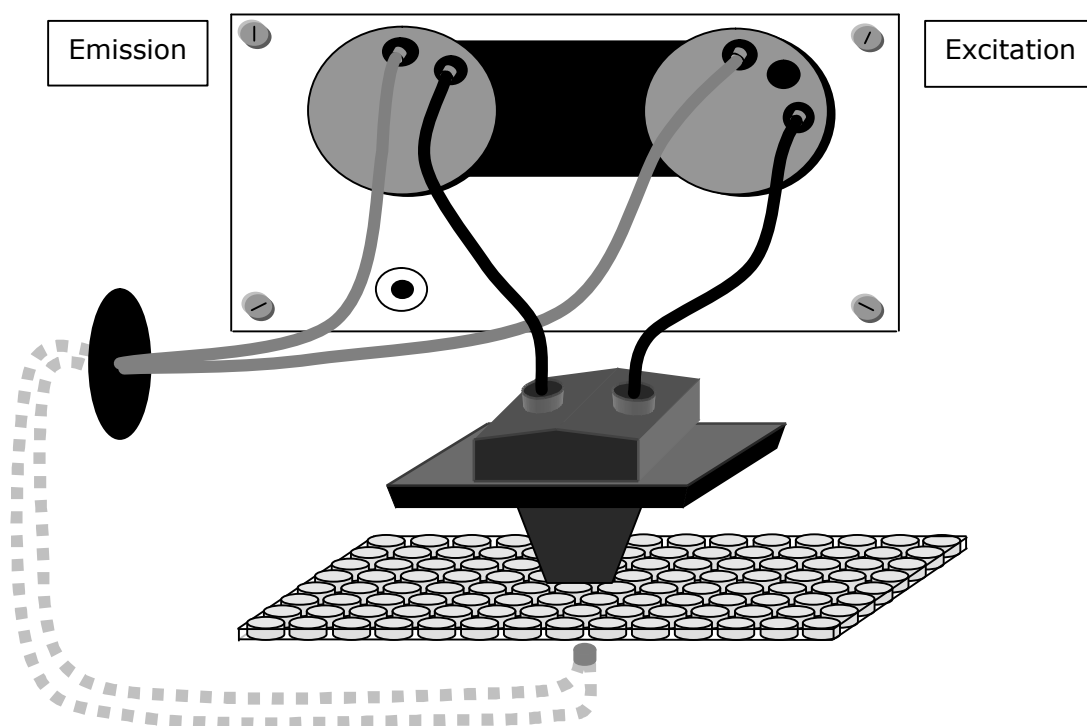


Figure 22: Principle drawing of bottom reading of a 96 well plate

4.3 384-Well Injection Head

A special injection head is required in order to inject into 384-well plates. The injection head is installed similarly to an optic (see section 4.2 Installation and changing of light guides).

This head allows injection from one pump into 384 well formats. The readings must be taken from the bottom. Therefore, both excitation and emission positioning wheels must be switched to the bottom optics. Place the injection needle from the desired pump into the injection head.

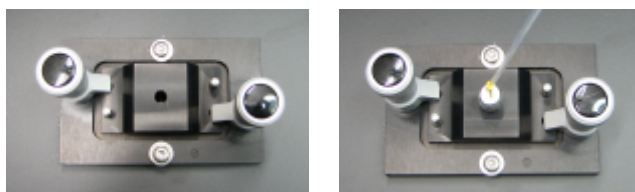


Figure 23: 384-well injection head, bottom reading

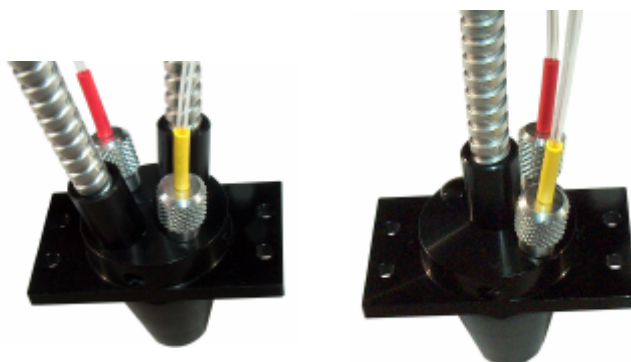


Figure 24: Injection in 384 well formats, top reading

There are 384-well injection optimized optics with the light guides turned in an angle that makes space for the injection to take place.

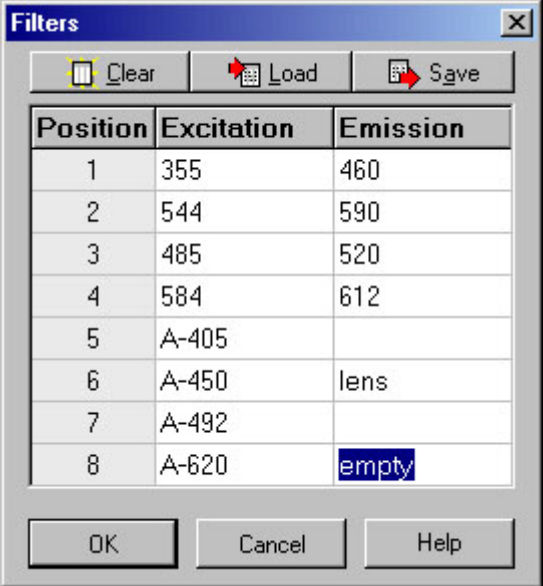
Note: Please be careful when positioning the needle.

4.4 Filters

In the FLUOstar Optima, 4 excitation and 4 emission filters are factory installed, in the POLARstar OPTIMA, 4 excitation and 5 emission filters are factory installed. (Filter selection varies with instrument configuration. If your unit is equipped with luminescence, then a lens will be installed. The absorbance option will also add filters.)

The position of the factory-installed filters can be found in the shipment information that follows the instrument.

After installation of the software, the filter must be typed in the filter table (figure 25). In the Control software, the filter table can be reached by choosing 'Setup | Filter' (see the software manual for additional information).



Position	Excitation	Emission
1	355	460
2	544	590
3	485	520
4	584	612
5	A-405	
6	A-450	lens
7	A-492	
8	A-620	empty

Figure 25: Filter table with example of entered values

In the control software you have to type in the filters that are installed ('Setup | Filters'). It is necessary to label the empty position in the emission wheel with a name e.g. "empty".

4.4.1 Filter Change and Installation

All BMG filters have an arrow printed on the side to indicate the direction in which they should be installed. The arrow should point in the same direction as the light. If the filter is being installed in the excitation wheel, the arrow should point outwards toward the front of the unit. If the filter is to be placed in the emission wheel, the arrow should face inward toward the back of the unit.

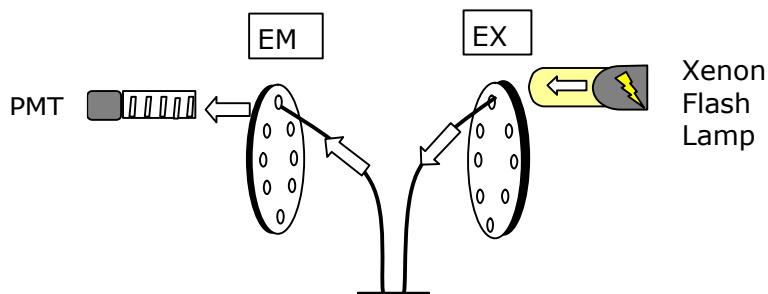


Figure 26: Direction of the light

The excitation filters and the emission filters are located in their respective filter wheels behind the filter wheel cover (figure 27 to figure 29). To access the filters, first remove the light guides. The filter wheel cover can then be removed by loosening the 4 thumbscrews (figure 28).



Figure 27: Filter change/Filter installation:
First remove the optic (valid for all readers)



Figure 28: Filter change/Filter installation:
Loosen the 4 screws

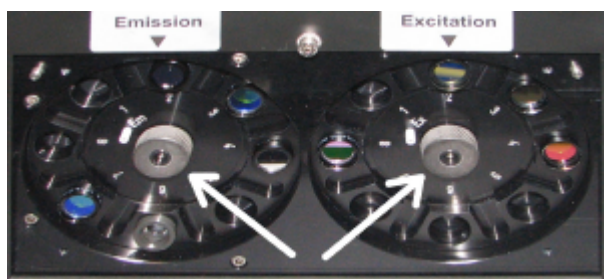


Figure 29: Filter change/Filter installation:
Loosen the filter wheel by loosening the nut in the middle (see text for detail description).

The filter wheel itself (figure 30) can be removed by loosening the large retaining nut in the center (figure 29). Put a finger on the filter wheel (careful not to touch any filters) and turn the nut counter clockwise. The filter wheel can be pulled straight out of the housing.

The filter positions are numbered 1 to 8. When installing new filters note the positions and enter the information in the filter table.

Once the filter is in place, it should be fixed with the Allen key and a small filter screw. Place the small screw in the hole on the side of the wheel and turn it only until it is snug (not too tight).

Note: Do not place a screw in a position that is not equipped with a filter; the screw may slowly loosen and fall out and cause damage to the filter wheel mechanism.



Carefully tighten (untighten) the small screw that holds the filter

Figure 30: Filter wheel

Next to the axle in the center of the housing is a small positioning pin. This pin must fit into one of the holes on the back of the filter wheel. Replace the filter wheel on the axle and push it in position (the axle should stick out 3mm). Turning the filter wheel quickly might help in position it correctly. Place the filter wheel nut on the axle and hand tighten it until it is snug. Spin the wheel again. The filter wheel should move freely and move without vibration. If the wheel seems to drag or wobble, tighten the nut more or remove and reposition the wheel.

Replace the cover and reconnect the light guides.



If the instrument makes a grinding sound it is very likely that the large filter wheel nut in the middle should be tightened better or that a filter screw is loose.

4.4.2 Fluorescence Filters

The fluorescence filters have a bandwidth that varies dependant upon type of fluorescence filter. Filters optimized for specific fluorophores can vary in the bandwidth from around 10 nm to around 30 to 40 nm (measured at ½ height). BMG-10 filters are symmetrical filters that in ½ height have a bandwidth of 10 nm and hence can be used in both in the excitation and the emission position.

4.4.3 Fluorescence Polarization Filters

The excitation filters can go in any available position in the excitation wheel. The emission wheel has two filters of the same wavelength for each channel. They should be positioned 180° from each other (for example position 3 and position 7 for the 520 filter). The filter configuration can be entered in 'Setup | Filters'.

4.4.4 Dual Emission Filters

As for fluorescence polarization, the excitation filter can be placed in any position. The emission filters to be used in dual emission measurement should be positioned 180° from each other (emission position 1 pairs with position 5, position 2 pairs with position 6, etc).

4.4.5 Absorbance Filters

Place the filter (either a BMG-10 or an "Abs" filter) that matches the wavelength of choice in any excitation position. For emission, there are two possibilities:

Empty: Define a filter position as empty so that all the light from the reaction will pass directly through to the PMT ("empty" needs to be typed in).

Use of a filter: If you know the wavelength of the emitting light, you may choose to use a filter to block any stray light. The filter can be used in any emission filter position.

4.4.6 Luminescence Filters

Like for fluorescence filters, the luminescence filters are designed to transmit as much light as possible. The bandwidth varies depending upon the type of assay (filters are optimized to return the best result) and can vary in the bandwidth from around 10 nm and up to around 100 nm (measured at ½ height).

4.5 Spacers

The FLUOstar, POLARstar and LUMIstar OPTIMA are designed for most plate formats. The height of some microplates exceeds the space allowed under the optics. The minimum space between the optics and microplate should be 1.5 mm. With 6, 24, 48-well plate formats, it will be necessary to raise the optics using the spacers provided in the service box.

The spacers are metal rectangular pieces with a hole in the center. Each spacer is 2 mm in height. They fit between the measurement head and the optics. The number of spacers used depends on how high the optics needs to be elevated.

Determination of the number of spacers:

If the height of the microplate exceeds the height of the left border of the plate carrier, (see figure 31) spacers need to be installed under the measurement head (see figure 32). There should be enough spacers so that the height of the left side of the plate carrier is slightly higher than the microplate.

3 spacers are needed in this example to make the height of the side of the plate carrier higher than the microplate

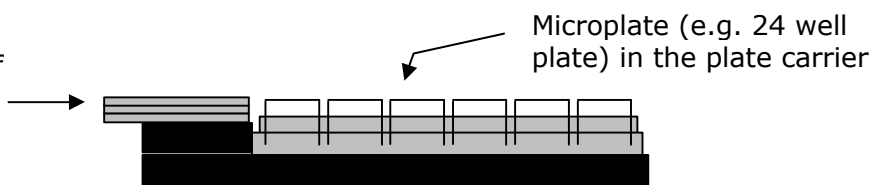


Figure 31: Front view of the plate carrier

Installation of spacers:

If you install spacers, then first remove the injection needles (if any) from the optics and then remove the optics.

Install the appropriate number of spacers, using the positioning pins as a guide.

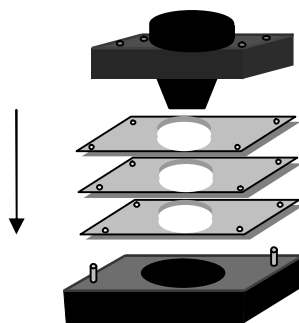


Figure 32: Example of spacers between measurement head and bottom of reagent box

As a cross check (to ensure that the microplate can pass under the optics), push the plate carrier manually into the instrument and slowly move it towards the optics. If there is approximately 1.5 to 2 mm of space between the optics and microplate, then enough spacers were installed.

4.6 Reagent Injectors

The FLUOstar, POLARstar as well as the LUMIstar OPTIMA can all be equipped with up to 2 reagent injectors (figure 33).

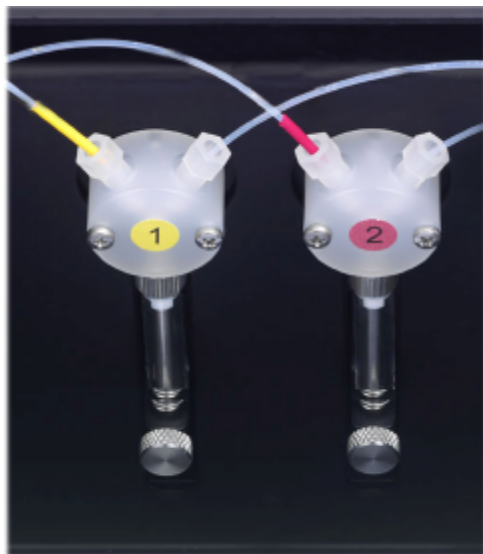


Figure 33: Reagent injectors

When the reagent injector(s) are not in use, the needle(s) can be placed in the needle holder (figure 8).

The reagent needles are made of stainless steel, the tubings and valve housing are made of Teflon and Kel-F, and the syringe barrel is made of glass. All materials are among the most chemical resistant materials available.

Of course, the needle tip plays a role regarding the pumps' accuracy. You should always treat the needles with care. That is, e.g. be careful when positioning the needles in the measurement head or in the needle holder.

For obtaining optimal performance of the reagent injectors, please see the following chapter.

4.6.1 Use and Maintenance of the Reagent Injectors

To remove cellular debris and viscous solutions from the syringe barrel:

Take off the syringe barrel and rinse it with distilled water. It may be useful to use the wire syringe cleaners (can be found in the service box) to scrape particles off the walls.

In order to obtain optimal performance from the reagent injectors, it is recommended to follow these guidelines in the use of the reagent injectors:

- **Do not use the syringes more than two cycles without liquid.**
- **After each use, thoroughly flush the syringes with distilled water.**
- **If the plunger is removed from the syringe barrel, it should be wiped with ethanol before replacing.**
- **Syringes should be cleaned each week using one of the following procedures:**
 - **Cleaning with weak detergent or 10% bleach**
 1. Fill the syringe with a weak detergent or 10% bleach solution
 2. Leave the solution in the syringe for 30 minutes
 3. Flush the syringe a minimum of 10 times with distilled water
 - **Cleaning with acid / base** (best procedure if cells are used in the syringe)
 1. Fill the syringe with 0.1M NaOH and leave it in the syringe for 10 minutes.
 2. Flush the syringe with distilled water.
 3. Fill the syringe with 0.1M HCl, and leave the solution in the syringe for 10 minutes.
 4. Flush the syringe a minimum of 10 times with distilled water.

5 Instrument Disinfection

Please follow all instructions carefully for a successful disinfection of the FLUOstar, POLARstar and LUMIstar OPTIMA.

All parts of the instrument, which have the possibility of contacting patient sera or positive samples, have to be handled as if they are hazardous. For this reason, it is recommended that gloves be worn while maintaining or working with the instrument.

It is very important that the instrument is thoroughly disinfected before maintenance or before removing the instrument from the laboratory. Be sure that the instrument is disinfected before you send it to your distributor or to the producer. For safety reasons, you have to fill out the Disinfection Certificate, or the instrument may not be accepted by the service centre or by customs authorities.

If the laboratory has no experience disinfecting the instrument, use the following solutions:

Formaldehyde solution	10%
Alcohol	70%

(Please ensure that the national regulations for the handling of formaldehyde are observed).

Authorized personnel wearing disposable gloves and protective clothing should only perform the disinfection procedure. The location should be well ventilated. Please note that formaldehyde may have influence on measurement results.

Disinfection Steps

1. Disconnect the instrument from the main power supply.
2. Remove the RS232 cable from the connector.
3. Clean all outside surfaces of the instrument carefully with cotton wool, which has been soaked in formaldehyde solution.
4. Place the instrument in a large plastic bag along with the cotton wool that has been soaked in formaldehyde. Ensure that the wool does not touch the instrument.
5. Close and seal the bag.
6. Keep the instrument in the plastic bag for at least 24 hours.
7. After the disinfection time has lapsed, remove the instrument from the plastic bag and clean all outside surfaces of the instrument with cotton wool that has been soaked in alcohol solution.
8. Repeat the procedure for disinfection on any accessories, which will be returned with the instrument.
9. Complete the Disinfection Certificate.

Disinfection Certification

This instrument and its inventory have never been in contact with any dangerous biological material, or if so, the instrument and its inventory have been disinfected according to the instructions of the operating manual of instrument.

Name: _____

Company: _____

Date, Signature: _____

OPTIMA

Software User Manual

Version 2.00

This manual was designed to guide OPTIMA users through the software features.

Although these instructions were carefully written and checked, we cannot accept responsibility for problems encountered when using this manual. Suggestions for improving this manual will be gratefully accepted.

BMG LABTECH reserves the right to change or update this manual at any time. The Revision-Number is stated at the bottom of every page.

Headquarters**Germany**

BMG LABTECH GmbH
Hanns-Martin-Schleyer Str. 10
77656 Offenburg
Phone: +49 781 96968-0
Fax: +49 781 96968-67
germany@bmglabtech.com

Subsidiaries**Australia**

BMG LABTECH Pty. Ltd.
P.O. Box 469
Mount Eliza, Victoria 3930
Phone: +61 3 5973 4744
Fax: +61 3 5973 4711
australia@bmglabtech.com

China

BMG LABTECH Co. Ltd.
RM2-201, Hua Fu Jing Yuan
NO11. An Ding Men Wai Avenue
Dongcheng District
Beijing, P.R.China 100011
Phone: +86 10 64240632
Fax: +86 10 64258679
china@bmglabtech.com

France

BMG LABTECH SARL
7, Rue Roland Martin
94500 Champigny s/ Marne
Phone: +33 1 48 86 20 20
Fax: +33 1 48 86 47 07
france@bmglabtech.com

Japan

BMG LABTECH Japan Ltd.
2F TS-1 Building, 1-6-2, Shimo-cho
Omiya-ku, 330-0844
Saitama City, Japan
Phone: +81 48 647 7217
Fax: +81 48 647 7218
japan@bmglabtech.com

United Kingdom

BMG LABTECH Ltd.
P.O. Box 73
Aylesbury, HP 20 2 QJ
United Kingdom
Phone: +44 1296 33 66 50
Fax: +44 1296 33 66 51
uksales@bmglabtech.com

USA

BMG LABTECH Inc.
2415 Presidential Dr.,
Bldg. 204, Suite 118
Durham, NC 27703
Phone: +1 919 806 1735
Fax: +1 919 806 8526
usa@bmglabtech.com

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1 Installation

1.1 System Requirements

- Computer with Pentium CPU (we recommend Intel Pentium 4 or higher with at least 2 GHz clock rate)
- Minimum 128 MB RAM (we recommend 256 MB or higher, especially when you are using Windows 2000 or XP)
- One free serial communication port
- 30 MB free hard disk memory for software installation
- Microsoft Windows 98 / ME or Windows NT / 2000 / XP
- Excel 97 / 2000 / 2002 / XP for data reduction

1.2 A version of OPTIMA or FLUOstar OPTIMA Software is already installed

Please go to section 1.3: Software Installation if there is not a previously installed version of the OPTIMA or FLUOstar OPTIMA software. The OPTIMA software is the successor of the FLUOstar OPTIMA software.

Before installing a new OPTIMA software version over an existing version (of OPTIMA or FLUOstar OPTIMA software), it is recommended to export (or to backup) your test protocols and microplate definitions (if any changes are made) and to export your measurement data. You will find the export function in the test selection window ('Test Setup | Test Protocol' for test protocols (use 'Setup | Microplates' for microplate definitions). Select all the definitions you would like to export and click the '**Export**' button. To export measurement data of already performed test runs, start the evaluation part of the software. The export function is in the OPTIMA or FLUOstar OPTIMA pull-down menu at the top of the Excel window ('**Export Test Runs**').

The database format for the **user data base** and for the **measurement results data base** has been changed since version 1.20-0. If you perform the installation using the Smart Update mode (or if you use the Custom Installation mode without selecting the groups "User Data Base" and "Measurement Data"), the format of the existing data bases will be converted automatically. Please use the Custom Installation mode if you want to replace the installed data bases.



The format for the **microplate definitions** has changed with version **2.00**. If you install the software using the Smart Update mode (see below), the microplate data base will be replaced. If you want to keep existing microplate definitions, please use a custom installation and deselect the group "Microplate Definitions". The data base format will then automatically be converted.

Notes: The installation program will create a backup of the existing test protocols, the user database, the filter table, microplate definitions and measurement data when the data base format has been converted or files have been replaced during the upgrade procedure. At the end of the installation procedure there will be a window telling you where to find the backup files. As long as the database format has not been changed (see above) it is possible to copy the backup files back to their original directory (in case you want restore previous data). But if there has been a format change, you can only use the backup files after re-installing the old program version.

For complete removal of an old software version see chapter 1.4. De-installation of a former version is generally not necessary, you can install the new version directly over an existing version.

1.3 Software Installation

The software needs to be installed in the following order:

1. Excel 2. OPTIMA - Control 3. OPTIMA - Evaluation

Please follow the next set of instructions carefully to ensure proper installation of the software.

1.3.1 Excel Installation

The Excel software should be installed prior to the OPTIMA software. There are certain components that must be included in the installation. If Excel 97, Excel 2000 or Excel 2002 / XP has not been previously installed, follow the instructions below for complete installation. If Excel 97 is already installed, follow the instructions below to ensure the necessary components have been installed.

- **Complete installation of Excel 97 / 2000 / 2002 / XP**

- Start the Office 97 / 2000 / 2002 / XP or Excel 97 / 2000 / 2002 / XP installation program.
- Choose Custom or Complete Installation - this option installs all Excel components.
Be sure that all components are ticked (you can be sure of this if click on select all).
- The Excel wizard will ask for the directory. The default directory is ~:\Program Files\Microsoft Office.
If an older version of Excel already exists, a new directory should be created to prevent a conflict between the two Excel versions.
- Click on 'OK' to install.

- **If Excel 97 already exists on the PC**

- Start the Office or Excel installation program.
- Choose add/remove components.
- In the section Microsoft Excel Program files select 'Change option' | 'Add-ins' | 'Change option' | select: 'Solver and analysis tool pack' | press 'OK'.
- Than choose: 'Data Access' | 'Change Options' | select 'Data Access Objects for Visual Basic' | select: 'Data Base Driver' | 'Change Option' | select: 'All' | press 'OK'.

A standard installation of Excel 2000 / 2002 / XP should contain all necessary parts (This might not be true for small business editions.).

Note: Excel installation should be prior to the OPTIMA software installation or run time errors can occur. If it is ever necessary to reinstall Excel it can be necessary to reinstall the OPTIMA software. Therefore, important data and definitions should be saved on a diskette or another directory prior to reinstallation.

1.3.2 OPTIMA Software Installation

There are two parts to the OPTIMA software: the **Control** part and the **Evaluation** part. Control setup installs the software necessary for configuring the instrument, for setting up test parameters and for executing measurement procedures. Evaluation setup installs the data reduction program (Excel macros). In a few words: the control part is used to get the results and the evaluation part to look at them.

Place the CD into the CD ROM drive. This should start the program automatically, but if CD auto start is disabled, then execute the program '**Start**' from the CD drive. An installation directory will appear:



From this install menu you can start the installation of the control and the evaluation part of the OPTIMA program, you can install the optional Stacker Control software or you can read the manuals online.

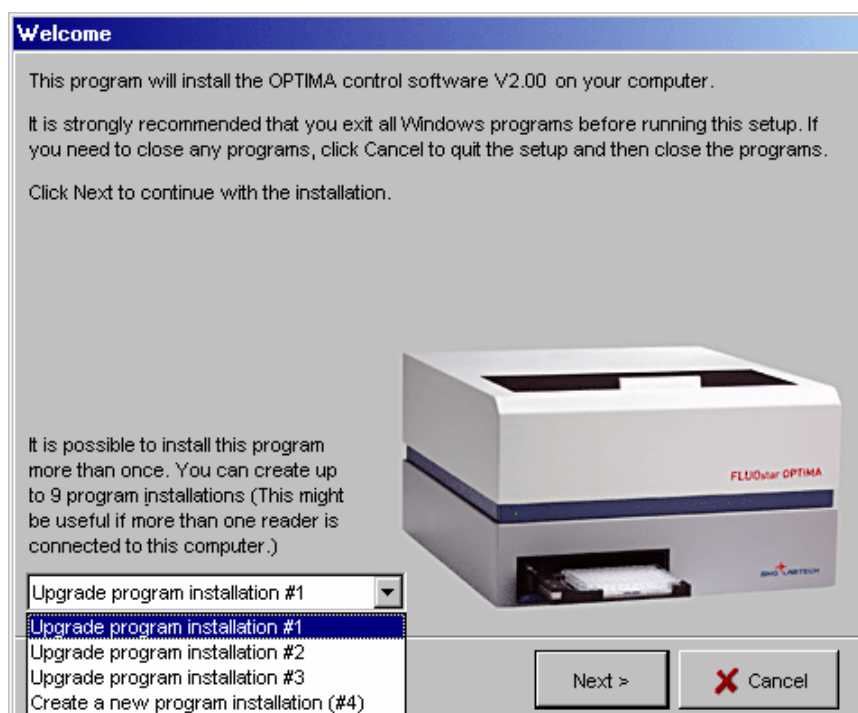
Note: If you are using Windows NT, Windows 2000 or Windows XP, it is necessary that the installation is performed by a user with administrative rights.

1.3.3 Control Part - Installation

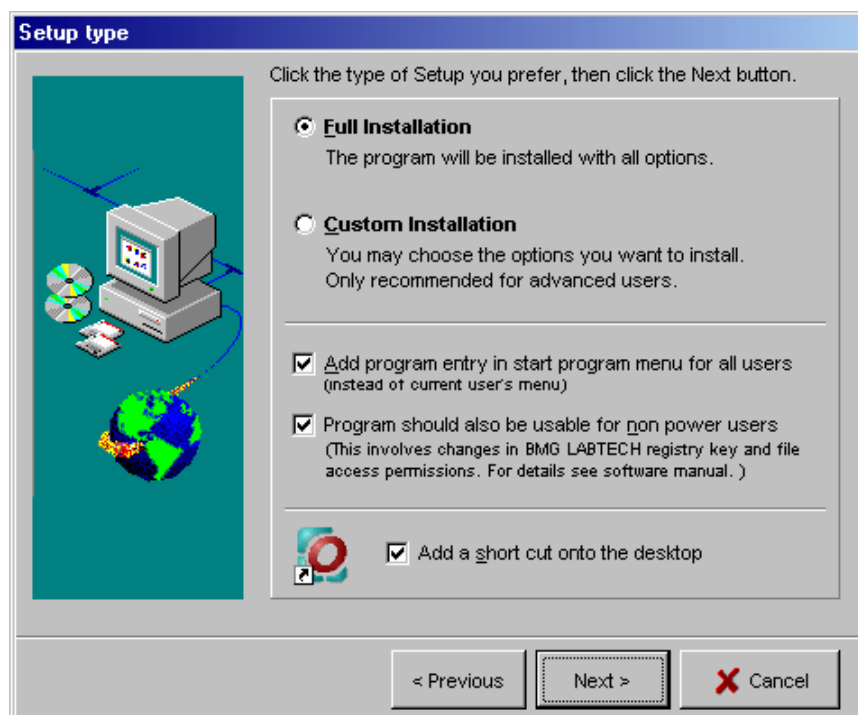
Click on '**Install Control Part**' and follow the instructions from the installation wizard.

It is possible to install this program more than once (see chapter 12 Using Multiple Installations). You can create up to 9 program installations. This will be useful if more than one reader is connected to one computer.

If the OPTIMA software is already installed on your computer, you can choose whether you want to upgrade/repair an existing installation or whether you want to create a new installation. If you already have installed an older OPTIMA / FLUOstar OPTIMA software version please perform an upgrade of the existing (first) installation first, otherwise create installation number one by performing a standard full installation (see below). To create a new installation (instance) please start the control part installation again. In the welcome screen you should now select 'Create a new program installation (#2)'.



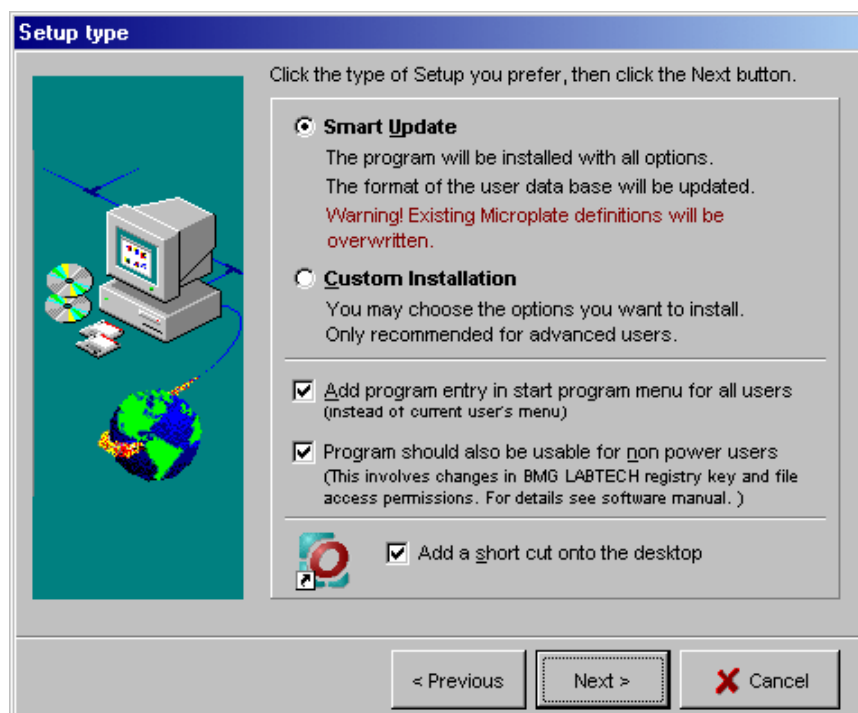
Using the next screen you can select the setup type:



If this is the first installation of the OPTIMA or FLUOstar OPTIMA software, you can choose either **Full Installation** or **Custom Installation**:

- Full installation will install the programs with all options. This is the installation option recommended for most users.
- Custom installation allows you to choose the options you want to install and is recommended for advanced users.

When you update from a previous version, you might decide between **Smart Update** and **Custom Installation**:



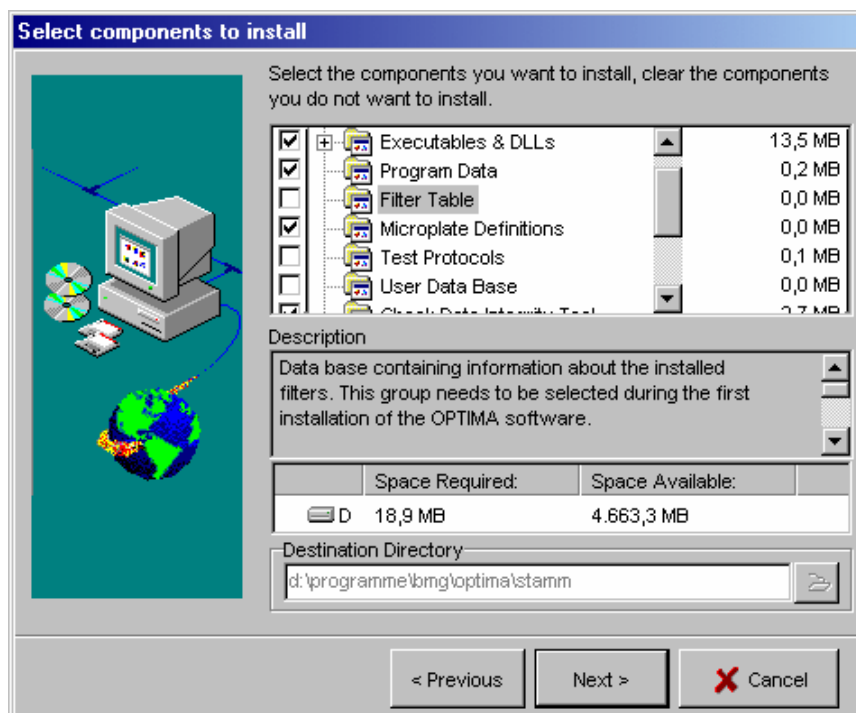
Using the Smart Update mode none of the existing user, filter, test protocol or measurement result data bases will be overwritten. If there has been a data base format change between the installed and the new version, the existing data bases will be automatically converted and a backup of the old files will be created. By default, only the microplate data base will be replaced, as BMG LABTECH might have added new microplate types. Please use the Custom Installation mode and deselect the 'Microplate Definitions' group, if you want to keep the existing microplate definitions. You should also use the Custom Installation mode if you want to replace any of the other data bases.

Using Windows NT / 2000 / XP you can choose whether you want to get the **start menu entry in the section for all users** or in the section for the current user only.

You may also decide whether the **program should be usable also for non-power users**. If you do not check this box, the program might not be usable for non-power users (depending on the operating system or the current setting of the system policies on your computer: beginning with Windows 2000 Microsoft changed the default access permissions for some registry keys and newly installed program files in a way that a non-power user is, by default, not able to use nearly any newly installed program other than some Microsoft certified programs). When you use this option, the access permission is changed for the BMG LABTECH registry keys and files to enable program usage for all users. For more information about access rights see chapter 13.5.

Custom Installation

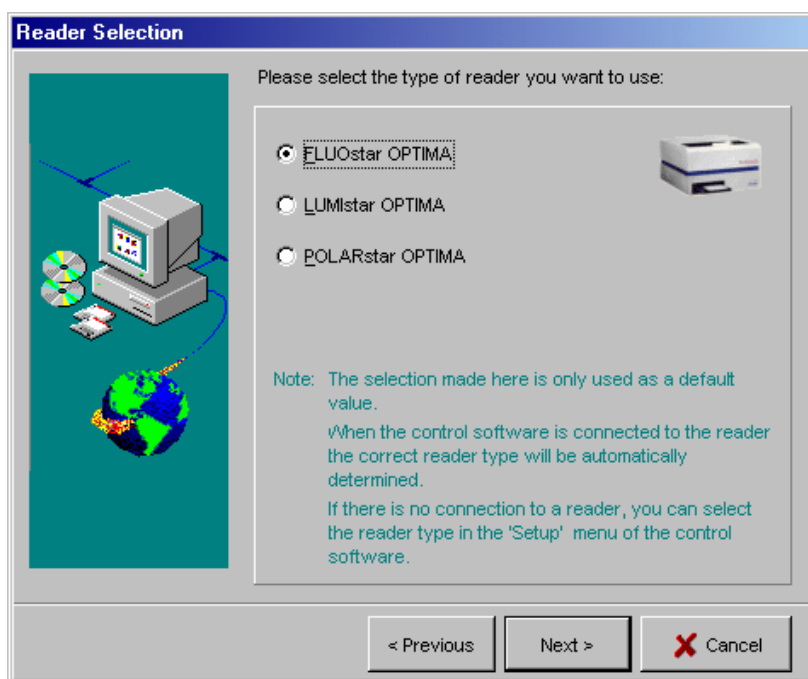
If you use the custom installation mode, the next window displays a list of the components that can be installed.



By selecting an item with a single mouse click, you can obtain a description of the individual software component. You can then decide if this feature needs to be installed. If the data base format of a group, which has not been selected, has been changed, it will automatically be updated.

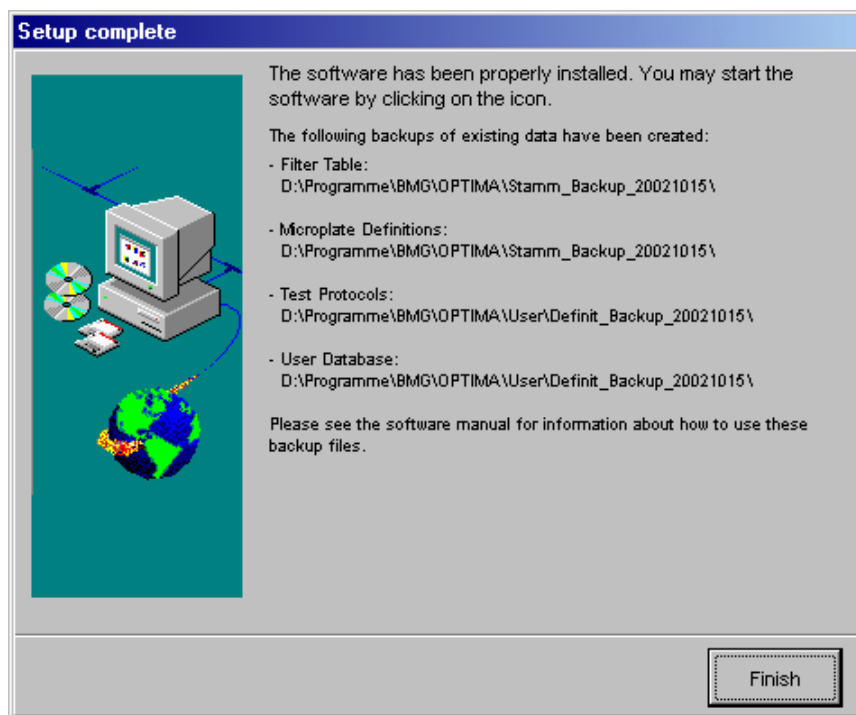
Reader Type Pre-Selection

During the installation you will be asked for the type of reader you want to use. The OPTIMA software can be used together with FLUOstar OPTIMA, LUMIstar OPTIMA and POLARstar OPTIMA readers. Depending on the reader type test protocols using different measurement methods can be defined and executed. The selection you make here is only used as a pre-selection for the software. If the reader is connected and switched on the control part of the software will automatically recognize the correct reader type.



Backup files

The installation program will create a backup of the existing test protocols, the user database, the filter table, microplate definitions and measurement data when performing an upgrade if files have been replaced or if the data base format has been changed. At the end of the installation procedure there will be a window telling you where to find the backup files.

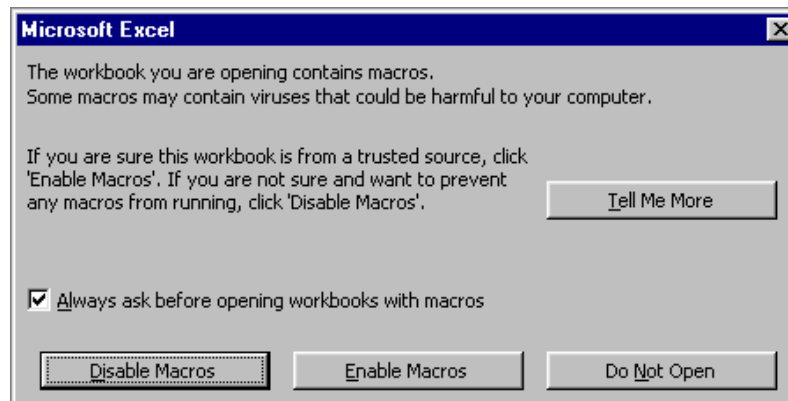


As long as the database format has not been changed (see chapter 1.2) it is possible to copy the backup files back to their original directory (in case you want restore previous data). But if there has been a format change, you can only use the backup files after re-installing the old program version.

1.3.4 Evaluation Part - Installation

After installation of the control software, the main menu will reappear. Click on 'Install Evaluation Part' and follow the instructions of the installation wizard to install the data reduction software (the installation procedure and options are similar to the control part installation, see above).

When the evaluation software in Excel is opened for the first time, the following screen might appear:



Deactivate the question 'Always ask before opening the workbooks with macros' by clicking on the box in front of the above statement. Then click on '**Enable Macros**'. The macros are safe for the computer.

For further details of macro security and the certification of macros, see chapter 10.12 Microsoft Office Macro Security .



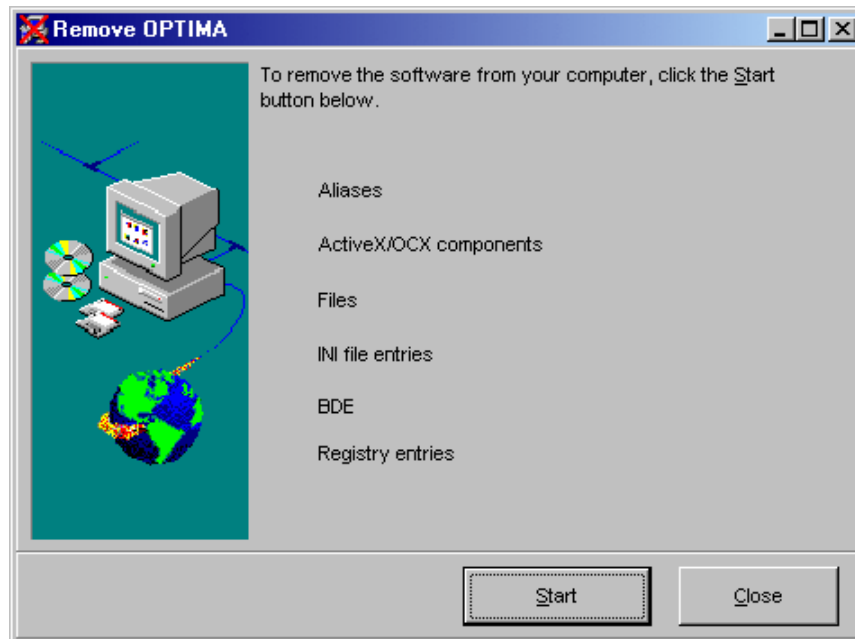
There are several bugs associated with Microsoft programs that effect the OPTIMA software. If error messages appear when the OPTIMA software is opened for the first time, please refer to chapter 13 of this manual for bug fixes.

1.4 Uninstalling the OPTIMA Software

An uninstall program has been included in the software package which allows you to delete all components of the OPTIMA software and all registry entries belonging to it.

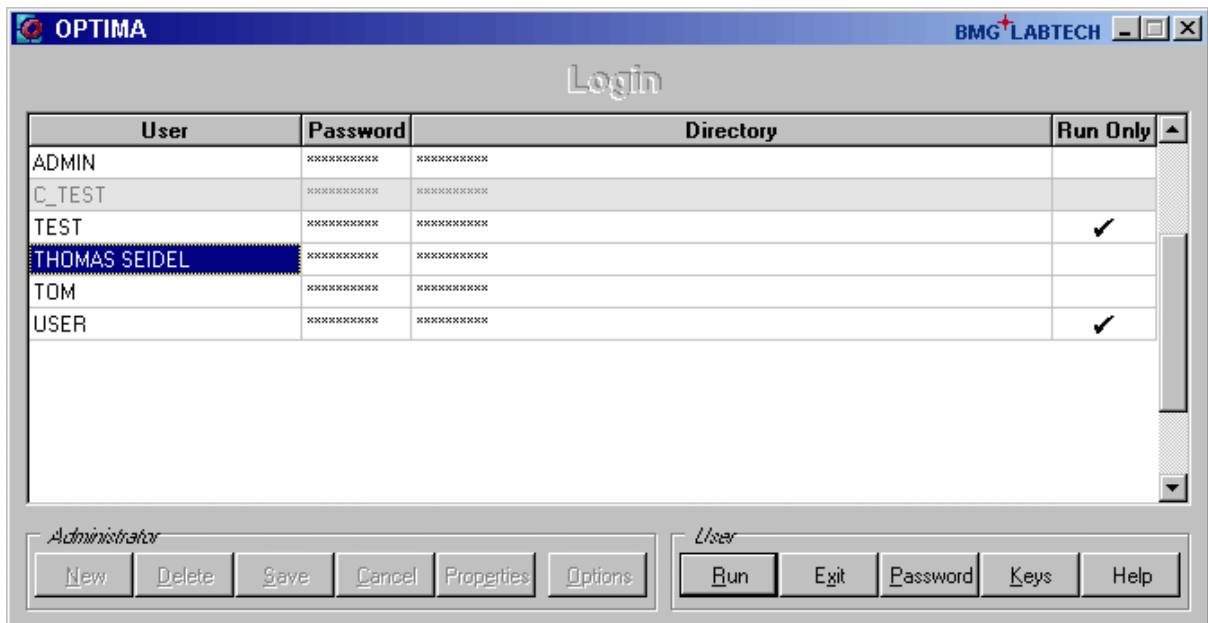
Select 'Start | Settings | Control Panel | Add/Remove Software'.

Select '**OPTIMA**' and click on '**Remove**'. In the '**Remove OPTIMA**' window, click on '**Start**'.



2 Login Screen

When the control or evaluation part of the software is opened, a login screen appears (The Login Screen function can be switched off, see chapter 4.3 Program Configuration). This feature allows more than one user to perform test runs on one PC. The data and test protocols are stored in an individual user folder. The user can also edit test parameters and some evaluation features without the changes applying to all users.



For logging in as the standard user 'USER', by default no password is necessary.

The initial administrator password is 'bmg'. When the password is entered, the **Administrator** buttons will become available and new users can be created or changes for existing user entries can be made (see chapter 2.2 Administrator Functions).

Run Only

If this field is checked, the respective user is only able to start pre-defined test protocols. The user can perform a gain adjustment and enter plate / sample IDs, but can not change or delete an existing test protocol or copy / import protocols or create new protocols. Changing offset or filter values or microplate definitions is also not possible for this type of user.

The Run Only property can only be set / unset by the administrator. When using this option, it is strongly recommended to select Run Only also for the default user 'USER' (or to define a password for this account) and to change the administrator password to something other than 'bmg'.

Note: Instead of setting / unsetting this option every time a protocol change is necessary, you can also create two user entries using the same directory, one of these entries with Run Only option and the other one without this option.

2.1 User Functions

Run

After clicking 'Run', the OPTIMA software will start using the test protocol and measurement results paths of the selected user. For all users other than the default user 'USER', you need to enter the password first.

Exit

Use this button to close the login screen without starting the program itself / without logging on.

Password

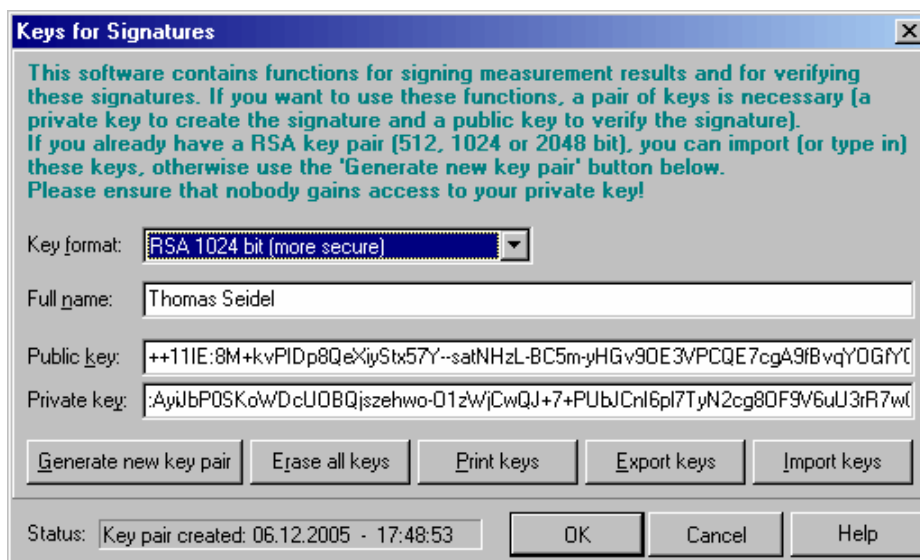
Use this button to change your password. Password restrictions might apply (see chapter 2.3.1 Password Policies and Password Aging).

Keys

Opens the 'Keys for Signatures' dialogue box (see below).

2.1.1 Signature Keys

If you want to digitally sign data records you need to have a pair of RSA keys: a private key for creating a digital signature and a public key for verifying this signature. A key pair can be generated or imported using the 'Keys' dialogue.



To **generate** a new key pair please select between 512, 1024 and 2048 bit key length and enter the **full name** which should appear as part of the signature. The longer the key, the more secure it will be, but creation of the key pairs and signing data records / verifying signatures will last longer. The largest amount of time will be necessary for generating the key pair, signing and verifying will only last a few seconds on a modern computer when using 2048 bit keys and less than a second when using 512 or 1024 bit keys. The keys will be stored inside the user data base. The private key will be encrypted, as everybody who has access to this key could sign in your name! Therefore, **please ensure that nobody gains access to your private key.**

If you already have a RSA key pair, you can **import** or type in these keys. An example of the expected file format is shown below.

When creating or importing a new key pair, existing public keys will not be overwritten. Old public keys will still be stored inside the user data base to be able to verify older signatures. Use the '**Erase all keys**' button if you want to remove keys.

It is possible to **print** out or **export** keys (you can decide whether you want to print/export your private or public key(s) or both).

Example of an exported key file:

```
[CurrentKeys]
Name=Thomas Seidel
Created=2003-07-22 - 16:17:14
Key Format=RSA, 1024 bit
PrivateKey=:QKUrnqlp3zpjFky+3O-M0QssGnRYRRJ9Hb8wEdDyYO8RDlwi5PtWpJnzc ...
PublicKey=++11Ik:cDGk7Ran3sVSxNkhjirqMNLDKXK-7qHoal-8T-vLo5xMYTe2SaRI ...

NumberOfOldPublicKeys=2

[OldKey1]
Name= Thomas Seidel
Created=2003-07-22 - 16:16:54
Key Format=RSA, 512 bit
PublicKey=++11Ik:h+BJtQm6NxBMDbIh4fpGZEs4ujF1GgOrCq+RWjaCygf3GcCee+6S ...

[OldKey2]
Name= Thomas Seidel
Created=2003-07-22 - 16:12:38
Key Format=RSA, 512 bit
PublicKey=++11Ik:p4SIFP6ngflvxM9v+CB5OF00zb3dNeOxcdOwG9OGmDybeUf0lxX6 ...
```

Note: Date and time information in this dialogue is displayed using the short date and long time format, which has been defined using the Windows Control Panel (regional settings).

2.2 Administrator Functions

The buttons of the 'Administrator' group will become available after entering the administrator password.

New

Click on 'New' to create a new user account. Enter the user name, the initial password (you can also leave this field empty and let the user define the password later). Select the directory for the user's data and test protocols. For directory options, click on the far right side under 'Directory' and a button appears [...]. After clicking this button, a user path dialog box appears where you can select the directory for storing test protocols and measurement data (see chapter 2.4 User Directories).

Delete

When an existing user entry needs to be deleted, highlight this entry and click 'Delete'.

Save

After clicking the 'Save' button all changes made will be saved in the user data base.

Cancel

If you click 'Cancel', all changes made after the last save will be discarded.

Properties

After clicking this button, a dialogue showing the properties of the selected user account will appear.

The screenshot shows a Windows-style dialog box titled "Account Properties". It contains the following elements:

- User name:** A text box containing "TOM".
- Account created:** A text box containing "Donnerstag, 21. August 2003 - 10:45:19".
- Password created:** A text box containing "Dienstag, 6. Dezember 2005 - 17:43:25" and a "Reset" button.
- Account Options:** A group box containing three checkboxes:
 - ☐ Account disabled
 - ☐ User can not change password
 - ☐ User must change password at next login
- Number of invalid login attempts:** A text box containing "0" and a "Reset" button.
- Buttons:** "OK", "Cancel", and "Help" buttons at the bottom.

Here you can disable / enable the user account, you can force the user to change his password at the next login or you select an option to not allow the user to change his password. In addition, you can reset the counter for invalid login attempts.

Note: Date and time information in this dialogue window is displayed using the long date and long time format, which has been defined using the Windows Control Panel (regional settings).

Options

After clicking the 'Options' button the 'Administrator Options' dialogue will appear (see chapter 2.3 Administrator Options).

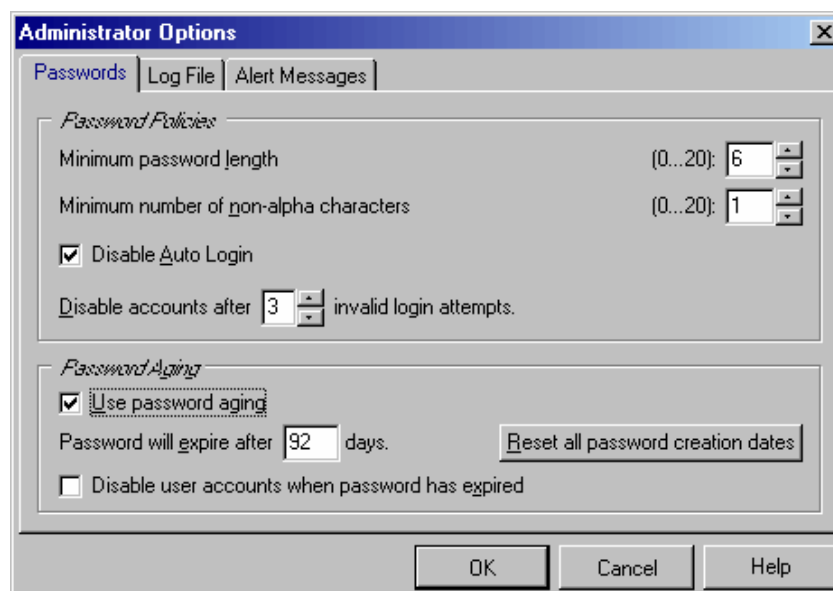
Note: Exporting the user table into a XLS (Excel), text or HTML file is possible after right clicking on the table.

2.3 Administrator Options

The Administrator Options dialogue box can be reached from the login screen.

2.3.1 Password Policies and Password Aging

In the first sheet the Administrator can define the policies for password. In addition it is possible to use password aging (passwords will be valid only for a defined time to force the users to change their passwords from time to time).



Password Policies

Here the required minimum length of passwords and the minimum number of non-alpha characters (numbers, special characters) can be specified. If you use 0 in both fields user accounts without passwords are possible.

The administrator can disable the 'Auto Login' function (see chapter 4.3.1 Program Configuration) here.

If you want an account to be disabled after a certain number of invalid login attempts (login attempts using a wrong password), select the number in the last line of the Password Policies box. If 0 is specified here, accounts will never be disabled.

When this software is used in an FDA 21 CFR part 11 compliant environment, BMG LABTECH recommends using a minimum password length of 6 characters including at least one non-alpha character. In this case, you should disable the auto login function and choose to disable account after 3 invalid login attempts. In addition, you should change the initial administrator password to something other than 'bmg' and you should also define a password for the default user 'USER'. See also chapter **Fehler! Verweisquelle konnte nicht gefunden werden. Fehler! Verweisquelle konnte nicht gefunden werden..**

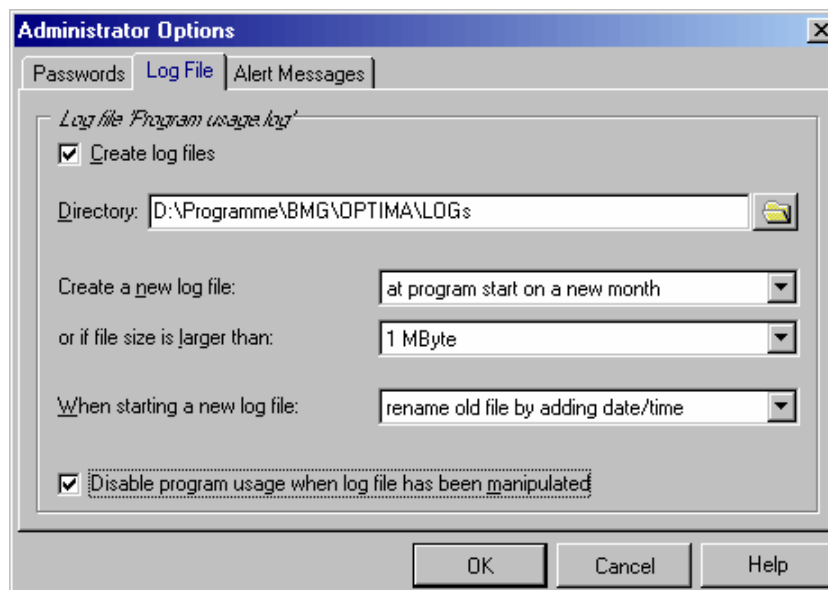
Password Aging

If passwords should be valid for only a certain amount of time, you can use the password aging function. The user will be prompted to change his password several days before the defined time is over. After the defined time, logging on is no longer possible. If the option **'Disable user accounts when password has expired'** has been selected only the administrator can reactivate the account (by deselecting the 'Account disabled' check box in the 'Account Properties' dialogue (see chapter 2.2 Administrator Functions), otherwise the user can reactive his account by changing the password (see chapter 2.1 User Functions). When changing the password aging settings you can use the **'Reset all password creation dates'** button to avoid that some existing passwords are already expired.

When this software is used in an FDA 21 CFR part 11 compliant environment, BMG LABTECH recommends using the password aging function with a setting of 92 days (3 months) or 183 days (half a year). See also chapter **Fehler! Verweisquelle konnte nicht gefunden werden. Fehler! Verweisquelle konnte nicht gefunden werden..**

2.3.2 Program Usage Log File

The BMG LABTECH software can create a log file (named 'Program usage.log') containing information about all important program actions, such as logging on, defining a test protocol, changing offset or filter values, performing a measurement and so on. This function can be switched on in the second sheet of the 'Administrator Options' dialogue box.



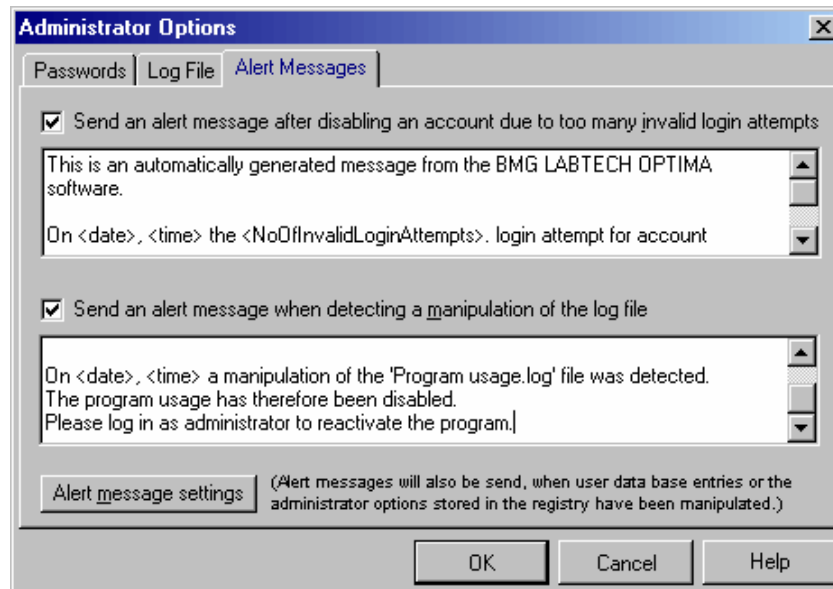
Here you can also specify the directory where this file is to be stored and under which conditions a new file should be started. When starting a new log file, the old file might be renamed or erased. The log file will be protected against manipulation by calculating a cryptographically secure hash value. The integrity of the log file will be checked at every program start. If the log file has been manipulated, the program usage can be disabled. To re-enable program usage the administrator needs to log in. You can also use the 'Check Data Integrity' tool (see chapter 11.3) to check the integrity of the log file.

When this software is used in an FDA 21 CFR part 11 compliant environment BMG LABTECH recommends using the log file function.

Note: There will be an additional run log file (see chapter 13.6 Run Log Window) which contains the communication between reader and computer. This file is intended to deliver background information in case of problems.

2.3.3 Alert Messages

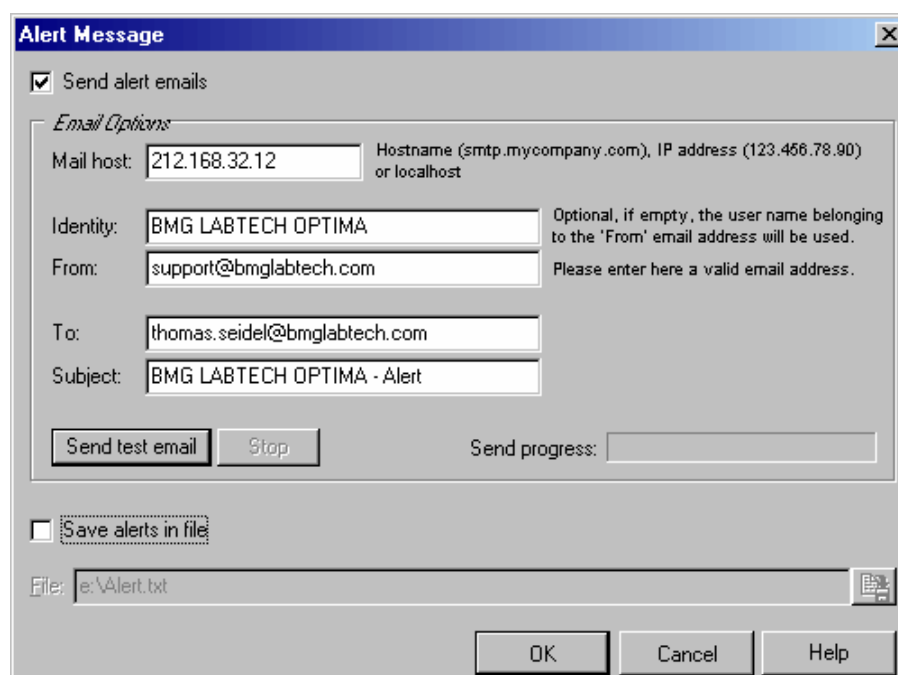
Using the third sheet of the administrator options dialogue, it is possible to define alert messages, which can be sent under certain circumstances via email (e.g. to the administrator) or saved into an alert message file.



You can define a message, which will be sent after disabling an account due to too many invalid login trials and you define a message, which will be sent after detecting a manipulation of the log file.

In addition, alert messages will be sent when user data base entries or the administrator options stored in the registry have been manipulated.

After clicking the 'Alert message settings' button a dialogue will appear, where you can define the way in which to send these messages.



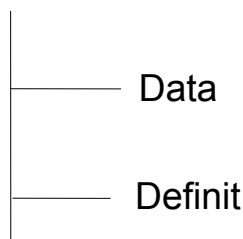
To be able to send emails, the computer needs to be connected to the internet. For the email options, you can use the same settings you are using in your normal email program. If you are not sure about these settings ask your system administrator.

It is also possible to add all alert messages to a text file. You can specify the directory and the file name in the lower part of this dialogue box.

2.4 User Directories

After the first login for a new user, two subdirectories will be automatically created under the defined user directory:

<Userdirectory>



Definit: In this directory test protocols will be stored.

Data: The measurement data is stored in this directory. Here you will find '<number>.dbf' (raw data) files and the 'Measure.db*' files (test runs overview data base table).

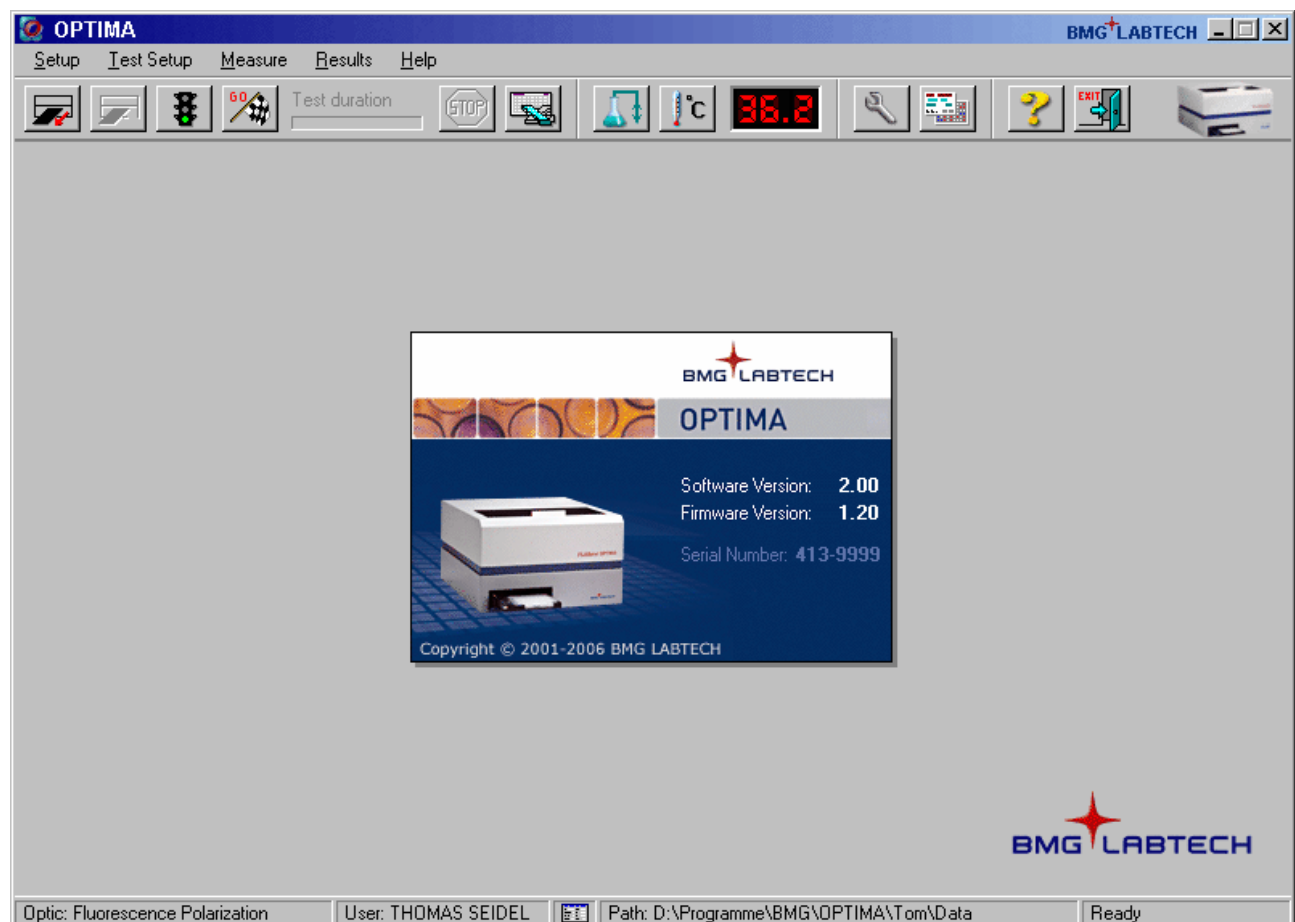
It is possible, at any time, to redirect the path of the data and to store the information in another directory. You can do this in the OPTIMA control software by selecting '**Setup**' and then '**Path**' or '**Login**' (see also Path and Login section 4.4).

3 Control Software Overview



3.1 Main Screen

After logging on, the main screen for the control software opens. An information screen appears in the middle containing details of the software and firmware versions.

Note: Write down the software version and firmware version so it is easily accessible if you need technical support. You can also retrieve this information by selecting '**Help**' and '**About OPTIMA**' in the main menu.



The status bar at the bottom of the main screen shows the currently installed optic (readers with auto optic switching) or the selected reading mode (other readers), the user logged in and the path used for storing the measurement results. You can change these settings by clicking the respective field of the status bar.

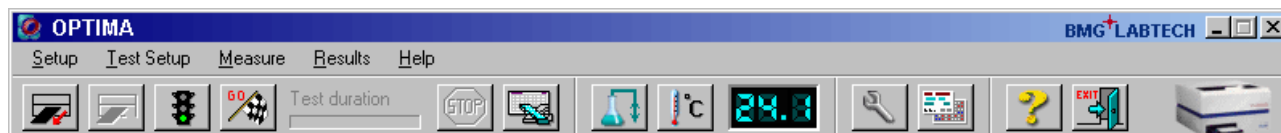
The little icon right of the user field shows whether the user is allowed to change test protocols  or if he is only allowed to run pre defined protocols  ('Run Only' mode).




In the last part of the status bar the current reader status will be shown.











Note: The first section does not exist when using a LUMIstar OPTIMA reader, as this can only measure in luminescence mode.

3.2 Icons and Menu Commands

The main menu is displayed across the top of the screen. Click the item to display the pull-down menu for additional options. You can also use the corresponding buttons of the tool bar that perform the same functions as the main menu items. The instrument must be turned on and connected for certain functions to be available.



<i>Menu command</i>	<i>Icon</i>	<i>Function</i>
Setup ...		Menu for configuring the instrument and program settings. Select the reader configuration (installed optic or measurement method), filters, change offsets values and select a directory for measurement data.
Setup Program Configuration	"	Changes program behavior, define options for ASCII export.
Setup Exit		Exits OPTIMA control software.
Test Setup Test Protocol		Defines the test parameters – e.g. number of flashes, intervals / cycles, content of the wells in the microplate, standard concentrations, injection volumes and times, etc..

Menu command	Icon	Function
Measure Plate Out		Moves microplate carrier out of the instrument.
Measure Plate In		Moves microplate carrier into the instrument.
Measure Measure		Performs a measurement using a pre-defined test protocol. Before the measurement starts, you can enter plate IDs and perform an automatic gain adjustment.
Measure Quick Start		Performs a simple end point measurement using the full plate without the need to define a test protocol before. Just select method, filters and plate type.
Measure Pause After Current Cycle		Stops the active test run after finishing the current cycle (only available for plate mode tests). *
Measure Stop Now		Stops the active test run or the execution of a script.
Measure Prime		Prepares the pumps for injection, if pumps are installed. Define pump speed.
Measure Temperature		Defines the temperature of the incubator.
Measure Script Mode	-	Opens the script mode window. **
Results Evaluation Part		Opens the BMG LABTECH evaluation software for data reduction.
Help Contents		Opens the online help.
Help Contact Addresses	-	Shows BMG LABTECH's contact addresses.
Help BMG Web Page	-	Starts an internet browser and makes a connection to the BMG LABTECH web page.
Help About OPTIMA	-	Shows the software and firmware version.
Help System Information	-	Shows information about the operating system, the CPU speed, the current Excel version and the amount of memory.

* The 'Pause After Current Cycle' function is not available when using the script mode.

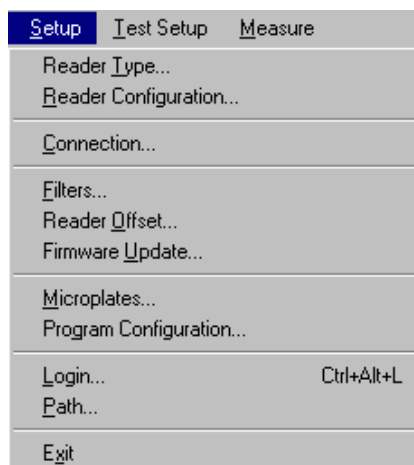
** The script mode is not available when the program is used in DDE mode, e.g. as part of a robotic system or in combination with Stacker Control.

4 Setup

4.1 Instrument Setup

The configuration settings for the instrument include the installed optic (reader equipped with auto optic switching) or the measurement method, the communication port, filter definitions, instrument offsets, definition of microplate dimensions and the path for measurement data.

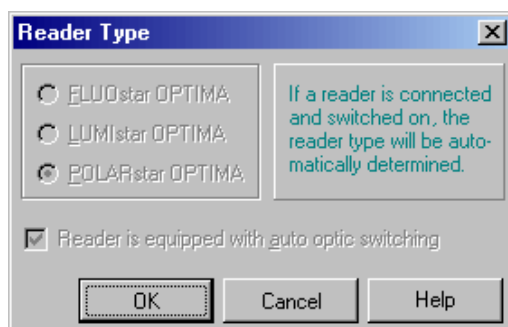
You can use the '**Setup**' pull-down menu



or the  button.

4.1.1 Reader Type

If a reader is connected to the computer and switched on, this window (accessible via '**Setup | Reader Type**') will show the type of reader which was automatically recognized. Depending on the reader type different measurement methods can be used.



The software remembers the last reader type used and will use this type if no reader is connected, but in this case you can change the reader type using this dialogue. This might be useful if you want to define a test protocol for a reader currently not available.

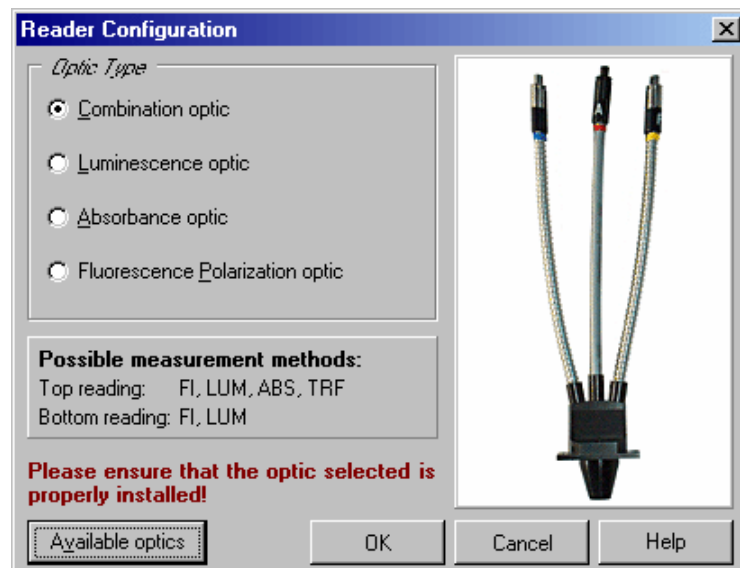
4.1.2 Reader Configuration

The LUMIstar OPTIMA can only measure in luminescence mode, therefore, the reader configuration dialogue is not available for this reader.

The FLUOstar OPTIMA can measure fluorescence intensity and time-resolved fluorescence and (optional) absorbance and luminescence. In addition, the POLARstar OPTIMA can read in fluorescence polarization mode. If the reader is equipped with auto optic switching, the reading methods possible depend on the installed optic. You can use the '**Setup | Reader Configuration**' function to define the optic type. If the reader is not equipped with auto optic switching, the reader configuration dialogue is used to select the reading method (see below).

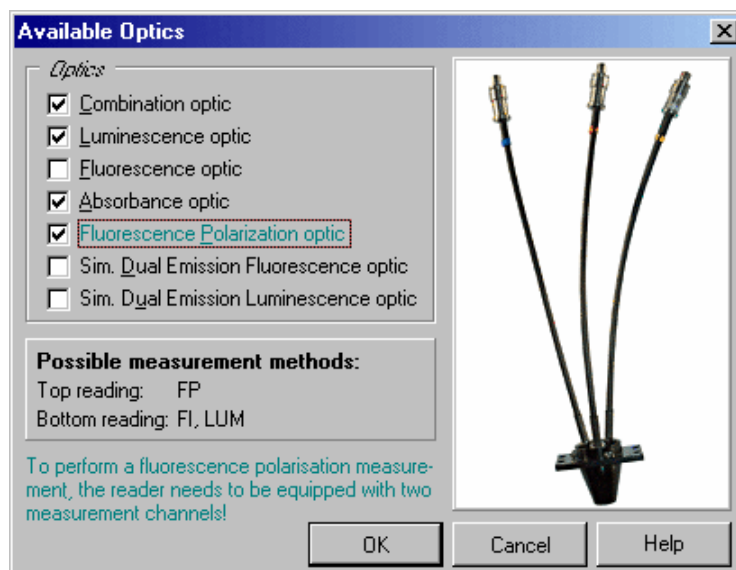
FLUOstar or POLARstar OPTIMA with auto optic switching

Using the '**Setup | Reader Configuration**' function, you can define which optic has been installed. You will see a picture of the optic selected on the right. For some measurement methods there are different optimized optics available, e.g. for absorbance measurement. If these optics look very different, the picture will show more than one optic.



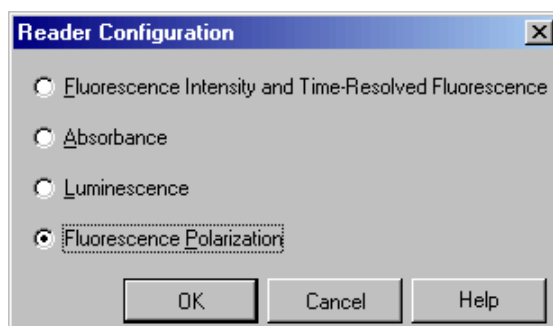
After selecting an optic, the reading modes possible with the reader connected (or selected using 'Setup | Reader Type') are listed on the left.

Use the '**Available Optics**' dialogue to define which optics you have available.



FLUOstar or POLARstar OPTIMA without auto optic switching

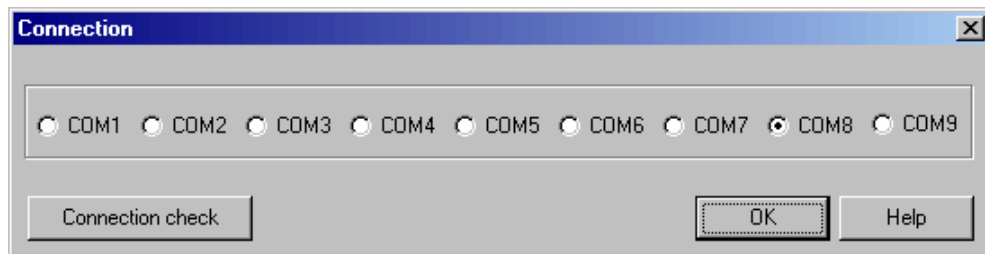
Using the '**Setup | Reader Configuration**' function, you can designate the measurement method.



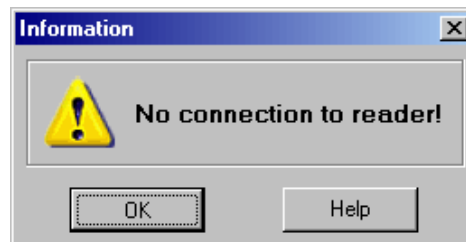
Please ensure that an optic suitable for the measurement method selected is installed and that you have switched to top or bottom reading, according to your needs.

4.1.3 Connection

You must select the corresponding communication port on the PC and check the communication status between the instrument and the PC. You can only select com ports which are available on the PC. If there is no communication between the PC and the instrument, check that the power to the instrument is switched on. If there is still no communication, try a different com port.



Connection check allows you to validate communication between the PC and the instrument. If there is no communication, a message box will appear:



4.1.4 Filters

The readers of the OPTIMA family can be configured with up to 8 excitation filters and up to 8 emission filters. Click on '**Setup | Filters**' to open the filter dialogue.

Position	Excitation	Emission
1	355	460
2	544	590
3	485	520
4	584	612
5	A-405	
6	A-450	lens
7	A-492	
8	A-620	empty

The position in the table corresponds to the filter position in the filter wheel (see picture below). Enter the filter wavelength values in the corresponding fields. For absorbance mode an empty position should be named on the emission side.



- Clear** After clicking this button, the table contents will be removed.
- Load** Loads filter information from an ASCII or Excel (.xls) file into the table. This might be useful if you are using more than one set of filter wheels.
- Save** Saves filter information from the table into an ASCII or Excel (.xls) file.
- OK** Saves the filter positions and returns to the main menu.
- Cancel** Closes this window without saving changes.

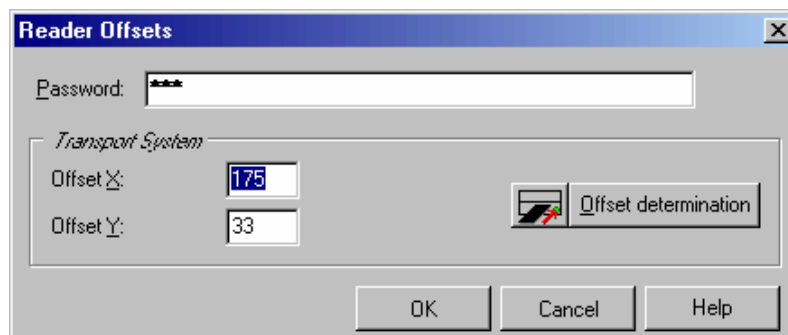
Notes: The table is common to all users.

Users with activated 'Run Only' option (see 2 Login Screen) are not allowed to change the filter settings.

4.1.5 Reader Offsets

Every OPTIMA reader is accurately calibrated at the factory and has individually defined offset values. The offset values correspond to the home position of the microplate carrier. Correct offset values are important for optimal measurement results. The offset values are stored in the reader EEPROM. The determination of the offset values should be carried out only by a qualified service technician. If the plate carrier is removed or replaced, the offset values should be re-calibrated.

The Reader Offsets screen can be reached by selecting '**Reader Offset**' in the '**Setup**' menu.



You can now enter new values. Do not perform an 'Offset determination' unless you are qualified to do so!

The  button will move the plate carrier to the defined offset position.

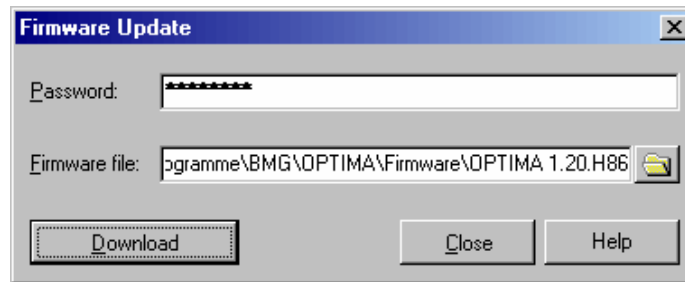
The '**Offset determination**' button is for service personnel only.

Click on '**OK**' to save the offset values into the reader EEPROM.


Note: Users with activated 'Run Only' option (see 2 Login Screen) are not allowed to change offset values.

4.1.6 Firmware Update

This function allows you to update the reader firmware (content of the built in Flash-EPROM).



If you received a new firmware file from BMG LABTECH, you should copy this file into the folder ~:\Program Files\BMG\OPTIMA\Firmware.

Make sure that the reader is connected to the computer and switched on. After typing in the correct password (you will get the password with the new firmware) select the firmware file using the  button, then press 'Download'. The OPTIMA program will bring the reader into the download mode and then start the download program 'FlashTools'. Downloading the new firmware is an automatic process, which will last a few minutes. Do not interrupt this process by switching the reader or the computer off or by closing the download program!

After the download is completed, the 'FlashTools' program will close automatically. Then the reader needs to be switched off and on again to activate the new firmware.

If the download process is interrupted, the reader will not work, but even in this state it is usually possible to repeat the download process (you need to restart the OPTIMA software after an interrupted download process).

4.2 Microplate Database

The OPTIMA software contains a data base with dimensions of microplates from most microplate manufacturers. While most plates have the standard spacing and footprint, some plates have slightly different dimensions, and must be positioned accordingly for optimal results.

In addition, new plates that are not on the current list can easily be defined by selecting '**Setup | Microplates**'. If microplate dimensions are edited, the changes will apply to all users.



Microplate	Length	Width	X (1)	Y (1)	X (n)	Y (n)	Plate Format
FALCON 3078 TISSUE CULT. PL. 48	127.80	85.60	18.70	10.50	109.20	75.20	48
NUNC 150687 MULTIDISH 48	127.60	85.60	16.60	9.00	111.10	76.50	48
BMG LABTECH 96	128.00	85.50	14.40	11.20	113.60	74.30	96
CORNING 25860 96	127.60	85.50	14.40	11.20	113.20	74.20	96
CORNING 25880 96	127.60	85.50	14.40	11.20	113.20	74.20	96
COSTAR 3695 96	127.90	85.60	14.40	11.20	113.50	74.40	96
CYTOPLATE 96	127.50	85.80	14.30	11.40	113.20	74.10	96
DYNATECH 96	128.00	85.80	14.40	11.20	113.60	74.10	96
DYNEX 6903 MICROFLUR 1 96	128.00	85.80	14.40	11.20	113.60	74.10	96
DYNEX 7521 MICROLITE TM 1 + 96	128.00	85.80	14.40	11.20	113.60	74.10	96
DYNEX 7571 MICROLITE TM + 96	128.00	85.80	14.40	11.20	113.60	74.10	96
FALCON 3072 96	127.60	85.80	14.30	11.40	113.40	74.40	96
FALCON 3075 CULTURE PLATE 96	127.70	86.00	14.40	11.50	113.30	74.50	96
FALCON 3872 CULTURE PLATE 96	127.70	86.00	14.40	11.50	113.30	74.50	96

To select the first microplate, beginning with e.g. 'B', simply press the key [B]. To select more than one microplate, press [Shift] together with [↑] or [↓] or press [Ctrl] and click on the desired microplate names with the left mouse button.

New

Click on 'New' to enter the plate dimensions for a new plate. A new window will appear that allows you to enter the necessary coordinates. Enter the plate format (well number) and the mechanical dimensions of the microplate (see Edit below).

Note: The dimensions should be measured from the center of the wells and need to be very accurate in order for the wells to be positioned exactly during measurements.

Edit

Select a microplate entry that you want to modify. A second window appears that displays the dimensions and plate format. All dimensions are in mm.

Name	Name of the new microplate
Plate Format	Select the total number of wells in the plate – 1536, 384, 96, 48, 24, 12 or 6 wells.
Length	The outer length of the entire microplate, from border to border.
Width	Outer width of entire microplate, from top to bottom.
X(1)	Distance from the center of the upper left well to the left border of the microplate.
Y(1)	Distance from the center of the upper left well to the top border of the microplate.
X(n)	Distance from the center of the lower right well to the left border of the microplate.
Y(n)	Distance from the center of the lower right well to the top border of the microplate.
Well shape	Select between round and square. This information is necessary for well scanning.
Well diameter	Diameter of a well. This information is necessary for well scanning.

Note: When you define a new microplate the values for X(n) and Y(n) will be pre-calculated as soon as the length and X(1) or the width and Y(1) are entered. The calculation is based on the assumption, that the distance to the border of the microplate is equal on both ends. It is still possible to override the pre-calculated values. Please note: this calculation will only take place as long as there are 0 values inserted for X(n) and Y(n).

Use the second sheet to define the Z dimensions. All dimensions need to be entered in mm. If the plate manufacturer does not provide these dimensions, you can use an accurately calibrated caliper to obtain the following dimensions. Please see the diagrams for the correct measurement of the dimensions.

Microplate

Name:

XY Dimensions | **Z Dimensions**

Plate height H: Please enter all dimensions in mm.

Well depth W:

Border height B:

Distance between plate and well bottom D:

Stack height S:

Comment:

OK Cancel Help

Plate height	Total height of the microplate.
Well depth	Depth of a well, measured in the middle of the well.
Border height	Height of the border of the microplate.
Distance between plate and well bottom	Distance between the bottom of the microplate and the outer bottom of the wells.
Stack height	Vertical distance between two microplates, if the plates are stacked. As the well bottom is higher than the plate bottom, this stack height is lower than the plate height.

You can use the **comment** field to store additional information about the microplate, for example order numbers or the microplate material.

Copy

Select a microplate that you want to copy. A window will appear allowing you to rename the microplate. The dimensions are copied exactly. This option allows you to edit a microplate's dimensions without changing the original.

Export

Select a microplate definition that you want to export to a disk or to a different directory. A new window will ask for the destination drive and directory. The file name will get an extension '.MPC'.

Import

Imports microplate definition from a disk or another directory and adds it to the existing data base. The extension for the microplate file must be '.MPC' or '.MPL' (microplate definitions exported from other / older BMG LABTECH programs).

Delete

Deletes an existing microplate and its dimensions from the data base.

Close

Returns to main menu.

Notes: Exporting the microplate table into a XLS (Excel), text or HTML file is possible after right clicking the table.

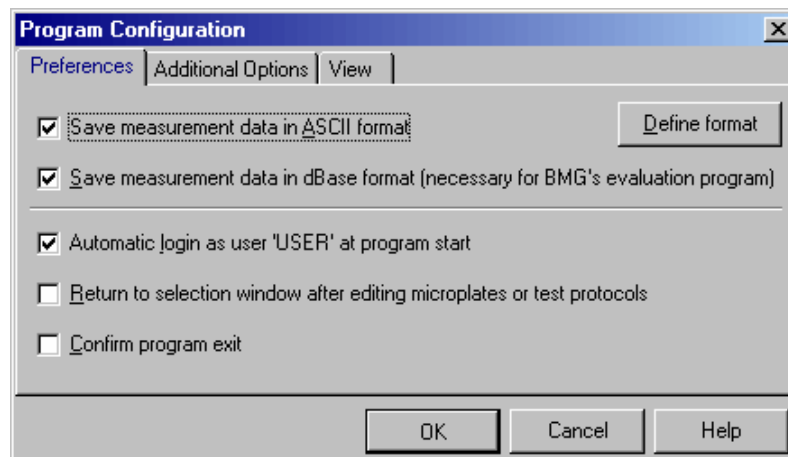
Users with activated 'Run Only' option (see chapter 2 Login Screen) are not allowed to edit, copy or import microplate definitions.

4.3 Program Configuration

Program Configuration allows the user to modify the appearance of the software. You can activate a feature by ticking the box next to the statement.

4.3.1 Preferences Sheet

In the first sheet you can select how the measurement data should be stored. Here you can also define some general program behavior settings.



Save measurement data in ASCII format

All measurement data is saved in the specified user directory, e.g. '~:\Program Files\BMG\OPTIMA\<Username>\Data'. The data is saved in a dbase file format that can be used with the macros in the evaluation software. If you would rather use a different evaluation software package, which will not work with dbase formats, then it is also possible to store the data in ASCII format. If you select this option then the '**Define Format for ASCII Export**' window becomes available (see chapter 4.3.4).

Save measurement data in dBase format

If this option is selected, all measurement data is stored in a set of dbase files in the data directory of the user logged in. The data is saved in a dbase file format that can be used with the macros in the evaluation software. If you would rather use a different evaluation software package, which will not work with dbase formats, then it is possible to get an ASCII file (see above).

Note: It is possible to use both formats simultaneously.

Return to selection window after editing microplates or test protocols

The selection window for tests and microplates contains previously defined setups and options to edit, export, import, etc.. By default, you can return to the main control software after creating or editing a test or microplate definition without returning to the selection window. If you wish to see the selection window for the microplates or test protocol definitions again, check the box in the 'Program Configuration' window.

Auto login

If you choose the option 'Automatically login as user "USER" at program start' there will be no login screen at program start, instead you will be logged in as the default user 'USER'. It is still possible to use the login function later ('Setup | Login').

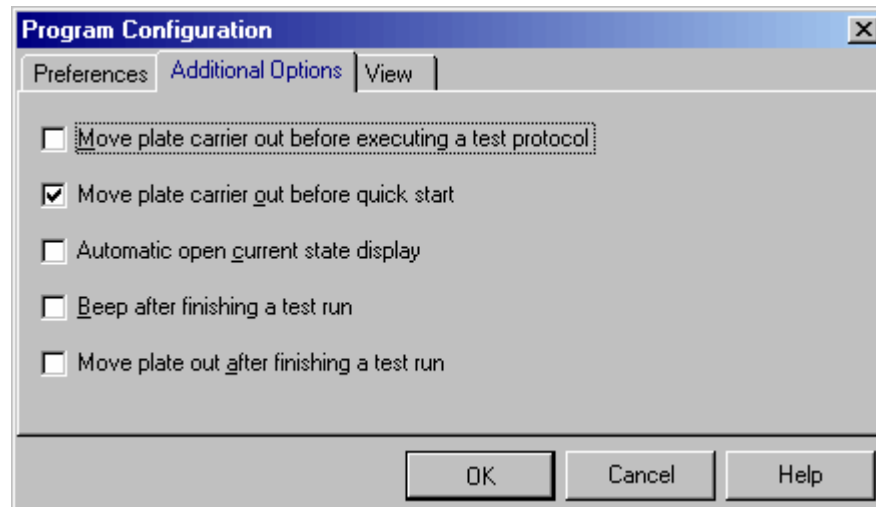
Note: Setting this option is only possible, if you are logged in as "USER" and if the administrator has not disabled this function (see chapter 2 Login Screen).

Confirm program exit

If you deselect this option there will be no confirmation window when exiting the program.

4.3.2 Additional Options Sheet

In the first sheet you can select how the measurement data should be stored. Here you can also define some general program behavior settings.



Move plate carrier out before executing a test protocol

Select this option if you want the plate carrier to be moved out when starting the execution of a test protocol. The plate carrier will be moved out after selecting the test protocol and opening the 'Start Measurement' dialogue.

Move plate carrier out before quick start

Select this option if you want the plate carrier to be moved out when opening the 'Quick Start' dialogue.

Automatic open current state display

If this option is selected the Current State display will be automatically opened after starting a measurement.

Beep after finishing a test run

If you select this option there will be a short acoustic signal after a test run is finished.

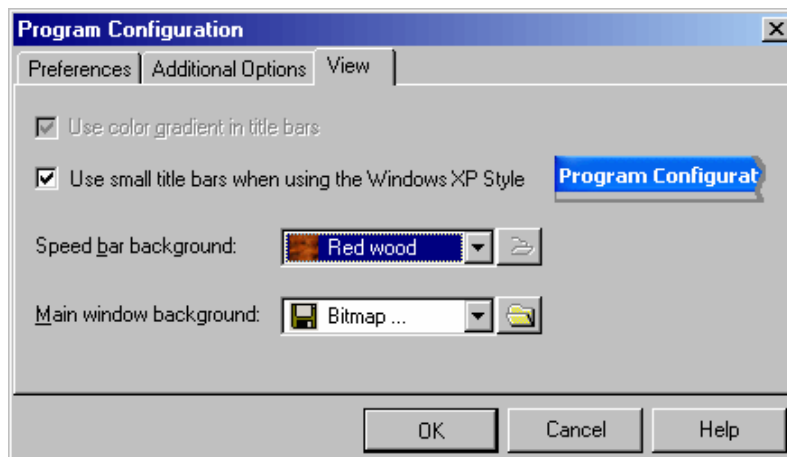
Move plate out after finishing a test run

If you select this option the plate will be automatically moved out of the reader as soon as the test run is finished, the current state window has been closed and the measurement data is saved.

Note: This option is not active if the software is used in DDE mode (e.g. in combination with Stacker Control) or in script mode (see chapter 8).

4.3.3 View Sheet

Using this sheet you can change the program look.



Use color gradient in title bar

The border or title bar at the top of the software can be either a solid color or a gradient from dark to light. This is only for appearance and does not affect any other feature of the software.

Note: In Windows 98, 2000 or XP, you define the title bar style using the windows control panel. Therefore, this option will be grayed out.

Use small title bars when using the Windows XP Style

Using this option you can decide whether you want to get small or large title bars for all program sub windows. The title bar of the main program window and of the login screen will not be changed. The style of Windows standard dialog boxes, e.g. for file name selection or for printing options, will also not be changed.

Note: This option is only available when Windows XP is used and the Windows XP style has been selected. It is not available when using the classic style.

Speed bar background

It is possible to define the style of the speed bar. Choose any of the pre defined background styles or use one of your own bitmap files (BMP-, GIF-, JPEG format).

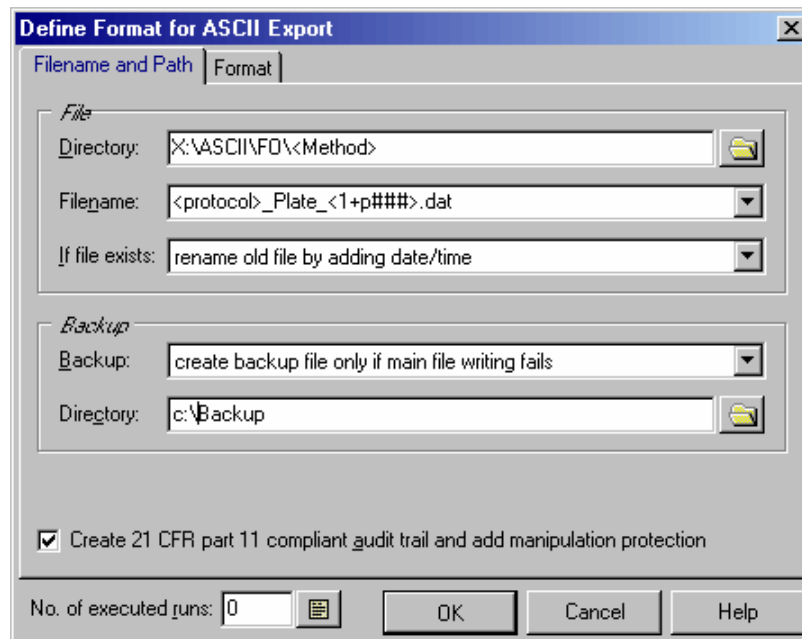
Main window background

Similar to the speed bar it is also possible to define the background of the main program window. Choose any of the pre defined background styles or use one of your own bitmap files.

Note: All settings from the program configuration window besides the Auto Login option are user specific, therefore, each user can select his preferences independently.

4.3.4 ASCII Export Function

If you click the 'Define format' button in the 'Program Configuration' window (see chapter 4.3.1) the '**Define Format for ASCII Export**' window will appear. In this window, you can choose the name and format of the file and where the data is saved.



File

Directory

The data path where the ASCII files will be stored. This can be a network directory, too. To create a new directory simply type the path you wish in. You can use the special functions <protocol> and <method> as part of the directory name to get your protocols sorted (explanation see below under Filename).

Filename:

The file name can be a constant name (something you type in) or you can use one of the following options given in the drop down menu:


- <protocol> Name of the used test protocol.
- <method> Name of the used method, e.g. 'Fluorescence Intensity' or 'Luminescence'.
- <ID1>...<ID3> The information written in the plate identification window before the measurement begins.

- **<1+#>** Tests will be assigned consecutive numbers. When the OPTIMA software is restarted the numbers begin again with '1'. You can change the start number; for example, enter the number 5 and the test numbers will increase consecutively starting at 5.

You can add a '#' character in order to increase the number of digits used. (<1+###> will produce file names 001, 002, 003, etc.)

If you want a number to be changed only after e.g. every tenths plate you should add this value in parenthesis before the > character, e.g. <1+###(10)>.

To use the 'Total no. of executed runs' instead of the number of executed runs after program start add a 'T' before the '#', e.g. <1+T####>.

To use the 'No. of executed runs for the used protocol (used test definition)' add a 'P' before the '#', e.g. <1+P###>. You can see a Run Statistics after pressing the  button.

In script mode (see chapter 8) you can add a 'B' before the '#' to get the plate numbers of the current batch run.

Counting down is also possible, use a '-' instead of the '+'.
 - **<A+#>** Identical to consecutive numbers except now the file name will be consecutive alphabetical letters. The number of letters used can be modified (i.e. <A+###> will result in AAA, AAB, AAC, etc).
 - **<date>** insert current date

You can specify the date format if you add a format description after "<date:" using yy or yyyy for the year, m or mm for the month and d or dd for the day:

yy	year with two digits (1999 => 99, 2000 => 00)
yyyy	year with four digits
m	one or two digits for the month (January => 1, December => 12)
mm	month with two digits (January => 01)
mmm	abbreviated name of the month (January => Jan.)
mmmm	full name of the month
d	day with one or two digits
dd	day always with two digits
ddd	abbreviated name of the day (Monday => Mo.)
dddd	full name of the day
dddddd	date in the format defined as "Short Date Format" under windows ('Settings Control Panel Regional Settings')
dddddd	date in the format defined as "Long Date Format" under windows

 Example: <date:yyyy_mm_dd>

 If you do not specify the format "yyyymmdd" will be used.

- `<time>` insert current time
 You can specify the time format if you add a format description after "`<time:>`":

h or hh	for the hour (one or always two digits)
m or mm	for the minute
s or ss	for the second
t	time in the format defined as "Short Time Format" under windows ('Settings Control Panel Regional Settings')
tt	time in the format defined as "Long Time Format" under windows
am/pm or a/p or AM/PM or Am/Pm	use 12 hours format and show am or pm (a or p...)

 Example: `<time:hh.mm.ss>`
 If you do not specify the format "hhmmss" will be used.
- `<ser_number>` Serial number of the reader used

You can use more than one of these parameters at one time in the filename (example: `<protocol> plate <1+###>.dat`). If a file extension is not specified then '.DAT' will be added automatically.

If file exists:

If a file with the same name already exists, then there are several options:

- ***Rename the old file by adding date and time*** to distinguish it from the more recent file.
- ***Overwrite old file.***
- ***Append*** the new ***data*** to the existing file (it will list separate tests in the same file; each test can be separated by a dashed line. See also the 'Separate data blocks with an empty line' option described below).

Backup

Here you can choose what to do if the data storage to the above defined directory fails:

- ***no backup file***
Show an error message when creation / writing into the defined ASCII file failed. The measurement results are still available in the database files (dBase format, for usage with e.g. Excel).
- ***create backup file only if main file writing fails***
If creation / writing into the defined ASCII file failed the ASCII file will be stored in the specified backup directory (same filename and behavior if file already exists as defined above). If writing into the backup file fails too, then there will be an error message. This setting is recommended if the main directory is on a network drive.
- ***always create backup file***
The ASCII file will be stored in the specified main directory. A second copy will be stored in the backup directory (same filename and behavior if file already exists as defined above). There will be no error message when writing into the main file failed. But there will be an error message if writing into the backup file fails.
- ***always create backup file (no error message when this fails)***
The ASCII file will be stored in the specified main directory. A second copy will be stored in the backup directory (same filename and behavior if file already exists as defined above). There will be no error message when writing into the main and/or backup file failed.

Directory

Specify the directory for storing the backup copy of the ASCII file.

Create 21 CFR part 11 compliant audit trail and add manipulation protection

If you use this option, there will be an additional file created for each ASCII file (same file name, but with additional file extension '.at'). This file will contain an anti manipulation hash value and the audit trail for the data stored in the connected ASCII file. The anti manipulation hash value will protect the whole ASCII file and the audit trail entries (see chapter **Fehler! Verweisquelle konnte nicht gefunden werden. Fehler! Verweisquelle konnte nicht gefunden werden.**).

The hash value will be created when the control part of the BMG LABTECH software creates the ASCII data file. It can be checked using the "Check Data Integrity" tool (see chapter 11.3).

Data Format

On the second sheet ('Format'), you can specify the data format:

The screenshot shows the 'Define Format for ASCII Export' dialog box with the 'Format' tab selected. The 'Data Format' section includes a 'Style' dropdown menu set to 'Table with well numbers, only measured wells'. Below it is a checkbox for 'Separate data blocks with an empty line'. The 'Separator' is set to '#9' and the 'Number format' is '#####0m'. The 'Non measured wells' is set to '-' and the 'Header' is 'Long header'. There are checkboxes for 'Include sample IDs' (unchecked) and 'Include cycle/interval start time values' (checked). The 'Calculation' section has checkboxes for 'Store blank corrected values' (unchecked) and 'Use average of all blanks from all groups for blank correction (if groups are used)' (unchecked). The 'Polarization tests' dropdown is set to 'store raw data and polarization values [mP]'. At the bottom, 'No. of executed runs' is set to 12, and there are 'OK', 'Cancel', and 'Help' buttons.

Style

The data can appear as a **table** (raw data side by side in a matrix) or a column (**list**). You can select the following options from the pull-down menu:

- List the data with the well number included.
- Include or exclude non-measured wells.

Tests that have more than one cycle will have data blocks representing the results from each cycle. Multichromatic measurements will appear with the data from the first filter set, followed by the data from the second filter set, etc.

If you choose the option '**List sorted by wells**', you will get the measurement results for all cycles in one line per well, but if your test contains more than one filter setting, you will still get a data block for each filter setting.

Separate data blocks with an empty line

If you use multichromatics or test protocols using two channels or if you use test protocols with more than one cycle / interval you will get more than one block of data in your ASCII file. By checking this box these blocks are separated by an empty line. There will also be an empty line separating the data from different test runs if you use the 'Append data' option (see above).

Separator

Select how the individual raw data number will be separated: for example, a comma, a semi colon or back slash. By selecting '#9', a tab step will be used.

Number format

Select the number of digits before and after the decimal point. A position described with a # symbol will be a number or a space; a position described with a zero will be a number or a zero.

For absorbance tests, you should specify a format of 0.000 because the OD values can range from 0.000 to 4.500. By adding a 'm' to the format string (e.g. '###0m') the numbers will be expressed in milliOD (0 to 4500 mOD).

Non-measured wells

Select the appearance of a non-measured well, for example: hyphen (-), backslash (/), or a zero (0).

Header

It is possible to include a description of the test run.

- **Short header**
Describes the test run in 5 lines (test name, date, plate IDs, number of cycles, and number of channels / chromatics).
- **Long header**
Contains the information of the short header but also includes the measurement mode, filters and the gain settings.
- **Full header**
Using the full header option all protocol settings including things like the layout, the used target temperature, the firm- and software versions and the reader serial number will be included in the ASCII file. BMG LABTECH recommends this option if you want to use ASCII files as data records according to the 21 CFR part 11 rule (see chapter **Fehler! Verweisquelle konnte nicht gefunden werden. Fehler! Verweisquelle konnte nicht gefunden werden.**).
- **Danish style**
If you use this option there will be no header, but in the first column of every data line the plate ID1 will be listed. This may be useful, if you combine data from more than one test run in one ASCII file and search, for example, for the highest result in any well of all plates. When you have found this result, you not only see the well name, you also see immediately the plate identification.

If you use the option 'Short', 'Long' or 'Full header' there will also be a line with the 'Channel / Multichromatic' number and a line with the 'Cycle / Interval' number before each data block.

Include sample IDs

Store the sample IDs (see 7.5 Sample IDs) also in the ASCII file (after the measurement value).

Include cycle/interval start time values

If this option is selected, the cycle start times (plate mode) or the interval start times (well mode) will also be included. The time values will be stored in a separate line in front of each measurement values block or in one line before the data when using a 'List sorted by wells' style. If you have decided to include a header, the time information is preceded by 'Time [s]: ', otherwise only the numbers will be stored.

Calculation

Store blank corrected values

If this option is selected, the measurement values stored in the ASCII file will be blank corrected.

Use average of all blanks from all groups for blank correction

This option is only important when different layout groups (see chapter 5.6.1 Using Layout Groups) are used. If this option is not selected the measurement values of a group will be corrected using only blanks from the same group. If you select this option, the correction will be done using the average of all blanks from all groups.

Polarization tests

For polarization tests you can decide whether you want the raw data for both channels to be stored or the blank corrected polarization or anisotropy values. If you choose the second option the polarization value will be calculated and stored in mP. In this case and for anisotropy values (stored in mA) you should use a number format with enough space for decimal numbers, e.g. '####0.000'.

Polarization values:

$$P = \frac{\text{Ch.A} - (\text{Ch.B})}{\text{Ch.A} + (\text{Ch.B})}$$

Anisotropy values:

$$A = \frac{\text{Ch.A} - (\text{Ch.B})}{\text{Ch.A} + 2(\text{Ch.B})}$$

Number of executed runs

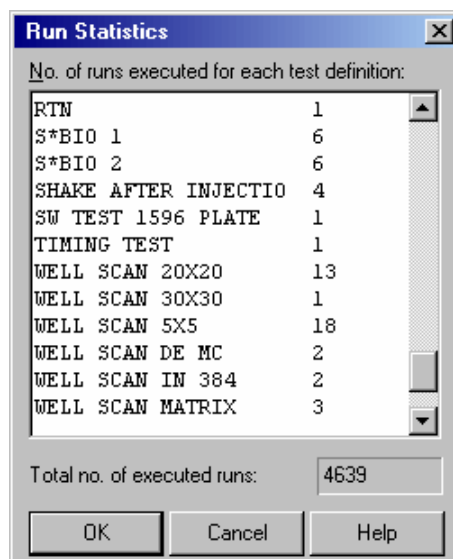
The 'counter' tracks the number of measurements that were performed after the program was started. You can manually type in a starting number. This number will be used in the filename options <1+#> and <A+#>.

To see a **Run Statistics** press the  button.

Note: All settings from the 'Define Format for ASCII Export' window are user specific, therefore each user can select his preferences independently.

4.3.5 Run Statistics

In this window, you see a list of all used test protocols together with a number showing you how often a protocol was executed. It is possible to edit these numbers or to delete a protocol from the list.



You can use this number together with the <1+P#> or <A+P#> option for defining the ASCII export filename or as part of a plate ID.

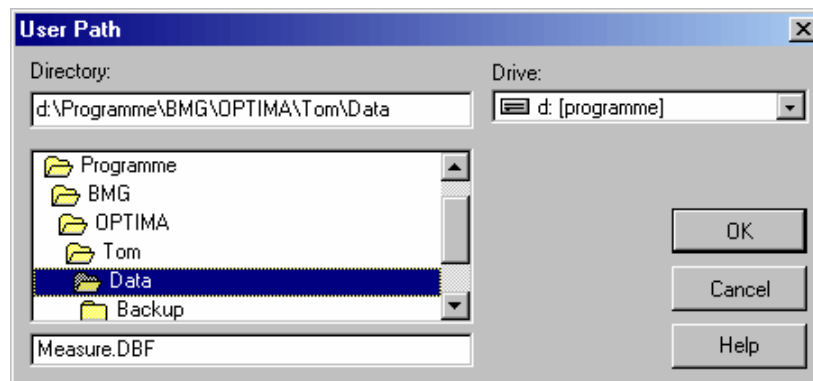
In this window, you also see the '**Total no. of executed runs**'. This is the total number of all tests executed using this computer after installing the OPTIMA software.

4.4 Login

The login function is described in chapter 2 Login Screen.


4.5 Data Path

This window allows you, to temporarily change the directory for your measurement results to a directory different than the one specified using the Login screen (see chapter 2). This might be useful, for example, to store the measurement results from different methods in different subdirectories.



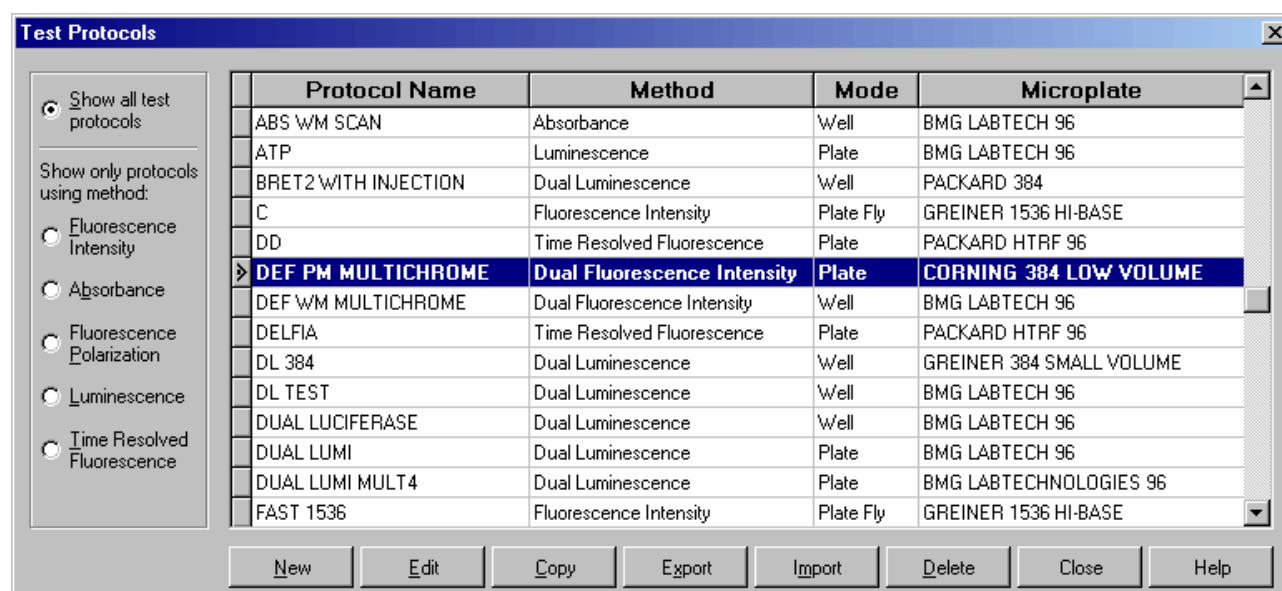
5 Defining Protocols

5.1 Protocol Selection Window

To create or edit a protocol click on  or select 'Test Protocol' from the 'Test Setup' menu.

The FLUOstar OPTIMA can measure fluorescence intensity and time-resolved fluorescence and (optional) absorbance and luminescence. In addition, the POLARstar OPTIMA can read in fluorescence polarization mode. The LUMIstar OPTIMA can only measure in luminescence mode.

The 'Test Protocols' window lists all previously defined tests and gives you options for creating new test protocols or modifying existing protocols. The window will show only protocols using a method which can be performed with the reader currently connected. If no reader is connected, the selection depends on the reader type used last or the reader type manually selected in the 'Reader Type' dialogue (see chapter 4.1.1).



By default the protocols are sorted by method first and then alphabetically by name. If you prefer sorting by name, only click the 'Protocol Name' part of the table headline. To switch back to the original sorting, click 'Method'. It is also possible to get the list sorted by the mode or the microplate used. Simply click on the 'Mode' or 'Microplate' part of the table headline. Besides displaying all available protocols, you might select to see only protocols using a certain measurement method by clicking one of the radio buttons on the left side of this dialogue box.

Note: As the LUMIstar OPTIMA can only measure using the luminescence method, the method selection box will not appear when using this type of reader.

To select the first test protocol, beginning with e.g. 'T', simply press the key [T]. To select more than one protocol (e.g. for export) use [Shift] together with [↑] or [↓] or press [Ctrl] and click on the desired protocol names with the left mouse button.

New

Define a new test protocol. See section 4.2.1.

Edit

Modify a previous test protocol. Select the test and click on 'Edit' or double click on the test name.

Copy

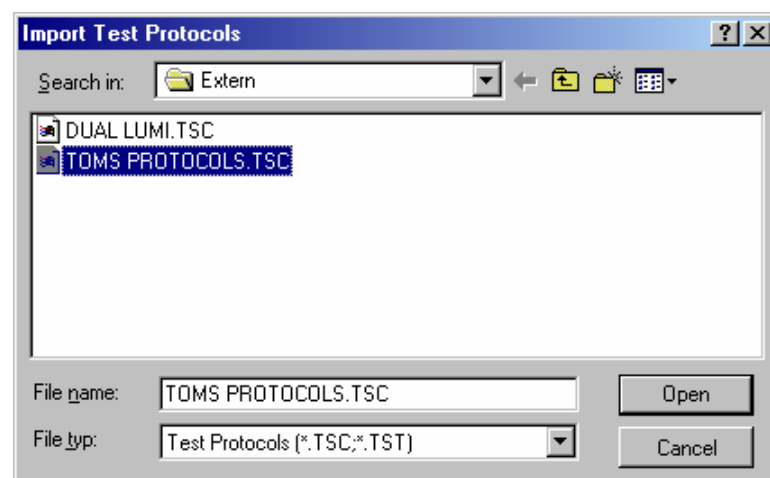
Select the protocol you want to duplicate. A new screen appears and asks for a name for the copy. The duplicate will appear on the test protocol list under the new name. Making a duplicate protocol means that you can modify a protocol without changing the original.

Export

You can copy a test protocol to a disk or another directory. Select the protocol(s) you want to export then click on 'Export'. A new screen will ask for the destination drive and directory and a name for the file.

Import

Import a test protocol from another drive and directory by clicking on 'Import'. Choose the directory. A list of files with extensions '.TSC' and '.TST' will appear; select the desired file and click on 'Open'. The imported test protocols will be added to the list of pre-defined test protocols.



Notes: It is possible to import test protocols from the NOVOSTAR or from the FLUOSTAR OPTIMA software.

It is also possible to import test protocols created using FLUOSTAR (old 403 series), FLUOSTAR Galaxy and LUMISTAR Galaxy. If you import a test protocol from one of these programs, the OPTIMA import function will ask you to import the layout definitions used by these tests immediately after importing the test definitions (In OPTIMA, the layout information is stored with the other test parameters, the above mentioned programs use different data bases for layout and test definitions.).

Delete

Remove a test protocol from the list by selecting the protocol and clicking on 'Delete'.

Close

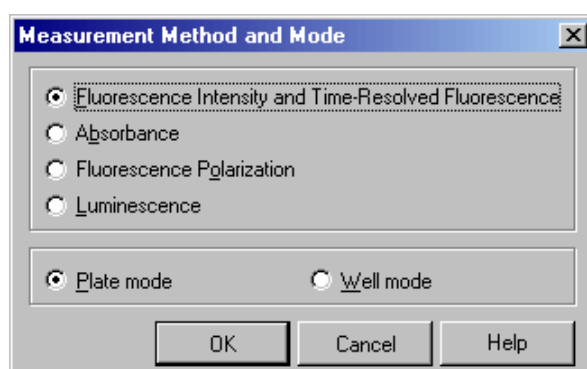
Return to the main menu.

Notes: Exporting the test protocols table into a XLS (Excel), text or HTML file is possible after right clicking the table.

Users with activated 'Run Only' option (see chapter 2 Login Screen) are not allowed to edit, copy or import test protocols.

5.2 Creating a New Test Protocol

Click on '**NEW**'; the next dialog box will ask for the measurement method and for plate mode or well mode. Select the method that is appropriate for the assay. The following sections will explain the difference between plate and well mode.



Note: Depending on the reader type not all measurement methods might be possible. As the LUMIstar OPTIMA can only measure using the luminescence method this dialogue will only provide a selection between plate and well mode when using this type of reader.

After the method and mode is selected, the next window allows you to enter the test protocol parameters.

5.3 Plate Mode Fluorescence Tests

Plate mode can be used for slow kinetics, i.e. the reaction lasts for an extended period of time, even after injection. All wells defined in the layout are read once during a plate cycle; it is possible to read up to 250 cycles. An injection can be defined for any cycle; all defined wells will receive an injection followed by a measurement cycle.

Key words:

Cycles	The number of times the entire plate will be measured. Each well is read only once per cycle if no multichromatics are used, otherwise it will be measured as many times as chromatics are used within each cycle.
Cycle time	The amount of time it takes to measure the plate during one cycle. You can use the Minimum cycle time as calculated by the instrument (fastest possible time) or enter a higher time if you want a delay between the cycles.

5.3.1 Basic Parameters – Plate Mode

Fluorescence Intensity - Plate Mode

Basic Parameters | Layout | Concentrations / Volumes / Shaking | Injection Timing | Timing Overview (One cycle)

Test name:

Microplate:

General Settings

Positioning delay (0.0...1.0 s):

☐ Flying mode

No. of kinetic windows (1...4):

Kinetic Window 1

No. of cycles (1...250):

Measurement start time (0...1200.0 s):

No. of flashes per well and cycle (0...200):

Cycle time (1...10000 s):

Optic

☒ Top optic ☐ Bottom optic

Filters and Integration

☒ Fluorescence Intensity ☐ Time resolved Fluorescence

No. of multichromatics (1...8):

☐ Simultaneous dual emission

Excitation filter: Emission filter: Gain (0...4095):

Integration: Start (0...1510 µs): Time (10...1510 µs):

Well Scanning

Minimum cycle time 1:

Pause before cycle (0...10): for seconds

Total measurement time:

Test Name

Assign a test name, as you would like it to appear on the list of test protocols.

Microplate

Use the pull-down menu to select the microplate used in the assay. All microplates defined under 'Setup | Microplates' will be listed. To select the first microplate, beginning with e.g. 'B', simply press the key [B] after opening the pull-down box (using e.g. [↓]).

General Settings

Positioning delay

You can define a waiting period after a well of the microplate moves to the measurement position and before the measurement begins. The delay time allows the liquid to settle and the surface to become stable so that the measurement is more accurate. For homogeneous fluorescence assays BMG LABTECH recommends a delay time of 0.2 s. For fluorescence measurements of cell assays, a delay time of 0.5 s is adequate. Liquid movements and hence delay time are influenced by viscosity.

Note: If you enter 0 here, the firmware will use 20 ms as smallest possible delay time. See also flying mode.

Flying mode

This is a time optimized parameter for plate mode. When this function is selected the measurement, using 1 or 3 flashes only, will occur at the exact moment that the center of the well is under the measurement head. The plate carrier does not stop as the well passes the measurement position. If you have defined injections, the injection cycles will be performed in non flying mode.

Number of kinetic windows

You can split the measurement into up to 4 kinetic windows. You can define the number of cycles, the measurement start time, the number of flashes and the cycle time independently for each kinetic window. Therefore, it is possible to have denser measurement points on more interesting parts of the kinetic curve. If you choose to use more than one kinetic window, a new sheet 'Kinetic Windows' (see chapter 5.3.3) will become available, where you can define the parameters for all kinetic windows. To switch to this sheet, click the arrow button or on the 'Kinetic Windows' tab. If you use only one kinetic window, you can define all necessary parameters on the 'Basic Parameters' sheet.

Kinetic Window 1

Number of cycles

This is the amount of times the entire plate will be measured for kinetic window 1. Each well defined in the layout will be measured once per cycle if no multichromatics are used, otherwise it will be measured as many times as chromatics are used within each cycle. You can define up to 250 cycles (250 is the total number for all kinetic windows).

Measurement start time

Defines the time when the measurement will start for all cycles belonging to kinetic window 1, relative to the time when the measurement position is reached plus positioning delay. Defining a measurement start time larger than 0 makes sense when i.e. injection or shaking is performed before the measurement.

Number of flashes per well and cycle

You can define up to 200 flashes per well and cycle. All the measurement values obtained for all flashes for a cycle are averaged for one intensity value per well. Therefore, the greater the number of flashes, the greater the accuracy. For fluorescence measurements, 10 flashes are usually adequate.

Note: Increasing the number of flashes also increases the minimum cycle time and, therefore, the reading time.

Cycle time

You can define the duration of each cycle from 1 to 10000 seconds. When the instrument is switched on, you can click the '**Check timing**' button and the cycle time is automatically validated by the instrument (You will see the minimum cycle time below this group box. If the cycle time you have defined is smaller than this minimum cycle time, it will be automatically corrected to the minimum time.). If you want to increase the cycle time (if you want a delay between cycles), you can manually enter a time greater than the minimum cycle time calculated by the 'Check timing' function.

For example, the instrument gives a time of 25 seconds per cycle, but you can change this to 85 seconds so that there will be a delay of 60 seconds between cycles.

Note: For endpoint tests (tests using only one measurement = one cycle), this time value does not matter, therefore, this input box will be disabled.

Optic

If your reader is equipped with auto optic switching you can select the **top** or **bottom optic** for fluorescence and luminescence mode protocols. If your reader is not equipped with auto optic switching you can select the top or bottom optic manually (see Instrument Manual for details).

Note: Bottom measurement is not possible in time-resolved fluorescence or in simultaneous dual emission mode.

Filters and Integration

Select **Fluorescence intensity** or **Time-resolved fluorescence** (time-resolved fluorescence involves a delay time between flash and measurement).

Number of multichromatics

There is the possibility to analyze 8 fluorophores per well. Enter the number of fluorophores to be analyzed, then click the arrow button or on the 'Multichromatic' tab to define the filter combination for each fluorophore to be analyzed.

Excitation and Emission filter

If you only want to analyze one fluorophore, you can select the filter combinations directly in this window. When you use the pull-down menu, the list of filters, as defined under 'Setup | Filters' (see chapter 4.1.4 Filters), is displayed.

Gain

This value will be used to adjust the sensitivity of the photo multiplier tube (PMT). A higher gain factor will increase the signal. Choose a gain value which will keep the measurement result of higher concentrations within the range of the instrument (i.e. 0 to 65000 relative fluorescence units). You can enter the gain settings here or perform an automatic gain adjustment before the measurement.

Integration Start and Time

This is for time-resolved assays. For integration start, enter the time in which the measurement should begin after the flash (see also chapter 5.3.2). This time should provide the maximum signal from the fluorophore after the flash has dissipated. The integration time is the length of the measurement (i.e. how long the PMT reads the emission light). To find the optimal times for the fluorophore, see section 5.9.1

Simultaneous dual emission

If your reader has two fluorescence measurement channels (using two PMTs) built in (POLARstar OPTIMA), you can use this option to measure light of two different wave lengths simultaneously. When switching on this option, a second emission filter box and a second gain box will appear.

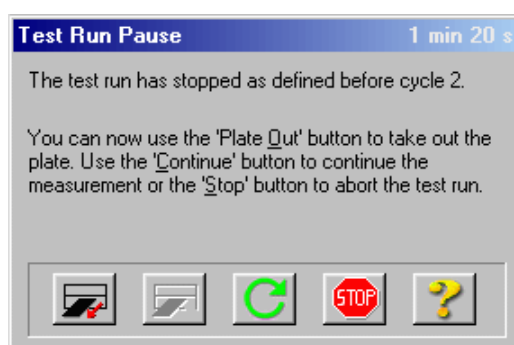
By selecting the emission filter for channel A, the filter for channel B is selected automatically. Due to mechanical constraints for channel B the filter 180° opposite to the A channel filter will always be used. This means, if you are using the filter installed in filter wheel position 1 for channel A, the filter installed in position 5 will be used for channel B.

Well Scanning

If you use microplates with up to 96 wells, instead of measuring one point in the middle of the well you might use any of the available well scanning modes (see chapter 5.5 Well Scanning).


Pause before cycle

You can define a pause for plate mode tests. The default setting is 0, which results in an uninterrupted measurement. If you want a pause, enter the cycle number before which the reader should pause. The reader will pause the measurement before the defined cycle. If you have defined 0s as pause time, the Test Run Pause window will appear, otherwise the measurement will automatically be continued after the defined pause time.



In the caption bar of the window you can see the time elapsed since the beginning of the pause.

The pause window provides the possibility of bringing the plate out (to make a manual injection, to incubate or to change the plate) or stopping the test run.

You can also pause after a measurement has started (plate mode only with more than one cycle). Go to 'Measure' and select 'Pause After Current Cycle' or use the  button.

Notes: It is possible to define a pause before the first cycle, e.g. to incubate the plate for a certain time.

The pause function is not available in script mode (see chapter 8).

Comment

Here you can enter a short description of the test protocol (up to 255 characters). This text will also be visible later in the comment field of the evaluation sheet of the OPTIMA evaluation software (see chapter 10.5 Evaluation Worksheet).

Check timing

Minimum cycle time 1

The minimum cycle time can only be calculated by the reader. After defining all necessary settings (do not forget the filters), click the button '**Check timing**'. The shortest possible cycle time will be displayed. If you have defined a cycle time, which is shorter than this minimum time, it will automatically be corrected. At the bottom of the test protocol definition window, the calculated '**Total measurement time**' for the entire plate will be displayed.

Note: The 'Check timing' function is only available if the reader is switched on.

After pressing the 'Check timing' button the 'Timing Overview' sheet will become available (see chapter 5.10).

Limitations for 1536 well plates

Your reader has to be specially prepared for measuring 1536 well plates. Measuring 1536 well plates is only possible in plate mode. The following limitations exist for the test protocol:

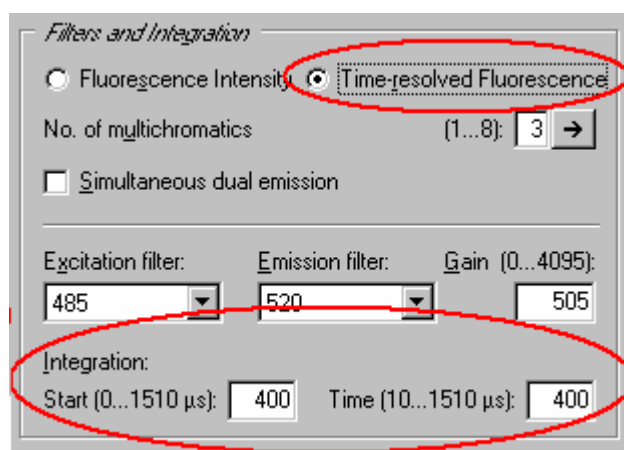
- only one kinetic window
- only horizontal bidirectional reading, starting top left
- no multichromatics
- no injection
- no shaking

Printing the Protocol

To print the protocol use the key combination [Shift]+[Ctrl]+[P]. A standard print dialog box will appear. Here you can decide, whether you only want to print the current sheet ('Selection'), all sheets ('All') or a selection of sheets ('Pages'). When using portrait format two sheets will be printed on one page, using landscape format only one.

5.3.2 Time-Resolved Test Protocols

In the 'Filters and Integration' box you can define the optimal integration time for a time-resolved assay.



If you have one time-resolved fluorophore you can define the same filter setting for up to 8 (multichromatic) tests and try different integration times (see chapter 5.9.1). You can then compare the measurement data to find the optimal time for sensitivity.

5.3.3 Kinetic Windows – Plate Mode

It is possible to split the measurement into up to 4 kinetic windows. You can define the cycle time individually for each kinetic window. Therefore, it is possible to have denser measurement points on more interesting parts of the kinetic curve.

Number	No. of cycles (0...250)	Meas. start time (0...1200 s)	No. of flashes (0...200)	Cycle time (1...10000 s)	Minimum cycle time
1	3	0.0	10	100	32 s
2	30	0.0	20	40	39 s
3	2	0.0	20	40	39 s
4	0	0.0	0	2	

☐ Equidistant kinetic cycles (all cycles are as long as necessary for the longest/latest injection or measurement to take place)

Total measurement time: 33m20s

Number of cycles

This is the amount of times the entire plate will be measured. Each well defined in the layout will be measured once per cycle if no multichromatics are used, otherwise it will be measured as many times as chromatics are used within each cycle. You can define up to a total of 250 cycles divided into up to 4 kinetic windows.

Measurement start time

Defines the time when the measurement will start for all cycles belonging to the respective kinetic window, relative to the time when the measurement position is reached plus positioning delay. Defining a measurement start time larger than 0 makes sense when i.e. injection or shaking is performed before the measurement.

Number of flashes (all modes except luminescence)

You can define up to 200 flashes per well and cycle. All the measurement values obtained for all flashes of a cycle sent to one well are averaged for one intensity. Therefore, the greater the number of flashes, the greater the accuracy. For fluorescence measurements, 10 flashes are usually adequate.

Note: Increasing the number of flashes also increases the minimum cycle time and, therefore, the reading time.

Measurement interval time (only luminescence mode)

Defines the measurement time (defines how long the light is measured). By increasing this time, you will get higher (and usually more accurate) measurement values.

Cycle time

You can define the duration of each cycle from 1 to 10000 seconds (=2hr46min40s). When the instrument is switched on, you can click the '**Check timing**' button and the cycle time is automatically validated by the instrument. If you want to increase the cycle time (if you want a delay between cycles), you can enter a time greater than the minimum cycle time calculated by the 'Check timing' function.

For example, if the instrument gives a minimum cycle time of 25 seconds and you change this to 85 seconds, there will be a delay of 60 seconds between cycles.

Equidistant kinetic cycles

If you use this option, the minimum cycle time for all cycles is as long as the cycle with the latest / longest injection or measurement requires.

If you do not use this option, the time for cycles with injection can be different from cycles without injection and the minimum cycle time for each kinetic window can be different. This allows very short sampling rates in kinetic areas with a fast change in signal.

Note: Even if you choose this option, it is possible to define different kinetic cycle times for different kinetic windows, but the software will ensure that the timing of all wells is equal by adding waiting times after processing each well.

Minimum cycle time

The minimum cycle times can only be calculated by the reader. If you do not use the Equidistant kinetic cycles option, the Minimum cycle time can be different in different kinetic windows (depending on the Measurement start time and the Number of flashes). After defining all necessary settings (do not forget the filters), click the button '**Check timing**'. The shortest possible cycle times will be displayed. If you have defined a cycle time in any of the kinetic windows which is shorter than the corresponding minimum time, it will automatically be corrected. At the bottom of the test protocol definition window, the calculated '**Total measurement time**' for the entire plate will be displayed.

Note: The 'Check timing' function is only available if the reader is switched on.

5.4 Well Mode Fluorescence Tests

Well mode tests should be selected for fast kinetics, i.e. if the reaction, usually initiated by an injection, occurs over a very finite amount of time. Measurements can then be performed in small intervals when the reaction occurs. In well mode, each well, as defined in the layout, will be measured individually for the defined number of intervals.

For example, a test can be defined with 3 intervals and an injection. Therefore, well A1 moves to the measurement position, a measurement is performed, then an injection, followed by two more measurements. When all defined measurement intervals have been performed on well A1 the microplate carrier moves to the next defined well for the same protocol. Once all the defined wells have been measured, the assay is finished.

Key words:

Intervals	Number of times the well is measured. Up to 250 intervals per well can be defined.
Interval time	The length of time, in seconds, for each measurement interval. This includes the flashes and the time for measuring the emission light.
Total measurement time per well	The amount time it takes to perform all the measurement intervals, including any injections, on one well. It is calculated by multiplying the number of intervals by the interval time (and taking into account all used kinetic windows and the measurement start times).

5.4.1 Basic Parameters – Well Mode

Fluorescence Intensity - Well Mode

Basic Parameters | Layout | Concentrations / Volumes / Shaking | Injection Timing | Timing Overview

Test name:

Microplate:

Optic

☒ Top optic ☐ Bottom optic

General Settings

Positioning delay (0.0...1.0 s):

No. of kinetic windows (1...4):

Kinetic Window 1

Measurement start time (0...1200 s):

No. of intervals (1...250):

No. of flashes per well and interval (0...200):

Interval time (0.02...100 s):

End time of kinetic window 1 (s):

Filters and Integration

☒ Fluorescence Intensity ☐ Time-resolved Fluorescence

No. of multichromatics (1...8):

☐ Simultaneous dual emission

Excitation filter: Emission filter: Gain (0...4095):

Integration: Start (0...1510 µs): Time (10...1510 µs):

Well Scanning

Comment: Example test protocol using well mode.

Min. interval time 1: Total meas. time/well:

Total measurement time:

Test name

Assign a test name, as you would like it to appear on the list of test protocols.

Microplate

Use the pull-down menu to select the microplate used in the assay. All microplates defined under 'Setup | Microplates' will be listed. To select the first microplate, beginning with e.g. 'B', simply press the key [B] after opening the pull-down box (using e.g. [↓]).

General Settings**Positioning delay**

You can define a waiting period after the microplate moves a well to the measurement position and before the measurement begins. The delay time allows the liquid to settle and the surface to become stable so that the measurement is more accurate. For homogeneous fluorescence assays BMG LABTECH recommends a delay time of 0.2 s. For fluorescence measurements of cell assays, a delay time of 0.5 s is adequate. Liquid movements and hence delay time are influenced by viscosity.

Number of kinetic windows

You can split the measurement into up to 4 kinetic windows. You can define the number of intervals, the interval time and the start time independently for each kinetic window. Therefore, it is possible to have denser measurement points on more interesting parts of the kinetic curve. If you choose to use more than one kinetic window, a new sheet 'Kinetic Windows' (see chapter 5.4.2) will become available, where you can define the parameters for all kinetic windows. To switch to this sheet, click the arrow button or on the 'Kinetic Windows' tab. If you use only one kinetic window, you can define all necessary parameters on the 'Basic Parameters' sheet.

Kinetic Window 1**Measurement start time**

= Start time for kinetic window 1. Defines the time when the measurement will start, relative to the time when the measurement position is reached plus position delay.

Example - measurement start time 5 seconds: After the reader plate carrier reaches the measurement position, the defined positioning delay time starts. After this, there will be an additional delay of 5 seconds before the measurement itself starts.

Using this measurement start time, it is possible to start the measurement after an injection.

Number of intervals

This is the amount of times a measurement will be successively taken on each well (equals the number of readings on each well). You can define up to 250 intervals (250 is the total number for all kinetic windows). This is typically used for kinetic assays, in which the dynamics of a reaction changes very quickly over time. Each interval is plotted as a kinetic point displaying the change over time.

Number of flashes per well and interval

You can define up to 200 flashes per measurement interval. All the measurement values from the flashes defined for one interval are averaged to produce one intensity value per well. The greater the number of flashes, the greater the accuracy. For fluorescence measurements, 10 flashes are usually adequate.

Note: Increasing the number of flashes also increases the reading time.

Interval time

You can define the duration of each interval from 0.02 to 100.0 seconds. When the instrument is active, you can click the 'Check timing' button and the interval time is automatically validated by the instrument. If you want to increase the interval time (if you want a delay between the intervals), you can manually enter a time. If you have defined an interval time in any of the kinetic windows which is shorter than the respective minimum interval time, it will automatically be corrected to the shortest possible value. If necessary, the start times for following kinetic windows will also be corrected.

The minimum interval time depends on the number of flashes (1 flash equals 0.02 seconds, 10 flashes equals 0.2 seconds, etc.) and on the usage of multichromatics.

For example, the instrument gives a minimum interval time of 0.4 seconds, but you can change this to 5 seconds so that there will be a delay of 4.6 seconds between intervals.

Note: For endpoint tests (tests using only one measurement = one interval), this time value does not matter, therefore, this input box will be disabled. In these cases the measurement of the one interval might also last longer than 100 seconds, for example when using a well scanning mode with a high number of scan points.

End time of kinetic window 1

This value is automatically calculated (Measurement start time plus Number of intervals multiplied by Interval time). The start time for kinetic window 2 needs to be higher than or equal this end time.

Optic

If your reader is equipped with auto optic switching you can select the **top** or **bottom optic** for fluorescence and luminescence mode protocols. If your reader is not equipped with auto optic switching you can select the top or bottom optic manually (see Instrument Manual for details).

Note: Bottom measurement is not possible in time-resolved fluorescence or in simultaneous dual emission mode.

Filters and Integration

Select **Fluorescence intensity** or **Time-resolved fluorescence** (time-resolved fluorescence involves a delay time between flash and measurement).

No. of multichromatics

There is the possibility to analyze up to 8 fluorophores per well. In well mode, all fluorophore settings will be measured in one cycle (one well at a time). Enter the number of fluorophores to be analyzed, then click the arrow button or on the 'Multichromatic' tab to define the filter combination for each fluorophore to be analyzed.

Excitation and Emission filter

If you only want to analyze one fluorophore, you can select the filter combinations directly in this window. When you use the pull-down menu, the list of filters, as defined under 'Setup | Filters', is displayed.

Gain

This value will be used to adjust the sensitivity of the photo multiplier tube (PMT). A higher gain factor will increase the signal. Choose a gain value which will keep the measurement result of higher concentrations within the range of the instrument (i.e. 0 to 65000 relative fluorescence units). You can enter a gain manually or perform an automatic gain adjustment before the measurement.

Integration Start and Time

This is for time-resolved assays. For integration start, enter the time in which the measurement should begin after the flash. This time should provide the maximum signal from the fluorophore after the flash has dissipated. The integration time is the length of the measurement (i.e. how long the PMT reads the emission light). A way to find the optimal times for the fluorophore is described section 5.9.1.

Simultaneous dual emission

If your reader has two fluorescence measurement channels (using two PMTs) built in (POLARstar OPTIMA), you can use this option to measure light of two different wave lengths simultaneously. When switching on this option, a second emission filter box and a second gain box will appear.

By selecting the emission filter for channel A, the filter for channel B is selected automatically. Due to mechanical constraints for channel B the filter 180° opposite to the A channel filter will always be used. This means, if you are using the filter installed in filter wheel position 1 for channel A, the filter installed in position 5 will be used for channel B.

Well Scanning

If you use microplates with up to 96 wells, instead of measuring one point in the middle of the well you might use any of the available well scanning modes (see chapter 5.5 Well Scanning).

Comment

Here you can enter a short description of the test protocol (up to 255 characters). This text will also be visible later in the comment field of the evaluation sheet of the OPTIMA evaluation software (see chapter 10.5 Evaluation Worksheet).

Check timing

Minimum interval time 1

The minimum interval times (this time might be different for different kinetic windows) can only be calculated by the reader. After defining all necessary settings (do not forget the filters), click the button '**Check timing**'. The shortest possible interval time will be displayed. If you have defined an interval time in any of the kinetic windows which is shorter than the respective minimum time, it will automatically be corrected. If necessary, the start times for all following kinetic windows will also be corrected. At the bottom of the test protocol definition window, the calculated '**Total measurement time**' for the entire plate will be displayed.

Note: The 'Check timing' function is only available if the reader is switched on.

Total measurement time per well

This time will be calculated using the settings in the protocol, i.e. the interval time, the number of intervals and the start time for all kinetic windows. If the defined interval time for the last interval (in the last kinetic window), is higher than the minimum interval time, the calculated total measurement time per well will show a value slightly too high, as the reader, when the last interval is finished will not wait for the defined interval time before it goes on to the next well. If you have defined an injection which will last longer than the last measurement (which usually makes no sense), this time will be too short. (The total measurement time calculated by the reader will always be correct.)

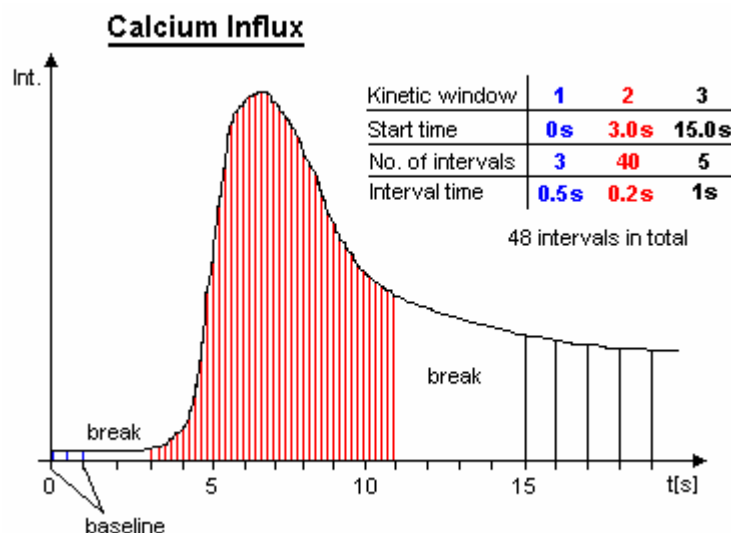
After pressing the '**Check timing**' button, the 'Timing Overview' sheet will become available (see chapter 5.10).

Click on '**OK**' to save the information and to add the test protocol to the list.

5.4.2 Kinetic Windows – Well Mode

It is possible to split the measurement into up to 4 kinetic windows. You can define the interval time independently for each kinetic window. Therefore, it is possible to have denser measurement points on more interesting parts of the kinetic curve.

Example:



Fluorescence Intensity - Well Mode

Basic Parameters | **Kinetic Windows** | Layout | Concentrations / Volumes / Shaking | Injection Timing | Timing Overview

Number	Start time (0...1200 s)	No. of intervals (0...250)	No. of flashes (0...200)	Interval time (0.02...100 s)	End time (s)	Minimum interval time
1	0.0	3	5	1.00	3	0.10 s
2	3.0	40	10	0.20	11	0.20 s
3	12.0	5	10	2.00	22	0.20 s
4	320.7	0	10	0.10	0	

Total meas. time/well: 22 s

Total measurement time: 27m39s

OK Cancel Help

Start time

Defines the time when the measurement for the selected kinetic window will start, relative to the time when the measurement position is reached plus position delay. The start time for a kinetic window needs to be higher than the end time of the kinetic window before.

Number of intervals

This is the amount of times a measurement will be successively taken on each well. You can define up to a total of 250 intervals.

Number of flashes (all modes except luminescence)

You can define up to 200 flashes per measurement interval. All measurement values obtained for all flashes defined for one interval are averaged to produce one intensity value per well. The greater the number of flashes, the greater the accuracy. For fluorescence measurements, 10 flashes are usually adequate.

Note: Increasing the number of flashes also increases the reading time.

Measurement interval time (only luminescence mode)

Defines the measurement time (defines how long the light is measured). By increasing this time, you will get higher (and usually more accurate) measurement values.

Interval time

You can define the duration of each interval from 0.02 to 100.0 seconds. When the instrument is switched on, you can click the '**Check timing**' button and the interval times are automatically validated by the instrument. If you want to increase an interval time (if you want a delay between the intervals), you can manually enter a time.

For example, the instrument gives a time of 0.4 seconds but you can change this to 5 seconds so that there will be a delay of 4.6 seconds between intervals.

End time

This value is automatically calculated (Start time plus Number of intervals multiplied by Interval time). The start time for the next kinetic window needs to be higher than this end time.

Minimum interval time

The minimum interval times (this time value might be different for different kinetic windows) can only be calculated by the reader. After defining all necessary settings press the button '**Check timing**'. The shortest possible interval times will be displayed. If you have defined an interval time in any of the kinetic windows which is shorter than the respective minimum time, it will automatically be corrected. If necessary, the start times for all following kinetic windows will also be corrected. At the bottom of the test protocol definition window, the calculated '**Total measurement time**' for the entire plate will be displayed.

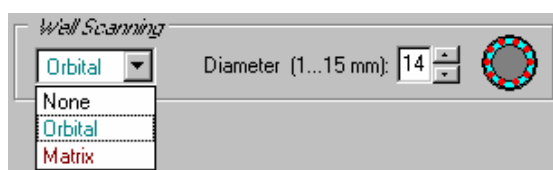
Note: The 'Check timing' function is only available if the reader is switched on.

5.5 Well Scanning

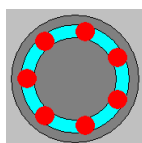
If you use microplates with up to 96 wells, instead of measuring one point in the middle of the well, you might use any of the available well scanning modes. This is useful if you use large wells and if the probe is not equally distributed, e.g. when using cell assays.

5.5.1 Orbital Scanning

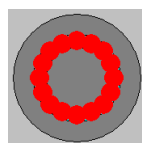
Using this mode, the measurement takes place on an orbit with definable diameter. In **absorbance, fluorescence and fluorescence polarization modes** the defined number of flashes will be equally distributed over the orbit. For each well there will be one orbital movement per cycle/interval (in multichromatic mode one movement per chromatic per cycle/interval). The maximum number of flashes possible depends on the diameter defined. In orbital scanning mode only the average measurement value of all scan points will be displayed.



A little icon inside the well scanning box will illustrate how the measurement points are distributed over the well:

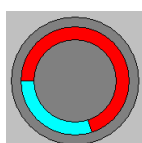


7 flashes

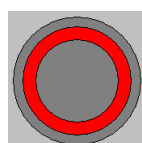


16 flashes, smaller diameter

In **luminescence mode** the measurement is done by continuously measuring the emitted light during the defined measurement interval time (see chapter 5.12 Luminescence Tests). As the time necessary for one orbital movement only depends on the selected diameter, it might happen, that the measurement occurs only during a part of the orbital movement. The little icon in the well scanning box will show you which part of the orbit is used for the measurement.



70 % coverage



100 % coverage

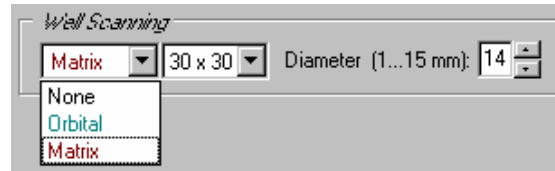
It is recommended to define the measurement interval as long as the orbital movement lasts (this time is shown as maximum possible time besides the measurement interval time input box). There will be a warning message if the defined measurement time covers less than 50% of the orbital movement.

Notes: As it is possible to use different numbers of flashes / different measurement interval times in different kinetic windows (see chapter 5.3.3), you will also see these well scanning icons in the Kinetic Windows sheet.

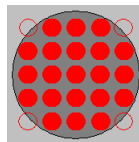
The defined positioning delay time occurs only once for each well in orbital scanning mode (opposite to matrix scanning, see below). There will be no positioning delay between the measurement of single scan points.

5.5.2 Matrix Scanning

Besides measuring during on orbital movement it is also possible to measure different points of a well in a matrix style. Using this mode you will also be able to see measurement values from the single scan points in the Current State Display (see chapter 7.7.4 Display of Well Scanning Data) and in the Evaluation software (see chapter 10).



You can define the matrix size and therefore the number of measurement points. When using a plate with round wells (see well shape in chapter 4.2 Microplate Database), only measurement points which are inside the circle with the defined diameter will be measured (in the example below 21 out of 25 possible points):

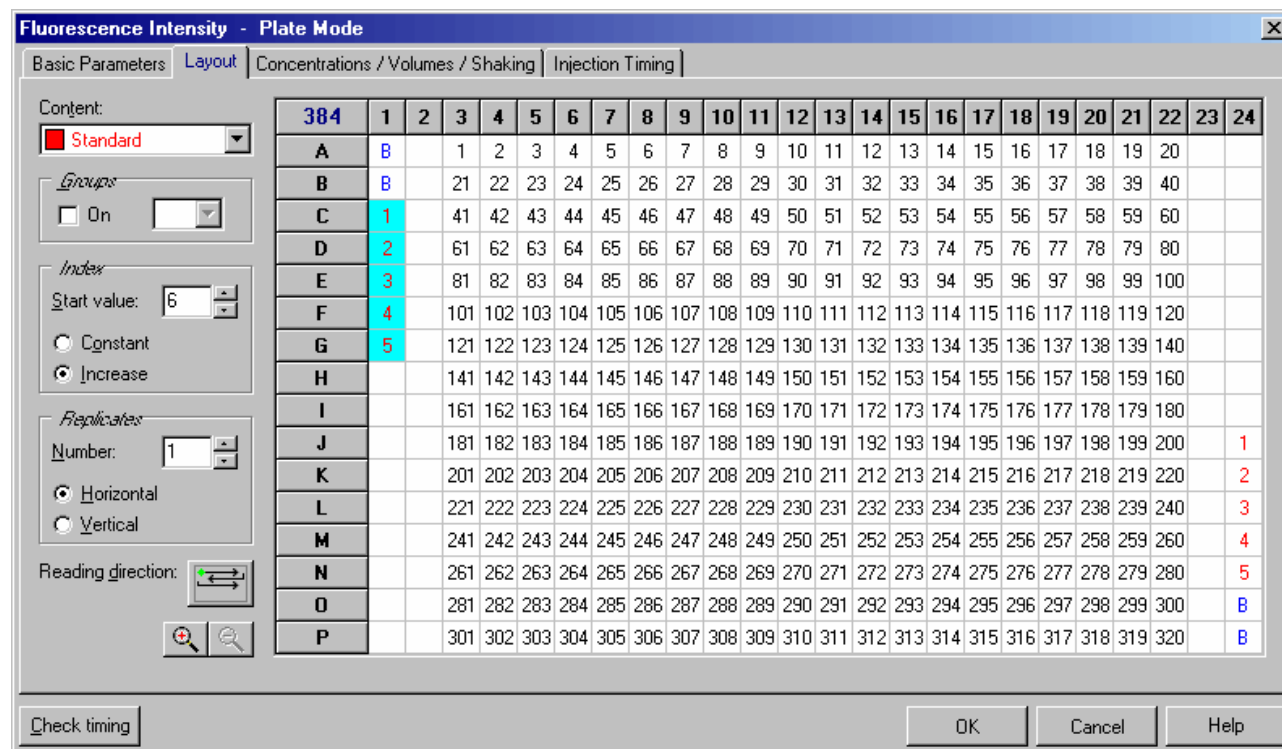



Notes: Matrix scanning is slower than orbital scanning.

The defined number of flashes or the defined measurement interval time in luminescence mode and the defined positioning delay time will be used for every single scan point!

5.6 Layout Definition

This sheet contains a grid representing the wells of the microplate that you selected. You can define wells containing samples, blanks, standards and controls.



If you edit a 384 or 1536 well layout, you can use the zoom buttons  to enlarge or reduce the layout display.

Content

In the content pull-down menu, you can select the type of sample that the well contains; select sample, standard, or blank.

Sample	(X) The well's content has unknown concentration.
Standard	(S) The well's content has a known concentration and is used to formulate a standard curve in the data reduction
Negative Control, Positive Control Control	(N) The well's content has known concentrations, but will not be used for the standard curve calculation. It can be used for (P) comparisons or for special evaluation calculations. (C)
Blank	(B) The well contains water or buffer for measuring background.

Index

The **Index** is the reference number of the sample or standard. The index box displays the number that will be used for the next sample or a standard. If **Increase** is selected, each sample or standard will be labeled with consecutive numbers. With **Constant** the number will remain the same, in the case of continuous replicates. In the example picture above, the index box shows that the next standard well number is 6.

Increase	For each well the next consecutive number will be used. The number shown in the index box is the number of the next well.
Constant	The number remains fixed; use this if the samples are identical.

Replicates


Replicates are the number of repeated samples. If you have duplicates of a sample on the microplate then you select '2' and whether they are labeled in the horizontal or vertical direction.

Methods of Labeling

1. Select the appropriate content from the contents box and then double click on each well of that type. Select in the index whether the samples should be labeled with increasing numbers or with the same number.
2. If the samples are in successive rows or columns, select increase if the samples should be labeled with consecutive numbers, or choose constant if they are continuous replicates. Click on the first well with the left mouse key and drag across the wells containing the samples, standards, controls or blanks.
3. If a row or column contains the same contents (samples, standards, ...) click the row letter or the column number and all wells of that row / column will be labeled.
4. To fill the entire microplate select the appropriate content (samples, standards, ...) and click on the format number (e.g. '96') in the top left corner.

Note: Exporting the layout grid into a XLS (Excel), text or HTML file is possible after right clicking the grid.

Reading direction

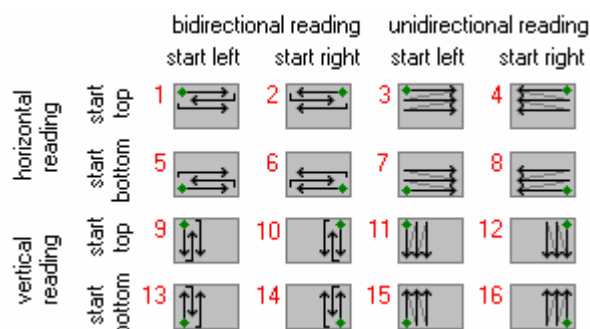
After pressing the reading direction button () , you can choose between **horizontal** or **vertical** reading (horizontal: the plate carrier will move from left to right reading across successive columns, vertical: the plate carrier will move up and down reading in successive rows).

It is also possible to select a **bidirectional** or **unidirectional** reading mode. Example: Using a bidirectional horizontal reading of a full 96 well plate, the reading will continue after reading A1 ... A12 with B12 and then go back via B11, B10, ... to B1. If you choose unidirectional reading, the plate carrier would move to B1 after A12 and continue reading with B2, B3,

It is possible to **start** the reading **from any of the four corners**.

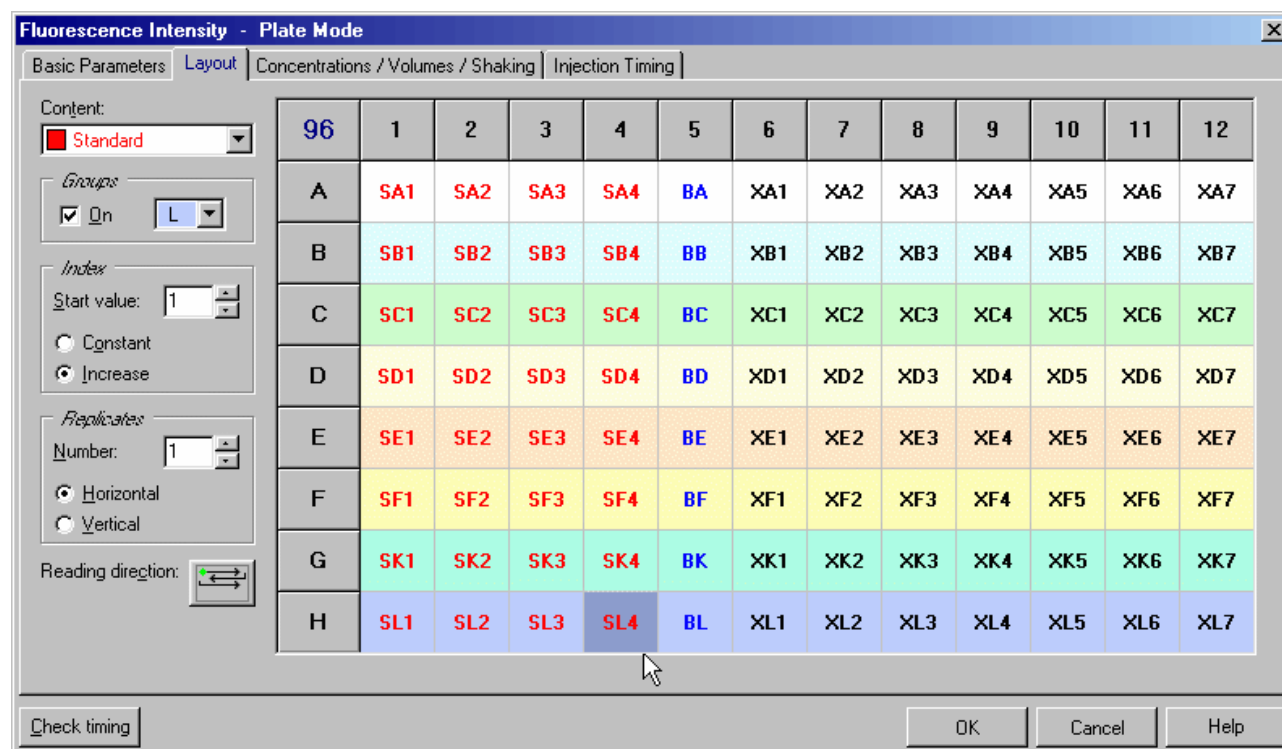
Note: The reading direction has no bearing on the measurements; it is intended to optimize the amount of time it takes to read the plate.

Possible reading modes:



5.6.1 Using Layout Groups

It is possible to use up to 12 independent layout groups (sets of samples, blanks, controls and standards). Each group can be individually blank corrected and it is possible to calculate a separate standard curve for each group (see 10.7 Standard Curve Worksheet).



To activate the usage of groups activate the 'On' check box in the groups box. Use the pull down box next to this check box to select one of the 12 possible groups (A...L). Now enter samples, blanks and standards as described above. The group will be shown in the layout grid using different background colors and by inserting the group letter between content type identifier (e.g. B for blank, S for standard and X for sample) and index.

Notes: When switching on the group mode, all existing layout entries will be converted to group A.

When switching off the group mode, all layout entries not belonging to group A will be erased.

5.7 Concentrations / Volumes / Shaking

To define the standard concentrations and the injection volumes, go to the '**Concentrations / Volumes / Shaking**' sheet. In this sheet you will see a table that lists contents and their reference numbers. Concentration refers to the known concentration of the standards. Volume 1 ... 4 refers to the injection volumes of volume groups 1 ... 4, respectively. The concentrations (of standards) and volumes for injection can be entered manually into each space or by using the auto function.

Concentration

Start concentration:

☒ Factor ☐ Increment ☐ Decrement

Volume [0...350 µl]

Start volume:

☐ Factor ☒ Increment ☐ Decrement

Shaking Options

Mode: ☒ Orbital ☐ Double orbital ☐ Linear

Shaking width (1...7 mm):

⇒ Rounds per minute:

Additional shaking:

Shaking time (1...3600 s):

Content	Concentr.	Volume 1	Volume 2	Volume 3	Volume 4
S1	1	0	50	0	0
S2	10	0	50	0	0
S3	100	0	50	0	0
S4	1000	0	50	0	0
B		0	50	0	0
X1		5	50	0	0
X2		10	50	0	0
X3		15	50	0	0
X4		20	50	0	0
X5		25	50	0	0
X6		30	50	0	0
X7		35	50	0	0
X8		40	50	0	0
X9		45	50	0	0

Pump to use: Pump 1 Pump 2 - -

Pump speed [µl/s]: 310 200 310 310

Shaking time [0...300 s]: 5 0 0 0

The **Auto function** can be used to define the concentrations and volumes without entering them manually. Define the start value and select Factor, Increment, or Decrement and type in a factor. Then click with the **right mouse button** on the table headline 'Concentration' or 'Volume 1' ... 'Volume 4'. The calculations are done automatically. You can also select a specific set of wells by finding the first well and clicking and scrolling down with the right mouse button or using the cursor keys [↓] and [↑] together with [Shift].

Concentration

The auto function will automatically calculate the concentrations using the given start concentration and a number to be multiplied (factor), added (increment) or subtracted (decrement). Click on '**Concentration**' at the top of the table or select a range of standards with the **right mouse button** and the calculations will automatically be entered.

Volume

Enter the starting injection volume. Indicate whether the injection volumes for the wells increase or decrease by a certain amount. Leave the factor as 1 if the volume is the same for every well. With the **right mouse button**, click the desired field in the table title row ('Volume 1' for volume group 1) and the volumes are entered automatically in all columns of this row, or select the desired columns using the right mouse button. It is not necessary to use all volume groups consecutive (you can for example use only volume 2 and 4).

You can enter the volumes in steps of 0.5 μl . If you prefer steps of 0.166 μl (1/6 μl = minimum step of the injectors) add the following line to the [Configuration] section of the 'FLUOstar OPTIMA.ini' file: MinVolumeStep=6, for minimum steps of 1 μl use MinVolumeStep=1. You will find this configuration file in the OPTIMA main installation directory, usually '~\Program Files\BMG\OPTIMA\'.

Note: Exporting this table into a XLS (Excel), text or HTML file is possible after right clicking on the content column of the table.

Pump to use

Select which pump should be used to inject the defined volume(s). It is possible to use the same pump in different volume groups.

Pump speed

The pump can dispense at different speeds. The injection speed is defined in μl / second. The default speed is 310 μl / second which should be appropriate for most assays. There are pre-defined speeds available by using the pull-down menu.

Factors determining pump speed:

- Viscosity:** Liquids, such as water and buffer, can be dispensed at higher speeds. For highly viscous solutions you should use a slower speed to ensure higher precision and lower the risk of air bubbles.
- Cells:** Solutions containing cells should have a lower speed because it causes less stress on the cells.
- Volume:** Higher speeds are necessary for small volumes (below 3...5 μl) to ensure the best performance.

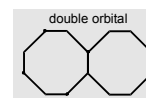
Shaking time

If you want shaking after an injection, add the desired shaking time here. It is possible to define different shaking times for different volume groups. The shaking width and shaking style (orbital or linear) will be defined for all volume groups (and for possible additional shaking) in the 'Shaking Options' group box on the left side of this sheet.

Shaking Options

Shaking mode

- Orbital:** Shaking mode is circular; mixing is more complete, especially around the edges of the microplate.
- Double orbital:** The shaking function is performed as orbital movement. The plate carrier makes a figure eight movement.
- Linear:** Shaking mode is from right to left.



Shaking width

The width (linear) or diameter (orbital) of the shaking motion. The range is 1 mm to 7 mm. The width depends on several conditions:

- Plate format:* For plates with larger wells, such as 6 or 24 well plates, you can use a larger diameter (slower speed) while you should use a smaller diameter (faster speed) for plates with smaller wells.
- Samples:* If the wells contain cells, the diameter should be larger so the force of the shaking does not stress the cells. For viscous solutions, you should also use a larger shaking diameter.
- Volume:* A smaller diameter is suggested for smaller volumes.

Additional shaking

If you want additional shaking (other than shaking after injection), you can choose in plate mode between shaking before or after each cycle or before / after the first cycle. In addition, it is possible to select shaking after inject cycle(s). Opposite to the shaking definable for every volume group (see above), this shaking does not occur immediately after each injection. It will occur after all injections of a cycle have been completed (When using a full 96 well plate this shaking does occur only once and not 96 times.).

In well mode, additional shaking is only possible before plate reading.

Shaking time

The duration of time for additional shaking is defined in seconds. The maximum shaking time is 300 seconds (5 minutes).

Limitations for Injection

The maximum amount per well (Volume 1 + Volume 2 + Volume 3 + Volume 4) is 500 µl (6, 12, 24 and 48 well plates), 350 µl for 96 well plates and 100 µl for 384 well plates respectively. Injection is not possible in 1536 well plates.

Note: It is necessary to prime all used pumps before performing a test with injections (see chapter 6.1 Priming).

Shaking after Injection without Injection

If you need shaking at a certain time, but no injection at this time, you can use any of the four volume groups. Please set all injection volumes in the table column belonging to this group to 0, but select a pump (which will not be used). You can then specify the shaking duration at the bottom of the table. The start time of this shaking procedure can be defined under 'Volume group X injection start time' in the 'Injection Timing' sheet (see chapter 5.8).

5.8 Injection Timing

5.8.1 Injection Timing – Plate Mode

Note: Only the input elements for volume groups, where a pump is defined in the corresponding layout definition, are available (see chapter 5.7).

Injection Cycles

Volume group 1 injection cycle

The cycle in which the injection of volume group 1 will be performed. The defined cycle number cannot be greater than the number of cycles defined.

Volume group 2 injection cycle ... Volume group 4 injection cycle

Same for volume group 2 ... 4.

Equidistant kinetic cycles

If you use this option, the cycle time for all cycles is as long as the cycle with the latest / longest injection or measurement requires – otherwise the time for all cycles without injection can be smaller.

Start Times

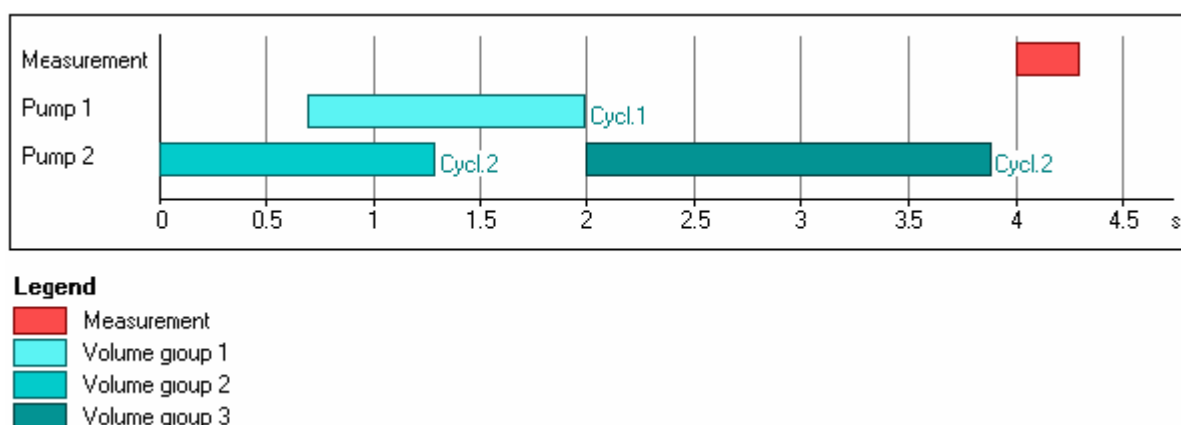
Using the Start Times box, it is also possible to meet special requirements regarding timing on one well in a slow kinetic. The start of the injection(s) or of the measurement (in Basic Parameters or Kinetic Windows sheet) can be delayed up to 1200 seconds on each well after the time when the well reaches the measurement position and the positioning delay is over.

Volume group X injection start time

Defines the time when the injection of volume group 1, 2, 3 or 4 is initiated, relative to the time when the measurement position is reached, plus positioning delay.

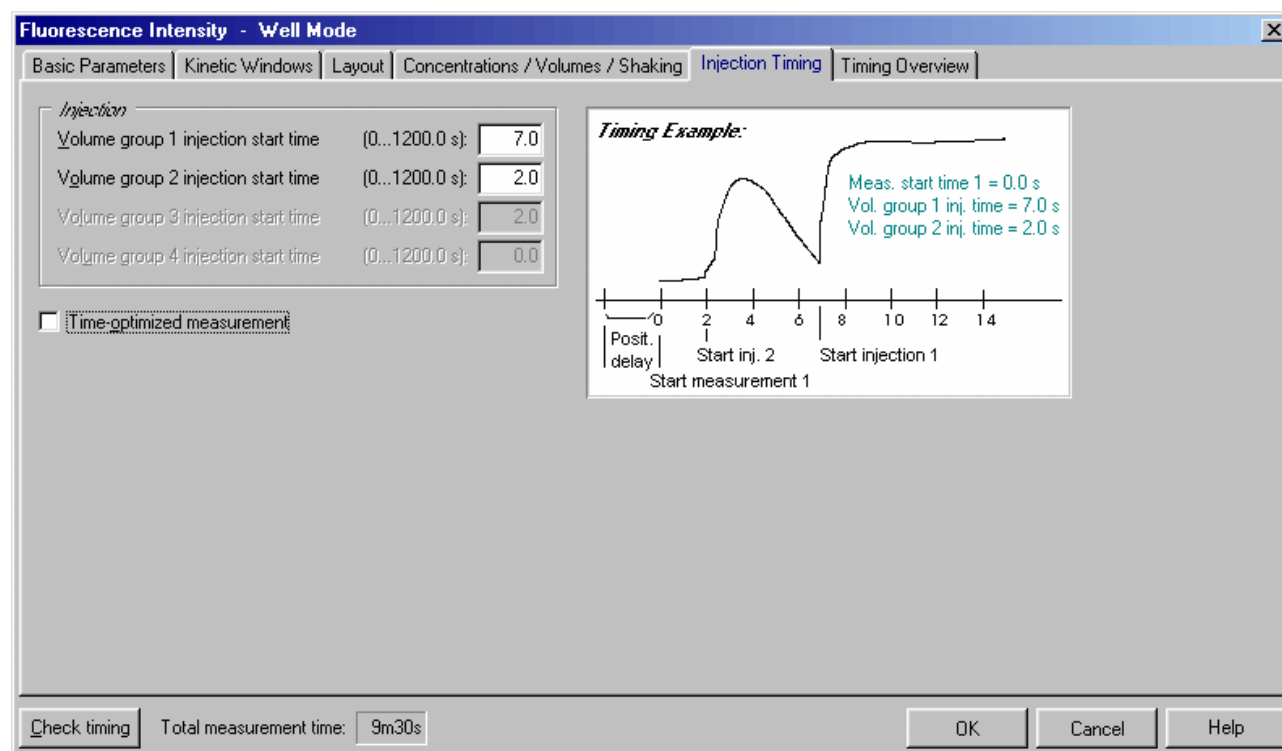
Example: The following scenario would be possible with this method:

- Inject the volumes defined in volume group 1 in the first cycle at 0.7 s
- An incubation time of 10 minutes could be realized by setting the cycle time of the first cycle (kinetic window 1) for 600 s.
- Inject the volumes from group 2 in the second cycle (10 minutes later) at 0 s and the volumes from group 3 in the same cycle at 2 s (which then will take place just after the volume group 2 injection).
- The measurement could be started at 4 s. This will then be directly after injection 3 (the measurement will start in all kinetic cycles at the same time).



5.8.2 Injection Timing – Well Mode

Click on the 'Injection Timing' tab in order to define the injection times:

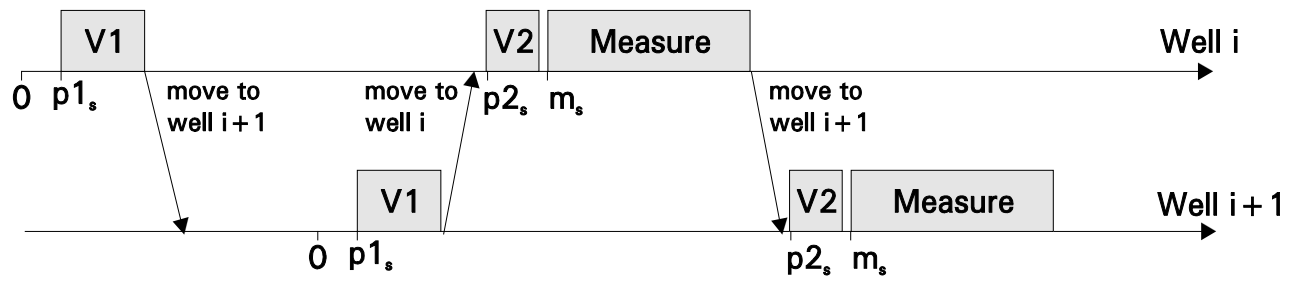


Volume group X injection start time

Defines the time the injection for volume group 1, 2, 3 or 4 is initiated, relative to the time when the measurement position is reached, plus positioning delay. The time can be before the beginning of the measurement (Measurement start time > Volume group X injection time), it can be during or after the measurement. You can only define the injection time for those volume groups, where volumes are defined and a pump is selected in the 'Concentration / Volumes / Shaking' sheet.

Time-optimized measurement

If a kinetic test run with time optimization is defined, then two consecutive wells are processed at the same time. This mode is especially useful when there is a long incubation time between e.g. an injection and the measurement. An injection into a well is, in this case, immediately followed by an injection into the next well. Then the first well is measured followed by the second well. By injecting into the next well in the sequence, the incubation time of that well is underway during the measurement of the first well. The ability to use Time-optimized measuring is contingent on the injection and measurement start times, as well as on the duration of each action. This mode is only possible when there is a time lag between different actions; this delay time must be longer than the time necessary to perform all actions.



Example for well mode test with time optimization

- V1 Injection of volume 1
- V2 Injection of volume 2
- $p1_s$ Volume group 1 injection start time
- $p2_s$ Volume group 2 injection start time
- m_s Measurement start time

5.9 Multichromatics

When you choose to analyze more than one fluorophore per well (well mode or plate mode), you must define the filter combinations for each fluorophore. First enter the number of fluorophores to be analyzed ('Basic Parameters' sheet) and then click the arrow key or the 'Multichromatic' tab. A new sheet will appear:

Number	Excitation filter	Emission filter	Gain (0...4095)	Integr. start (0...1510 µs)	Integr. time (10...1510 µs)
1	320	405	1000	0	12
2	390	460	800	0	12
3	485	460	950	0	12
4			0	0	12
5			0	0	12
6			0	0	12
7			0	0	12
8			0	0	12

Check timing Total measurement time: 33m20s OK Cancel Help

Now you can use the pull-down menu in each row to define the filters. The filters shown in the pull-down menu are listed as they are defined in the 'Setup | Filter' section (see chapter 4.1.4 Filters).

It is possible to define the gain for each filter combination. The gain is the amplification of the signal in the PMT. This function allows you to optimize the sensitivity for each assay. Enter the gain manually (from 0 to 4095) or perform an automatic gain adjustment for each filter combination before the measurement (see chapter 7.4 Gain Adjustment).

5.9.1 Multichromatics in Time-Resolved Fluorescence

Using the Multichromatics function, you can determine the optimal integration time for a time-resolved assay. Using one time-resolved fluorophore, you can define the same filter combinations for all 8-filter settings and define 8 different integration times. You can then compare the data in the evaluation part to find the optimal time for the fluorophore.

For example, if you were using Eu^{3+} , you would define all 8-filter settings as TR-EX and TR-EM. You can keep the integration time constant and vary the integration start time in order to find the optimal start time. You can then keep the integration start time constant and vary the integration time to find the optimal measurement time.

Fluorescence Intensity - Plate Mode

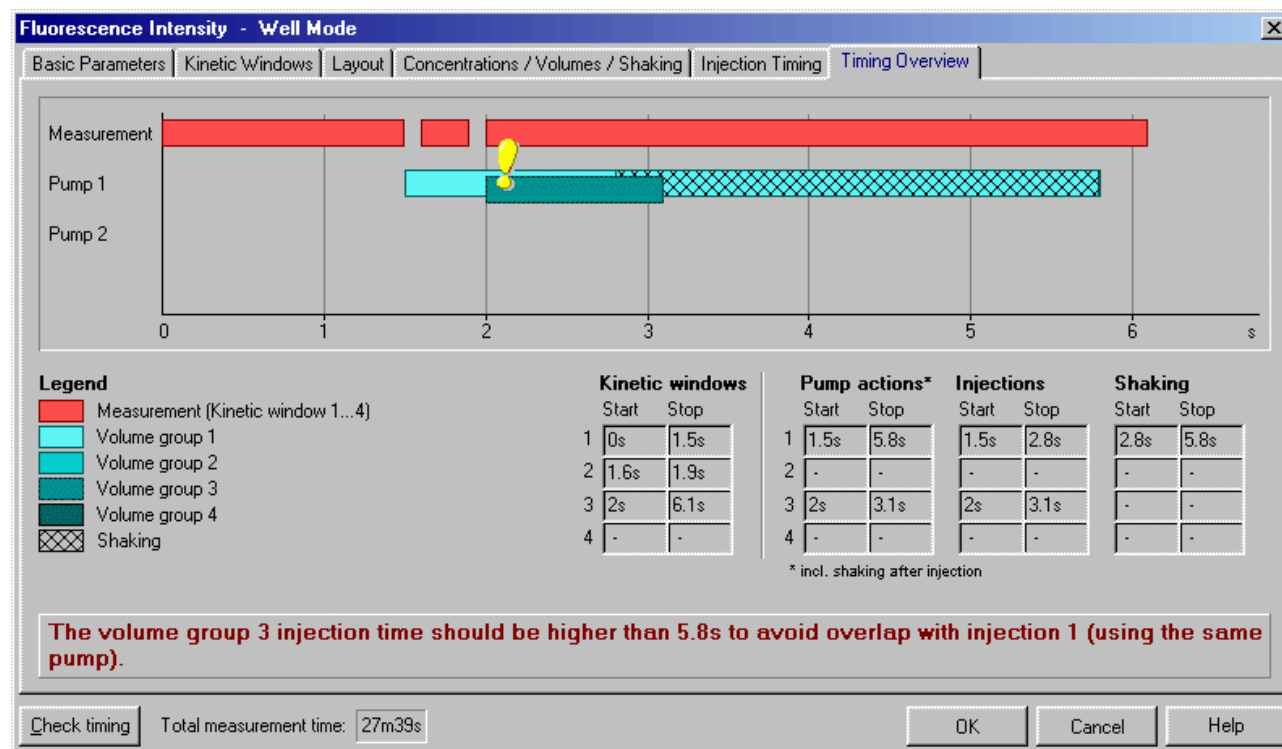
Basic Parameters | Layout | Concentrations / Volumes / Shaking | Injection Timing | **Multichromatic** | Timing Overview (One cycle)

Number	Excitation filter	Emission filter	Gain (0...4095)	Integr. start (0...1510 μs)	Integr. time (10...1510 μs)
1	trex	trem	0	400	100
2	trex	trem	0	400	200
3	trex	trem	0	400	300
4	trex	trem	0	400	400
5	trex	trem	0	400	500
6	trex	trem	0	400	600
7	trex	trem	0	400	700
8	trex	trem	0	400	800

Total measurement time: 3m59s

5.10 Timing Overview

After clicking the 'Check timing' button (lower left corner of the test protocol definition window), the following sheet will become available:



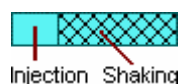
In this sheet, you can see a graphic overview of the measurement and all injections for one well. (For plate mode tests, all actions are displayed in the timing overview of one cycle. The displayed injections may occur in different cycles, as defined under 'Injection / Timing'. The cycle used will be listed behind each injection bar.)

If there is a timing problem, e.g. overlapping injections using the same pump this will be marked by an exclamation mark and a description will inform you about the origin of the timing problem. X

In addition, you will see tables containing the timing for measurements and the injection times. If you use the 'Shaking after injection' option (definable in the 'Concentrations / Volumes / Shaking' sheet), you will see these times as well.

Note: The pump action times listed include time for shaking.

An injection action can contain different steps:



Injections are not possible during shaking.

5.11 Absorbance Tests

Most test parameters are similar to those listed in fluorescence mode (see chapter 5.3 Plate Mode Fluorescence Tests or 5.4 Well Mode Fluorescence Tests). You can still choose Well mode or Plate mode.

Absorbance - Plate Mode

Basic Parameters | Layout | Concentrations / Volumes / Shaking | Injection Timing | Timing Overview (One cycle)

Test name: ABS PLATE

Microplate: BMG LABTECH 96

General Settings

Positioning delay (0.0...1.0 s): 0.5

☐ Flying mode

No. of kinetic windows (1...4): 1

Filter Settings

No. of multichromatics (1...8): 1

Excitation filter: A-492

Emission filter: empty

Kinetic Window 1

No. of cycles (1...250): 1

Measurement start time (0...1200.0 s): 0.0

No. of flashes per well and cycle (0...200): 20

Cycle time (1...10000 s): 28

Well Scanning: None

Minimum cycle time 1: 28 s

Pause before cycle (0...1): 0 for 0 seconds

Comment

Check timing Total measurement time: 28 s

OK Cancel Help

Filter settings

Important parameter for absorbance mode is the filter setting. The absorbance filter should be in a position on the excitation side. The emission side should have a position defined as 'empty'.

No. of flashes

In absorbance mode the number of flashes should be defined as at least 20 to minimize the deviation from flash to flash.

Positioning delay

The positioning delay should be defined as at least 0.5 seconds.

Multichromatics

Define up to 8 filter settings for multiple absorbance analysis. Select 280 and 260 excitation (absorbance) filters for DNA absorbance.

5.12 Luminescence Tests

Luminescence can be measured in plate or well mode. Most parameters are similar to fluorescence protocols (see chapter 5.3 Plate Mode Fluorescence Tests or 5.4 Well Mode Fluorescence Tests). Multichromatic measurements are possible. If the reader is equipped with two luminescence measurement channels simultaneous dual emission measurements are also possible.

Luminescence - Plate Mode

Basic Parameters | Kinetic Windows | Layout | Concentrations / Volumes / Shaking | Injection Timing | Timing Overview (One cycle)

Test name:

Microplate:

Optic:
☒ Top optic ☐ Bottom optic

Comment

General Settings
 Positioning delay (0.0...1.0 s):
 No. of kinetic windows (1...4):

Filter Settings
 No. of multichromatics (1...8):
☐ Simultaneous dual emission
 Emission filter: Gain (0...4095):

Well Scanning

Time to normalize the results (0.02...100 s, 0=off):

Minimum cycle time 1:

Pause before cycle (0...11): for seconds

Total measurement time:

Positioning delay

At least 0.2 seconds are recommended for luminescence tests.

Measurement interval time

Defines the measurement time (defines how long the light is measured). By increasing this time, you will get higher (and usually more accurate) measurement values.

Interval time (only in well mode)

You can define the duration of each interval from 20 ms to 100.0 seconds. When the reader is switched on, you can click the 'Check timing' button and the interval time is automatically validated by the reader. If you do not use multichromatics, the minimum interval time is equal to the defined measurement interval time. If you use multichromatics, the minimum interval time is equal to the defined measurement interval time multiplied by the number of multichromatics plus the time necessary to switch the filters.

If you want to increase the interval time (if you want a delay between the intervals), you can enter a time manually. If you have defined an interval time in any of the kinetic windows which is shorter than the respective minimum interval time, it will automatically be corrected to the shortest possible value. If necessary, the start times for following kinetic windows will also be corrected.

For example, the instrument gives a minimum interval time of 0.1 seconds, but you can change this to 0.2 seconds so that there will be a delay of 0.1 seconds between intervals.

Note: For endpoint tests (tests using only one measurement = one interval), the time value defined here does not matter. In these cases you can also use times larger than 100 seconds, for example when using a well scanning mode with a high number of measurement points.

Optic

If your reader is equipped with auto optic switching you can select for luminescence (and fluorescence) mode protocols the top or bottom optic. If your reader is not equipped with auto optic switching you can select the top or bottom optic manually (see Instrument Manual for details).

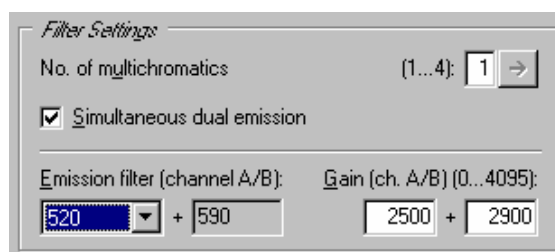
Note: Bottom measurement is not possible in simultaneous dual emission mode.

Emission filter

It is possible to choose an emission filter or use an 'empty' position to measure without filter.

Simultaneous dual emission

If your reader has two luminescence measurement channels (using two PMTs) built in, you can use this option to measure light of two different wave lengths simultaneously. When switching on this option, a second filter box and a second gain box will appear.



By selecting the filter for channel A, the filter for channel B is selected automatically. Due to mechanical constraints for channel B the filter 180° opposite to the A channel filter will always be used. This means, if you are using the filter installed in filter wheel position 1 for channel A, the filter installed in position 5 will be used for channel B.

Time to normalize the results

As the measurement values depend on the measurement interval time, it is usually a good idea to normalize the measurement values. This will allow you to compare the measurement values of test runs using different measurement interval times. This will also allow you to compare the results of different kinetic windows using different measurement times. To switch off the normalization enter 0 here.

Example: When you set the *Time to normalize the results* to 1.0s (to get the results in counts per second) and use a *Measurement interval time* of 0.1s the raw measurement values will be multiplied by 10.

5.13 Fluorescence Polarization Tests

In fluorescence polarization mode, 2 PMTs (photo multiplier tubes) are used for measuring two different channels. Channel A measures vertically polarized light and channel B measures horizontally polarized light.

Fluorescence polarization can be measured in plate or well mode. Most parameters are similar to fluorescence protocols (see chapter 5.3 or 5.4). Multichromatic measurements and injections are not possible.

Fluorescence Polarization - Plate Mode

Basic Parameters | Layout | Concentrations / Volumes / Shaking | Injection Timing | Timing Overview (One cycle)

Test name:

Microplate:

Comment
This is a polarization test using fluorescein (35 mP).

General Settings

Positioning delay (0.0...1.0 s):

☐ Flying mode

No. of kinetic windows (1...4): →

Kinetic Window 1

Ng. of cycles (1...250):

Measurement start time (0...1200.0 s):

No. of flashes per well and cycle (0...200):

Cycle time (1...10000 s):

Filters and Integration

☒ Standard ☐ Time-resolved

Excitation filter:

Emission filters (Channel A/B): /

Gain (0...4095):

Channel A: Channel B:

Integration:

Start (0...1510 μs): Time (10...1510 μs):

Minimum cycle time 1: Pause before cycle (0...1): for seconds

Total measurement time:

Filters

For polarization measurement, two identical emission filters should be installed in the filter wheel positioned 180° from each other. When you select one filter, the other filter is automatically selected.

Gain

You can specify a gain value for both channels. The necessary gain may differ slightly between the channels.

It is recommended to perform an automatic gain adjustment right before test start (see chapter 7.4.3 Gain Adjustment – Fluorescence Polarization).

No. of flashes

BMG LABTECH recommends 50 for the number of flashes for the most accurate results. All flashes are averaged together for one intensity value.


Positioning delay

The positioning delay in fluorescence polarization tests is recommended to be 1 second, so that the surface of the liquid is stable before measurement.

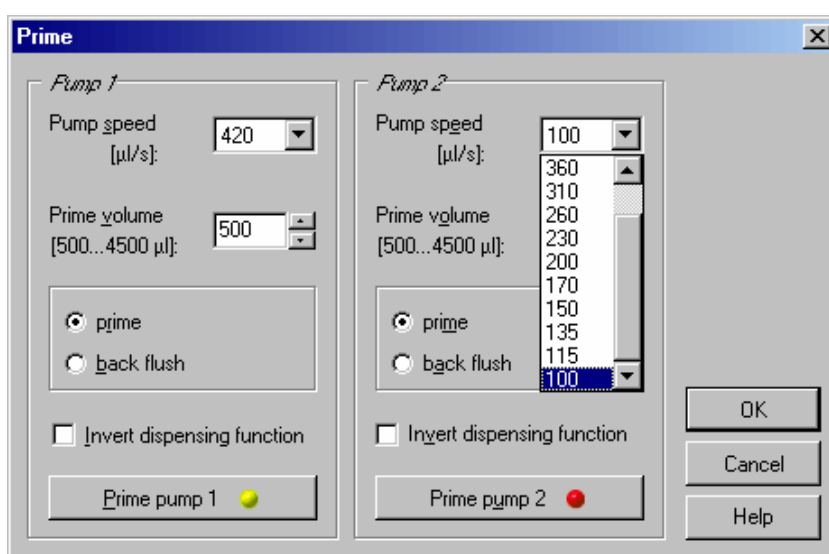
6 Priming / Incubation

6.1 Priming

The reader may have up to 2 pumps. Before starting a test run it is necessary that all used injector pumps are initialized. This can be achieved by using the prime function. The priming procedure needs to be repeated once after each program start. Solutions for injections can be placed inside the reagent box.

To open the prime window, use the  button or choose the menu command '**Measure | Prime**'.

Note: Before priming a pump, the injection needle must be removed from the measurement head to prevent contamination of the instrument.



Pump speed

The pump speed selected here will only be used for priming. The injection speed for test protocols is defined in the 'Concentrations / Volumes / Shaking' sheet of the test protocol definition window (see chapter 5.7).

Priming

It is necessary to fill the tubing with the dispensing solution prior to starting a measurement. Place the tubing in the solution to be dispensed and place the injection needle in a waste container. Do not leave the needle in the measurement head or you will risk contamination of the instrument. Select the prime volume from 500 to 4500 µl. The volume of the syringe is 500 µl, and a prime volume of at least 1000 µl is recommended for priming the tubings and the syringe. A higher volume can be used for washing the tubing after the measurements are complete.

After clicking on a 'Prime pump' button, a message will appear to remind you to remove the injection needle from the measurement head. Repeat the procedure for all pumps you want to use.

Back flush

If the pumps have been in use, it is important to flush out any solutions that could be considered a contamination reagent (non contaminating reagent could be i.e. distilled water or a water / alcohol solution). The back flush feature also allows you to conserve expensive reagents, since the entire syringe can be emptied after use.

Invert dispensing function


The default position of the plunger is at the bottom of the syringe barrel. In this position, the injection starts with the liquid in the syringe barrel being pumped through the tubing and then the syringe is refilled as the plunger comes back down.

It is possible to change the order of the plunger's movements by clicking on '**Invert dispensing function**'. The plunger will begin at the top of the syringe barrel and the syringe barrel will, in this case, first fill and then dispense through the tubing.

The invert function is helpful in cases where the solutions have particles or cells that may settle at the bottom of the syringe barrel, and, therefore, will not be dispensed uniformly.

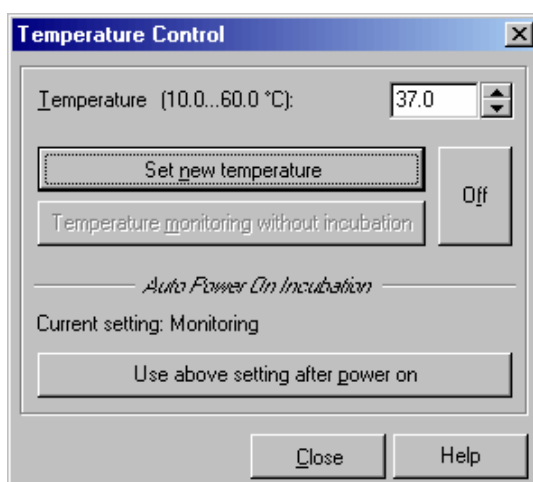
6.2 Incubation

6.2.1 Temperature Control

The incubation can be activated through the temperature button  or by selecting 'Temperature' under the 'Measure' menu.

The temperature range of the incubator is 25°C to 45°C (optional up to 60°C). The chosen temperature must be higher than the ambient temperature. The temperature can be set in 0.1°C increments. The temperature can also be monitored without activating the incubator (see chapter 6.2.2 Temperature Monitoring Feature below).

You can enter the temperature manually or toggle to the desired temperature using the arrow buttons.



Click on 'Incubator on'. The temperature indicator in the toolbar of the control software will be activated. The indicator will be red until the selected temperature is reached, then the indicator turns green.

It is possible to perform a measurement before the target temperature is reached. In the Excel data reduction, the current temperature for each kinetic point during the measurement is displayed in the raw data worksheet.

The 'Incubator on' button changes to 'Set new temperature' if you select a new target temperature during incubation.

6.2.2 Temperature Monitoring Feature

It is possible to monitor the instrument's temperature without using the incubator. The temperature sensor of the incubator will be activated and update the temperature display.

Click the 'Temperature monitoring without incubation' button. The temperature will appear on the temperature display in the control software. When using this function, the indicator color will be cyan. The temperature will also be stored with the measurement data and displayed in the Raw Data Worksheet of the evaluation software (see chapter 10.3).

6.2.3 Auto Power On Incubation

When you click the **'Use above setting after auto power on'**, the currently selected temperature value will be used as the default target temperature for the incubator.

If you have defined a value within the allowed temperature range, the built-in incubator will be automatically switched on the next time the reader is switched on. Using this function, the incubator will be turned on even without starting the OPTIMA software.

By using a target temperature of 00.1°C (selected e.g. by clicking the 'Temperature monitoring without incubation' button), only the temperature monitoring function is switched on by default, the heating plates will not be used.

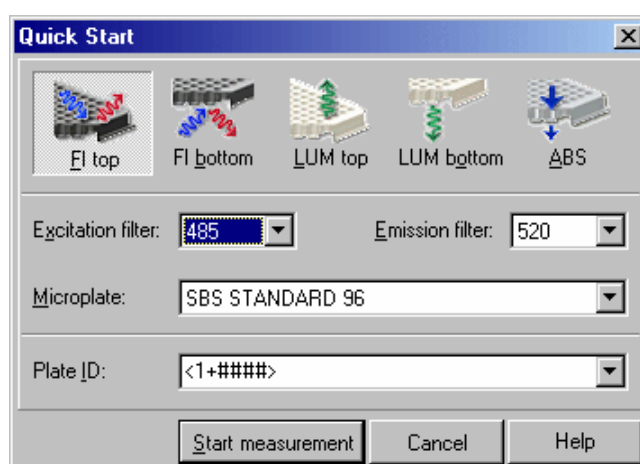
To switch off the auto power on incubation function, select a target temperature of 0°C (either by clicking the 'Off' button or by manually entering this value), and click the 'Use above setting after auto power on' button.

7 Performing a Measurement

7.1 Quick Start

The Quick Start function can be used to measure a plate without defining a test protocol in fluorescence intensity, luminescence and absorbance mode. Using this function the full plate will always be measured as an endpoint test. Please use the standard measurement method based on pre-defined protocols (see chapter 5) if you want to measure only a part of the microplate, if you want to perform a kinetic measurement, if you want to use dual emission / multichromatics, if you need injections or shaking ... or if you want to measure in fluorescence polarization mode. The measurement of 1536 well plates is also not possible using the quick start function.

After using the menu command '**Measure | Quick Start**' or after clicking the  button the following dialogue will appear:



Please select the **measurement method** first by clicking the appropriate button.

Note: If your reader is not equipped with auto optic switching, the method selection is done using the 'Reader Configuration' dialogue (see chapter 4.1.2 Reader Configuration).

The **filter** and **microplate** selection is specific for the method and top/bottom selection done on top of the dialogue.

You have the option to add one **plate identifier**. Here you can use the same special functions as when executing a pre-defined protocol (see chapter 7.3 Plate Identification).

After clicking '**Start measurement**' an automatic gain adjustment will be performed in fluorescence intensity and absorbance modes. In luminescence mode, a fixed gain of 3400 will be used.

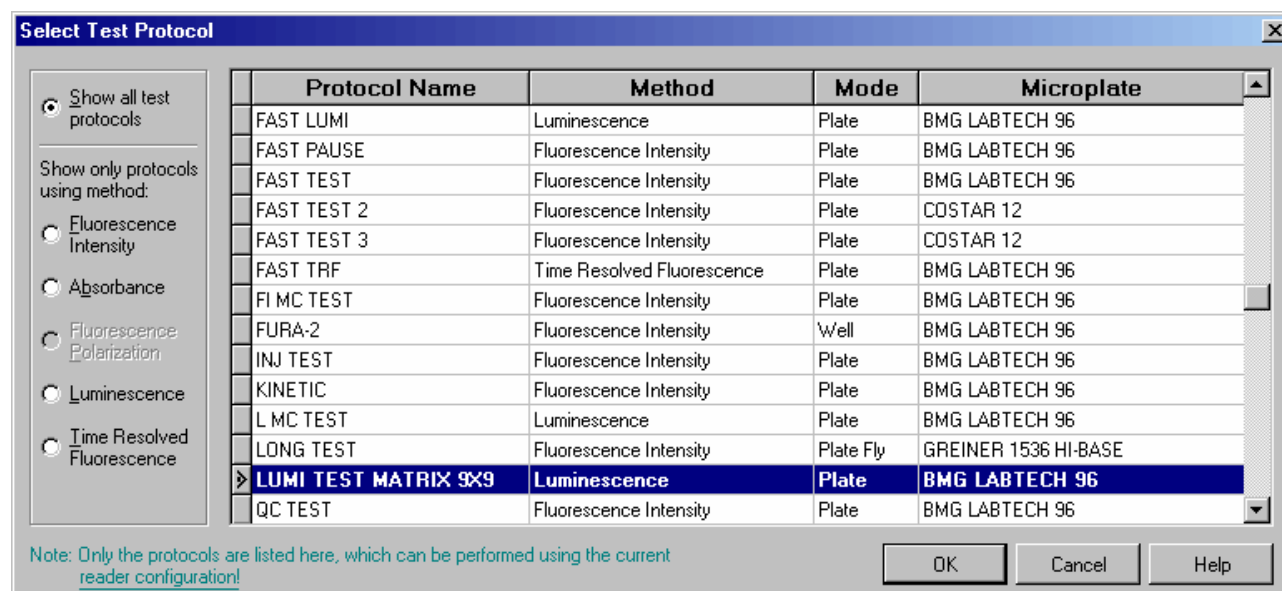
As soon as the measurement itself has been started, you can open the Current State display (see chapter 7.7) to have a look onto the measurement values. You can also setup the program to automatically open the Current State display (see chapter 4.3.2 Additional Options Sheet).

Note: To use the Quick Start function firmware version 1.20 (or newer) needs to be installed.

7.2 Executing Pre-Defined Test Protocols

A selection window displays all the defined protocols which can be performed using the installed optic, if the reader is equipped with auto optic switching.

If the reader is not equipped with auto optic switching you will see here only the protocols using the measurement method currently selected, i.e. fluorescence method. Use the 'Reader Configuration' dialogue to change the measurement method.



By default the protocols are sorted by method first and then alphabetically by name. If you prefer a sorting by name only click the 'Protocol Name' part of the table headline. To switch back to the original sorting click 'Method'. It is also possible to get the list sorted by the mode or the used microplate. Simply click on the 'Mode' or 'Microplate' part of the table headline.

Besides displaying all available protocols you might select to see only protocols using a certain measurement method by clicking one of the radio buttons on the left side of this dialogue box.

Note: As the LUMIstar OPTIMA can only measure using the luminescence method the method selection box will not appear when using this type of reader. This box will also not appear if the reader is not equipped with auto optic switching.

To select the first protocol, beginning with e.g. 'T', simply press the key [T].

Double click on the desired protocol or select the protocol and click '**OK**'. Click '**Cancel**' to return to the main menu.

After selecting the protocol the next window gives you the option to add identifiers to the test, to define dilution factors, to perform a gain adjustment and to start the measurement.

7.3 Plate Identification

After selecting the test protocol a window appears, where you can add identifiers to the test. This is an optional feature that allows you to give the plate specific identifying features such as a title, a description, or a number. Three identifiers with up to 100 alphanumeric characters are possible.

The drop down menu for each identifier allows you to choose several options for the IDs or you can manually type in a description.

ID Options


<protocol> The name of the used test protocol will be entered.

<method> Name of the used method, e.g. 'Fluorescence Intensity' or 'AlphaScreen'.

<1+#> Consecutive numbers for each test run (with the same test name) will be automatically entered. You can manually change the start number in the space 'No. of executed runs since program start.' The number of digits to be used can be changed by adding '#' characters, i.e. using <1+###> 001, 002, 003, etc. will be generated.

To use the number of loop executions in a script mode run instead of the number of executed runs after program start, add a 'B' before the '#', e.g. <1+B####>.

To use the 'Total no. of executed runs' instead of the number of executed runs after program start, add a 'T' before the '#', e.g. <1+T####>.

To use the 'No. of executed runs for the used test protocol', add a 'P' before the '#', e.g. <1+P###>. You can see a Run Statistics after pressing the  button (see section 4.3.5).

Counting down is also possible, use a '-' instead of the '+'.

<A+#> Same concept as consecutive numbers using the alphabet.

Example: <A+####> → AAA, AAB, AAC ...

<date> The date of the test run.

You can specify the date format if you add a format description after “<date:” using yy or yyyy for the year, m or mm for the month and d or dd for the day:

yy	year with two digits (1999 → 99, 2000 → 00)
yyyy	year with four digits
m	one or two digits for the month (January → 1, December → 12)
mm	month with two digits (January → 01)
mmm	abbreviated name of the month (January → Jan.)
mmmm	full name of the month
d	day with one or two digits
dd	day with two digits
ddd	abbreviated name of the day (Monday → Mo.)
dddd	full name of the day
dddddd	date in the format defined as “Short Date Format” under windows (‘Settings Control Panel Regional Settings’)
dddddd	date in the format defined as “Long Date Format” under windows

Example: <date:yyyy_mm_dd>

If you do not specify the format, the date format defined as “Short Date Format” under windows will be used.

<time> Time of the test run.

You can specify the time format if you add a format description after “<time:”:

h or hh	for the hour
m or mm	for the minute
s or ss	for the second
t	time in the format defined as “Short Time Format” under windows (‘Settings Control Panel Regional Settings’)
tt	time in the format defined as “Long Time Format” under windows
am/pm or a/p or AM/PM or Am/Pm	use 12 hours format and show am or pm (a or p...)

Example: <time:hh.mm.ss>

If you do not specify the format, the time format defined as “Long Time Format” under windows will be used.

<ser_number> Serial number of the reader used.

Clear IDs

Delete the plate IDs that are entered.

Get last IDs

Recall the ID settings used for the last test run performed by the user currently logged in.

Automatically enter the previous plate IDs when using same test protocol


If this box is checked, then the same plate identifiers will be entered automatically when using the same protocol. You can still edit the identifiers or use the ‘Clear IDs’ button.

7.4 Gain Adjustment

By clicking the tab for Gain Adjustment, the sheet with the gain settings and the layout defined will appear.

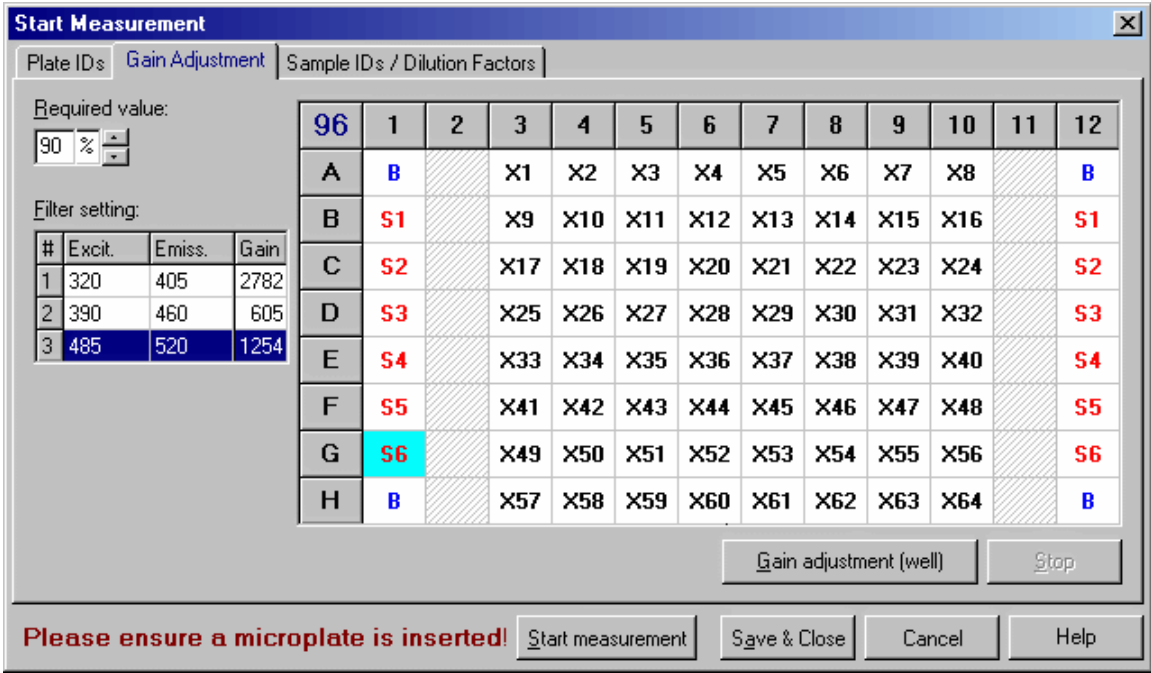
The purpose of a gain adjustment is to optimize the signal amplification so that the results have the maximum sensitivity and dynamic range. The gain is usually performed on the well containing the standard with the highest concentration of fluorophore (highest intensity). This sets the gain so there is no overflow in the higher intensity wells (an overflow means the relative fluorescent units of a well exceeds the maximum range, i.e. 65,000 rfu).

Zoom Feature

For 384 and 1536 well formats, the gain adjustment screen has a zoom function, allowing you to zoom in a certain section of the layout. To zoom in and out of the screen use the  icons.

The gain adjustment window is specific for each configuration. The parameters for different test modes are described on the following pages.

7.4.1 Gain Adjustment – Fluorescence and Luminescence Mode



Start Measurement

Plate IDs | **Gain Adjustment** | Sample IDs / Dilution Factors

Required value:
90 %

Filter setting:

#	Excit.	Emiss.	Gain
1	320	405	2782
2	390	460	605
3	485	520	1254

	96	1	2	3	4	5	6	7	8	9	10	11	12
A	B			X1	X2	X3	X4	X5	X6	X7	X8		B
B	S1			X9	X10	X11	X12	X13	X14	X15	X16		S1
C	S2			X17	X18	X19	X20	X21	X22	X23	X24		S2
D	S3			X25	X26	X27	X28	X29	X30	X31	X32		S3
E	S4			X33	X34	X35	X36	X37	X38	X39	X40		S4
F	S5			X41	X42	X43	X44	X45	X46	X47	X48		S5
G	S6			X49	X50	X51	X52	X53	X54	X55	X56		S6
H	B			X57	X58	X59	X60	X61	X62	X63	X64		B

Gain adjustment (well) Stop

Please ensure a microplate is inserted! Start measurement Save & Close Cancel Help

The filter settings are displayed on the left (in luminescence mode there are no excitation filters, only emission filters). If the protocol is multichromatic, then all filter combinations used will be displayed (you can perform a gain adjustment for each filter set).

Automatic Gain Adjustment for one Well

Select a well for gain adjustment, then click the '**Gain adjustment (well)**' button. The instrument samples the well eleven times in 3 seconds to find the optimal gain. The optimal gain value appears in the box next to the filter settings.

'Stop' will terminate the gain adjustment process.

Automatic Gain Adjustment for the entire Plate

There are occasions when a well gain adjustment is not possible, for example, when the well with the highest intensity is not known. You can then do a gain adjustment on the entire plate. The instrument finds the well with the highest intensity and determines the gain. Click on the blue microplate format number in the top left corner of the layout (i.e. 96 or 384). All wells are selected and the caption of the gain adjustment button changes to '**Gain adjustment (plate)**'. Click this button and the instrument will return a gain value in the box next to the filter settings.

Note: An automatic gain adjustment for the entire plate is not possible for 1536 well plates.

Gain Adjustment for Simultaneous Dual Emission Protocols

When using a simultaneous dual emission protocol the gain adjustment is always done simultaneously for the two channels (A and B). Nevertheless it is possible to define different required (target) values and, therefore, to define a target ratio.

In addition, it is still possible to type in values manually for each channel.

Gain Adjustment Plate is not possible in simultaneous dual emission mode.

The screenshot displays the software's gain adjustment interface. At the top, it shows 'Required value (Ch.A/B):' with two input fields: '80 %' for Channel A and '40 %' for Channel B. Below this is a section for 'Filter setting / channel:' containing a table with four rows and three columns: '#', 'Emiss.', and 'Gain'. The rows are labeled 1A, 1B, 2A, and 2B. The 'Emiss.' column contains values 520, 590, 460, and 510 respectively. The 'Gain' column contains values 2998, 2047, 3059, and 1693. The row for 2A is highlighted in blue. At the bottom, there are two input fields for 'Raw results:' labeled 'Channel A' and 'Channel B', with values 1599710 and 798280 respectively.

#	Emiss.	Gain
1A	520	2998
1B	590	2047
2A	460	3059
2B	510	1693

Channel A: 1599710 Channel B: 798280

7.4.2 Gain Adjustment – Absorbance Mode

In absorbance mode, the gain is determined when the instrument measures the full transmission of light (0% absorbance). The gain is dependent on the filter wavelength used.

During the gain adjustment procedure, the plate carrier moves out of the way of the optics and the instrument samples the full transmission of light.

Beginning with firmware version 1.20 the gain adjustment in absorbance mode is done automatically as part of the measurement, therefore, you will not see this sheet if your reader uses firmware 1.20 or newer.

The screenshot shows the 'Start Measurement' dialog box with the 'Gain Adjustment' tab selected. The 'Required value' is set to 80%. The 'Measurement value for 0% absorption (100% transmission)' is 51382. The 'Filter setting' table is as follows:

#	Excit.	Emiss.	Gain
1	A-492	empty	395
2	A-405	empty	327
3	A-620	empty	409

At the bottom, there is a red warning message: 'Please ensure a microplate is inserted!'. Below this are four buttons: 'Start measurement', 'Save & Close', 'Cancel', and 'Help'. In the top right corner of the dialog, there are two buttons: 'Gain adjustment' and 'Stop'.

The filter setting is displayed in the gain adjustment window; there should be a filter for excitation and the emission side should usually say 'empty'. If you use a multichromatic protocol perform a gain adjustment for all filter sets used. Please select the desired filter pair, before clicking the 'Gain adjustment' button.

Required value for absorbance is recommended at 80%.

'Stop' will terminate the gain adjustment process.

7.4.3 Gain Adjustment – Fluorescence Polarization

The gain adjustment window for fluorescence polarization protocols looks a little bit different.

Start Measurement

Plate IDs | **Gain Adjustment** | Sample IDs / Dilution Factors

Target mP: (0...500)

Use advanced options >>

Gain

Channel A:

Channel B:

	96	1	2	3	4	5	6	7	8	9	10	11	12
A		S1	S1				S1	S1				S1	S1
B		S1	S1				S2	S2				S1	S1
C							S3	S3					
D							S4	S4					
E							S5	S5					
F							S6	S6					
G		S1	S1				S7	S7				S1	S1
H		S1	S1				B	B				S1	S1

Raw results: Channel A: Channel B:

Gain adjustment Stop

Please ensure a microplate is inserted! Start measurement Save & Close Cancel Help

There are two PMTs (photo multiplier tubes) involved in polarization, one for channel A (vertical light) and one for channel B (horizontal light). As with standard fluorescence, you want to perform the gain adjustment on the well with the highest concentration of free fluorophore. Due to the fact that the relationship between channel A and B determine the fluorescence polarization value, it is important that the two channels are optimized for the best results. Select the desired well and click on '**Gain adjustment**'. In this mode it is not possible to perform a gain adjustment on the entire plate.

The gain settings for both channels needed to reach the defined Target mP value (see below) are determined during the automatic gain adjustment. For the adjustment procedure a required value (target raw measurement value) of 10 percent of the measurement range is used by default. This value should work fine for most assays. If you want to use a different required value click on the '**Use advanced options**' button. An input field for this value will appear:

Target mP: (0...500)

Required value: %

<< Use standard options

The value you enter in this window is used as a target value for channel A. An optimal value for channel B is calculated automatically, based on the entered Target mP (see below).

Target mP

It is recommended to use free fluorescein for gain adjustment, but it is possible to use other fluorophores or labeled biomolecules. The theoretical polarization value for free fluorescein is 35 mP. Enter this value in the field 'Target mP'. In case you use a different fluorophore or a labeled biomolecule, you need to use the theoretical mP value of this molecule as 'Target mP'.

Now, when you perform a gain adjustment on the well with the highest concentration of free fluorophore, the result of that well will become equal to the target value entered (so for fluorescein the polarization value of the well is equal to 35 mP).

Custom Target mP value

If you are using a different fluorophore or a fluorescein-labeled peptide (bound fluorescein), you must change the 'Target mP' value. If you do not know what this value should be, you can start with, for example, 35.

Perform a gain adjustment as described above.

Start the measurement. Use the current state display (see chapter 7.7) or the OPTIMA evaluation software (see chapter 10) to check the measurement values. If the results are negative, then you need to increase the Target mP value.

The results using a randomly selected target value are not absolute numbers, but it is still possible to see the binding curve and the inflection point from the polarization data.

7.4.4 Required Value

The measurement range for fluorescence is 0 to 65,000. If an overflow occurs, the measurement value will be 65,000. The measurement range for luminescence depends on the measurement interval time (max. 200,000,000 for 100 s). To avoid an overflow, it is necessary to set that the gain so the values stay within the range of the instrument.

In the top left corner of the gain adjustment screen is a '**Required Value**' number. This value refers to the percentage of the maximum value of the dynamic range. The default value is 90%. Therefore, when you perform a gain adjustment on the well with the highest concentration of fluorophore, the result will be 90% of the maximum fluorescence value, i.e. $65,000 \times 0.90$. This prevents an overflow in case of deviation of the intensities over the plate.



If the measurement is an endpoint test, then the required value can remain at the default value of 90%.

For a kinetic assay, an injection can increase the intensity values over the entire plate. If you do not know the final results you should lower the required value to 50% (or less) of maximum value in order to have enough space for higher intensity values and to prevent an overflow.

7.5 Sample IDs / Dilution Factors

You may enter identification values for each well in this sheet. These values will be shown in the Sample IDs sheet and in the Evaluation sheet (when 'Sample IDs' is selected in the pull down box) of the OPTIMA evaluation part. It is also possible to store these IDs together with the measurement results in an ASCII file (see chapter 4.3.4 ASCII Export Function).

In addition, it is possible to define dilution factors for all wells which do not contain blanks or standards. The dilution factor will be taken into account when calculating the unknown concentrations (see chapter 10.7 Standard Curve Worksheet).

Note: If you are using replicates, the sample ID and the dilution factor you entered for one well will be used for all replicates.

Well	Content	Sample ID	Dilution
A1	XA1	Sample 008/1	1
A2	XA2	Sample 008/2	1
A3	XA3	Sample 008/3	1
B1	XB1	Sample 009/1	2
B2	XB2	Sample 009/2	2
B3	XB3	Sample 009/3	2
C1	XC1	Sample 010/1	2.5
C2	XC2	Sample 010/2	2.5
C3	XC3	Sample 010/3	2.5
D1	XD1	Sample 011/1	5
D2	XD2	Sample 011/2	5

Options

- ☒ Automatically enter sample IDs previously used with this protocol
- ☒ Export / Print also lines without sample ID entry
- ☒ Include dilution factors when exporting / printing

Dilution Factor Auto Fill Function

Start dilution:

☒ Factor ☐ Increment ☐ Decrement

Use the right mouse button to select the cells to be filled out in the dilution factor column of the table or right click the caption for all cells.

Please ensure a microplate is inserted!

Start measurement Save & Close Cancel Help

Clear IDs

Pressing this button will clear all ID fields.

Get last IDs

By clicking this button you can recall the last IDs used for the selected test even if you did not use the option 'Automatically enter sample IDs previously used with this test protocol' (see below).

Table sort order

You can choose between sorting for rows, columns or well content. The selected sort order will also be used for printing or exporting the sample ID / dilution factor list.

Print ID list

You can print out the ID list on any available printer.

Import IDs

Import sample IDs / dilution factors from a text file (created using the export function or using a text editor like Notepad) or from a XLS (Excel format) file.

Format for Sample ID text files:

In the first column of such a file, there is the well number (e.g. 'A1'), the sample ID for this well should be in the same line beginning with position 12. If you want to include dilution factors, these values should be entered beginning at position 44.

Format for Sample ID Excel files:

	A	B	C	D
1	Well	Content	Sample ID	Dilution
2	B2	X1	Probe 007.1	2
3	B3	X2	Probe 007.2	2
4	B4	X3	Probe 007.3	0.5
5	B5	X4	Probe 007.4	1
6	C2	S3	Standard 50%	1
7	C3	S4		1
8	C4	B	Blank	1
9	C5	B	Blank	1

The well order in the files does not matter. If there are no dilution factors included, the dilution factors entered so far will not be changed.

Export IDs

Export current sample IDs (and if the appropriate option described below is selected also the dilution factors) into a text or XLS (Excel format) file.

Options

Automatically enter sample IDs previously used with this test protocol

If this option is checked, then the last used sample IDs for the selected test automatically will be reentered as default values.

Export / Print also lines without sample ID entry

If this option is checked all lines will be exported or printed, otherwise only the lines where a sample ID was entered would be exported or printed. When exporting into an Excel file, all lines will always be exported.

Include dilution factors when exporting / printing

If this option is checked the dilution factors will also be exported or printed.

Dilution Factor Auto Fill Function

If the dilution factor is equal for a high number of wells, you can use the auto fill out function. Enter the desired dilution factor inside the group box 'Dilution Factor Auto Fill Function'. Then select the corresponding cells in the table by right clicking a cell or by selecting a range of cells using the right mouse button. To use this factor for all wells (besides standards and blanks) right click the caption of the dilution column of the table. You might also specify a factor or increment / decrement value to automatically increase / decrease the dilution values.

Note: Exporting the table into a HTML file (in addition to the XLS or ASCII format) is possible after right clicking the table.

The settings from this window are user specific, therefore each user can select his preferences independently.

7.6 Measurement

After entering IDs / performing the gain adjustment, you have the following options:

Start measurement: Begins the measurement using the defined gain.

Save & Close: Saves the results of the gain adjustment, the ID settings and the dilution factors and return to the main menu without performing a measurement.

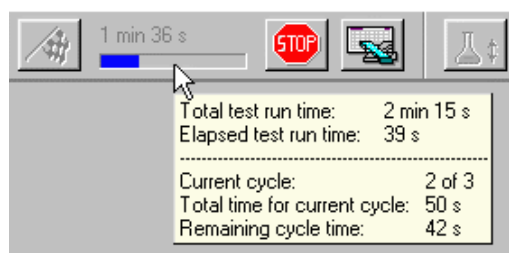
Cancel: Closes this window without saving the gain adjustment results and ID settings and without starting the test.



7.6.1 Time Duration

On top of the main program window, there is a time gauge indicating the elapsed time of the test. The remaining time will be displayed above the time gauge. It is updated dynamically during the measurement.




If you move the mouse cursor to the time gauge, a small window with additional timing information will appear.



If you perform a plate mode kinetic measurement the quick start button () will change into a pause button (). This button has the same function as the menu entry 'Measure | Pause After Current Cycle': the active test run will pause after finishing the current cycle and a pause / continue window will appear (see chapter 5.3.1).


7.6.2 Stopping the Test Run

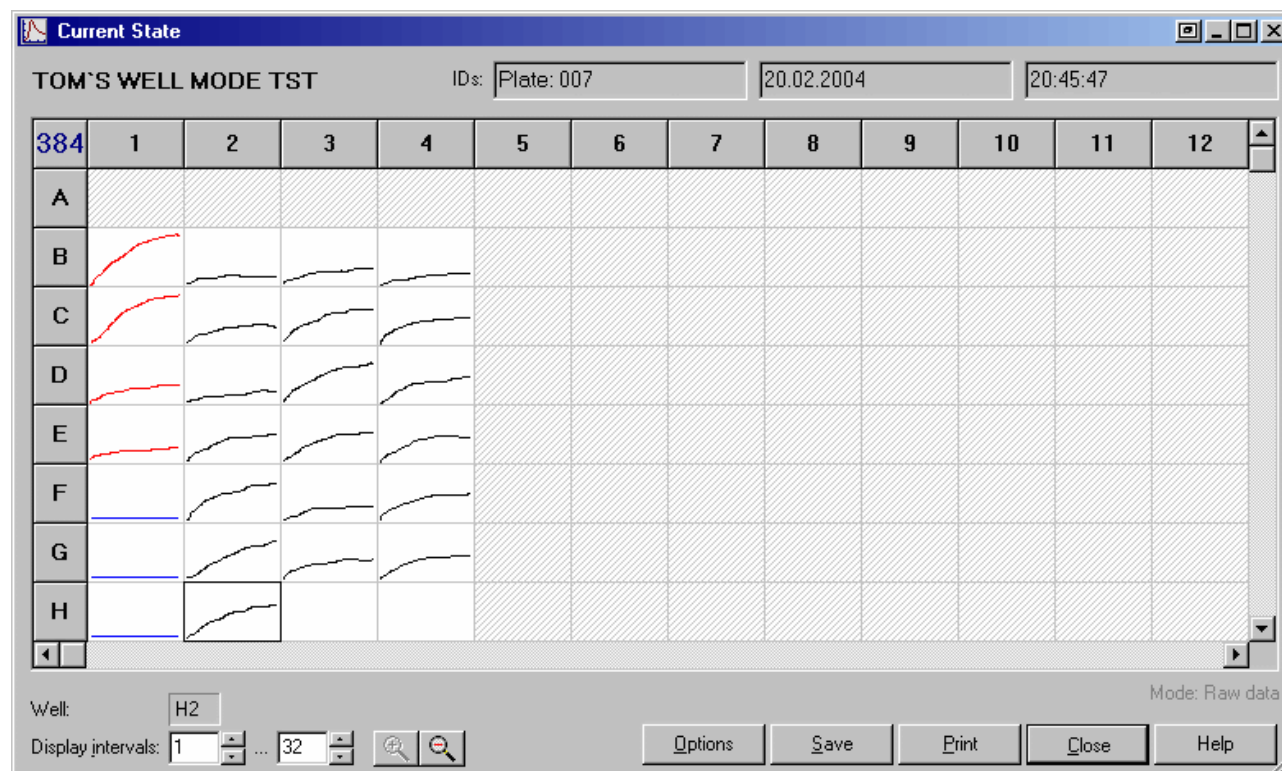
It is possible to terminate a measurement procedure after it has started by pressing the stop button  or by using the menu function 'Measure | Stop Now'. If there is already measurement data available, a dialog box will ask if you want to save the incomplete data.

In script mode (see chapter 8), you can use this function to immediately stop any activity.

7.7 Current State Graphics


7.7.1 Current State Overview

When the measurement has started, the 'Start Measurement' button will change to the 'Current State Graphics'  button. This function allows you to view raw data graphically as the measurement occurs.



The Current State window displays a grid of the microplate format. The measurement results can be displayed as curves: Each measurement value is represented by a dot and you can see the relative position of the values. You can choose between a curve of points or lines. It is also possible to display the measurement values of the last measured cycle / interval numeric or use colors for a fast overview (see chapter 7.7.2 Current State Options).

It is possible to change the size of this window. The software will remember the last used size and position user specific.

When using 384 or 1536 well plates it is possible to zoom in and out using the zoom buttons .

- Current cycle:** Shows the number of the cycle currently being measured (only for plate mode tests).
- Well:** Shows the name of the current well (only for well mode tests).
- Display cycles / intervals:** Kinetic points that will be displayed. This can be changed manually, otherwise the default number of cycles / intervals as defined in the test protocol will be used.
- Options:** Opens the 'Current State Options' dialog box.
- Timing:** Opens the 'Timing Overview' window.

- Save:** Saves the data as bitmap (windows BMP format or JPEG format).
- Print:** Prints the screen on any available printer.
- Close:** Closes the current state window.

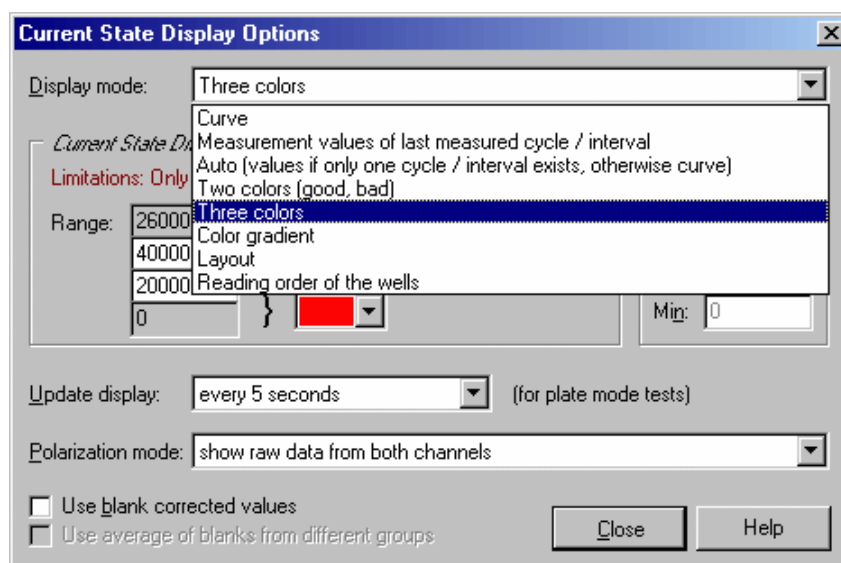
If you are using layout groups (see chapter 5.6.1), the layout grid will be displayed using the background colors belonging to the layout groups used. When you move the mouse pointer over a well, the well content (e.g. 'SA1') will be shown for a few seconds.

For test protocols with well scanning in matrix mode (see chapter 5.5.2 Matrix Scanning) the single scan points will be displayed, if you use the two or three colors display mode or when using the color gradient display mode (see chapter 7.7.4 Display of Well Scanning Data).

You can double click on a square to get a zoomed view of the measurement values (see chapter 7.7.3).

7.7.2 Current State Options

After clicking the 'Options' button in the Current State window, the following dialog box will appear:



Display Mode

The measurement results can be displayed as:

Curve

The measurement results can be displayed as a curve of points or lines. If there is more than one chromatic used (multichromatic, more than one filter pair), you will see the results of all chromatics displayed together.

Measurement values of last measured cycle / interval

You will see the last measurement values in numeric style. Due to space limitations, only the results for up to two chromatics (filter pairs) will be shown (the first two). To see values from an earlier cycle / interval, you can change the number in the Cycles / Intervals input box of the Current State window.

Note: When you use this option in plate mode with an update display setting (see below) of more often than *'only after a cycle is completed'*, for all wells, which are not yet measured in the current cycle, '...' will be displayed to avoid that measurement values of different cycles can be mixed up.

If you want to see the last available measurement value for each well, regardless whether this measurement value is from the current cycle or still from the previous cycle, add the following setting to the [Configuration] section of the 'FLUOstar OPTIMA.ini' file: `ClearBeforeUpdateInMValuesMode=False`. You will find this configuration file in the OPTIMA main installation directory, usually '`~\Program Files\BMG\OPTIMA\`'.

Auto

If you use this option, you will get numeric values for all tests with only one cycle / interval (endpoint tests) and a curve display for all other tests.

Two colors

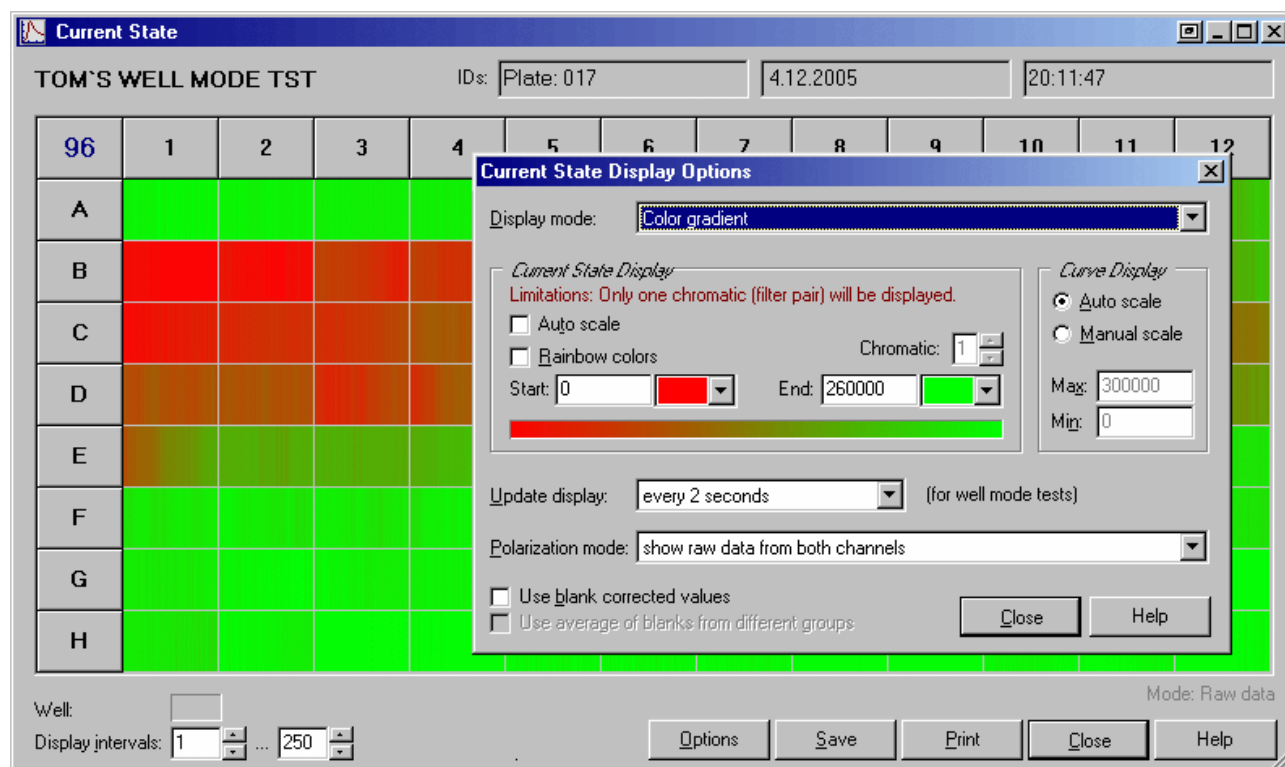
If you are only interested in a good / bad decision, you should choose the option to display different colors for all values under a certain limit and for all values above the limit. You can select the two colors and change the limit value. Only the results from one chromatic / channel will be displayed, but if your test uses multichromatics you can decide which chromatic should be used for the current state display.

Three colors

Same concept as 'Two colors', but here you can also define a range 'in-between' to be displayed in a third color.

Color gradient

The measurement values will be displayed in different shades of colors or gray levels.



You can select a start and an end color. It is also possible to use colors from the rainbow spectrum. If your measurement values use only a part of the total measurement range (i.e. 0...300000), it might be useful to change the start and/or end value to enlarge the used range of the color gradient. Alternatively use the auto scale option. Similar to the Two and Three colors option, it is only possible to display the results from one chromatic / channel.

Note: For this option, it is important to use a graphic mode with more than 256 colors (windows control panel).

Layout

Shows which wells are used for samples, standards and blanks.

Curve Display

The settings in this group will be used for the Curve display in the Current State window and for the Current State Zooming window.

Auto scale: The limits for the graph will be selected automatically according to the measurement results.

Manual scale: This options allows you to personally specify the graph limits. You can use this function to enlarge parts of the graph of special interest.

Max: Maximum value displayed

Min: Minimum value displayed

Update display

Here you can specify how often the display is updated. In well mode the display is updated at least after finishing the measurement of a well, in plate mode it is updated at least after each cycle. If you select any of the 'Update every X seconds' options, there will be updates even for unfinished cycles / wells showing the already available measurement values.

Notes: For updating the Current State Overview and the Current State Zooming window, a large amount of computing power is necessary, especially for tests with a large number of cycles / intervals or multichromatics. If the computer is too slow to redraw the current state display between two measurement values, the update sequence will be lowered automatically.

The update display modes for well mode tests and plate mode tests are independent.

This option is not available for well mode endpoint test runs without multichromatics, as here only one value per well (two for simultaneous dual emission protocols) will be transmitted. When using the well scanning feature all scan points will also be transmitted at once, therefore, the update function does not make sense in these cases.

Absorbance mode

The measurement results from absorbance mode test runs can be displayed in

- **OD units**
- **mOD units**

Polarization mode

The measurement results from polarization tests can be displayed as

- **Raw data from both channels**
- **Polarization values in mP units**

These values are calculated from the results of channel A and channel B:

$$P = \frac{\text{Ch.A} - (\text{Ch.B})}{\text{Ch.A} + (\text{Ch.B})}$$

- **Anisotropy values in mA units**

$$A = \frac{\text{Ch.A} - (\text{Ch.B})}{\text{Ch.A} + 2(\text{Ch.B})}$$

Use blank corrected values

If you use this option, all results will be blank corrected before display.

Notes: If you use this option during well mode tests, measurement results cannot be displayed before the first “Blank” well is measured. Therefore, it might be a good idea to place at least one blank at the beginning of reading (depending on the selected reading direction, see chapter 5.6.1 Using Layout Groups) if you are interested in the current state display. As soon as the measurement values of additional blanks become available, the entire current state display will be recalculated and updated.

If you use this option during plate mode tests, the current state display will only be updated when a cycle is finished (regardless of the selection under ‘Update display’).

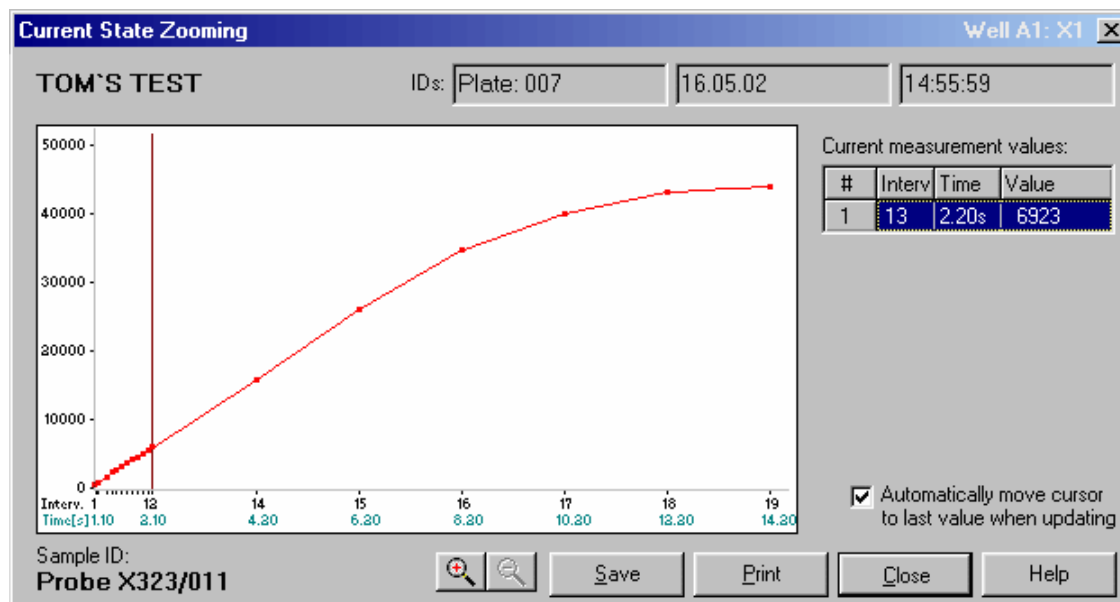
Use average of all blanks from all groups for blank correction

This option is only available when different layout groups (see chapter 5.6.1 Using Layout Groups) are used. If this option is not selected, the measurement values of a group will be corrected using only blanks from the same group. If you select this option, the correction will be done using the average of all blanks from all groups.

Note: All settings from this window are user specific, therefore each user can select his preferences independently.

7.7.3 Zoom Feature

After double clicking on a well in the Current State window, you get a zoomed view of the measurement values. Here the measurement values are always displayed as a curve (when using well scanning in matrix mode the display might look different, depending on the selected display mode, see chapter 7.7.4). There is also a table on the right side of the window where you can see measurement results for all used filter pairs in numeric style. When you move the mouse pointer to the filter setting number column of the table a little hint box, containing the filter setting details, will appear.



You can click and drag the cursor line to each kinetic point to see the measurement value(s) of this point. It is also possible to move the cursor by using the following keys: [←], [→], [PgUp], [PgDn], [Pos1] and [End].

Using the   keys you can zoom into the curve and out again.

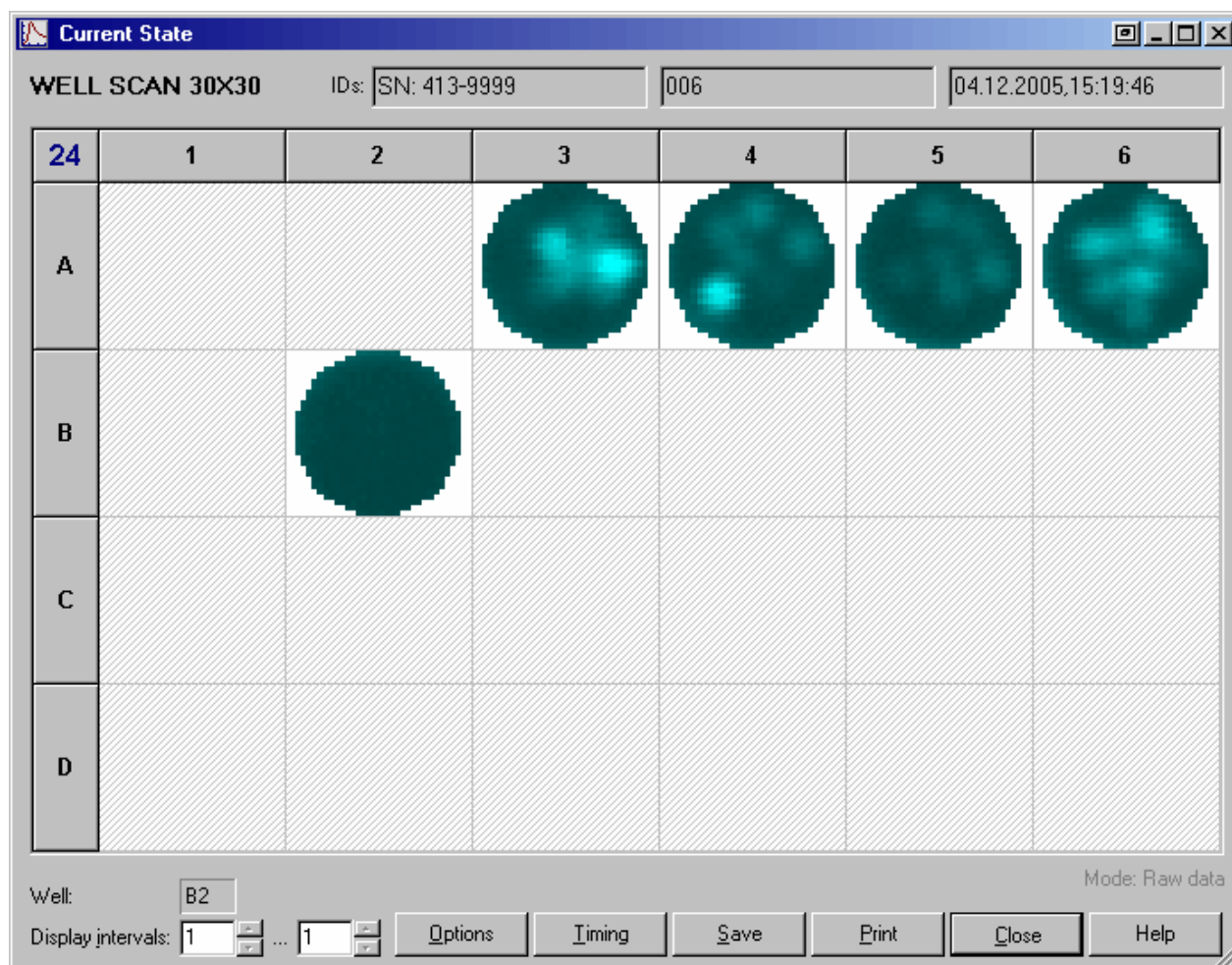
When the option '**Automatically move cursor to last value when updating**' is selected, the cursor will automatically move to the newest value when the measurement result of a new cycle becomes available. The table on the right side will show the new value(s) numerically.

If you have defined a Sample ID for the selected well, this ID will be displayed below the graph.

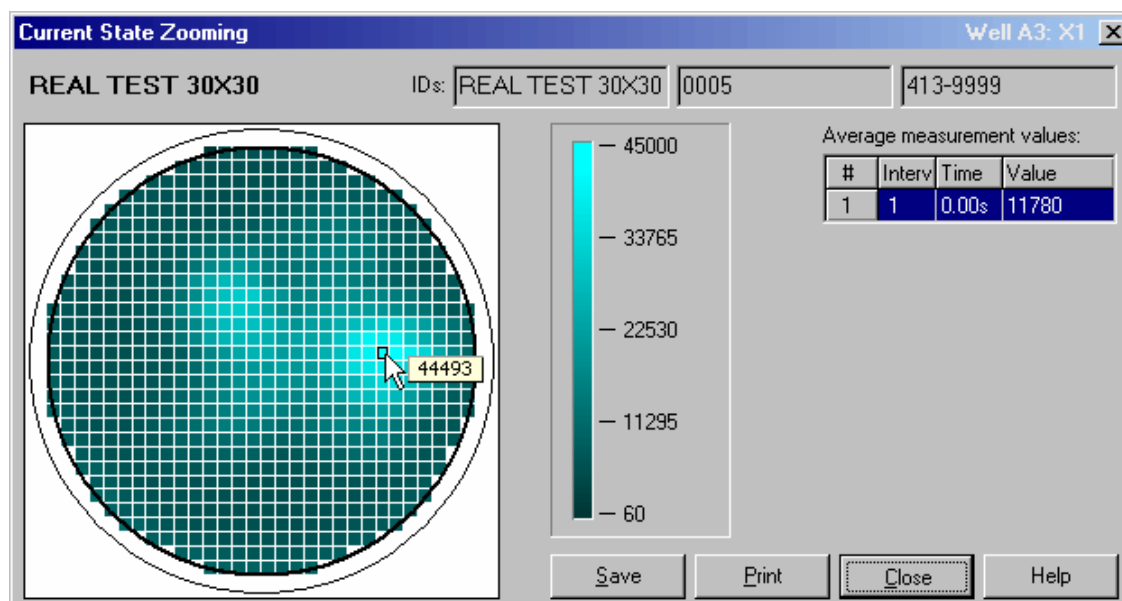
- Save** Saves the data as bitmap (windows BMP or JPEG format) on the hard drive.
- Print** Creates a print out of the screen on any available printer.
- Close** Closes this window.

7.7.4 Display of Well Scanning Data

When performing a protocol using matrix well scanning, you can see the single scan values in two and three colors and color gradient display mode (see chapter 7.7.2 Current State Options). Example using the color gradient display mode:



Clicking one well will open the Current State Zooming window:

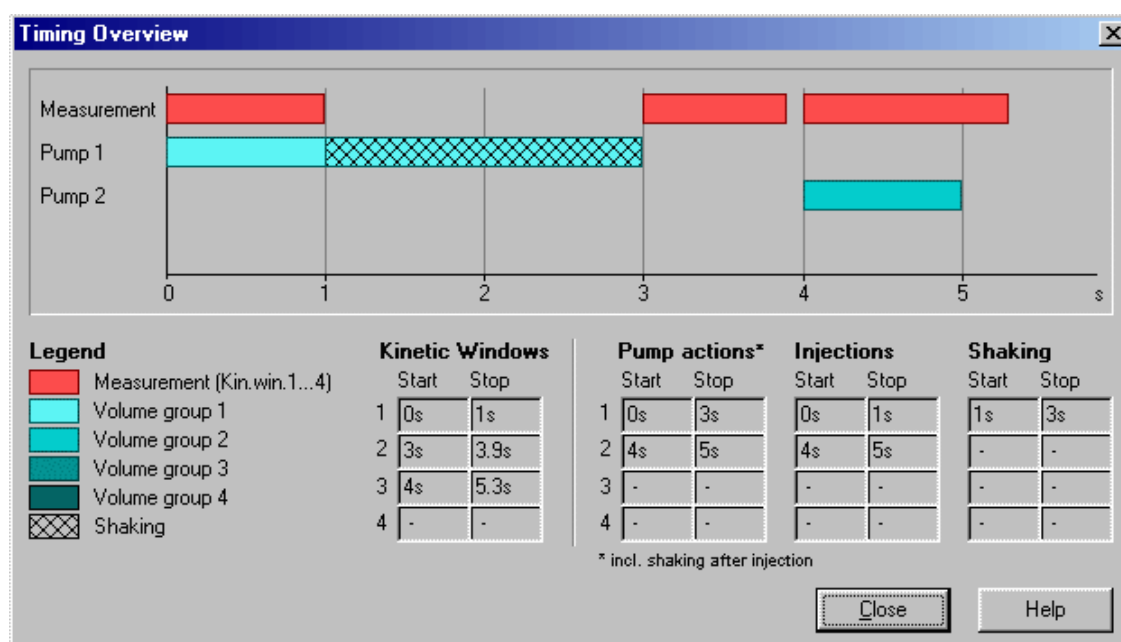


If you move the mouse cursor over the well display, the measurement value of the selected scan point will be displayed. The table on the right shows the average measurement value of the well.

Note: If you use a multichromatic or dual emission protocol, the values of the chromatic / channel selected in the Current State Options dialogue will be displayed. The table on the right of the zooming display will show the average measurement values of all chromatims / channels. The selected chromatic / channel will be marked by a blue bar.

7.7.5 Timing Overview

After clicking the 'Timing' button in the Current State window, you will get a window showing an overview of the timing of the test currently running.

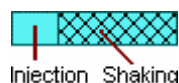


In this window, you can see a graphic overview of the measurement and all injections for one well. (For plate mode tests all actions are displayed in the timing overview of one cycle. The displayed injections may occur in different cycles. The used cycle will be listed behind each injection bar.)

In addition, you will see tables containing the measurement and the injection times.

Note: The listed pump action times include the time for shaking after injection.

An injection action can contain different steps:



8 Script Mode

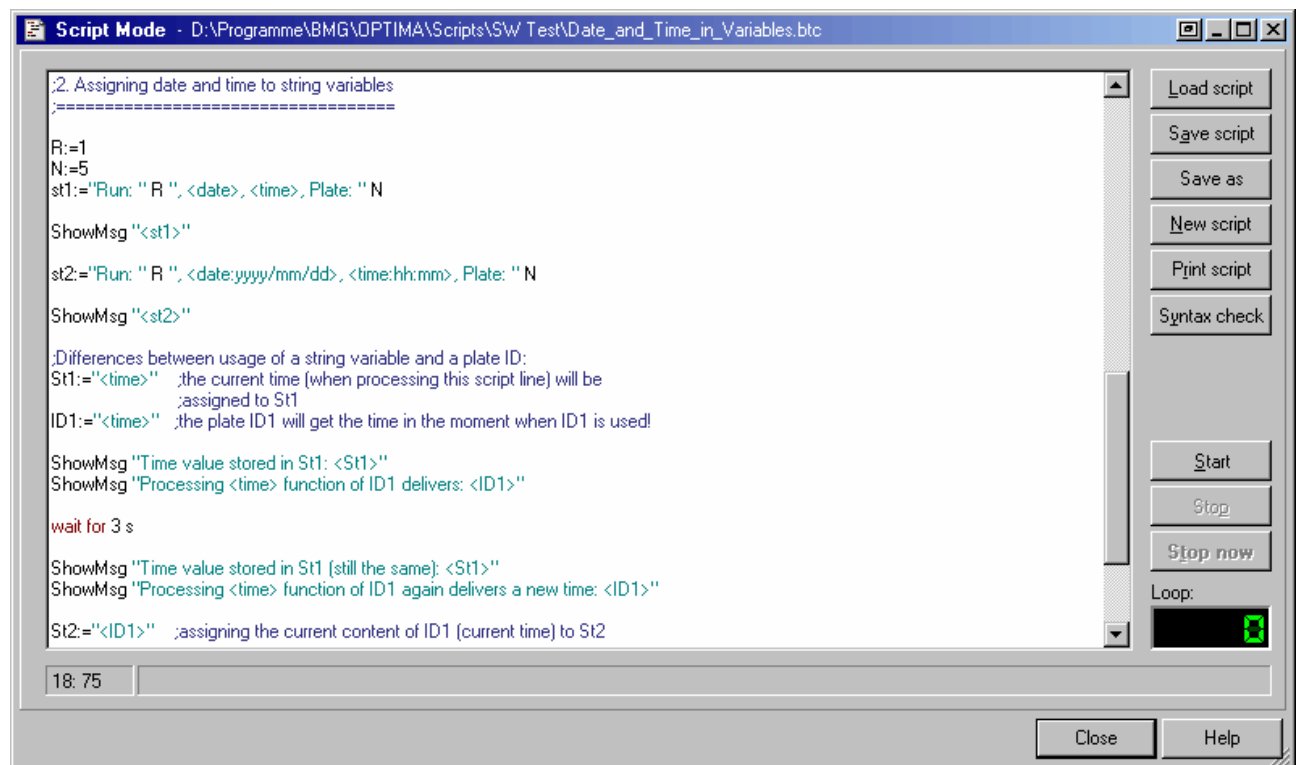
8.1 Script Mode Window

Using this window you can define your own run scripts, which gives you **unlimited flexibility**. You can use this function, for instance, to use different test protocol definitions in one batch run or to perform more than one measurement on a plate.

All available script language commands are described in chapter 9 The Script Language.

Note: The script mode is not available when the program is used in DDE mode, e.g. as part of a robotic system or in combination with Stacker Control.

8.1.1 Control Elements



Load script: Loads a script file. Using the mouse you can also move a script file directly from an explorer window into the script window (drag and drop).

Save script: Saves all changes.

Save as: Saves the current script under a new file name.

New script: Clears the script editor window to allow entering a new script.


Print script: Prints the script on any available printer.

Syntax check: Performs a syntax check. If there is a syntax error in the script you will get a message. It will also be checked, whether the protocols specified as parameter for e.g. the R_Run command exists and whether optional path parameters are correct. There will be a range check performed for all numeric parameters. *Hotkey:* [Ctrl]+[F9]

Start: Starts the execution of the script. *Hotkey:* [F9]

Stop: Stops the execution of the script after finishing the current command.

- Stop now:** Stops the execution of the script immediately (the command currently processed will be terminated). *Hotkey:* [Ctrl]+[F2]
- Close:** Closes this window. You can close (and reopen) this window even when the script is currently executed.

Clicking the  button beside the 'Minimize' and 'Maximize' button or using the system menu function 'Default position and size' will bring the script mode window back to its default size and position. To open the system menu, use [Alt]+[], click on the form's icon or right click on the form's caption. You can also double click the form's background to activate this function.

In the left part of the script mode window status section you will see the current cursor position (line: column) while editing the script.

The software will automatically remember the last script used (user specific) and will reopen the script the next time you open the script mode window.

The script mode window is resizable. The size and position used will be stored user specifically.

8.1.2 Editor Functions

To **undo** the last change of the script use the key combination [Ctrl]+[P] or [Alt]+[Back].

Block indent / outdent function: After selecting one or more lines and using the key combination [Ctrl]+[K] or [Ctrl]+[Tab], two spaces will be inserted on the beginning of the selected lines. (The selected block will be indented by 2 characters.) To outdent a block, use [Ctrl]+[U] or [Ctrl]+[Shift]+[Tab].

It is possible to **search text** forward or backward beginning from the cursor position or from the top / end of the editor content. The following key combinations can be used to activate the search function:

1. Borland Style:

To open the search parameters window use [Ctrl]+[Q], [F], to repeat the last search (in the defined search direction) use [Ctrl]+[L]. Use [Shift]+[Ctrl]+[L] to search against the defined direction, e.g. to go back to the previous occurrence.

2. Microsoft Style:

To open the search parameters window use [Ctrl]+[F], to search the next occurrence in forward direction use [F3], for backward direction use [Shift]+[F3].

If you select something before using the [Ctrl]+[Q], [F] / [Ctrl]+[F] key combination, this selection will be used as default search string, otherwise if the cursor is positioned onto a word this word will be taken as default value.

Note: Searching something, which includes line breaks, is not possible. You might change the width of the window to remove soft line breaks.

All above mentioned functions are also available using the **pull-up menu** (right click into the editor window).

[Ctrl]+[Y] will **delete** the cursor line, [Ctrl]+[Q],[Y] will delete everything in the current line after the cursor position.

[Ctrl]+[F1] will provide **context sensitive help**, as this key combination will open the Script Language chapter of the online help. If the word at the cursor position is a known command / language element, the page of the online help containing this element will be opened (it might be necessary to scroll down some lines to see the description).

8.1.3 Syntax Highlight Function

To ease the reading of script code, a syntax highlight function has been implemented. Reserved words (as for example for, to, do, begin, end) are displayed in **red**. String constants are shown using **cyan**. **Blue** will be used for comments.

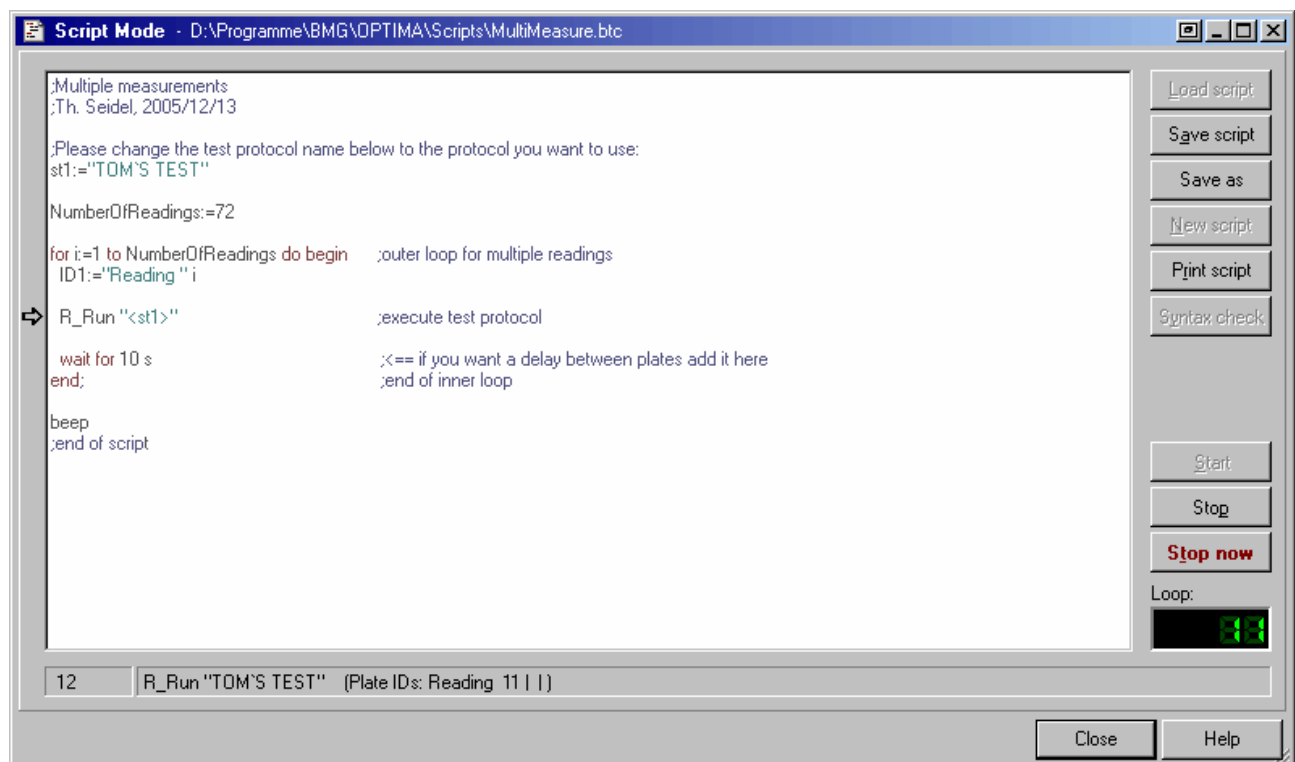
Example:

```
for i:=1 to NumberOfReadings do begin ;loop for multiple readings
  ID1:="Reading " i ;define plate ID
  R_Run "TOM'S PROTOCOL" ;execute test protocol
end; ;end of loop
```

If you use a color printer for printing a script, the colors of the syntax highlight function will also be used for the print out. When using other printers the colors will be replaced by different levels of gray and bold or non-bold fonts.

8.1.4 Executing Scripts

When executing a script a pointer (⇒) on the left side of the script mode window will indicate the line currently processed.



In the left part of the status section you will see the number of the script line currently processed. In the remaining part of the status section, you will see the currently processed command and, for many commands, some additional information.

You will also find all script commands listed in the run log file / run log window (see chapter 13.6).

The **loop** display on the lower right side of the window shows the value of the loop variable if the script execution is currently inside a loop (see 'for ... to' loop in chapter 9.2.2). If you use nested loops, this loop counter will show the value of the inner most loop.

8.2 Script Auto Run

It is possible to optionally specify a script, which will automatically be executed after starting the program (immediately after initialization and reading the EEPROM content). To use this feature, specify the name of the script in the command line after the program name preceded by **/s**, e.g.

```
FLUOstar.exe /s"C:\Program Files\BMG\OPTIMA\Scripts\Autorun.btc" /v.
```

If you also add **/v** to the command line, the script editor will be opened and the script specified will be loaded into this window and executed there. Without the **/v** parameter the script will be executed in the background. When specifying only **/v**, the script mode window will be automatically opened and the last used script will be loaded, but the script execution will not be started.

Notes: If no path is specified, the script will be searched inside the 'ExeDll' subfolder of the OPTIMA main directory. It is possible to omit the file extension ".btc", but it is not possible to use files with extensions different than ".btc". If the path or file name contains spaces, you need to put it into quotation marks (").

9 The Script Language

9.1 Introduction

Scripts can either be automatically generated or written by the user using the 'Script Mode Window' (see chapter 8.1).

You will find some example scripts in the subfolder `Scripts` of the OPTIMA installation directory (usually `c:\Program Files\BMG\OPTIMA\`).

The script language is case insensitive. Nevertheless, it is recommended to follow the writing style from this manual to ease the reading of script code.

All **string** values / string parameters need to be enclosed in double quotes `"`. Everything behind a semicolon `;` is considered to be a **comment**. During execution of the script these comments will be ignored.

Note: If all parameters of a command do not fit in one line, you should enter a `/` at the end of the line and continue in the next line, e.g.:

```
R_GainWell "DUAL EMISSION FLUO" /
           "D:\Program Files\BMG\OPTIMA\Tom\Definit" /
           2 5 80 40 1
```

9.1.1 Typographical Conventions

<code>{ }</code>	Curly brackets are used to characterize a place holder for a command parameter. Replace the expression with an appropriate numeric or string value (depending on the parameter type) or with a variable.
<code>()</code>	Round brackets enclose optional items.
Boldface	Boldfaced words represent elements of the script language (commands or reserved words).
<code>Monospace type</code>	Example code is displayed using a monospace font type (fixed pitch font).

Note: When items are shown enclosed by `< >`, these signs need also to be entered - opposite to the signs `{ }` and `()`. Items enclosed by `< >` will be replaced by the item values when the script line containing the item is processed. These signs are used to mark system constants or variables and for conversion functions, like `<IntToStr(n)>`.

9.2 Batch Control Commands

9.2.1 Wait Commands

wait for {n} s

Wait for {n} seconds before proceeding with the next line. You can specify the time in 0.1 s steps.

Example:

```
wait for 3.5 s
```

wait for temp >= {n}

Wait for the built in incubator of the reader to reach the specified temperature before proceeding with the next line (you can specify the temperature in 0.1 °C steps).

You should use this command after switching on the incubator using the R_Temp command.

Example:

```
R_Temp 37  
wait for temp >= 36.9
```

wait for temp <= {n}

Wait for the temperature of the reader to reach or fall below the specified temperature before proceeding with the next line.

You can use this command after setting the incubator's target temperature to a lower value using the R_Temp command or after switching off the incubation. To get temperature values transmitted even when the incubator is switched off, use the temperature monitoring function (see chapter 6.2.2) by sending a 'R_Temp 0.1' command.

9.2.2 Loop Commands

for i:={n} to {m} do begin
 {commands}

...

end;

for ... to - loop: All commands between the for...to- and the end-line will be repeated {m-n+1} times. A maximum of 100 nested loops ('begin ... end' blocks used in combination with 'for ... to' or 'if ... then') is possible.

Example:

```
for i:=1 to 5 do begin  
    R_Run "MY TEST"  
end;
```

break

Leave a loop and continue operation with the first command after the loop end. (When used outside a loop, this command will end the script execution.)

9.2.3 Dialogue Commands

ShowMsg {Message} {Type} ({Title})

Show the defined message in a small window. Depending on the {Type} (Info, Warning, Error), the window will contain an appropriate icon. Optional: you can specify the window title (caption). If you do not specify a title, 'Information', 'Warning' or 'Error' will be used (depending on the specified {Type}).

Use '&' inside the message text to specify the start of a new line.

The processing of the script will be continued when the user clicks the 'OK' button (or uses the enter key).

Example:

```
ShowMsg "This is an example message.&&Press enter to continue." Info
```

Ask {Question} ({Title}) (Yes: {cmd}) (No: {cmd})

Open a question window which displays the {Question} text and contains a 'Yes', a 'No' and a 'Cancel' button.

Optional: You might enter a title; otherwise 'Question' will be used.

When the user clicks the 'Cancel' button, the script operation will be terminated.

After **Yes:** you can enter a command to be performed when the user clicks the 'Yes' button.

Similarly, you can enter a command to be performed after clicking the 'No' button after **No:.**

Example:

```
Ask "Continue operation?" No: Halt
```

9.2.4 Comparisons

if ID1="value" **then** ...

if ID2="value" **then** ...

if ID3="value" **then** ...

Perform the command after 'then' only if the ID1 (2, 3) string is equal the declared value. *

if st{n}="value" **then** ...

Perform the command after 'then' only if the value of the string variable st{n} (st0 ... st9) is equal the declared value. *

if st{n}=st{m} **then** ...

Perform the command after 'then' only if the values of both string variables are equal. *
Instead of a string variable you can also use one of the ID values.

if {variable}=value then ...

Perform the command after 'then' only if the (numeric) value of the variable is equal the declared value. *

if {variable1}={variable2} then ...

Perform the command after 'then' only if the (numeric) value of the variable1 is equal the value of variable2. *

* Instead of '=' you can also use '<>' for not equal, '>' for greater than, '>=' for greater than or equal, '<' for smaller than and '<=' for smaller than or equal.

If you want to perform more than one command after the comparison, you can use '**begin**' and '**end;**' to define a block of commands.

Example:

```
if A>5 then begin
  beep
  ShowMsg "Information: A is larger than 5"
end;
```

A maximum of 100 nested 'begin ... end' blocks (used in combination with 'if ... then' or 'for ... to') is possible.

Notes: The string comparisons work case insensitive, e.g. "ABC"="abc".

9.2.5 Other Batch Control Commands

beep

This command produces a short acoustic signal (you can use this for example, to announce the end of a batch operation).

restart

Restart operation with first script line (useful e.g. for endless demos).

halt

End the execution of the script.

terminate

End the execution of the script and terminate the software. This might be especially useful in combination with the script auto run function (see chapter 8.2).

9.3 Reader Commands

9.3.1 Init Command

R_Init ({InitMode})

This command will initialize the reader.

Using the optional {InitMode} parameter you can change the behavior. This parameter is binary coded (see table for the high byte). To the low byte the TestMode from the configuration file will be added.

Bit 0	move plate carrier to transport lock position
-------	---

Examples:

R_Init 0 *normal initialization*

R_Init \$0100 *initialization including a movement of the plate carrier to the transport lock position*

Note: The high byte of the InitMode parameter will only be used if a firmware version 1.20 or higher is installed.

9.3.2 Plate Carrier Movement

R_PlateOut

Move plate carrier out of the reader to stack position 1.

R_PlateOutR

Move plate carrier out and to the right side (stack position 2).

R_PlateIn

Move plate carrier into the reader.

R_PlateUser {x} {y}

Move plate carrier to position x, y.

9.3.3 Incubator Control

R_Temp {n}

Switch the built in incubator of the reader on and set {n} as target temperature. The allowed temperature range for the standard incubator is 25.0 ... 45.0. If your reader is equipped with an extended incubator you can use the range 10.0 ... 60.0.

Use R_Temp 0 to switch the incubator off.

Use R_Temp 0.1 to switch the **temperature monitoring function** on without using the incubator.

Notes: The target temperature should be at least 5 degrees above the ambient temperature.

You can use this command together with a **wait for temp** command.

When the incubator is switched on or the temperature monitoring function is used, the current temperature will be displayed inside the icon bar of the main program window.

9.3.4 Gain Adjustment

Notes: Gain adjustment is not necessary / possible for absorbance protocols. Gain adjustment over the whole plate (R_GainPlate) is not available for fluorescence polarization or dual emission protocols.

R_GainPlate {Protocol name} ({Path to protocol definition}) {Desired raw result for channel 1} {Desired raw result for channel 2} {Chromatic number}

Perform a gain adjustment for all measurement points of the plate using the declared parameters.

The parameter {Path to protocol definition} is optional. Use this, if you want to use a test protocol belonging to a different user account.

Please specify the desired raw result (required value) in % of the measurement range.

Example:

```
R_GainPlate "QC Test" 80 0 1
```

R_GainWell {Protocol name} ({Path to protocol definition}) {Well column} {Well row} {Desired raw result for channel 1} {Desired raw result for channel 2} {Chromatic number} ({Target mP value for fluorescence polarization protocols})

Perform a gain adjustment on the specified well using the declared parameters.

The parameter {Path to protocol definition} is optional.

Example:

```
R_GainWell "FAST TEST" 5 2 80 0 1
```

(Perform gain adjustment for channel A (req. value 80%) with the first filter setting used in the protocol (chromatic 1) on well B5.)

R_GainWell in Fluorescence Polarization Mode

In fluorescence polarization mode you can either define the required values for both channels or you can define the required value for channel A and a target mP value (to use this mode set the required value for channel B to 0). When using the second option, the software will calculate the necessary required value for channel B itself.

Example:

```
R_GainWell "FP PLATE" 2 2 80 0 1 35
```

Note: In fluorescence polarization mode a fine adjustment between the two channels will be performed automatically.

R_GetKFactor {Protocol name} ({Path to protocol definition}) {Well column} {Well row} {Chromatic No.} {Target polarization value}

Calibrate the ratio between the two measurement channels (fine adjustment for fluorescence polarization tests) using the declared parameters.

Note: This fine adjustment between the two channels will also be performed automatically during the R_GainWell command.

R_SetGain {Protocol Name} ({Path to protocol data base}) {Chromatic} {channel} {gain value}

Change the gain value defined in a test protocol to the specified value (range 0...4095).

Example:

```
R_SetGain "FP PLATE" 1 "B" 222
```

(Sets the gain value for chromatic 1 (=filter setting 1), channel B to 222.)

9.3.5 Run Commands

n:=R_CalculateTestDuration {Protocol name} ({Path to protocol definition})

Calculate the duration of executing a test protocol and stores this value (in seconds) in variable n.

ID1:=, ID2:=, ID3:=

Change the ID values used by the R_Run command. You can enter <Loop> to use the current value of the loop variable (For ...To command), e.g.: ID1:="Batch: <Loop>".

You can also combine numerical variables with strings, e.g.: ID1:="Temperature=" Temp1.

Here you can use all special functions that are described in the chapter 7.3 Plate Identification.

R_Run {Protocol name} ({Path to protocol definition}) ({Path for measurement data})

Start a measurement using the declared parameters.

Specifying the path to the protocol definition and specifying the path for measurement data is optional (useful, if you want to perform a protocol belonging to a different user), but it is not possible to only specify the path for measurement data. If you omit these parameters, the path belonging to the user currently logged in will be used.

9.4 System Variables

There are a few predefined variables you can use in scripts.

9.4.1 Numerical System Variables

<Temp> current temperature of the incubator built into the reader (floating point value with 0.1 °C resolution)

Note: To use this variable it is necessary to switch the incubator on (R_Temp {n}) or to use the temperature monitoring function (R_Temp 0.1).

After Gain Adjustment:

<GainA> gain value for channel A obtained during gain adjustment

<GainB> gain value for channel A obtained during gain adjustment

<RawResultA> row result obtained during gain adjustment for channel A

<RawResultB> row result obtained during gain adjustment for channel A

<GainAdjWellColA> column of well with highest signal found during R_GainPlate (gain adjustment over the whole plate) for channel A

<GainAdjWellRowA> row of well with highest signal found during R_GainPlate for channel A

<KFactor> K factor (fine adjustment between the two channels) obtained during R_GainWell or R_GetKFactor in fluorescence polarization mode (floating point value)

Note: To convert a column and row value into a well name (e.g. column 5, row 3 => C5), use the function **<WellName(Column, Row)>**.

9.4.2 Boolean System Variable

<PlateCarrierOut> true, if the reader plate carrier is outside the reader

You can use this boolean variable in combination with the 'if ... then' command.

Examples:

```
if <PlateCarrierOut> then ...
```

(perform the command after 'then' only if the reader plate carrier is outside the reader)

```
if Not(<PlateCarrierOut>) then ...
```

(perform the command after 'then' only if the reader plate carrier is inside the reader)

Note: It is not possible to use this boolean variable anywhere else than in comparisons; it is not possible to use this variable in calculations or as part of strings.

In addition, it is currently not possible to define your own boolean variables and use these for comparisons in the above style (use numeric variables and comparisons in the style 'if MyVar<>0 then ...' or 'if MyVar=0 then ...' instead of boolean variables).

9.5 Numerical Variables

You can use up to 100 numerical variables (type double float, 8 bytes precision) in your scripts. In addition, up to 10 string variables can be used (see chapter 9.6). You do not need to declare the variables. A variable will be automatically initialized with a value of 0.0 when it is used first time.

Variable Names

A variable name can be up to 50 characters long. You can use letters and numbers for the name, but it must start with a letter. Example for correct variable names: i, Temp, Temp2.

Variable names are case insensitive.

Do not use any of the reserved words: begin, break, do, empty, end, for, halt, ID1, ID2, ID3, if, next, no:, NotLastPlate, plate, restart, StopperZPosIsDifferent, TestPlateOnReaderCarrier, TestPlateOn-StackerTable, terminate, then, to, wait, yes:.

Please also do not use any of the command names for variables, which means anything beginning with R_ and S_ or Ask, Beep and ShowMsg / ShowMessage or any of the names of system variables: Barcode, LastPlateDetected, Magazine1In, Magazine2In, PlateCarrierOut, PlatesIn1 or PlatesIn2. In addition, do not use st0 ... st9, as these are the predefined names for the string variables.

Mathematical Operations

Available operations: addition (+), subtraction (-), multiplication (*) and division (/). Only one operation per line is allowed.

Limitations

Use only one command / mathematical operation per line.

It is not possible to combine calculations with other commands.

Wrong:

```
R_Temp Temp + 1
```

Correct:

```
Temp := Temp + 1
```

```
R_Temp Temp
```

One operation between 'for' and 'to' is allowed, but not after 'to'.

Wrong:

```
for i:=1 to 3*a do begin
```

Correct:

```
b := 3*a
```

```
for i:=1 to b do begin
```

Also allowed:

```
for i:=4*c to d do begin
  ...
  if <Barcode_String>="abc" then break ;continue with first command
                                         ;after end;
end;
```

9.5.1 Converting Numerical Values into String Values

To use a numerical value as part of a string value, e.g. for the test name parameter of the R_Run command, you need to use the **<IntToStr(n)>** or **<FloatToStr(n)>** conversion function.

Examples:

```
for i:=1 to NoOfRuns do begin
  R_Run "Test <IntToStr(i)>"
end;
```

```
Temp:=36.5
st1:="<FloatToStr(Temp)>"
```

When the variable *n* contains a real number, **<IntToStr(n)>** will return the integer part of *n*; that is, *n* rounded toward zero, and then converted into a string.

<FloatToStr(n)> will return the real number converted into a string.

For example

```
X:=2.5
ID1:="Plate <IntToStr(X)>"
ID2:="Plate <FloatToStr(X)>"
```

will result in ID1=Plate 2 and ID2=Plate 2.5.

You can use the **<IntToStr(n)>** and **<FloatToStr(n)>** functions in your scripts on all places where a string is used. These functions need to be inserted inside the quotation marks.

9.6 String Variables

You can use up to 10 string variables in your scripts. The name of these variables need to start with 'st' followed by a number '0'... '9' (st0, st1, ... st9). You do not need to declare the variables. The string variables will be initialized with an empty string.

To assign a value to a string variable you can use string constants, string variables, numerical variables or any combination of these.

Examples:

```
st1:="This is a string."
st2:="Current temperature: <Temp>°C"
st3:="<st1> <st2>"
st4:="The value of n is " n "." or
st4:="The value of n is <FloatToStr(n)>."
```

You can use a string variable for all commands where a string parameter is expected. You need to enter the name of the string variable in <brackets> inside the quotation marks.

Examples:

```
ShowMsg "<st3>!" info
R_Run "<st4>"
```

It is also possible, to compare strings.

Examples:

```
if st1="value" then halt
if st2<>st3 then R_Run "TOMS TEST"
```

Note: Everything, which can be done with string variables, can also be done with the three plate identifiers ID1 ... ID3. The only difference is, that you can use some special functions for the plate IDs, as for example ID1:="Date: <date>" (see chapter 7.3).

9.7 Measurement Data

To get access to the measurement data you can use the **R_GetData** command. This is useful, if you want to perform different actions in your script depending on the measurement values.

Syntax:

Variable := **R_GetData** {well name} {cycle/interval} {chromatic} {channel}

or

Variable := **R_GetData** {well column} {well row} {cycle/interval} {chromatic} {channel}

When the well name is computed (e.g. inside a loop) the numeric version is easier to use, but the alphanumeric version is easier to read. When using the alphanumeric version, the well name is case sensitive (the rows in 1536 well plates are named A...Z and a...f).

The cycle/interval, the chromatic and the channel parameters are optional. If you do not specify these parameters, cycle/interval 1, chromatic 1 and channel A will be used.

Examples:

Value:=R_GetData "A1" 1 2 "B" *Get the measurement value for well A1, cycle 1, chromatic 2, channel B.*

Value:=R_GetData "B09" 3 *Get the measurement value for well B9, cycle 3, chromatic 1, channel A.*

Value:=R_GetData 4 5 3 *Get the measurement value for well E4, cycle 3, chromatic 1, channel A.*

Value:=R_GetData "a12" *Get the measurement value for well a12 (1536 well plate), cycle 1, chromatic 1, channel A.*

Note: The R_GetData command uses the dBase format measurement files and will therefore only work if the 'Save measurement data in dBase format' option has not been switched off (menu command 'Setup | Program Configuration')!

9.8 Date and Time Functions

9.8.1 Using Date and Time in Numerical Variables

It is possible to assign the current date and time to numeric variables by using the following functions:

N:=<now> The integral part of the delivered value is the number of days that have passed since 12/30/1899. The fractional part is the fraction of a 24 hour day that has elapsed since midnight.

T:=<time> delivers the fraction of the current day that has elapsed since midnight

D:=<date> delivers the number of days that have passed since 12/30/1899

Using these functions, it is possible to measure run times and to write scripts where certain actions need to take place at a defined time after other actions.

Example:

```
Tstart:=<now>
R_Run "WHATEVER"
Tend:=<now>
DeltaT:=Tend-Tstart
DeltaT:=DeltaT * 86400 ;convert fractions of a day into seconds
WaitTime:=600 - DeltaT
wait for WaitTime s
;Next action ...
```

This example script will perform one measurement and will then wait the remaining time to 600 seconds before continuing with the following commands. This way you can achieve a distance of 10 minutes between the start of the first measurement and the following action.

Note: It is not allowed to combine the <now>, <date> and <time> commands with calculations.

Wrong:

```
DeltaT:=<now>-Tstart
```

Correct:

```
Tend:=<now>
DeltaT:=Tend-Tstart
```

or

```
DeltaT:=<now>
DeltaT:=DeltaT-Tstart
```

9.8.2 Using Date and Time in String Variables

It is also possible to assign the current date and time to string variables by using the <date> and <time> function.

<date> will be replaced by the date and **<time>** will be replaced by the time in the moment when this script line is processed. The date and time format can be specified in the same way as you can do when using <date> and <time> for the plate IDs. If you do not specify the format the “Long Date Format” / “Long Time Format” as defined in the windows control panel will be used.

Example:


```
R:=1
N:=5
st1:="Run: " R ", <date>, <time>, Plate: " N
st2:="Run: " R ", <date:yyyy/mm/dd>, <time:hh:mm>, Plate: " N
```

Note: If you assign <date> or <time> to any of the plate IDs, the date and time value will be assign in the moment, when this ID is used, e.g. when performing a R_Run command.

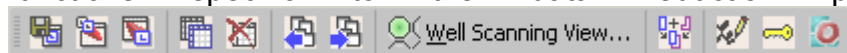
Assigning a time value to a string variable allows to use this value several times (it will not change unlike the time in ID1:="<time>").

10 Evaluation Software - Data Reduction

The data reduction package provides powerful Excel macros for easy data calculations as well as all the functions possible with the Excel software. Once a measurement is performed the data is automatically saved as a database file in Excel. The test run will be present in a list of all saved test runs on the first worksheet in Excel.

You can access the data by clicking on the Excel  icon in the tool bar or selecting 'Results | Excel' from the main menu bar. Or you can go directly to Excel from the Windows Start menu: 'Programs | BMG LABTECH | OPTIMA | OPTIMA – Evaluation'. If the evaluation software is opened from the start menu then a login screen will appear as with the control software. The same user path and password applies.

At the top of every worksheet is the normal Excel menu with all the functions from the Excel program. In addition there is a OPTIMA menu on the far right with some special functions specific to the data reduction package. A toolbar box



also appears there (see chapter 10.2.2). The functionality is shortly explained with tooltip texts appearing when you move the cursor over the buttons.

Note: The worksheets are designed for a screen resolution of 800 x 600 pixels or higher.

10.1 The Worksheets

The OPTIMA evaluation software will be opened with up to 8 worksheets:

Test Runs

This worksheet is displayed when Excel is opened. It lists all the test runs that have been performed along with the 3 identifiers, the layout, microplate, date, time and database file number. Select the test run you want by double clicking on the test run.

Raw Data

This worksheet gives the raw results from the measurement. You can select the data you want to include in the signal curve sheet and define the ranges for kinetic calculations.

Signal Curve

You can view the curve formed by the kinetic points for a single well or a group of wells. If you only have one kinetic point (only one column of measurements), the signal curve sheet will not appear.

Evaluation

View the results on three tables; you can select how the data is presented on the table, i.e. averages, raw data, blank subtraction, standard concentrations, etc.

Sample IDs

Contains a list of all sample IDs. This sheet is only available if sample IDs are defined.

Standard Curve

Plot of the curve from the standards and the results of the unknowns according to the curve. This worksheet is only available if standards are defined.

Result List

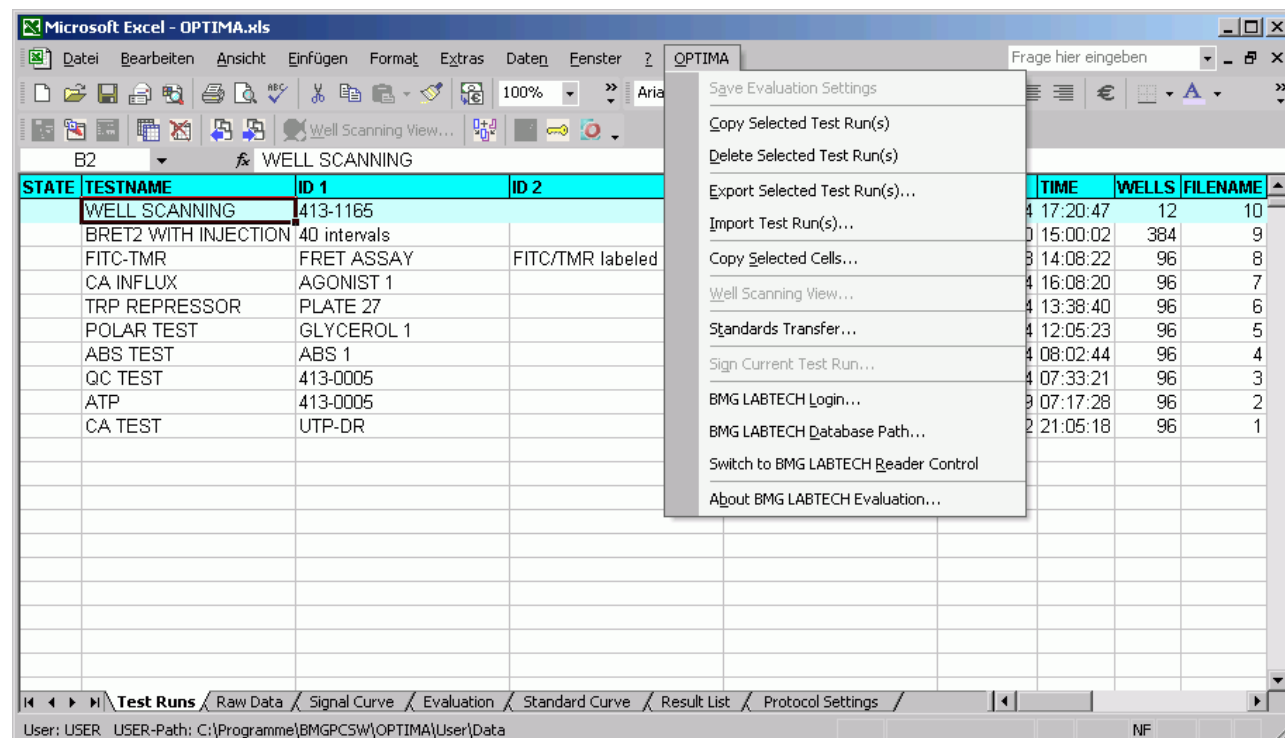
Shows the raw data, the average, the standard concentration, SD, %CV values of replicates and the recalculated sample concentrations in list form. This worksheet is only available if standards are defined.

Protocol Settings

In this sheet you can see the used protocol settings for your test run, also two fields with the audit trail and signature entries.

10.2 Test Runs Worksheet

The Test Runs worksheet is automatically displayed when Excel is opened. It lists all the test runs that have been saved or imported. At the bottom of the worksheet, the user name and the user path are shown; the test runs for that user only are displayed on the worksheet.



State

The state field appears on the very left and describes the history of a test run. The following states are defined:

- O: test run has been imported from an older version of the database; this means, in its history no validation checks have been made.
- C: test run has been copied.
- M: modified test run (e.g. wells have been taken out (see Save under 10.3) or Sample IDs (see chapter 10.6) have been changed).
- X**: manipulations have been detected since the generation of the test run (manipulations done outside the evaluation software).
- S: signed test run (it is not possible to save further changes to this test run). It can also appear combined with other markers, e.g. 'MS'.

If the state field is empty, the test run is still in its original state.

Testname

The testname is listed first and appears as it is defined in the protocol definition.

ID1 / ID2 / ID3

These are the plate identifiers that were created before the measurement (see chapter 7.3 Plate Identification).

Date and Time

The date and time that the measurement took place.

Wells

Plate format (number of wells) of the microplate.

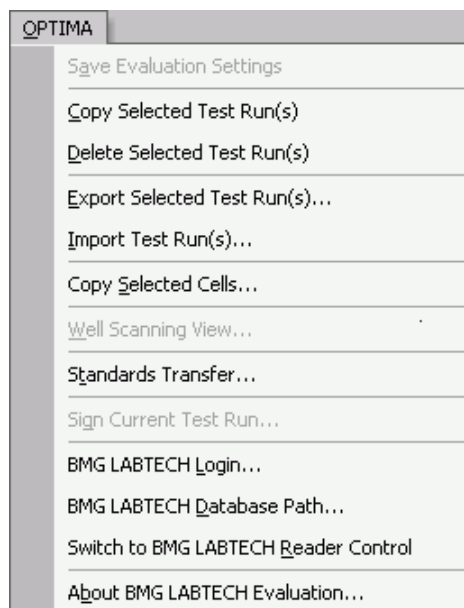
Filename

The number of the database file assigned to the test run.

Select the test run which results you want to view and double click it. You can also select the test run and select one of the options from the OPTIMA pull-down menu located in the toolbar.

10.2.1 OPTIMA Pull-Down Menu

The following options are located under the OPTIMA menu entry at the top of the Excel worksheets.



Save Evaluation Settings

Here you can save the changes you have made during an evaluation session. Your program settings (evaluation methods, control states etc.) and the changes you made to values (changed sample Ids, wells marked as 'deleted', changed comments) will be saved and used again for this test run when you reopen it.

This option is disabled, until you have made changes to your session or if you have signed the test run. When you make changes during a session, you have also the possibility to save and store them before choosing another test run or before signing a test run.

Each time you save your changes, an audit trail entry is made. The audit trail entries can be seen on the Protocol Settings sheet.

Copy Selected Test Run(s)

This feature allows you to copy an entire test run - a reproduction of all the raw data, layout, etc. It is then possible to modify the data and save the changes without losing the original data. Highlight the test run you wish to copy by clicking the test name, then choose 'Copy Selected Test Run(s)'. The copied test run is listed on the test run list with an 'C' to the left of the name, if the test has no other marker.

For example, if you want to remove raw data from the calculations, you can copy the original test run and then remove the data you do not want to use and save these modifications.

Delete Selected Test Run(s)

If you want to delete a test run, select it and choose 'Delete Selected Test Run(s)'. You can select and delete several test runs at a time.

Export Selected Test Run(s)...

With the export function you can copy the test run onto a diskette, a new drive or directory. Highlight a test run or a group of test runs then select 'Export Selected Test Run(s)...'. A new window will ask for the destination drive and directory as well as the file name. The extension for exported test run files is '.RUC'.

Import Test Run(s)...

With the import function, you can copy a test run from a disk or another drive. Click on 'Import Test Run(s)...', and the next window will ask for the file name, the directory and drive where it is located. Highlight the test run and select 'OK'. The test run is added to the list on the Test Runs worksheet. To be compatible to previous versions of the BMG software, also files with the extension '.RUN' resp. '.RUM' will be accepted for import.

Well Scanning View...

If the opened test run contains well scanning data you can open the window to view the data. For more details about the presentation of the well scanning data see chapter 10.10 Display Well Scanning Data.

Copy Selected Cells...

Select the data you want to copy in a worksheet and choose 'Copy Selected Cells...'. A new workbook is created and the selected data will be copied into a sheet within the new workbook. This workbook can be saved under a new name and can be used like any Excel workbook.

Standards Transfer...

This opens the Standards Transfer Wizard, see chapter 10.11.

Sign Current Test Run...

You can sign a test run, if you have opened it. You can make more than one signature for each test run. An audit trail entry is made for each signature. The signatures can be seen on the Protocol Settings sheet.

If you have signed a test run, it is not possible to save further changes. If you would like to make changes to a signed test run, you must make a copy of it with the 'Copy Selected Test Run(s)' menu point. The signature will be removed in the copied version.

For more details about signatures look in chapter Digital Signature (see Chapter 11.2).


BMG LABTECH Login...

This allows you to change the logged in user. The functions are the same as in the control part (see chapter 2 Login Screen).

BMG LABTECH Database Path...

A destination directory for the test runs can be specified. The change is valid only for the user who is logged in. The directory must be a subdirectory of the user directory. Select the drive and directory in the user path window (see chapter 4.5 Data Path).

Switch to BMG LABTECH Reader Control

With this function you can switch back to the OPTIMA control software or use the  button in the toolbar. The evaluation software will stay open in the background.

About BMG LABTECH Evaluation...

This opens an information window about the evaluation software.

To access the Raw Data, Signal Curve and Standard Curve worksheets for a test run, double click on the desired test run.

10.2.2 OPTIMA Toolbar

The OPTIMA toolbar contains twelve buttons.



Save Evaluation Settings

Same functionality as the correspondent menu option, see chapter 10.2.1



Copy settings from Test Run / Paste settings to Test Run

When you leave a test run, it is possible to save the settings you made during your session, e.g. evaluation methods, sorting options etc. When the test run is opened again, the evaluation software will be restored with the same adjustments.

If you would like to copy this settings to a series of test runs, first select this test run on the test run sheet and press the 'Copy settings from Test Run' button. The settings are now stored in the memory. If you now select a series of test runs and press the 'Paste settings to Test Run' button, your settings will be copied to these test runs.



Copy Selected Test Run(s)

Same functionality as the correspondent menu option, see chapter 10.2.1



Delete Selected Test Run(s)

Same functionality as the correspondent menu option, see chapter 10.2.1



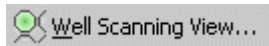
Export Selected Test Run(s)

Same functionality as the correspondent menu option, see chapter 10.2.1



Import Test Run(s)

Same functionality as the correspondent menu option, see chapter 10.2.1

**Well scanning view**

Same functionality as the correspondent menu option, see chapter 10.10 Display Well Scanning Data.

**Standards Transfer**

If you would like to apply a standard measurement to another test run with samples, you can use this feature. A detailed description can be found in chapter 10.11.

**Sign Current Test Run**

Same functionality as the correspondent menu option, see chapter 10.2.1

**BMG LABTECH Login**

Same functionality as the correspondent menu option, see chapter 10.2.1

**Switch to BMG LABTECH Reader Control**

This button enables you to directly switch from the evaluation software to the control part.

10.3 Raw Data Worksheet

This worksheet displays all the raw data for each interval or cycle from the measurement. Each row represents the data from a specific well. Each column represents the data at a particular interval (well mode) or cycle (plate mode).

Test Name **GROUP A STANDARD** ID 1: **Rhodamin** ID 2: ID 3: 2001.09.12 11:42:17

☒ Order by rows
☐ Order by columns

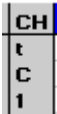
Calc. Range Start 1 Stop 1
 Calc. Range Start 2 Stop 2

All

Well	Cont.	Cycles	2	3														
	t	0	143	286														
	C	0,0	0,0	0,0														
A01	SA1	1	56668	57495	57194													
A01	SA1	2	249	248	247													
A01	SA1	3	1565	1516	1564													
A01	SA1	4	62	59	59													
A02	SA2	1	19777	19642	19119													
A02	SA2	2	136	134	137													
A02	SA2	3	894	902	910													
A02	SA2	4	42	42	44													
A03	SA3	1	8902	8630	8453													
A03	SA3	2	69	72	68													
A03	SA3	3	532	564	559													
A03	SA3	4	35	42	37													
A04	SA4	1	4762	4514	4431													
A04	SA4	2	34	40	43													
A04	SA4	3	354	376	351													
A04	SA4	4	36	32	34													
A05	SA5	1	2947	2845	2729													
A05	SA5	2	21	23	24													
A05	SA5	3	258	290	287													
A05	SA5	4	37	35	31													

Description of the chart:

Order by rows	The order of the wells appears sorted by rows on the microplate (default).																								
Order by columns	The order of the wells appears sorted by columns on the microplate.																								
Well	The coordinates of the well in the microplate (A01= row A, column 1)																								
Cont.	Content of the well as labeled in the layout definition.																								
<table border="1"> <thead> <tr> <th>Well</th><th>Cont.</th></tr> </thead> <tbody> <tr><td>A01</td><td>S1</td></tr> <tr><td>A02</td><td>S2</td></tr> <tr><td>B03</td><td>S3</td></tr> <tr><td>B04</td><td>S4</td></tr> <tr><td>B05</td><td>S5</td></tr> <tr><td>B06</td><td>S6</td></tr> <tr><td>B07</td><td>S7</td></tr> <tr><td>B08</td><td>S8</td></tr> <tr><td>B09</td><td>S9</td></tr> <tr><td>B10</td><td>S10</td></tr> <tr><td>B11</td><td>S11</td></tr> </tbody> </table>	Well	Cont.	A01	S1	A02	S2	B03	S3	B04	S4	B05	S5	B06	S6	B07	S7	B08	S8	B09	S9	B10	S10	B11	S11	<p>Here you can select a specific well or a group of wells to be displayed in the Signal Curve sheet by highlighting the well name(s) in the Cont. Column. If you do not select anything here the signal curve for all wells will be displayed, although with a maximum of 253 wells.</p>
Well	Cont.																								
A01	S1																								
A02	S2																								
B03	S3																								
B04	S4																								
B05	S5																								
B06	S6																								
B07	S7																								
B08	S8																								
B09	S9																								
B10	S10																								
B11	S11																								

<p>CH</p> 	<p>Chromatic/Channel: Number of the used filter setting. The measurement channels are marked with 'A' resp. 'B', the chromatic numbering will always start with 1, which corresponds to the first defined filter combination in the multichromatic sheet (see chapter 5.9 Multichromatics).</p> <p>t: In the channel column, the "t" stands for time. For more than one cycle or interval the "t" row shows the time in seconds in which the cycle / interval occurred.</p> <p>C: In the channel column, the "C" stands for Celsius. If the incubator was used during the measurement or if the Temperature Monitoring Feature is switched on (see chapter 6.2), this row displays the temperature of the instrument at the particular kinetic cycle (only plate mode). In well mode, an additional column "Temp" appears, which gives the starting temperature at each measurement of a well.</p>
Cycles / Intervals	Lists the number of the particular interval (well mode) or cycle (plate mode).

Calc. Range Start 1 / Stop 1

Calc. Range Start 1  Stop 1 

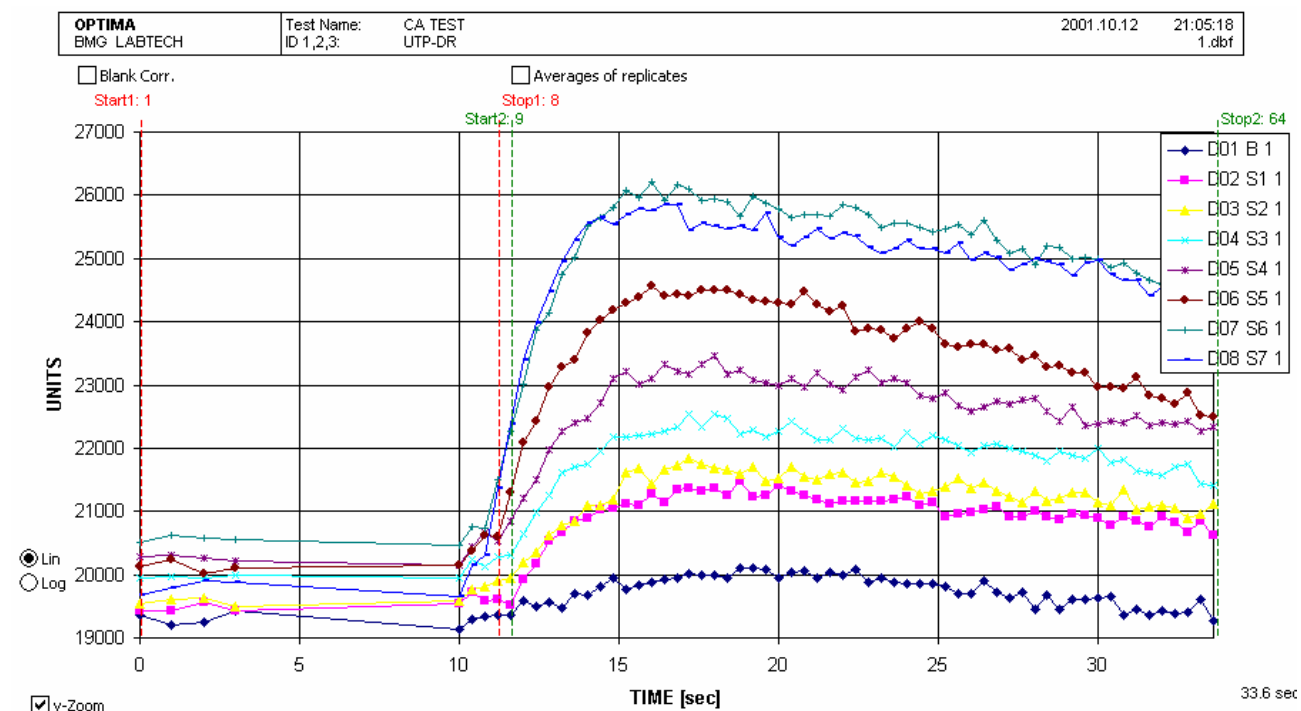
You can select the kinetic intervals / cycles that will be included in the data calculations by entering the interval that you want to start with and the interval you want to stop at. The cycles / intervals you select will be highlighted in red.

Calc. Range Start 2 / Stop 2

Here you can select a second range of kinetic intervals / cycles. With this function it is possible to calculate the difference or the quotient of range 1 and range 2. The cycles / intervals selected for range 2 will be highlighted in green.

10.4 Signal Curve Worksheet

The signal curve worksheet graphically plots the data points for one well or a group of wells selected in the Raw Data Worksheet, see chapter 10.3 (Cont. description). This sheet only appears if you have more than one kinetic point.



In plate mode, you can also include the temperature line. The temperature will be displayed on the right axis of the chart.

You can change the scale for the units by clicking on the scale in the lower left corner.

If threshold is the evaluation type (selected in the Evaluation Worksheet, see chapter 10.5), then the legend will show a threshold line (as 'The') and the desired threshold number.

Blank Corr.: Appears only if blanks are defined. If checked, the average of the blanks will be subtracted from each data point of the corresponding signal curves. The blank curve itself will not be shown.

Averages of replicates: If checked, the averages of each selected content and channel will be calculated and displayed as signal curve. In the legend the names of the contents appear instead of the names of the wells.

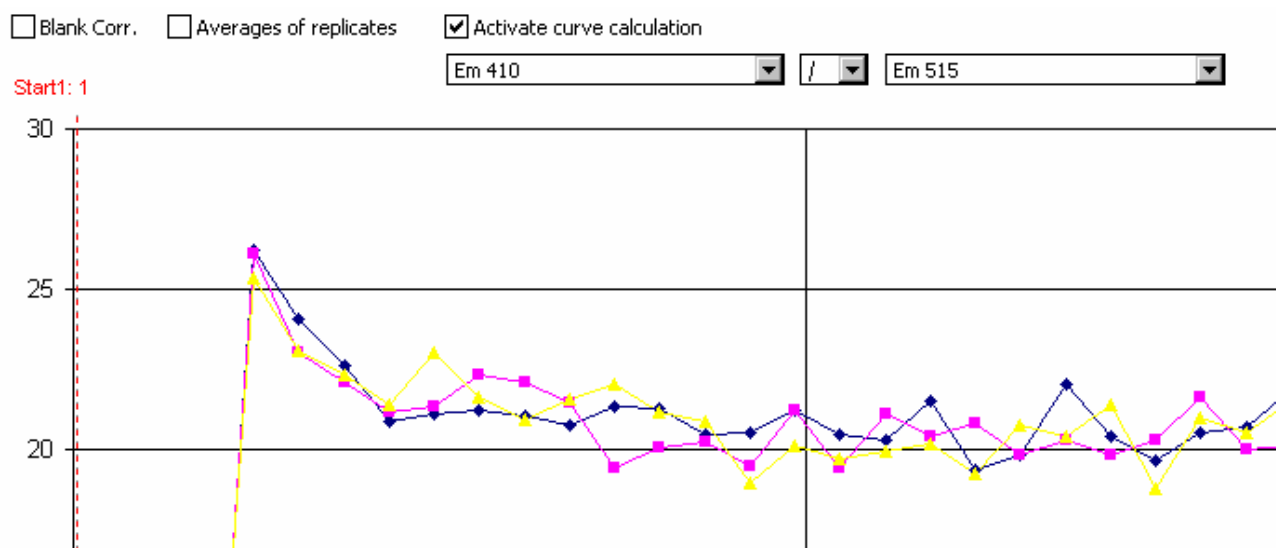
Y-Zoom: A checkbox to zoom the signal curve in y-direction. The lowest and highest measurement data will be used as lower/upper limits.

Lin / Log: Buttons to switch between linear and logarithmic scaling of the y-axis.

Curve calculation:

This feature is useful for calculations between different chromatic datasets, e.g. FURA-2 applications. It is available for all measurements with more than one chromatic or more than one channel. The wavelength calculation is disabled if polarization or anisotropy values are selected on the raw data sheet.

If an appropriate test run is chosen, a checkbox 'Activate curve calculation' appears. If it is checked, three dropdown boxes appear. In the first and third you can select the wavelength as operand, in the second you can choose the operator. You can calculate the ratio, the product, the difference or the sum between the values of two chromatic datasets.




10.5 Evaluation Worksheet

You can specify the evaluation method in the evaluation worksheet. It contains tables where you can see calculated data.

You can see the most important settings from the used protocol definition on the top portion of the evaluation sheet. You can see all settings in the Protocol Settings sheet (see chapter 10.8).

Note: The header section of 6-, 12-, 24-, 48- and 96-evaluation sheets is fixed for better general view of the results. If you use the scrollbar at the left of the window, only the tables move.

OPTIMA		Testname: GROUP A+B+C STANDARD		2001.09.12 12:09:07		<input type="checkbox"/> Hide protocol settings	
BMG LABTECH		ID 1,2,3: Rhodamin+4 Methylumb +Coumarin				66.dbf, imported	
Fluorescence, plate mode equidistant				Kinetic window	1	2	3
Microplate: BMG LABTECHNOLOGIES				No. of cycles	3	-	-
				Cycle time [s]	143	-	-
				Meas. start time [s]	0,0	-	-
				No. of flashes	10	-	-
No.	Excitation	Emission	Gain	Volume group	1	2	3
1	544	590	118	Volume [µl]	20	-	-
2	355	460	048	Injection cycle	1	-	-
3	340	440	116	Shaking after inject. [s]	-	-	-
4	485	520	093				
Pos. delay [s]: 0,2				Calculation Start1: 1 Stop1: 2		Start2: 3 Stop2: 3	
Reading direct. 1 							
Comment: 110 µl color reagent per well							

Calculation:	Sum	<input type="checkbox"/> Use average of blanks of all groups					Chromatic	Table content				
Table 1	Sum	Range1					1	Layout				
A	SA1		SA4	SA5	SA6	SA7	SA8	SA9	SA10	SA11	BA	
B						XA2		XA1				
C	SB1		SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11	BB	
D						XB13		XB12				
E	SC1		SC4	SC5	SC6	SC7	SC8	SC9	SC10	SC11	BC	
F						XC13		XC12				
G	XD1		XD4	XD5	XD6	XD7	XD8	XD9	XD10	XD11	BD	
H						XD13		XD12				
	1	2	3	4	5	6	7	8	9	10	11	12

Hide protocol settings

It is possible to hide the header area and the comment to give better overview for the data by checking this checkbox.

Reading Direction Icon Legend

		bidirectional reading		unidirectional reading	
		start left	start right	start left	start right
horizontal reading	start top	1	2	3	4
	start bottom	5	6	7	8
vertical reading	start top	9	10	11	12
	start bottom	13	14	15	16

Comment

It is possible to insert a comment on the worksheet by typing the comment into the comment field between header and table 1. You can save the comment permanently if you use the 'Save Evaluation Settings' option of the OPTIMA menu (see chapter 10.2.1). If you leave the test run without saving, you are prompted for to save.

10.5.1 Calculations on the Three Tables in the Evaluation Sheet

The data is presented on three different tables. Each table can have different information based on what you choose from the table content menu on the right side above each table.

A table represents the wells of the microplate and data is presented on the table according to the layout defined in the test protocol.

OPTIMA	Testname: GROUP A+B+C STANDARD	2001.09.12 12:09:07	<input checked="" type="checkbox"/> Hide protocol settings
BMG LABTECH	ID 1,2,3: Rhodamin+4 Methylumb +Coumarin		66.dbf, imported

Calculation: Sum ☐ Use average of blanks of all groups Chromatic: 1 Table content: Layout

Table 1 Range1 1

A	SA1	SA2	SA3	SA4	SA5	SA6	SA7	SA8	SA9	SA10	SA11	BA
B							XA2		XA1			
C	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11	BB
D							XB13		XB12			
E	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	SC9	SC10	SC11	BC
F							XC13		XC12			
G	XD1	XD2	XD3	XD4	XD5	XD6	XD7	XD8	XD9	XD10	XD11	BD
H							XD13		XD12			
	1	2	3	4	5	6	7	8	9	10	11	12

Table 2 Range1 1 Standard concentration

A	10	5	2,500	1,250	0,625	0,313	0,156	78,13E-3	39,06E-3	19,53E-3	9,77E-3	
B												
C	1000	500	250	125	62,500	31,250	15,625	7,813	3,906	1,953	0,977	
D												
E	10000	5000	2500	1250	625	312,500	156,250	78,125	39,063	19,531	9,766	
F												
G												
H												
	1	2	3	4	5	6	7	8	9	10	11	12

Responsible for standard curve

Table calculation: None Range1 1 Raw data - blank

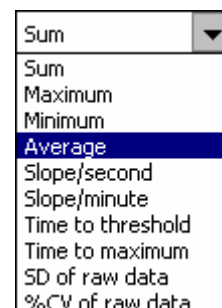
A	112705	35996	14515	6802	4006	2345	1494	1298	920	766	907	
B							424		6006			
C	117	94	65	70	250	91	-24	138	94	20	5	
D							-89		-91			
E	8	-28	59	27	-28	19	-5	73	48	41	44	
F							-90		-15			
G	977	566			68	91	174	47	48	12	38	
H							-97		41			
	1	2	3	4	5	6	7	8	9	10	11	12

Gray fields contain deleted values

Calculation Pull-Down Menu for Kinetic Evaluations

These calculations are for kinetic assays where there are more than one cycle/ interval. They are related to the selected intervals / cycles defined by the calculation start 1/stop 1 resp. start 2/stop 2 controls on the Raw Data sheet. The selection in this menu is valid for all three tables.

You can add all the kinetic points of the selected range of cycles / intervals together for each well. The sum will be used for the tables in the evaluation sheet. Sum is the default method of calculation for both well mode and plate mode, and is selected the first time the evaluation software is entered. After that, the evaluation program will remember the last settings used and automatically re-use these settings the next time the worksheet is entered.



Maximum

Finds the maximum value for each well (for the selected range of intervals / cycles). The maximum of the selected range will be listed in the tables of the evaluation sheet.

Minimum

Finds the minimum value for each well (for the selected range of intervals / cycles). The Minimum of the selected range will be listed in the tables of the evaluation sheet.

Average

Calculates the average of all readings for each standard, sample and/or blank replicate in the selected range of intervals / cycles. The average of the selected range will be listed in the tables of the evaluation sheet.

Slope/second

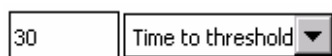
Calculates the linear regression curve and gives the corresponding slope per second value for each well (for the selected range of intervals / cycles). The slope of the selected range will be displayed in the tables of the evaluation sheet.

Slope/minute

Calculates the linear regression curve and gives the corresponding slope per minute value for each well (for the selected range of intervals / cycles). The slope of the selected range will be displayed in the tables of the evaluation sheet.

Time to threshold

When you select threshold over time, an additional box appears next to the pull-down menu.



You must enter the threshold that you are interested in in this field. The time it takes for the threshold to be reached is then indicated in the evaluation sheet tables.

Time to maximum

Gives the time until the maximum value in the selected range of cycles / intervals is reached.

SD of raw data

Calculates the standard deviation of the raw data for each well for the selected range of cycles / intervals.

$$SD = \sqrt{\frac{n \sum x^2 - (\sum x)^2}{n^2}}$$

%CV of raw data

Calculates the standard deviation of the raw data for each well over the selected range of cycles / intervals divided by the average of the raw data for this well / range, expressed in percent.

Use average of blanks of all groups

☐ Use average of blanks of all groups

Here you can collect the blank values from different groups as average instead of using an individual blank value for each group. This box appears only if the test protocol contains blanks and more than one group is defined. If it is checked, then the average of all blanks is used for calculation.

Range Pull-Down Menu

You can select the range you are interested in for each table.

Range1	▼
Range1	
Range2	

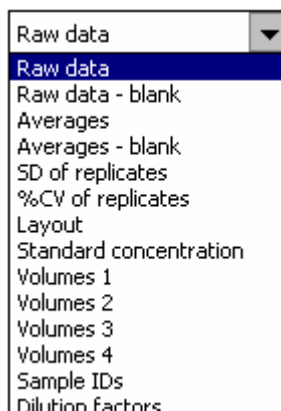
Range 1

The measurement values of range 1 (Calc. Range start 1 ... Stop 1 on the raw data worksheet) will be used in the corresponding table.

Range 2

The measurement values of range 2 (Calc. Range start 2 ... Stop 2 on the raw data worksheet) will be used in the corresponding table.

10.5.2 Table Content Pull-Down Menu



Raw Data

The raw data with no calculations is displayed.

Raw Data - blank

The average of the blanks (background) subtracted from the raw data is displayed.

Averages

The mean of the replicates is displayed.

Averages - blank

The average of the blanks subtracted from the average of replicates is displayed.

SD of replicates

The standard deviation of the replicates and the blanks is displayed. This is important for determining limit of sensitivity.

$$SD = \sqrt{\frac{n \sum x^2 - (\sum x)^2}{n^2}}$$

%CV of replicates

Calculates the standard deviation of the raw data for replicates and blanks divided by the average of the raw data for replicates and blanks, expressed in percent.

Regression coeff. (r)

The regression coefficient as calculated from the linear regression equation is displayed. Appears only if 'Slope/second(minute)' is chosen in the calculation pull down menu.

Layout

The contents (standards, samples, blank) as defined in the layout section of the test protocol, are displayed.

Standard concentration

The concentrations of the standards that were defined the test protocol ('Concentrations / Volumes / Shaking' 5.7) is displayed.

Volumes 1, 2, 3, 4

The injection volumes for volume group 1 ... 4 as defined in the test protocol ('Concentrations / Volumes / Shaking' 5.7) is displayed.

Sample IDs

Shows the Sample ID for each well (as defined before test start, see chapter 7.5 Sample IDs / Dilution Factors)

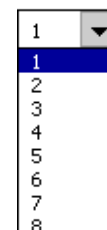
Note: If you use very long sample IDs you will only see a part here (approximately up to 10 characters). To see the full sample ID, use the Sample IDs worksheet (see chapter 10.6).

Dilution factors

Shows the dilution factor for each well (as defined before test start, see chapter 7.5 Sample IDs / Dilution Factors).

Multichromatic Data

If more than one filter setting was used in the test protocol (multichromatic), it is possible to view the data for each setting by using the channel drop-down box. The box contains numbers corresponding to the order of the filter combinations. The number is in the order, in which the filters were defined in the protocol definition (see chapter 5). If you use a polarization test, the channels are displayed as 'A' and 'B'. If you use a dual luminescence test, the chromatic settings are displayed as 1A, 1B, 2A, 2B etc. (see chapter 5.9 Multichromatics).



Choose the number in the box that corresponds to the raw data you want to view. You can choose different numbers for each of the three tables to make comparisons of the data.

Which filter setting corresponds to which position can be seen in the header of the evaluation sheet. The pull-down box will be empty if only one filter pair has been used.

10.5.3 Data for Standard Curve

Table 3 is used for defining the data used for the Standard Curve. If you choose 'Raw data – blank' then the results, if valid, will be plotted on the Standard Curve worksheet. This standard curve will be the basis for calculating the unknowns.

The data selection that is valid for plotting the standard curve includes raw data (minus blank) and averages (minus blank).

A standard curve cannot be formed from volumes or layout information. If the data from table three cannot form a graph, the standard curve worksheet will be blank. If a value in table 3 is negative, the logarithmic scale for the standard curve cannot be selected.

Calculations in the three tables

There is an additional pull-down menu on the left, between the second and third table. You can select optional calculations between the tables here. When a calculation is chosen then all three tables will convert to the same type of data (i.e., 'Raw Data', 'Averages', etc). If no calculation is possible the third table will be gray.

Responsible for standard curve

Table calculation:	Polarization	▼
141.0732	144.1	None
		Table1 / Table2
885.912	966.8	Table2 / Table1
		Table1 - Table2
		Table2 - Table1
537.3699	-220	Polarization
		Anisotropy
		Intensity

Description	Explanation
None	All 3 tables are independent
Table1 / Table2	The content of table 1 is divided by the content of table 2, results are shown in table 3.
Table2 / Table1	The content of table 2 is divided by the content of table 1, results are shown in table 3.
Table1 - Table2	Values from table 2 are subtracted from the values from table 1 and the results are shown in table 3
Table2 - Table1	Values from table 1 are subtracted from the values from table 2 and the results are shown in table 3
*Polarization	The polarization values in mP units are calculated using channel A and channel B, results are shown in table 3.
*Anisotropy	The anisotropy values are calculated using channel A and channel B, results are shown in table 3.
*Intensity	The intensity values are calculated using the values from both channels, results are shown in table 3.

Notes: The items marked with an asterisk appear only in polarization mode.

If there is a division by zero, the respective value will be marked with 'DivByZero' in the evaluation tables.

Removing data: If you want to eliminate the results of a well from the data reduction, highlight them in one of the three tables and press 'Delete'. The content name of this well will now appear in lower case letters. Its value will not be used in calculations. Pressing 'Delete' again will restore the data value.

Save changes: If you remove data in the described way, you can permanently save the changes by using the 'Save Evaluation Settings' option from the OPTIMA menu. If you leave the test run without saving, the software will ask you if you want to save your changes or not.

Note: Saving changes is only possible if the test run has no signature (see chapter 11.2). If you want to make changes to a signed test run, you must make a copy of it.

10.6 Sample IDs Worksheet

This worksheet is only available if you have defined Sample IDs before starting the test run (see section 7.5).

It contains a list with all sample IDs. You can choose between sorting for rows, columns, well content or sample IDs.

<input type="radio"/> Sort column up				
<input type="radio"/> Sort column down				
<input checked="" type="radio"/> Sort row up				
<input type="radio"/> Sort row down	<input type="radio"/> Sort up	<input type="radio"/> Sort down	<input type="radio"/> Sort up	<input type="radio"/> Sort down
Well	Contents	Sample IDs		
A12	BA	Blank A		
C12	BB	Blank B		
E12	BC	Blank C		
G12	BD	Blank D		
A01	SA1	RG 100%		
A02	SA2	RG 80%		
A03	SA3	RG 60%		
A04	SA4	RG 40%		
A05	SA5	RG 20%		

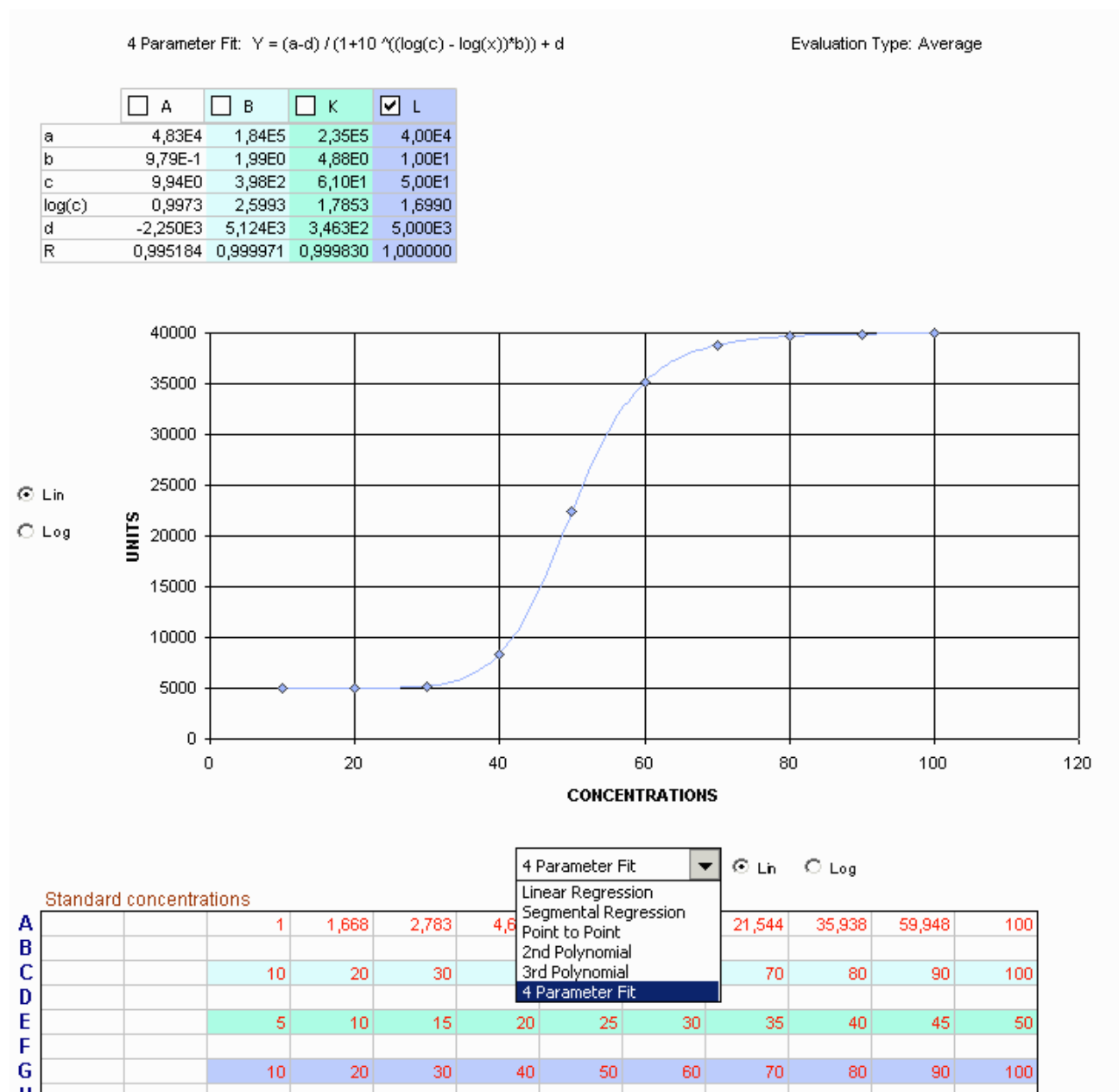
It is possible to change the sample IDs here by editing the respective fields in the Sample ID column. You can save your changes permanently by choosing the 'Save Evaluation Settings' option from the OPTIMA menu. If you leave the test run without saving, you are prompted for to save.

Note: Saving changes is only possible if the test run has no signature.

10.7 Standard Curve Worksheet

After the information in table three of the Evaluation Worksheet is selected, click on the tab for standard curve at the bottom of the screen. At the top of the sheet you will see the parameter table where the parameters for each group are listed. You can select the groups you wish to see by clicking the corresponding checkbox above the parameter table. Below, you find the standard curve graph, which displays the concentration and measurement units of your standard replicates. The graph can be plotted in linear or logarithmic scale by selecting the button below the graph.

Note: If one of the values equals zero or lower, logarithmic scaling is not possible.



The calculated unit values for the standard concentrations defined in the test setup are plotted in a 'Standard curve'. The graph can be plotted on linear or logarithmic scale in x or y direction by clicking the 'Lin' or 'Log' button of the correspondent axis. You can select the groups to be plotted with the checkboxes above the graph.

Curve Fits

There is a pull-down menu for selecting one of six curve fits below the graph. You can choose the one that best fits the data and provides the best results for calculating the unknowns.

Note: a) The Segmental Regression curve fit is useful especially for solubility applications. It tries to split the data range into two regions with optimal regression fit, displaying also the coordinates of the intersection point. If the parameter r for the whole data range is bigger than 0.98, only one regression line will be drawn.

b) The 4 Parameter Fit option is not available if the data is not suitable for this type of curve fit. Values which are greater than the maximum or lower than the minimum of the 4 Parameter Fit asymptote are marked with an asterisk.

Calculation of the Unknowns

Below the graph there are two tables:

Standard concentrations												
A					0.000001	5E-07	2.5E-07	1.25E-07	6.25E-08	3.13E-08		
B					0.000001	5E-07	2.5E-07	1.25E-07	6.25E-08	3.13E-08		
C					0.000001	5E-07	2.5E-07	1.25E-07	6.25E-08	3.13E-08		
D					0.000001	5E-07	2.5E-07	1.25E-07	6.25E-08	3.13E-08		
E					1E-07	5E-08	2.5E-08	1.25E-08	6.25E-09	3.13E-09		
F					1E-07	5E-08	2.5E-08	1.25E-08	6.25E-09	3.13E-09		
G					1E-08	5E-09	2.5E-09	1.25E-09	6.25E-10	3.13E-10		
H					1E-08	5E-09	2.5E-09	1.25E-09	6.25E-10	3.13E-10		
	1	2	3	4	5	6	7	8	9	10	11	12
Calculated concentrations												
Table calculation: None					Range1				Channel 1		Raw data - blank	
A			*	*	9.5E-07	2.89E-07	2.04E-07	1.42E-07	9.97E-08	*		
B			*	*	*	2.86E-07	1.89E-07	1.26E-07	9.67E-08	3.5E-08		
C												
D												
E												
F												
G												
H												
	1	2	3	4	5	6	7	8	9	10	11	12
*: The signal of the respective sample is lower or higher than the asymptote min or max values!												
Used for calculation:												
	<input checked="" type="radio"/> Group	<input type="radio"/> A	<input type="radio"/> B	<input type="radio"/> C	<input type="radio"/> D							

First Table: Standard Concentrations

This table contains the standard concentrations as defined in the layout (Concentration / Volumes / Shaking, see chapter 5.7).

Second Table: Calculated Concentrations

This table contains the calculated concentrations based on the curve fitting method chosen for the standard concentrations. They are calculated by using the formula for linear regression, segmental regression, point to point, 2nd and 3rd polynomial or the 4-parameter fit.

Used for Calculation

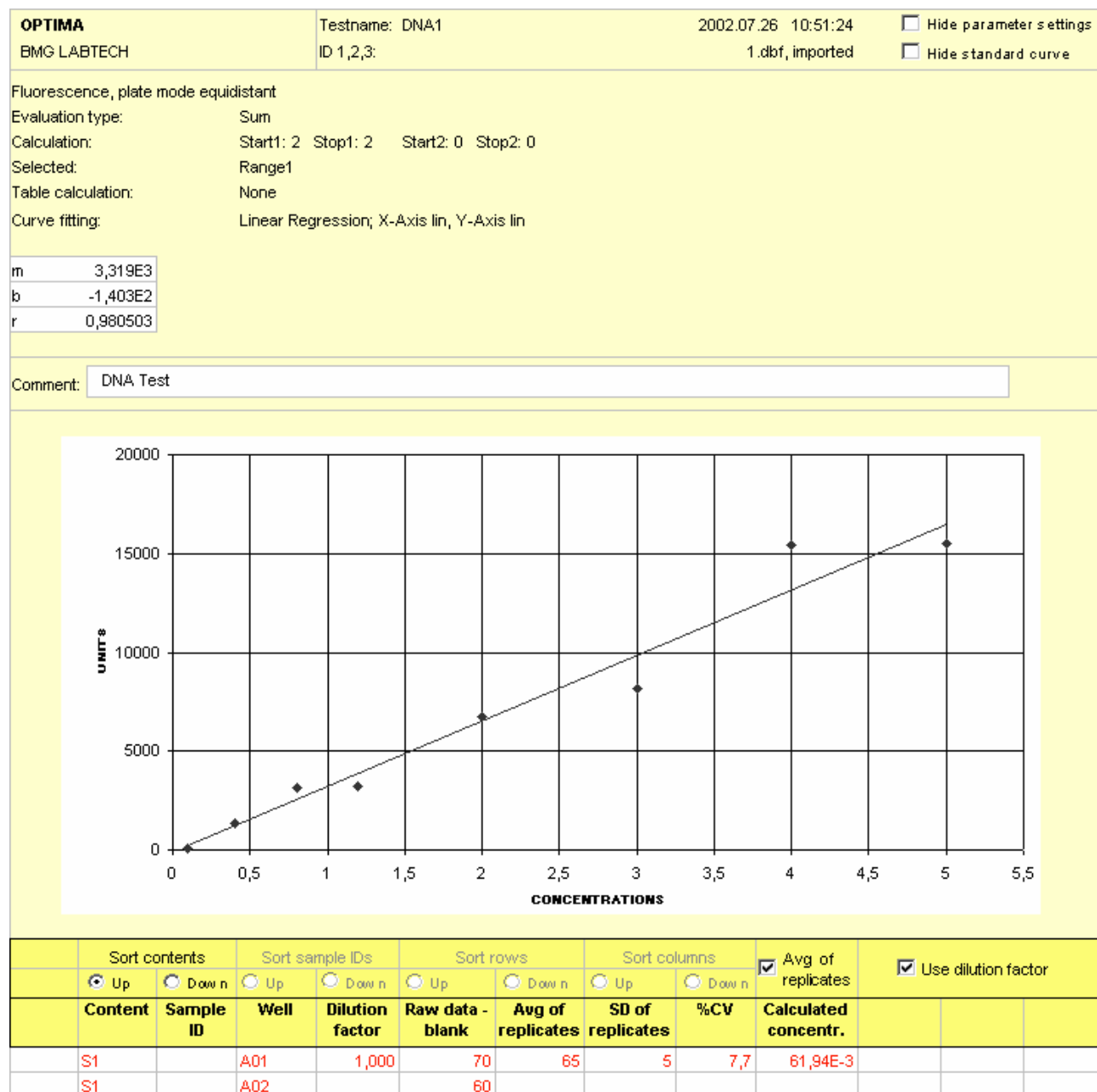
You can select the standard of the group, which is used for calculating the sample values with these buttons. With the button 'Group' selected, which is activated by default, every group uses its own standard for calculation.

Note: In the case of 2nd and 3rd degree polynomial equations, it is possible that there are problems calculating the unknowns. More than one concentration could be possible or the point could be out of the range of the curve. In these cases, the table will contain asterisks in place of the data.

*	The signal of the respective sample is out of the signal range of the standards. No extrapolation is possible.
*	The signal of the respective sample is lower or higher than the asymptote min or max values (can only appear if 4-parameter-fit is chosen).
**	There is more than one concentration possible for the signal (units). No clear relationship between signal and concentration.

10.8 Result List Worksheet

This worksheet is only available if the standard curve worksheet is visible. This means that standards must be defined in the layout, and in the third table of the Evaluation Worksheet one of the following selections must be used: 'Raw data' (minus blank) and 'Averages' (minus blank).



The worksheet contains a list of all raw data based on the selection you made for the third table on the evaluation sheet. The data values are grouped by replicates. The calculated concentrations are also shown using the curve fitting method of the current standard curve worksheet. The most important measurement parameters, the parameter table for the standard curve and the graph of the standard curve appear in the header. Both can be hidden by checking the checkboxes 'Hide parameter settings' and 'Hide standard curve' to get a better overview of the data.

The data can be sorted by plate rows, plate columns, well contents or sample IDs in upward or downward direction using the corresponding checkboxes.

If you check the box 'Use dilution factor' the calculated concentrations are multiplied with the corresponding dilution factor.

OPTIMA			Testname: DNA1			2002.07.26 10:51:24			<input checked="" type="checkbox"/> Hide parameter settings		
BMG LABTECH			ID 1,2,3:			1.dbf, imported			<input checked="" type="checkbox"/> Hide standard curve		
	Sort contents		Sort sample IDs		Sort rows		Sort columns		<input checked="" type="checkbox"/> Avg of replicates	<input type="checkbox"/> Use dilution factor	
	<input checked="" type="radio"/> Up	<input type="radio"/> Down	<input type="radio"/> Up	<input type="radio"/> Down	<input type="radio"/> Up	<input type="radio"/> Down	<input type="radio"/> Up	<input type="radio"/> Down			
	Content	Sample ID	Well	Dilution factor	Raw data - blank	Avg of replicates	SD of replicates	%CV	Calculated concentr.		
	S1		A01	1,000	70	65	5	7,7	61,94E-3		
	S1		A02		60						
	S2		B01	1,000	1315	1330	15	1,1	0,443		
	S2		B02		1345						
	S3		C01	1,000	2630	3125	495	15,8	0,984		
	S3		C02		3620						
	S4		D01	1,000	3277	3247	30	0,9	1,021		
	S4		D02		3217						
	S5		E01	1,000	5046	6720	1674	24,9	2,067		
	S5		E02		8394						
	S6		F01	1,000	7973	8149	176	2,2	2,498		
	S6		F02		8324						
	S7		G01	1,000	11068	15460	4392	28,4	4,700		
	S7		G02		19851						
	S8		H01	1,000	15318	15542	224	1,4	4,725		
	S8		H02		15765						
	X1		A03	1,000	5033	5002	31	0,6	1,550		
	X1		A04		4971						
	X2		B03	1,000	8965	9069	104	1,1	2,775		
	X2		B04		9173						
	X3		C03	1,000	1941	1964	23	1,1	0,634		
	X3		C04		1986						
	X4		D03	1,000	2140	2142	2	0,1	0,688		
	X4		D04		2144						
	X5		E03	1,000	2763	2805	42	1,5	0,887		
	X5		E04		2846						
	X6		F03	1,000	28519	28726	207	0,7	8,698		
	X6		F04		28932						

10.9 Protocol Settings Worksheet

Here you can see all settings defined in the protocol used for the current test run. You can also see the history of changes for a test run in the audit trail text box. If the test run has been signed, the signatures will appear in the signature text box.

OPTIMA	Testname: MULTI DEL PT INJ	2005.12.15 10:24:45
BMG LABTECH	ID 1,2,3:	3.dbf

Simultaneous dual luminescence, plate mode equidistant

Plate type: BMG LABTECH 96

Top optic used

Kinetic window	1	2	3	4
No. of cycles	1	-	-	-
Meas. start time [s]	0,0	-	-	-
Meas. interval time [s]	0,10	-	-	-

Chromatic No.	1A	2A	3A	4A	1B	2B	3B	4B
Gain	2351	4095	2679	4095	2447	4095	2427	1758
Emission filter	520	590	544	612	544	lens	520	empty

Required value A[%]: 40

Required value B[%]: 40

Positioning delay [s]: 0,2

Shaking width [mm]: 1

Shaking mode: double orbital

Additional shaking: 1s before each cycle

Volume group	1	2	3	4
Volume [µl]	2	2	2	-
Used pump	1	2	1	-
Injection speed [µl/s]	310	230	170	-
Injection cycle	1	1	1	-
Injection start time [s]	0,0	31,0	75,0	-
Shaking after inject. [s]	30	40	-	-

Target temperature [°C]: 28,9

Reading direction: 1 

Well scanning: None

Calculation Start1: 1 Stop1: 1

Start2: 0 Stop2: 0

Comment:

Software version control: 2.00 P1

Software version evaluation: 2.00 P1 B:0008

Serial number: 413-0445

User: USERRC16

Audit trail

Donnerstag, 15. Dezember 2005 - 10:26:21, User 'USERRC16': Data record created by performing test protocol 'MULTI DEL PT INJ' (started: Donnerstag, 15. Dezember 2005 - 10:24:45) using reader 413-0445.
Donnerstag, 15. Dezember 2005 - 10:30:25, User 'USERRC16': Automatic initialization of evaluation settings done during first opening of test run.

Signatures

All settings are described in chapter 5 Defining Protocols.


Notes: The 'Last required value [%]' is the last required value which has been used to perform an automatic gain adjustment using this test protocol. A gain can also be typed in manually and hence the required value [%] is not taken into account.

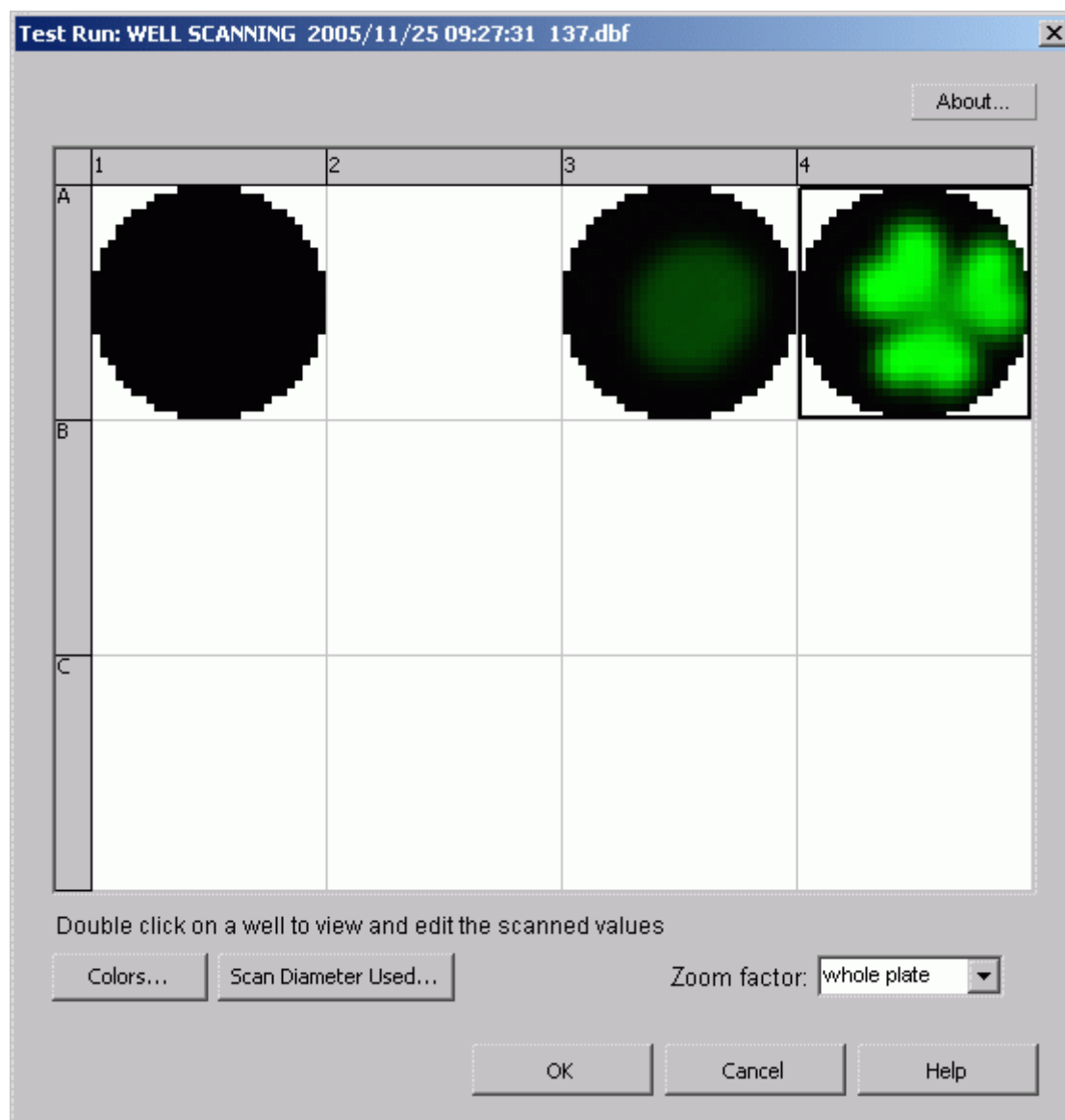
Date and time information for audit trail and signatures is displayed using the format as defined in the Windows Control Panel (Regional Settings) under long date and time format.

10.10 Display Well Scanning Data

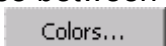
If the opened test run contains well scanning data, (see chapter 5 Defining Protocols) you can display this data in the evaluation software.

10.10.1 Well Scan Plate View

After you've selected the OPTIMA Pulldown Menu 'Well Scanning View...' or you've pressed the Toolbar Button  Well Scanning View..., a new window will be opened.



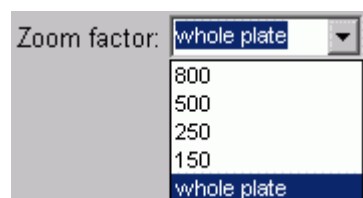
The Well Scanning View window displays the measured well scanning data graphically in a grid according to the microplate format. The header of the window contains the test run name, the date and time that the measurement took place and the filename assigned to the test run.

You can choose between three different display modes. To change the mode and the color settings press  (see 10.10.2 Color Settings).

If you are using layout groups, the layout grid will be displayed using the background colors belonging to the layout groups used.

You can double click on a well to get a zoomed view of the measurement values with additional information and to edit the measurement values (see 10.10.4 Detailed View of Well Scanning Data for a Selected Well).

Press **Scan Diameter Used...** to change the diameter of the circle/rectangle which defines the valid data points (see 10.10.3 Change Scan Diameter Used).



Use this control to zoom to the selected value in percent (whole plate is equal to 100%).

If the measurement was performed with two channels or more than one chromatic, an additional control appears to select the channel/chromatic to be displayed:

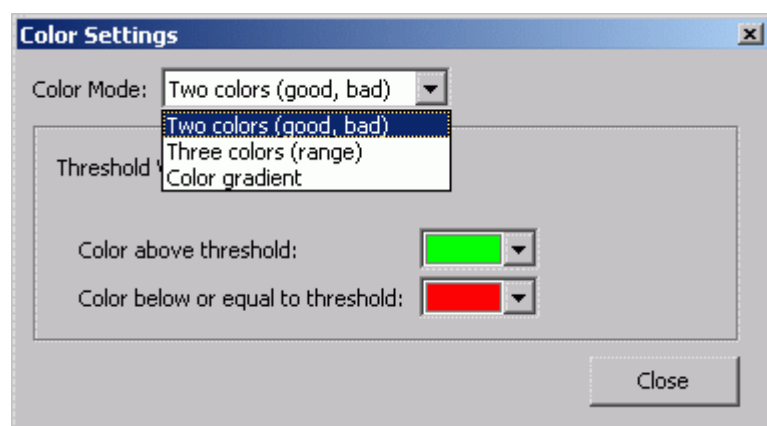


If you press the OK button, the changes performed will be assumed and saved. To revoke the changes, press the Cancel button. In both cases the window will be closed.

Only the average value of all used data points in the well (see also 10.10.3 Change Scan Diameter Used and 10.10.4 Detailed View of Well Scanning Data for a Selected Well) will be displayed in the 10.3 Raw Data Worksheet and used for further calculation.

10.10.2 Color Settings

After pressing **Colors...** in the Well Scanning View window or the Detailed View window, the following dialog box will appear:



The selected settings affect the way the well scanning data will be displayed in both the Well Scanning View window and the Detailed View window.

You can change the display mode by selecting one of the three color modes available:

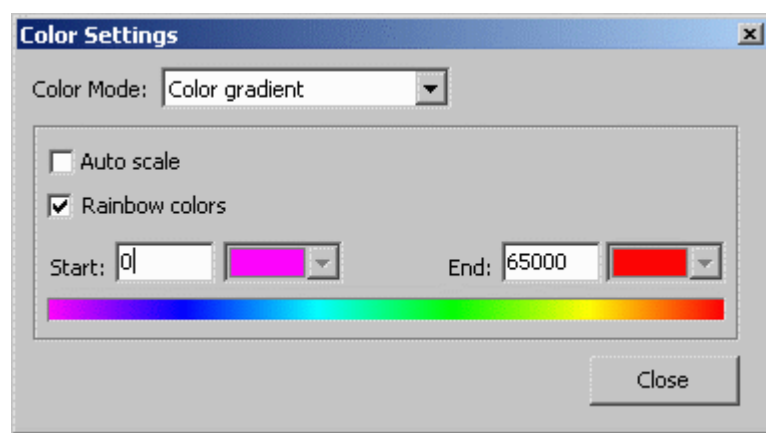
Two colors (good, bad)

If you are only interested in a good / bad decision, you should choose the option to display different colors for all values under a certain threshold and for all values above the threshold. You can select the two colors and change the threshold value.

Three colors (range)

Same concept as 'Two colors', but here you can also define a range 'in-between' to be displayed in a third color.

Color gradient



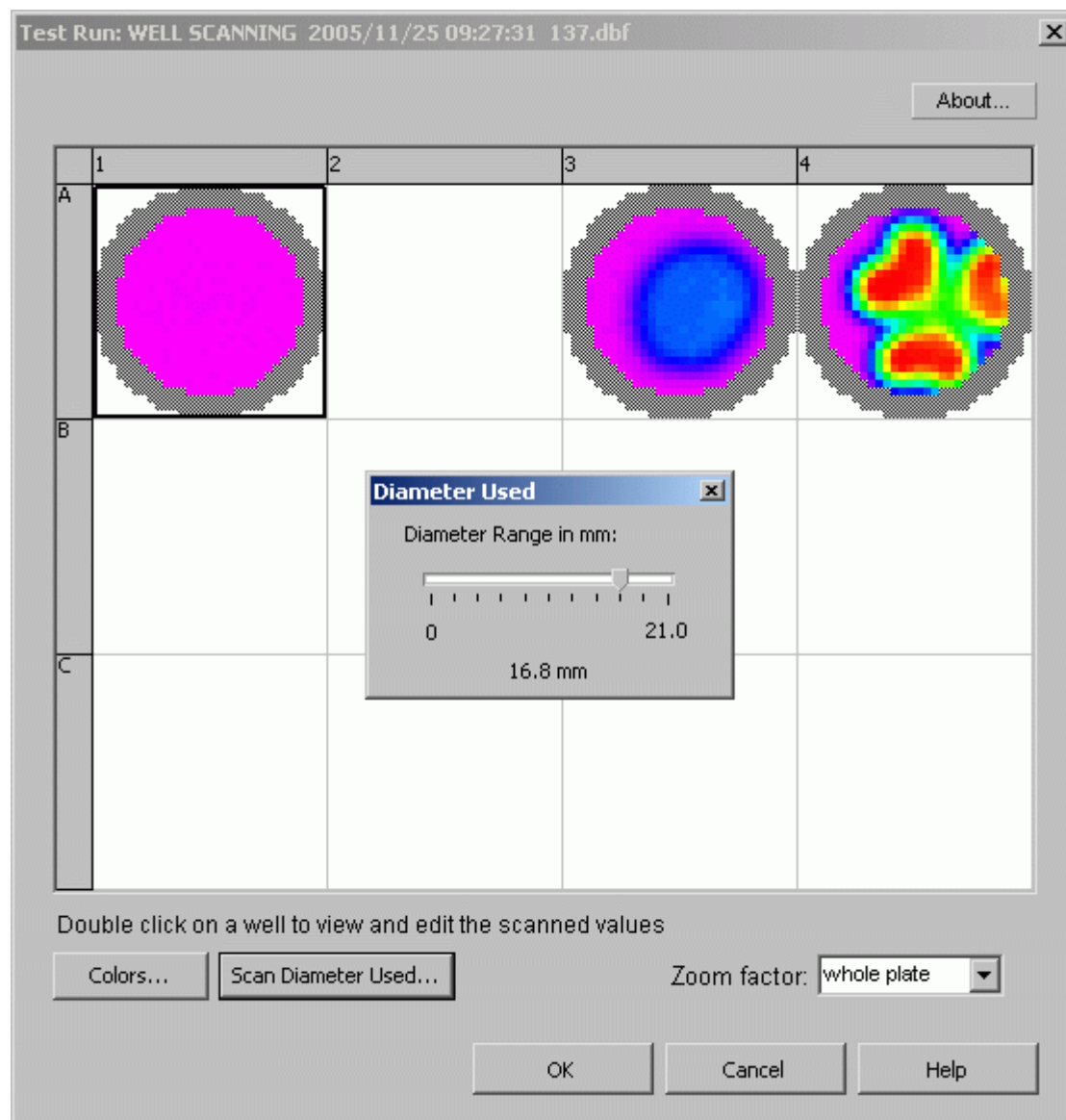
The measurement values will be displayed in different shades of colors or gray levels. You can select a start and an end color. It is also possible to use colors from the rainbow spectrum. You can define the start and the end values to enlarge the range of the color gradient used.

Use the auto scaling function to set the start and the end values automatically to the minimum and maximum measurement value of the selected Channel/Chromatic for the whole plate.

Note: For this option, it is recommended to use a graphic mode with more than 256 colors (windows control panel)

10.10.3 Change Scan Diameter Used

After pressing **Scan Diameter Used...** in the Well Scan Plate View or the Detailed View window, the Diameter Used window opens.



The Scan Diameter Used describes the diameter of a circle (for a round well shape) or of a rectangle (for a rectangular well shape) that defines the area within which the measured data points are used for further calculation.

Changing the diameter size allows you to reduce this area (i.e. if you find out that the results measured near the border of the well are not certain).

The used diameter window contains a slider control to change the diameter used.

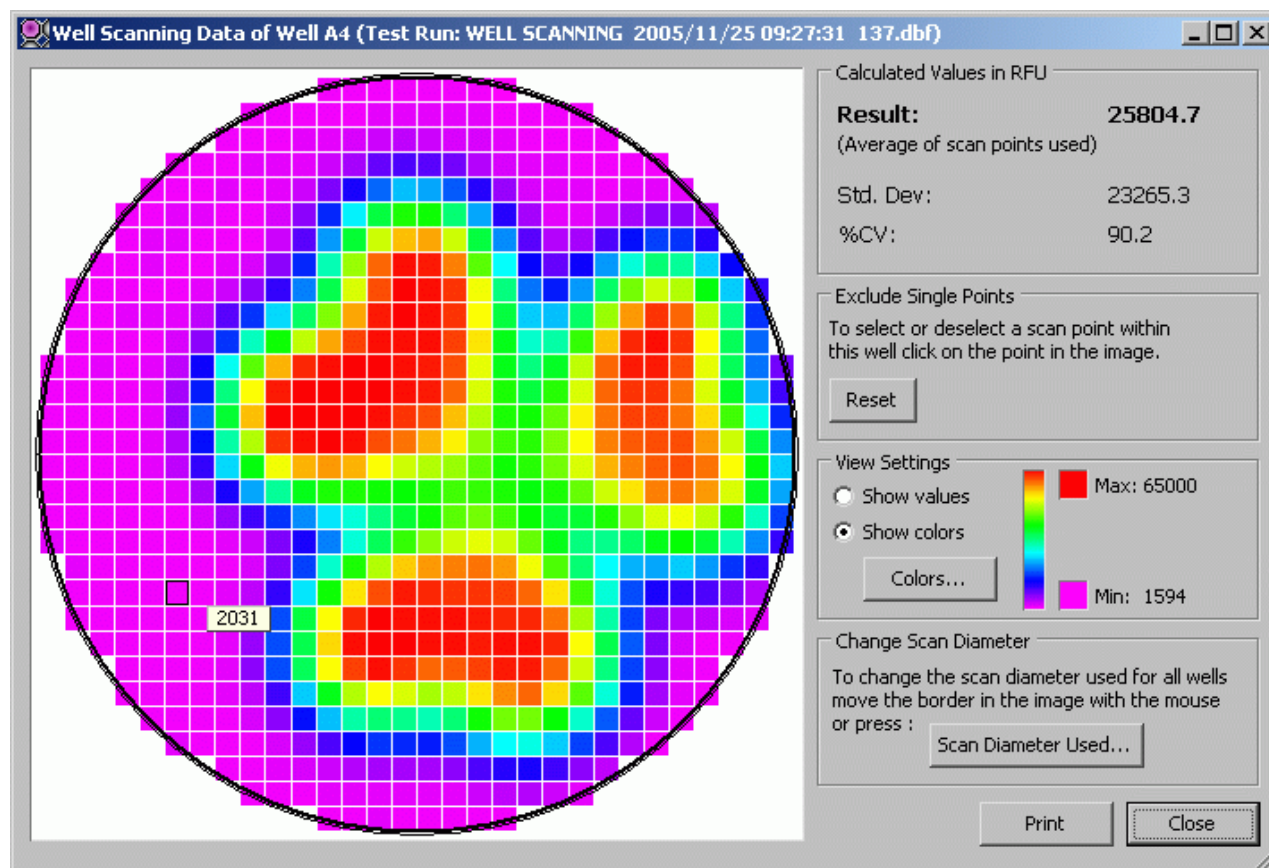
Move the slider with the mouse to change the diameter. The data points outside the area will be displayed in a gray pattern that indicates, that these points will not be used for calculating the average value of the well scanned.

You can also change the diameter in the Detailed View of a well.

Note: Changing the diameter always effects all wells, even if you change it in the Detailed View of a well.

10.10.4 Detailed View of Well Scanning Data for a Selected Well

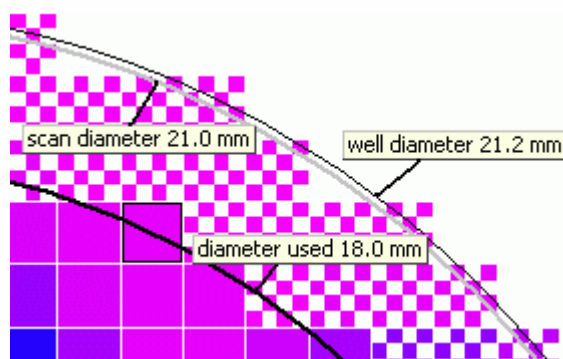
The window with a detailed view of the measured data for one well can be opened if you double click on the well in the Well Scanning View window of the whole microplate.



In this window you can see the selected well in a zoomed view with additional information.

If you move the mouse over a scan point, a hint with the measured value will be displayed.

The picture contains three circles (for a round well shape) or rectangles (for a rectangular well shape) with the following meaning:



Fat line, black: Shows the scan diameter used. All scan points outside are marked as not used and therefore displayed in a grid pattern.

Fat line, gray: Shows the physical scan diameter. This is the diameter used by the reader as limit when the well is scanned. Only scan points of the defined matrix whose center lies inside the area defined by this diameter are measured. You can define the scan diameter in the protocol settings of a test run in the reader control software.

Thin line, black: Shows the border of the well as it is defined in the microplate database.

If you move the mouse over one of these border lines, a hint with the identifier and the size of the border will be displayed.

Description of the Dialog

Calculated Values

Result

This value is calculated as average of all scan points used. It will be displayed in the Raw Data Worksheet and used as base value for further calculations.

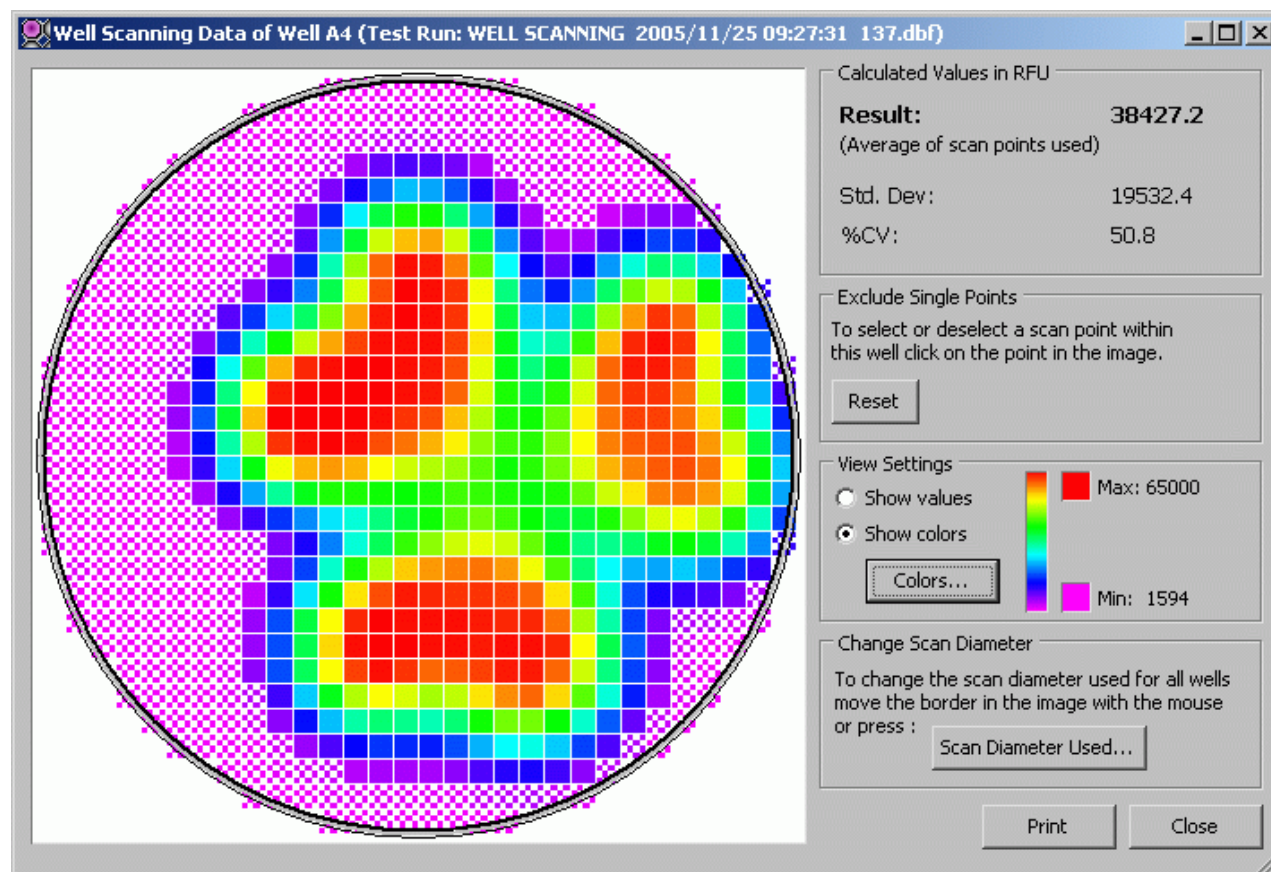
Std. Dev

The calculated standard deviation of the scan points used.

%CV

The calculated standard deviation, divided by the average of the scan points used, expressed in percent.

Exclude Single Points



You can exclude single scan points of the selected well, by clicking on it in the image. If you click on an excluded scan point, the exclusion will be revoked.

Excluded (unused) scan points are drawn in a grid pattern.

Note: If a scan point is excluded by the *Scan Diameter Used*, it is not possible to revoke this exclusion by clicking on it. Therefore you have to increase the *Scan Diameter Used*.

Pressing the **Reset** button will change back the state of each scan point to 'used', if its center lies inside the area defined by the *Scan Diameter Used*.

View Settings

To display the values of each measured scan point, select **Show values**. The image changes and the value is shown instead of a colored rectangle. It is recommended to maximize the window if you use this function, so that the font can be displayed in a readable size.

To change back to the selected color mode, select **Show colors**.

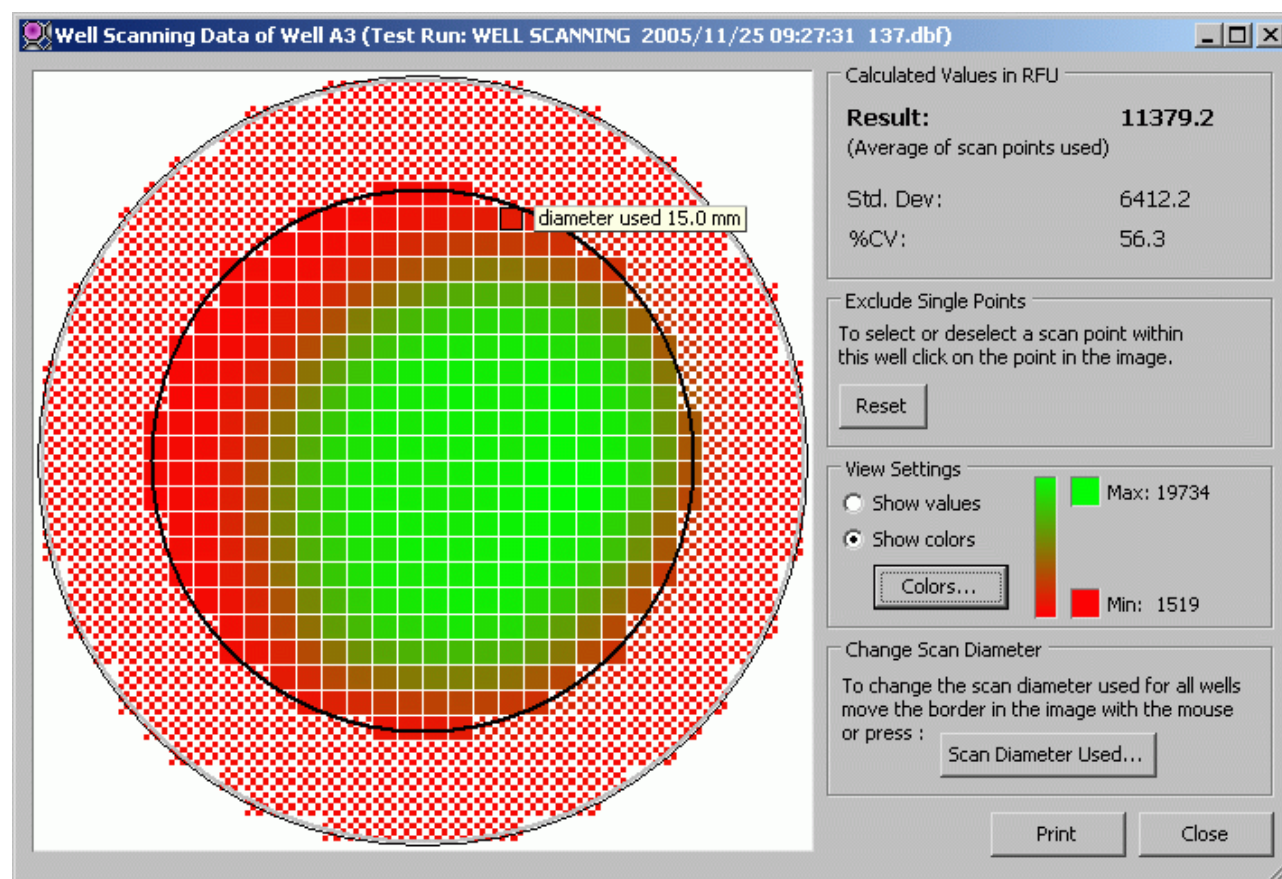
Press  to change the selected color mode and its settings (see Color Settings)

The color legend shows the color gradient between the minimum value (**Min:**) and the maximum value (**Max:**) of the selected well.

Change Scan Diameter

Press **Scan Diameter Used...** to change the diameter of the circle/rectangle which defines the valid data points (see Change Scan Diameter Used).

Alternatively you can move the border in the image with the mouse to change the diameter. Move the mouse over the fat black line in the image until the hint shows *diameter used xxx mm* and the mouse cursor changes to two arrows. Then press the mouse button (the color of the border changes to blue), move it to the desired size and release the mouse button. Note, that the new diameter concerns all wells!



Print: Prints the screen on any available printer.

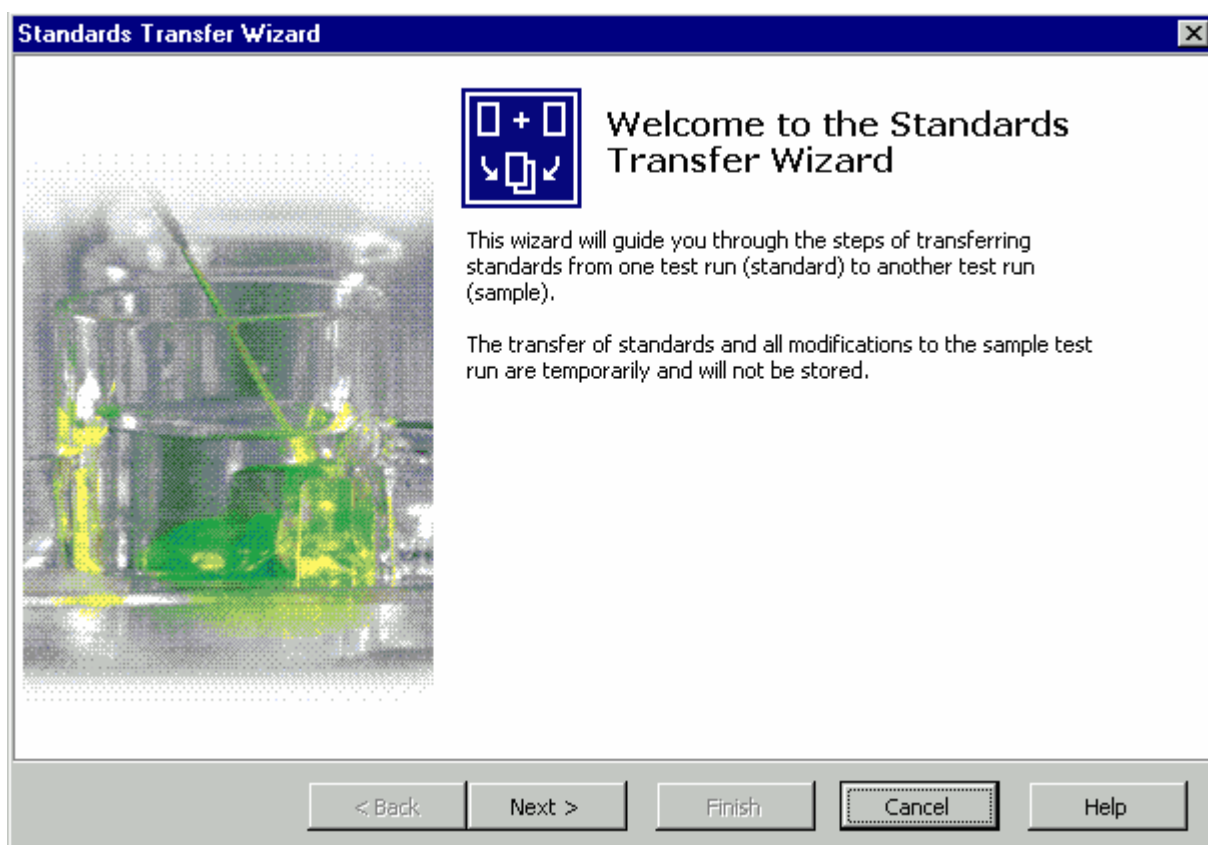
Close: Closes the detailed view window.

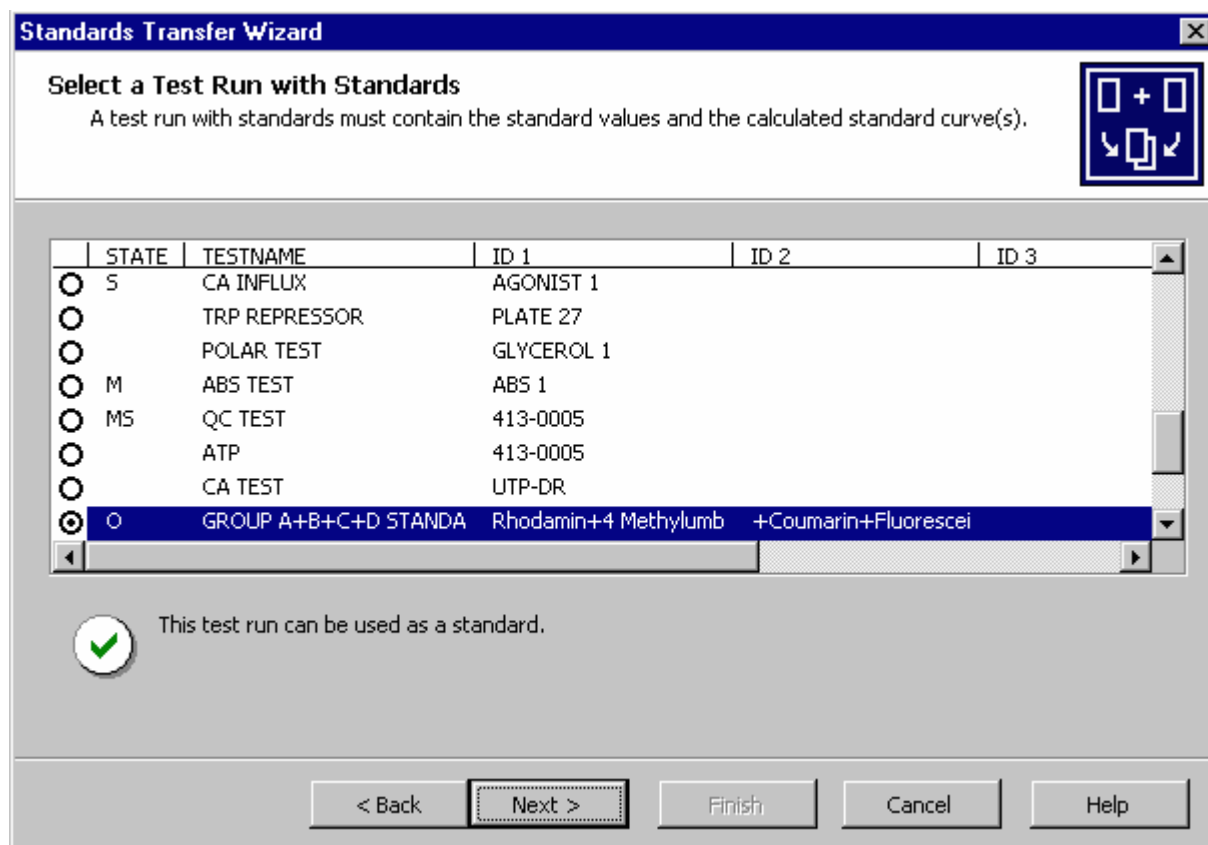
10.11 Standards Transfer Wizard

This feature is made for applying standard parameters gained from a plate with standards (called standard model in the following text) for a following series of plates with samples.

Note: The measurement parameters relevant for the calculation (e.g., gain value, cycle time, etc.) of your standard model must match the ones from your sample test. If they don't match, the software will not allow you to use the respective sample test.

To use this feature, you should first edit your standard test run and choose the calculation methods you are interested in. Save the changes you made and press the button 'Standards Transfer'. The following user dialogs will open:

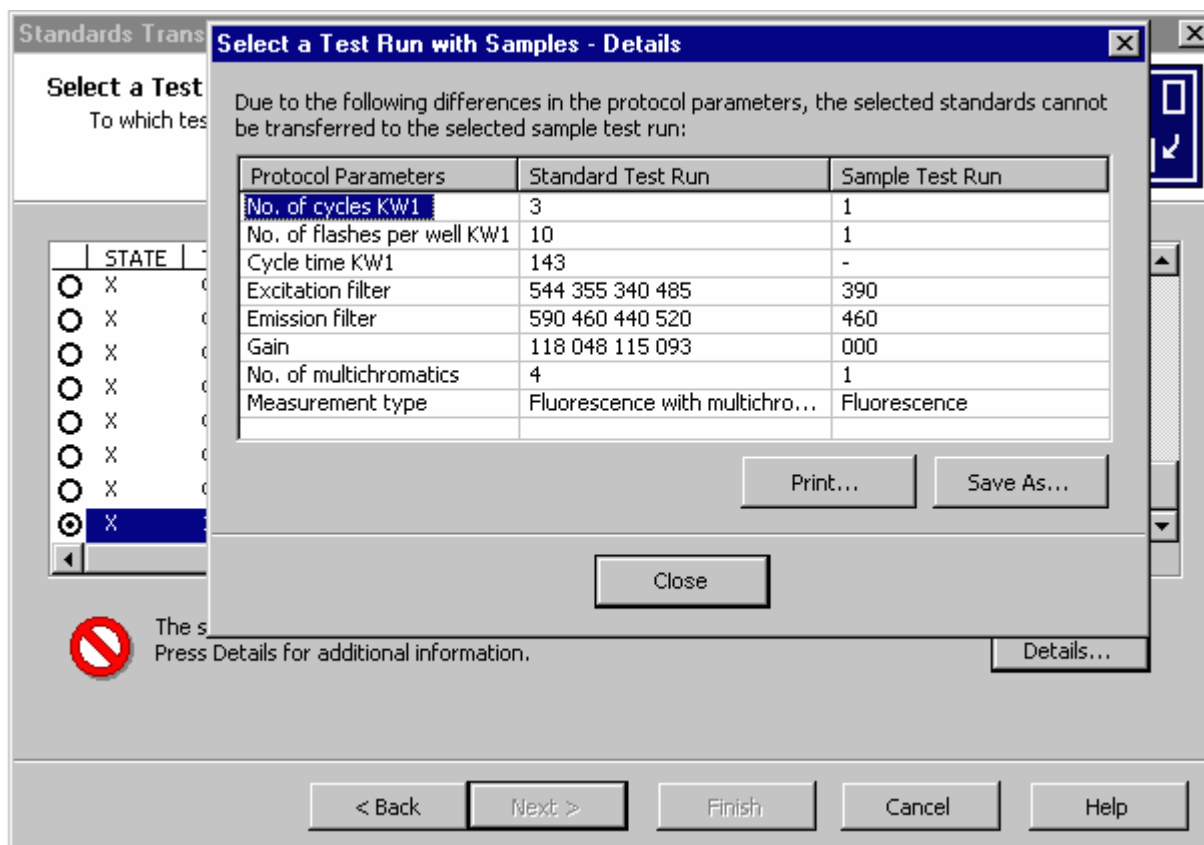




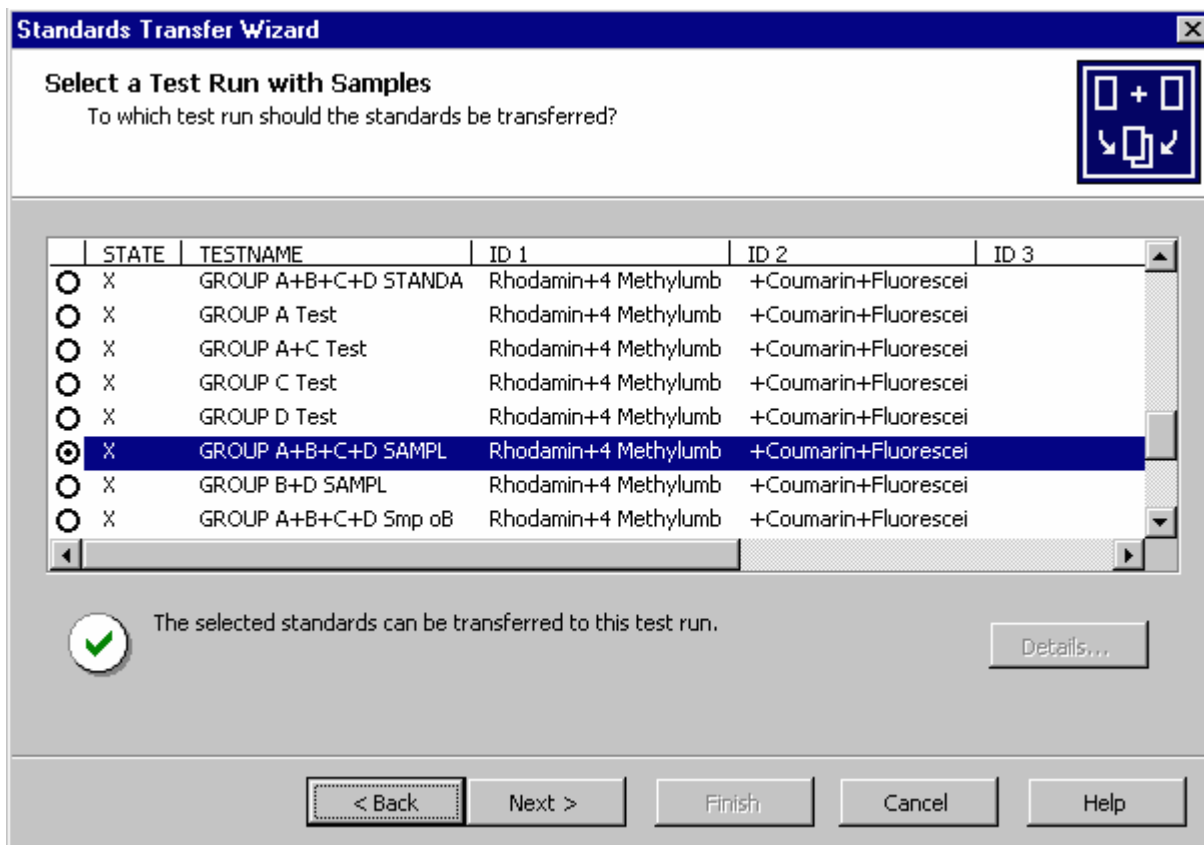
The test runs of your user directory appear in the list box. You can select them with cursor or mouse. Below the list box, a field with information whether or not the current test run can be used as standard appears. Choose the test run with the standards you wish to apply and press the 'Next' button. The test run will be loaded as standard model and the parameters for the curve fitting method will be calculated.

On the next screen, you can choose the sample test you like to combine with the loaded standard model.

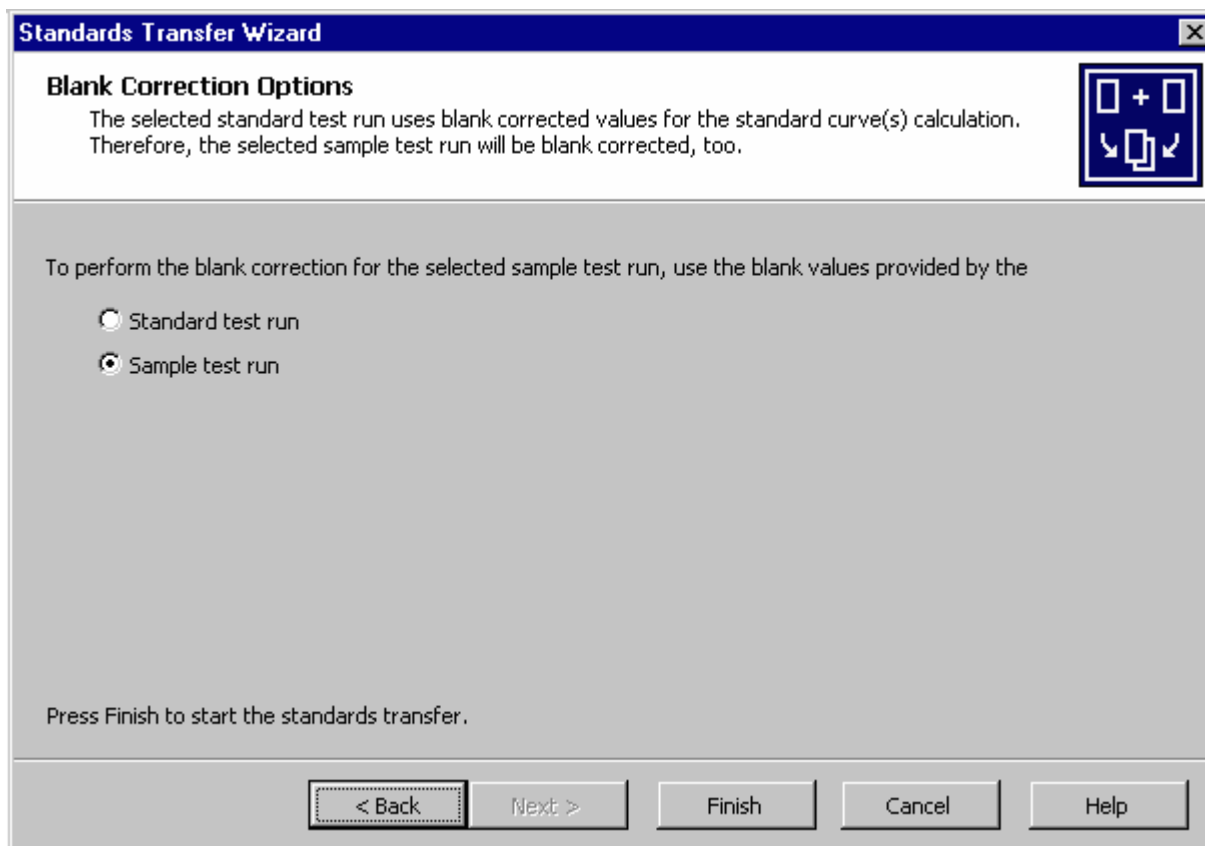
If the sample test run you have chosen is not applicable for your standard model, the 'Details' button is enabled. By pressing it, you can see a list of the measurement parameters not matching to the ones of your standard model. If you need it, you can print the list or store it in an ASCII file.



If you have chosen a test run which is applicable to your standard model, the wizard will appear like this:



If both the standard plate and your sample test run contain blanks, the following screen will appear where you can choose if to use the blanks of the standard plate or the blanks of your test run for the calculation:



By default, the blanks on the sample plate will be used. If you press apply, the software will switch to the standard curve sheet.

The design of the 'Standard concentration' table has now changed compared to the normal view. A table with the list of standard concentrations from the standard plate and their corresponding measurement units will appear. The second table contains the calculated concentrations of your samples. The header areas of all sheets appear now with light green background color. Below the measurement data tables appear a green text box with the information about your standard and sample plate.

Blank corrected standard concentrations of standard plate GROUP A+B+C+D STAND A (11.dbf) - 2001/09/12, 13:01:20

Content	Standard	Units	Content	Standard	Units	Content	Standard	Units
SA11	9.77E-3	403	SB11	0.977	13	SC11	9.766	16
SA10	19.53E-3	383	SB10	1.953	19	SC10	19.531	11
SA9	39.06E-3	423	SB9	3.906	66	SC9	39.063	24
SA8	78.13E-3	616	SB8	7.813	30	SC8	78.125	46
SA7	0.156	710	SB7	15.625	-2	SC7	156.250	-2
SA6	0.313	1103	SB6	31.250	59	SC6	312.500	14
SA5	0.625	1855	SB5	62.500	170	SC5	625	0
SA4	1.250	3189	SB4	125	34	SC4	1250	-4
SA3	2.500	6893	SB3	250	37	SC3	2500	30
SA2	5	17356	BB		389	SC2	5000	-33
SA1	10	54709				SC1	10000	14
BA		383				BC		402

Calculated concentrations using GROUP A+B+C+D STAND A (11.dbf) as standard plate

Table calculation: None						Range1		Channel 1		Raw data - blank	
A	B	C	D	E	F	G	H	1	2	3	4
						0.369		0.862			
						0.288		0.297			
						0.296		0.300			
						0.292		0.308			

Used for calculation:

☐ Group
 ☒ A
 ☐ B
 ☐ C
 ☐ D
 ☐ G
 ☐ H

You are currently using standards from another test run for calculation.

Standard test: GROUP A+B+C+D STAND A (11.dbf) 2001/09/12 13:01:20

Sample test: GROUP A+B+C+D SAMPL (25.dbf) 2001/09/12 13:01:20

Blank correction options:

For the blank correction of the samples the blank(s) of the sample plate are used.

Note: The controls on the Raw Data - and the Evaluation – sheet responsible for the standard calculation are now disabled.

10.12 Microsoft Office Macro Security

Many features in Microsoft Office are created in or depend on the Office integrated **Visual Basic for Application (VBA)** programming language. Since VBA is a potential mean to create and deploy macro viruses, Microsoft Office programs allow the usage of VBA features only in one of three **Security Levels**. These three Security Levels provide an adjustable protection against macro viruses.

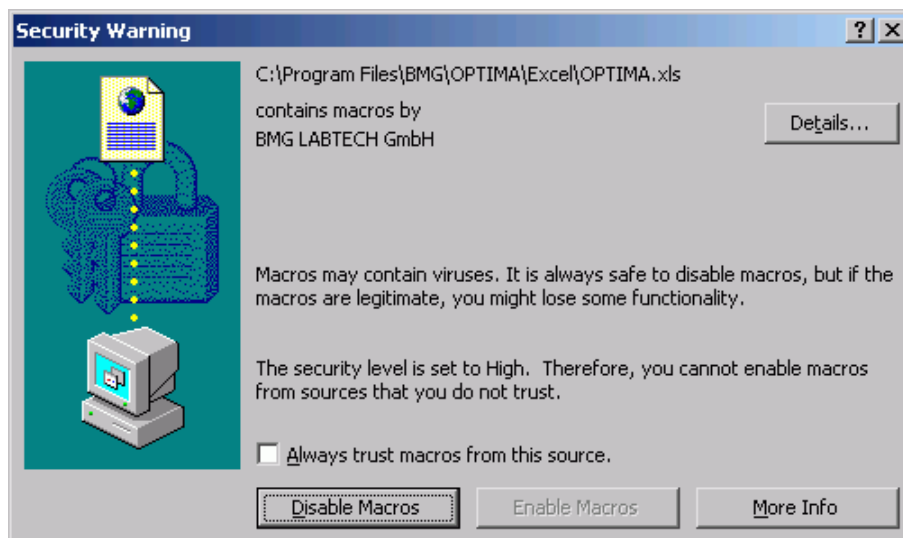
For the highest level of protection, Microsoft recommends setting the macro Security Level to High or Medium and using digital signatures.

A digital signature on a Microsoft Office VBA macro is like a wax seal on an envelope: it confirms that the macro originated from the signer and that the macro has not been altered since it was signed.

When you open an Excel workbook or load an add-in that contains a digitally signed VBA macro, the digital signature appears on your computer as a certificate. The certificate names the macro's source, plus additional information about the identity and integrity of that source.

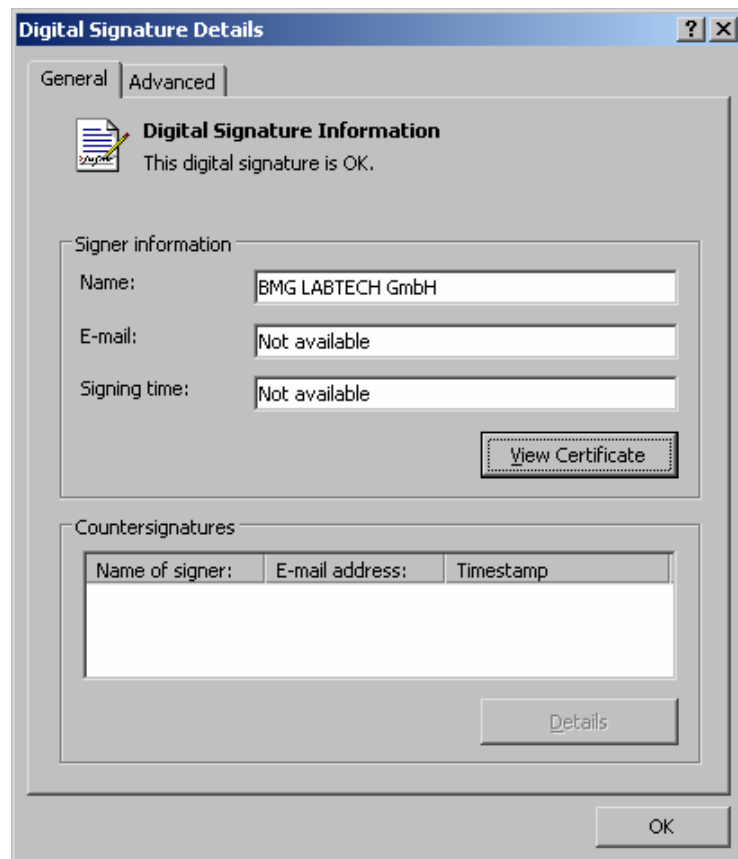
The BMG LABTECH Evaluation Excel workbook uses VBA macros as well to implement its features. In order to assure the highest security, all BMG LABTECH VBA macro codes are digitally signed.

If you have set the Excel **Security Level** to **High** or **Medium** and open the BMG LABTECH Evaluation Excel workbook for the first time, you will see a **Security Warning** dialog box regarding the digitally signed macro with BMG LABTECH GmbH as author specified:



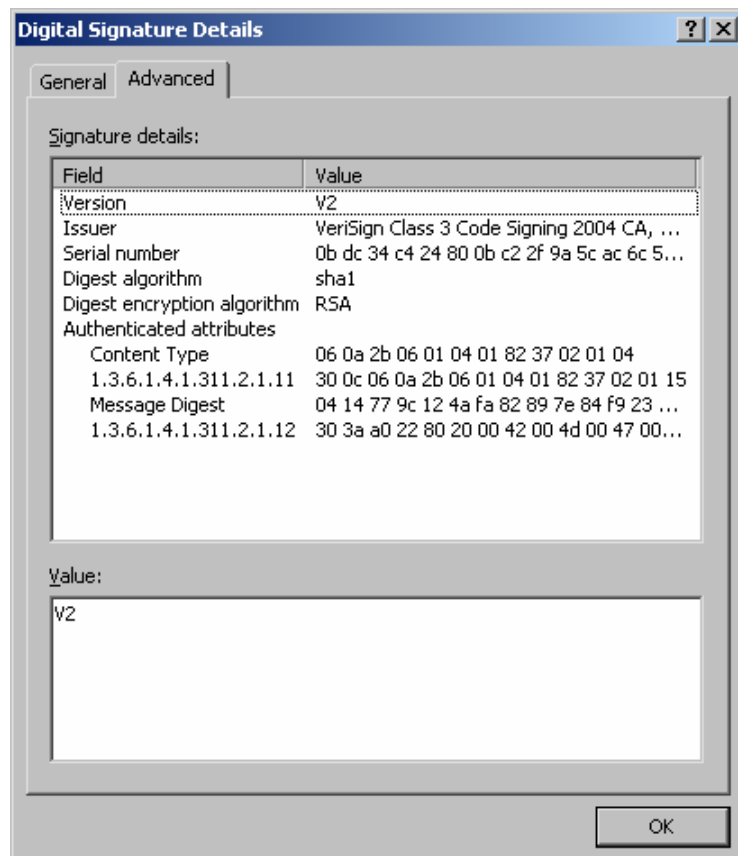
Security Warning dialog box for macros by BMG LABTECH.

To view details about the digital signature, click Details.... This displays the Digital Signature Details dialog box as shown in the next figure.



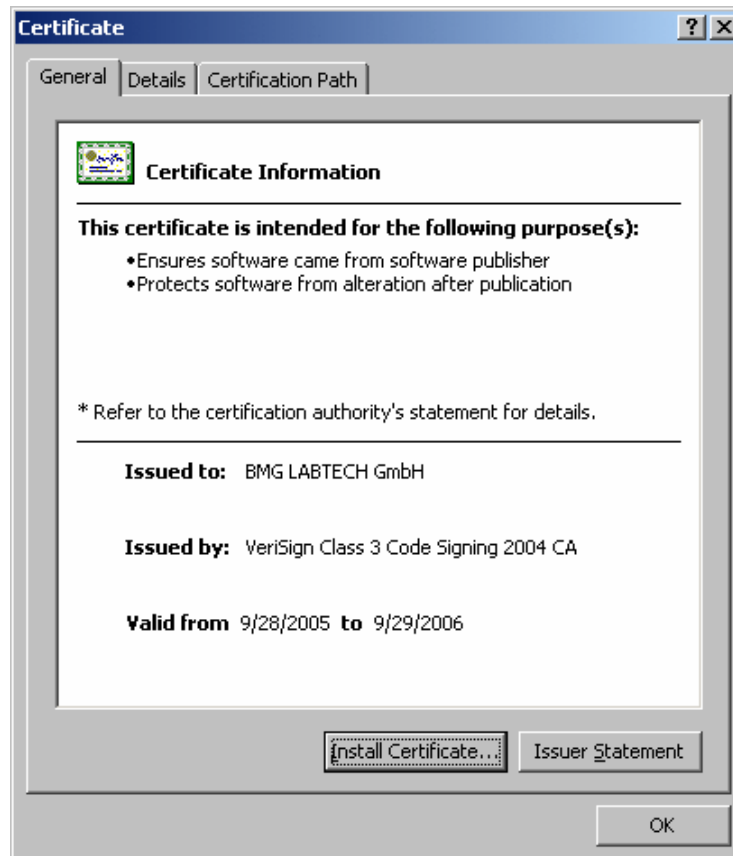
Digital Signature Details dialog box.

If you click on the **Advanced** tab, you will be able to view the **Signature** details as shown below.



Digital signature details.

To view the certificate, click **View Certificate** on the **General** tab.



Digital certificate issued to BMG LABTECH.

Back in the **Security Warning** dialog box, note that the **Enable Macros** button is disabled since BMG LABTECH is at this very moment an unknown, and therefore, not trusted source, which means you cannot enable VBA macros from sources that you do not trust. To enable it, you first have to trust the source of the VBA macro. To trust a source, select the **Always trust macros from this source** check box. This makes the Enable Macros selection available.

Click **Enable Macros**. This will load the signed BMG LABTECH VBA macro. In addition, BMG LABTECH will be added to your **Trusted Sources**. You can verify this in the **Trusted Sources** list (to get there, click **Tools**, point to **Macro**, and then click **Security**. In the **Security** dialog box, click the **Trusted Sources** tab).

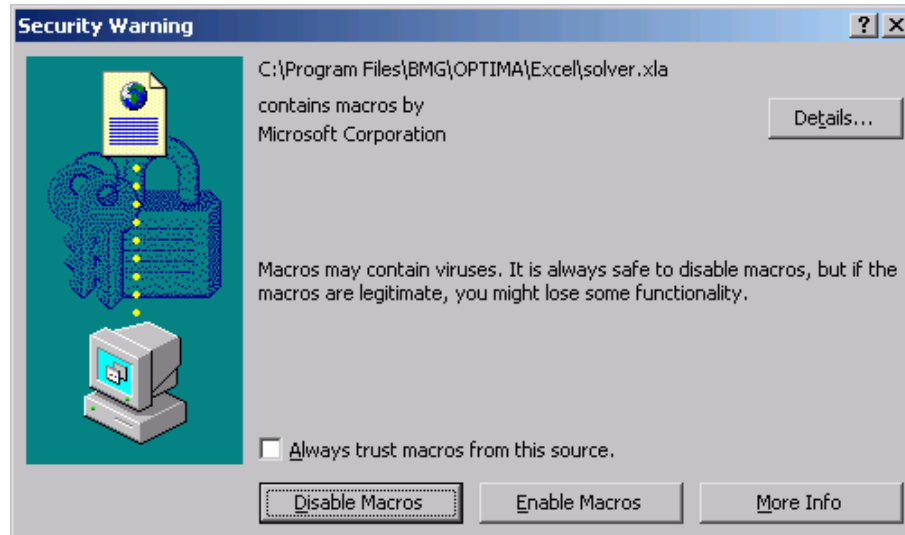
When you open the BMG LABTECH Evaluation workbook the next time, you will find that you won't be prompted and asked whether or not you want to enable the BMG LABTECH VBA macro. Instead, the VBA macro will be loaded without any user intervention. This is because the BMG LABTECH VBA macro was digitally signed using a certificate that corresponds with one that is now in the list of **Trusted Sources** certificates.

If you click **Disable Macros**, the signed BMG LABTECH VBA macro is disabled and won't be loaded.

The BMG LABTECH Evaluation Excel workbook uses VBA macros from two sources:

- BMG LABTECH GmbH and
- Microsoft® Corporation.

Therefore, you will be prompted two times: once for BMG LABTECH, as shown above, and once for the referenced VBA macros from Microsoft:



Security Warning dialog box for macros by Microsoft.

For further details on security and virus protection please see the Office help or www.microsoft.com/security.

11 FDA 21 CFR part 11 compliance

11.1 Overview

The OPTIMA software contains all features necessary to establish a FDA 21 CFR part 11 compliant system, but keep in mind, the software is only a part of the system. In addition to the software, you need people, who are trained regarding 21 CFR part 11 requirements (and regarding GLP / GMP ... requirements) and it will usually be necessary to set up a set of Standard Operational Procedures (SOPs).

11.1.1 What is 21 CFR part 11?

CFR stands for Code of Federal Regulations, which is a collection of regulations issued from federal authorities of the U.S.A.. The title 21 contains the regulations issued by the FDA (Food and Drug Administration). Part 11 of this title contains the FDA regulations regarding electronic records and electronic signatures.

21 CFR part 11 contains rules, **HOW** to create and handle electronic records and signatures. Aim: Electronic records and electronic signatures should be used equal to paper based documents and signatures.

It does **NOT** describe **WHAT** needs to be documented and signed. You will find this kind of information in predicated rules, like 21 CFR part 58 (GLP) or part 211 (GMP).

The rule 21 CFR part 11 has been a law since 20. September 1997 and is, therefore, legally binding in the U.S.A.. It is also binding for products which are intended for the US market.

There are other similar documents like, for example, the OECD GLP Consensus Document No. 10 ("The application of the principles of GLP to computerized systems", issued by the Organisation for Economic Co-Operation and Development, 1995) or the German ChemG GLP Appendix 1 (Anhang "Archivierung"). The requirements regarding record handling described in these rules are very similar to the requirements stated in 21 CFR part 11. The EPA (Environmental Protection Agency of the U.S.A.) has published the CROMERR (Cross Media Electronic Reporting and Record-keeping Rule) draft. The record-keeping part will be presumably finished in 2004. The requirements known so far are similar or identical to FDA 21 CFR part 11.

11.1.2 Definitions

The following definitions are taken from **21 CFR p. 11 Subpart A – General Provisions**.

☐ **Electronic Record**

"Electronic record means any combination of text, graphics, data, audio, pictorial, or other **information** representation in digital form **that is created, modified, maintained, archived, retrieved, or distributed by a computer system.**" §11.3 (6)

Concerning the BMG LABTECH software an electronic record means one measurement data set creating by performing a test protocol (Test Run), stored in dBase and / or ASCII format.

☐ **Electronic Signature**

"Electronic signature means a computer data compilation of any symbol or series of symbols executed, adopted, or authorized by an individual to be the **legally binding equivalent of the individual's handwritten signature.**" §11.3 (7)

❑ **Digital Signature**

“Digital signature means an **electronic signature based upon cryptographic methods** of originator authentication, computed by using a set of rules and a set of parameters such that the identity of the signer and the integrity of the data can be verified. §11.3 (5)

The BMG LABTECH software contains a function to digitally sign electronic records and to verify these signatures using the RSA (cryptographic) method (see chapter 11.2 Digital Sign Function and 2.1.1 Signature Keys).

11.1.3 Requirements

This chapter will list the requirements established in the FDA 21 CFR part 11 rule and how these requirements can be fulfilled using the *BMG LABTECH software*.

21 CFR p. 11 Subpart B - Electronic Records

❑ § 11.10 Controls for closed systems

Procedures and controls shall include:

- ❑ Validation of systems to ensure accuracy, reliability, consistent intended performance, and the **ability to discern invalid or altered records**. Protection of records to enable their accurate and ready retrieval throughout the records retention period.

1. dBase format:

The BMG LABTECH software will protect the data against manipulation by adding a hash value (SHA1, 160 bit) to each data record. This hash value will protect the record in the measurement data base overview table (Measure.dbf) and the whole content of the raw data file (<Number>.dbf). This hash value also includes the audit trail and any potential signatures (see below).

The hash value will be created when the control part of the BMG LABTECH software creates the data record. It will be updated every time a change of the data has been performed using the evaluation part of the BMG LABTECH software, e.g. by changing a sample ID, by removing outliers from the evaluation procedure or by changing the comment field. It will also be updated, when a change of the audit trail has taken place or after adding a signature. This hash value is stored with the data inside the measurement data base.

The hash value will be checked every time a data record is opened in the BMG LABTECH evaluation software. In addition, there is a tool (‘Check Data Integrity’) as part of the BMG LABTECH software package available that can be used to verify this hash value anytime you like (see chapter 11.3).

2. ASCII format:

The protection of the ASCII format data is optional (see chapter 4.3.4 ASCII Export Function). If you use this option, there will be an additional file created for each ASCII file. This file will contain an anti manipulation hash value and the audit trail for the data stored in the connected ASCII file. The SHA1 hash value will protect the whole ASCII file and the audit trail entries.

The hash value will be created when the control part of the BMG LABTECH software creates the ASCII data file. It can be checked using the above mentioned ‘Check Data Integrity’ tool.

- ❑ The ability to generate accurate and complete **copies of records in both human readable and electronic form** suitable for inspection, review, and copying.

Measurement data can be stored in dBase and / or in ASCII format. The ASCII format is human readable (using any text editor program, like Notepad) and can easily be printed. The dBase format can be used in a wide range of programs, including BMG LABTECH's own evaluation program. From there, it can be printed out or saved in XLS (Excel) format or converted into PDF (Acrobat Reader) format (when the Acrobat Distiller or a similar program has been installed).

- ❑ **Limiting system access** to authorized individuals.

This is achieved in the BMG LABTECH software by using a login function (see chapter 2 Login Screen). Setting up user accounts with different privileges (administrator, standard user, user with only permission to execute pre-defined protocols = run only user) is possible. The administrator can define password policies, as for example a minimum length of passwords and a minimum number of non-alpha characters. The user data base is protected against manipulation, passwords will not be stored anywhere (the data base only contains a cryptographically secure hash value); there are options for password aging and alert messages.

Recommendation for program usage in a 21 CFR part 11 compliant environment: *Change the administrator password to something other than 'bmg', define a password for the default user 'USER', disable Auto Login, define a minimum password length of 6 characters including at least one non-alpha character, use the disable accounts function after three invalid login attempts, use password aging (92 days = 3 months or 183 days = half a year) and switch on the alert message for invalid login attempts (see chapter 2.3 Administrator Options).*

- ❑ Use of secure, computer-generated, time-stamped **audit trails** to independently record the date and time of operator entries and actions that create, modify, or delete electronic records.

*All important user actions, such as logging on, defining a test protocol, changing offset or filter values, performing a measurement and so on, can be logged into a **program usage log file**, see chapter 2.3.2. It is possible to start a new log file at every program start or at program start on a new day / week / month / year or after the file has reached a certain size (100 KByte / 1 MByte / 10 MByte). When starting a new log file, the old log file can be renamed or erased. This log file is protected against manipulation by storing a hash value (160 bit SHA1) in an additional file ("Program usage.hv"). The integrity of the log file will be checked at every program start. If manipulation has been detected, the program usage can be blocked and an alert message can be sent (administrator selectable, see chapters 2.3.2 and 2.3.3). In addition, the 'Check Data Integrity' tool (see chapter 11.3) can be used to check the integrity of the log file.*

*The **audit trail for dBase data records** will be stored inside the measurement data base. The first entry of the audit trail will be produced by the control part of the BMG LABTECH software at the same time as the control part creates this data record (immediately after finishing the measurement and transferring the raw data from the reader to the computer). Additional entries will be produced by the evaluation part of the BMG LABTECH software when changes of the data have taken place or after copying or signing a data record. The Audit trail will be shown on the 'Protocol Settings' sheet of the BMG LABTECH evaluation software. In addition, you can use the 'Check Data Integrity' tool to display or print the audit trail.*

*The **audit trail function for ASCII format data** is optional (as is the anti manipulation protection), see chapter 4.3.4. If you use this option, the audit trail will be stored in an additional hash value and audit trail file (see the data protection chapter above) created for each ASCII file. You can use the 'Check Data Integrity' tool to display, print or check the audit trail. In addition, you can use any text editor program, like Notepad, to display the audit trail.*

- ☐ Use of operational system checks to enforce permitted sequencing of steps and events, as appropriate.

Where appropriate, the BMG LABTECH software / firmware checks that necessary sequences of steps are done in the right order (e.g. priming the pumps before performing a test protocol which uses injections).

- ☐ Use of device checks to determine the **validity of the source of data input or operational instruction**.

The measurement data is generated by the BMG LABTECH reader and transmitted to the computer using a proprietary communication protocol. The serial number of the reader used is stored with the measurement data (for ASCII format files use the 'Long header' or 'Full header' option, see chapter 4.3.4).

The log file 'Program usage.log' can be used to keep track of who logged on and modified or performed a test protocol or changed settings, etc..

- ☐ Determination that persons who develop, maintain, or use electronic record / electronic signature systems have the education, training, and experience to perform their assigned tasks.

Users' responsibility.

- ☐ The establishment of, and adherence to, written policies that hold individuals accountable and responsible for actions initiated under their electronic signatures, in order to deter record and signature falsification.

Users' responsibility.

- ☐ Use of appropriate controls over systems documentation including:
 - (1) Adequate controls over the distribution of, access to, and use of documentation for system operation and maintenance.
 - (2) Revision and change control procedures to maintain an audit trail that documents time-sequenced development and modification of system documentation.

BMG LABTECH keeps track of the distribution and uses a version / revision management system for the documentation delivered with the software (Software User Manual, DDE Manual), but keep in mind that the BMG LABTECH software is only a part of the whole system, which is the responsibility of the user.

❑ § 11.30 Controls for open systems

- ❑ Persons who use open systems to create, modify, maintain, or transmit electronic records shall employ procedures and controls designed to ensure the **authenticity, integrity, and, as appropriate, the confidentiality** of electronic records from the point of their creation to the point of their receipt. Such procedures and controls shall include those identified in § 11.10, as appropriate, and additional measures such as document encryption and use of appropriate digital signature standards to ensure, as necessary under the circumstances, record authenticity, integrity, and confidentiality.

Our products are targeted for use in closed systems. The data records will be protected to ensure authenticity and integrity and the BMG LABTECH software uses a cryptographically secure digital signature system, but the data itself is not encrypted. If confidentiality is required, only closed systems should be used.

❑ § 11.50 Signature manifestations

- ❑ Signed electronic records shall contain information associated with the signing that clearly indicates:
 - the **name** of the signer
 - the **date and time** when the signature was executed
 - the **meaning**.

All above mentioned items will be included when using the built-in sign function (see chapter 11.2 Digital Sign Function). In addition the signer can add a comment.

To sign dBase format data records use the OPTIMA menu in the evaluation part, see chapter 10.2.1 or use the 'Check Data Integrity' tool, see chapter 11.3.

Using the 'Check Data Integrity' tool you can also sign ASCII files.

The above mentioned signature parts will be protected against manipulation as the signed data itself. These signature parts will be displayed in the Protocol Settings sheet of the evaluation software (see chapter 10.9) or using the 'Check Data Integrity' tool. A print out is possible.

❑ § 11.70 Signature/record linking

- ❑ Electronic signatures and handwritten **signatures** executed to electronic records **shall be linked to their respective electronic records** to ensure that the signatures cannot be excised, copied, or otherwise transferred to falsify an electronic record by ordinary means.

The BMG LABTECH software uses a public / private key system (RSA with 512, 1024 or 2048 bit key length). The electronic (digital) signature(s) are stored as part of the data record.

Signatures of data records in dBase format will be verified every time the data record is opened in the BMG LABTECH evaluation software. In addition, you can use the 'Check Data Integrity' tool anytime you would like to view and verify signatures. This tool can also be used for verifying or displaying signatures of ASCII files.

21 CFR p. 11 Subpart C - Electronic Signatures

❑ § 11.100 General requirements

- ❑ Each electronic signature shall be **unique** to one individual.

This will be ensured by the BMG LABTECH software. The software uses digital signatures created by a cryptographic public / private key system (see chapter 2.1.1 Signature Keys). The keys are stored for each user account inside the user data base. The private key (necessary to create a signature) is stored in an encrypted version, and can therefore only be used by its owner (after logging on, see below). A signature is verified using the public key of the signer. When verifying a signature it will also be checked whether the public key (and therefore the signature) belongs to the signer named inside the signature.

Recommendation:

To prevent, that a user name is reused, you should not delete user accounts which are no longer needed (but might have been used in the past to sign data records), just disable these accounts (see chapter 2.2).

- ❑ Before an organization establishes, assigns, certifies, or otherwise sanctions an individual's electronic signature, or any element of such electronic signature, the organization shall **verify the identity of the individual**.

Users' responsibility (SOP).

- ❑ Persons using electronic signatures shall, prior to or at the time of such use, certify to the agency that the electronic signatures in their system, used on or after August 20, 1997, are intended to be the legally binding equivalent of traditional handwritten signatures.

Users' responsibility.

❑ § 11.200 Signature components and controls

- ❑ Electronic signatures that are not based upon biometrics shall:
 - Employ at least **two components** such as an id code and password.
 - **Be used only by their genuine owners.**
 - Be administered and executed to ensure that attempted use of an individual's electronic signature by anyone other than its genuine owner requires collaboration of two or more individuals.

*The BMG LABTECH software uses a cryptographic public / private key system. Before signing, the user needs to log on using his **user name** and **password** (see chapter 11.2 Digital Sign Function). Both components are required for all signings.*

The private keys of the users (necessary for signing) are stored inside the user data base in an encrypted version. A private key will only be decrypted for the sign procedure after the user has logged on using his user name and password. The password itself is not stored anywhere in the system. Without knowing the user password even the administrator can not gain access to the private key of a user or to the sign function.

❑ § 11.300 Controls for identification codes/passwords

Persons who use electronic signatures based upon use of identification codes in combination with passwords shall employ controls to ensure their security and integrity. Such controls shall include:

- ❑ Maintaining the **uniqueness of each combined identification code and password**, such that no two individuals have the same combination of identification code and password.

Done by the Login function.

- ❑ Ensuring that identification code and password issuances are periodically checked, recalled, or revised (e.g., to cover such events as **password aging**).

Password aging options are available as part of the Login function (see chapter 2.3.1 Password Policies and Password Aging). If a user leaves, the account can be disabled or deleted.

- ❑ Following loss management procedures to electronically **deauthorize** lost, stolen, missing, or otherwise **potentially compromised password information**, and to issue temporary or permanent replacements using suitable, rigorous controls.

The administrator can disable user accounts or select a function, which will ask the user at next login to change his password (see 'Account Properties' in chapter 2.2 Administrator Functions).

- ❑ Use of transaction safeguards to **prevent unauthorized use** of passwords and/or identification codes, and to detect and **report in an immediate and urgent manner any attempts at their unauthorized use** to the system security unit, and, as appropriate, to organizational management.

There is an option to disable user accounts after a pre-defined number of invalid login attempts (see chapter 2.3.1 Password Policies and Password Aging). Optionally there will be an alert email and / or an alert file after such attempts (see chapter 2.3.3 Alert Messages).

***Recommendation** for program usage in a 21 CFR part 11 compliant environment: Use the function to disable a user account after three invalid login attempts and switch on the alert message for this case.*

- ❑ Initial and periodic testing of devices, such as tokens or cards, that bear or generate identification code or password information to ensure that they function properly and have not been altered in an unauthorized manner.

The user data base is protected against manipulation. When detecting a manipulated user entry, this account will automatically be disabled and an alert message (see chapter 2.3.3) will be sent.

Manipulation of the administrator settings (e.g. the password restrictions or alert message settings) will also be automatically detected. In such cases, the program usage will be disabled. To re-enable the program usage the administrator needs to log on.

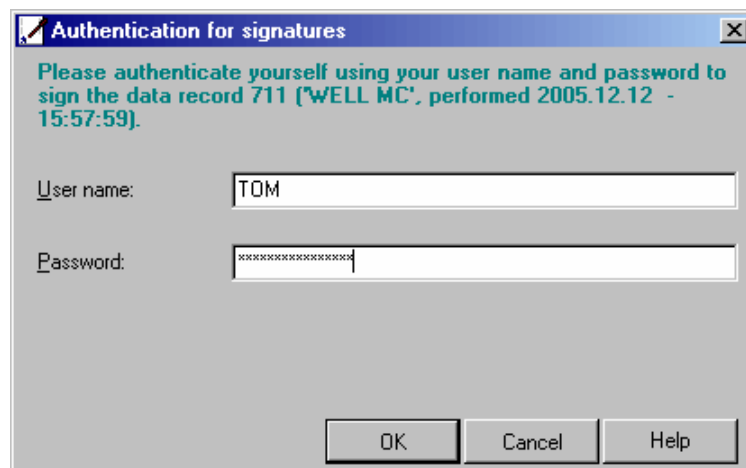
11.2 Digital Sign Function

If you want to sign a data record in dBase format use the 'Sign current test run' function from the 'OPTIMA' menu (see chapter 10.2.1) of the evaluation part or use the 'Check Data Integrity' tool (see chapter 11.3). Using this tool you can also sign ASCII files.

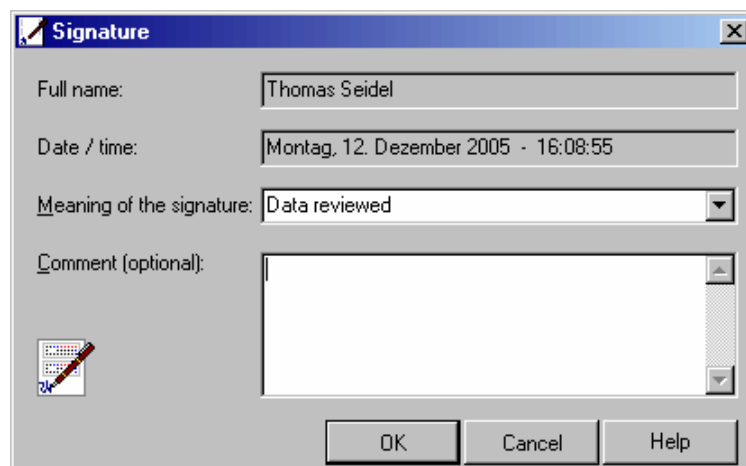
After selecting the sign function an authentication window appears. Please log in using your user name and password as defined using the 'Login' screen (see chapter 2).

Notes: It is possible, that another person signs the current data record than who is currently logged on.

You need to have a pair of RSA keys to be able to use the sign function (see chapter 2.1.1 Signature Keys).



After you have successfully logged in, the following dialogue box will appear:



The full name of the signer (as defined in the keys dialogue) and the current date and time will be automatically added to the signature.

You need to define the meaning of the signature, e.g. 'Data audited' or 'Data released'. You can select from the pre-defined list or you can type something in. In addition you can add a comment.

Note: The date and time is displayed here using the format as defined in the Windows Control Panel (Regional Settings) under long date and long time format.

11.3 Check Data Integrity Tool

With the BMG LABTECH software a tool 'Check Data Integrity' will be installed. You can use this tool to check the integrity of test run data (dBase and ASCII format) produced by BMG LABTECH software.

If there are signatures attached to the data, these signatures can be verified as well.

It is also possible to add new signatures.

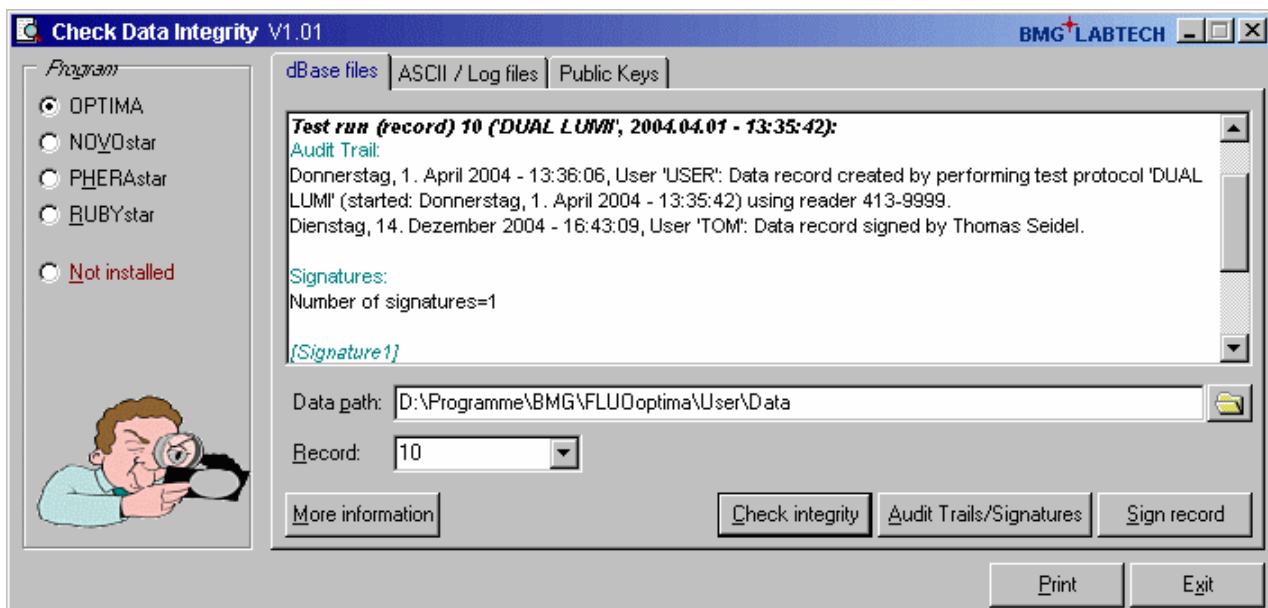
You can also check the integrity of the program usage log files.

To start this tool use the 'Start | Programs | BMG LABTECH | OPTIMA | Check Data Integrity' entry from the Windows start menu.

Please select the BMG LABTECH program used to create the data on the left side of the program window. If you are using the 'Check Data Integrity' tool on a computer, where the BMG LABTECH software itself has not been installed, choose 'Not installed' in the program selection box (see also the explanation for 'stand alone usage' at the end of this page).

11.3.1 dBase Files

The 'Check Data Integrity' tool contains three sheets. Use the first sheet to check data records in dBase format.



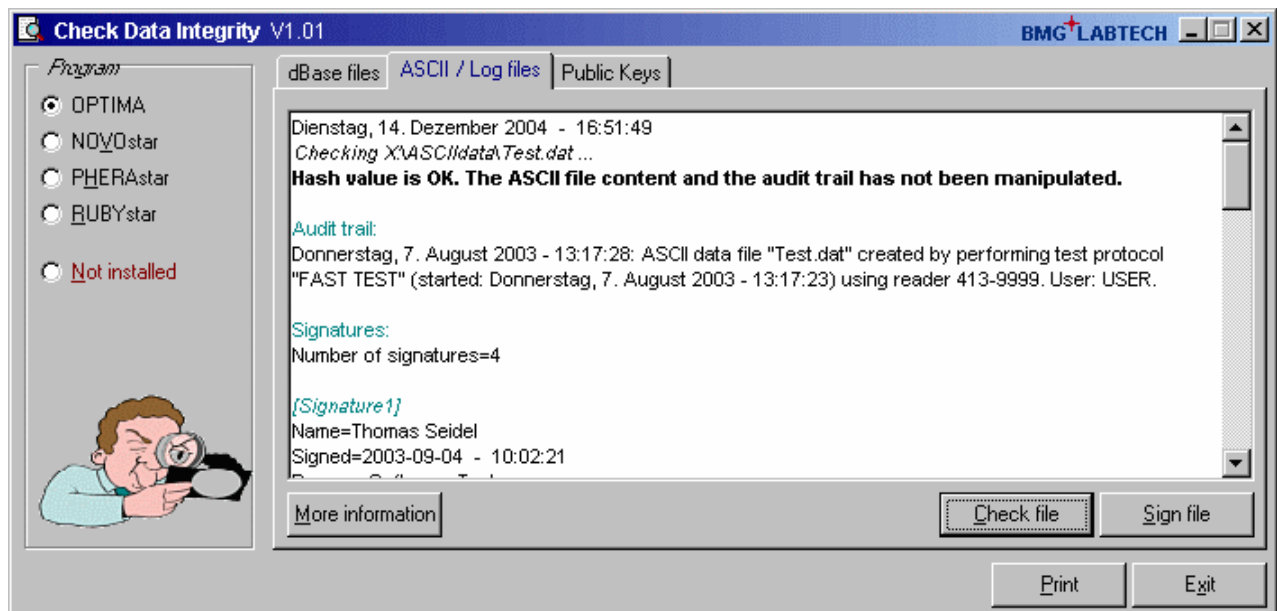
After selecting the **data path** you can choose which **record** you want to check or you can select 'All records'. To check the integrity of the data and the audit trail and to verify the signatures (if signatures exist) click the '**Check integrity**' button. To display the audit trail and the signatures use the '**Audit Trails/Signatures**' button.

Use the 'Sign record' button to sign a data record. The same signature dialogue (see chapter 11.2) will appear as when using the 'OPTIMA | Sign current Test Run' menu command of the evaluation part (see chapter 10.2.1) is used.

Note: It is not possible to sign more than one record at a time.

11.3.2 ASCII / Log Files

The second sheet of the 'Check Data Integrity' tools allows you to check data records in ASCII format and to check the program usage log files.

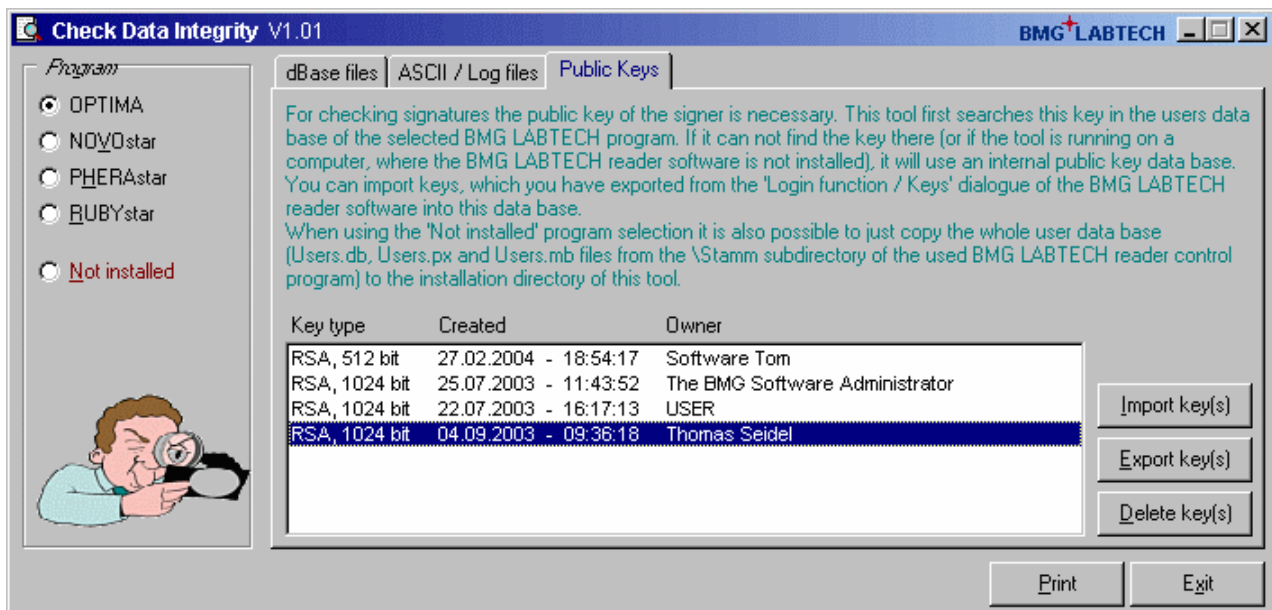


To check the integrity of the data and the audit trail of an ASCII or log file and to verify the signatures (if signatures exist) click the '**Check file**' button. The audit trail and the signatures will be displayed together with the check result.

Use the 'Sign file' button to sign a file. A signature dialogue (see chapter 11.2) will appear.

11.3.3 Public Keys

To verify a signature the public key of the signer is necessary. The BMG LABTECH software stores this key together with the signature, therefore verifying a signature is always possible. Nevertheless, checking the authenticity of the public key is a critical part of verifying digital signatures. The signature verify function of the BMG LABTECH software will try to check the authenticity of the public key by searching this key in the user data base. The 'Check Data Integrity' tool contains a separate public key data base, which is used in addition to the user data base of the reader control program. Using this data base, the 'Check Data Integrity' tool can also be used on computers where the BMG LABTECH reader software has not been installed. It is possible to export keys from the user data base and to import these keys in the public keys data base of the 'Check Data Integrity' tool.



In the Public Keys sheet you can see which public keys are stored in the public key data base. Use the '**Import key(s)**' button to add keys to this data base.

You can also select keys and **export** them (as a backup or to use them on another computer) or you can **delete** keys, which are no longer necessary.

11.3.4 Stand Alone Usage

If you want to use this tool on a computer, where the BMG LABTECH reader software is not installed, copy all files from the '~:\Program Files\BMG_CheckDataIntegrity\' folder and its subfolders into a folder of your choice on the target computer. The Borland Database Engine (BDE) needs to be installed on this computer. Alternatively you can use the normal OPTIMA installation CD-ROM. You only need to perform one installation (Control or Evaluation part). Please perform a custom installation, where you deselect everything besides the 'Borland Database Engine' and the 'Check Data Integrity' tool.

It is recommended to copy the files from the subfolder '..._CheckDataIntegrity\AdditionalFilesForStandAloneUsage\<Program Name>' into the directory, where you have installed the 'Check Data Integrity' tool (otherwise this program will ask you to specify the location of the DLLs).

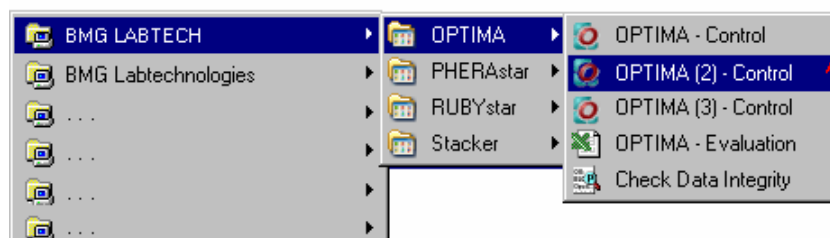
If you want to verify signatures, you should export the public keys from the user data base (see chapter 2.1.1 Signature Keys) and import these keys into the public keys data base of this tool (see chapter 11.3.3). Alternatively you can copy the whole user data base (all 'Users.*' files from the '~:\Program Files\BMG\OPTIMA\Stamm' folder into the directory, where you have installed the 'Check Data Integrity' tool.

If you want to sign, you need to copy the user data base to the target computer.

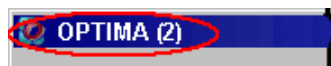
Note: This tool displays date and time information using the format as defined in the Windows Control Panel (Regional Settings) under long or short date and long time format.

12 Using Multiple Installations

It is possible to install the control part of the OPTIMA program more than once. You can create up to 9 program installations. This will be useful if more than one reader is connected to one computer. Please see chapter 1.3.3 Control Part - Installation for a description how to create a new installation. If you have created more than one installation you will find a separate desktop icon and a separate start menu entry for each installation:



You can use the different program installations totally independently. All settings, e.g. the communication port to be used, the test protocols and the measurement data will be stored independently. The program installation (instance) number 2 to 9 will show the installation number in the caption of the main program window and of the login screen.



This number will also be displayed in the Windows task bar.



The evaluation part can be installed only once, nevertheless it is possible to use this one installation to perform data reduction tasks for data created by all control part installations. You just need to setup accounts in installation number one using the different data directories, e.g. create a user called 'USER2' with 'c:\Program Files\BMG\OPTIMA2\User' as root directory to access the data created by control part installation number 2.

If you use the ASCII export function, you can define the target directories independently in all program installations.

13 Known Problems and Solutions

There are (at least) four possible errors caused by Microsoft (1. – 3., 5.):

1. Error 429 'ActiveX component can't create object or return reference to this object'
2. Error 1004 'VBA initialization failed'
3. Error 40009 'No current row'
4. Disabled Plate In / Out Buttons
5. Access Rights Problems under Windows NT / 2000 / XP

For solutions see the following chapters.

13.1 Error 429 'ActiveX component can't create object or return reference to this object'

If this message appears when you open the Excel evaluation sheet, you should use the Fix429 program from the installation CD-ROM (under ~\OPTIMA V1.xx\Evaluation\).

This program checks a registry key responsible for ActiveX data access objects delivered with Microsoft programs (Office (Excel), Visual Basic, Internet Explorer...) regarding presence and correct value.

Key: HKEY_CLASSES_ROOT\LICENSES\F4FC596D-DFFE-11CF-9551-00AA00A3DC45
Value: mbmabptebkjcldgtjmskjwtshbjbmkmwtrak

A wrong value of this key will cause the error 429 ('ActiveX component can't create object or return reference to this object') at startup of the evaluation software.

- If the key does not exist, it will be created (after pressing the 'Correct Error' button).
- If the key exists but has a wrong value, the old value will be saved under the name 'backup' in the same key and the key value will be changed to the correct value. Should you encounter problems with other programs, you can restore the old key value using the 'Restore' button of the Fix429 program.

Note: The patch program 'Fix429' will be called automatically during the installation of the OPTIMA evaluation part, so this error should occur (after installation) only if you install a new Microsoft program, which may have deleted or changed this registry key.

13.2 Error 1004 'VBA initialization failed'

When you get this error message after starting the evaluation part, some Excel add-ins are missing or are not enabled, e.g. the VBA macro language.

Please start the evaluation part directly from the windows start menu (and not using the Excel button in the control part). There will be a message telling you what parts are missing and asking you whether you want to install these Excel parts. Please answer 'Yes'.



Using Excel XP it is also necessary to set the security level to medium or low as the high level will not allow execution of our set of macros. To do so, please use the Excel menu command 'Extras | Security'.

13.3 Error 40009 'No current row'

If this message appears when you open the evaluation worksheet, you must update the Dao350.dll file installed on your computer.

- Close Excel and open a Windows Explorer.
- Open `~:\Program Files\Common Files\Microsoft Shared\Dao\`.
- Rename the existing Dao350.dll file in the folder to Daoold.dll.
- From the OPTIMA installation CD-ROM go to the directory `~:\OPTIMA Vx.xx-x\Evaluation\ Excel97_RunTimeError40009\`. Copy the file Dao350.dll from the CD and paste it in the `~\Microsoft Shared\Dao\` folder.
- Reopen the software. There should be no error messages.

13.4 Disabled Plate In / Out Buttons

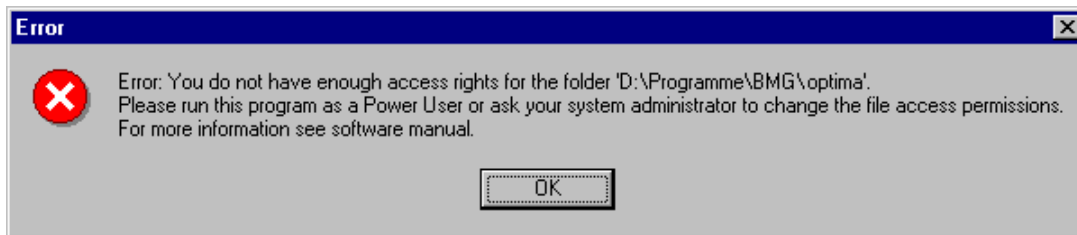
If both plate buttons (, ) are disabled and there is no test run active and the communication to the reader is OK, then it is most likely that there is a wrong setting in the configuration file '`~\Program Files\BMG\OPTIMA\FLUOstar OPTIMA.ini`'. The value behind '`DisablePlateCmds=`' should be '`False`'. This parameter will be set to '`True`' if the reader is connected to a robotic/stacker system and the plate in/out movement will be controlled only using the robotic software.

13.5 Access Rights under Windows NT / 2000 / XP

13.5.1 File Access Permissions

Microsoft changed the default access permissions for all program files newly installed. In all Windows versions before Windows 2000, any standard user has access to the files installed using a standard installation program. Beginning with Windows 2000, a normal user (non-power user) has only read access by default. As we store important information in data base files, all users of the BMG LABTECH software need to have write access to certain files in the program directory (usually `~\Program Files\BMG\OPTIMA`).

If you have used the option '**Program should be usable also for non power users**' during installation (see chapter 1.3.2 Control Part - Installation), the write access rights to the necessary files will be set during the installation. If you did not use this option or if you have added new users / user groups after installation, the following error might appear:

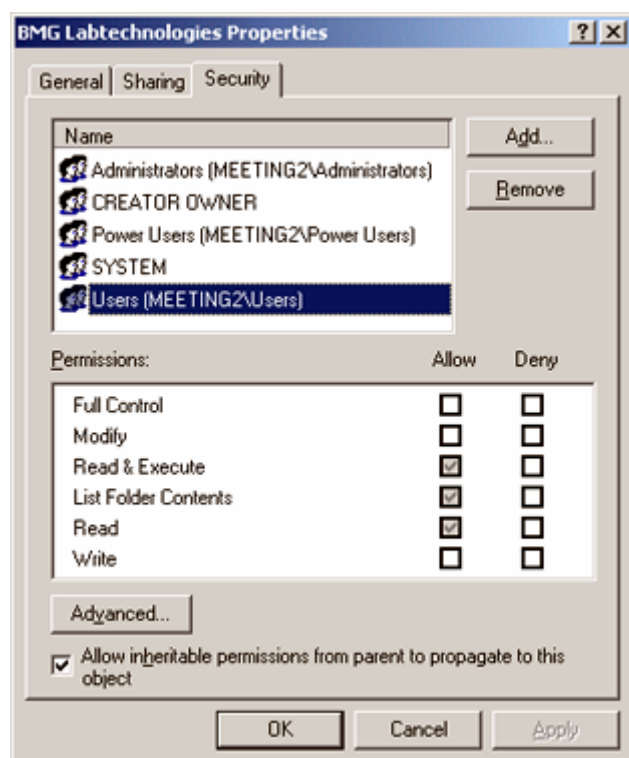
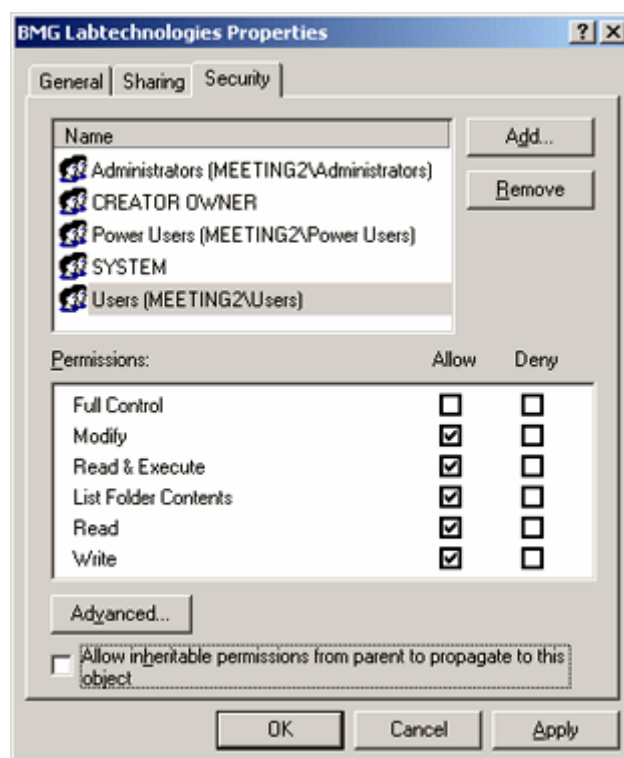


Solution

Logon as administrator (or user with administrative rights).

Run the program '**SetPermission**' from the OPTIMA CD-ROM (folder `~:\OPTIMA Vx.xx\`).

Alternatively, you can also manually change the permissions: Open the Windows Explorer and right-click the folder where the BMG LABTECH software is installed (usually `~:\Program Files\BMG\OPTIMA`). Choose 'Properties', then 'Security'. Check the 'Modify' permission, 'Write' permission will be checked automatically. Uncheck the box 'Allow inheritable permissions from parent to propagate to this object'. Now all users should be able to write to the BMG LABTECH directory and work with the software.

Default settings:*Customized settings:***Listing of files where write access is necessary**

The following files / directories need to have full access to write (temporary) data.

BMG LABTECH Software		
Directory	File	Usage
~:\Program Files\BMG\OPTIMA\	FLUOstar OPTIMA.ini * OPTIMA.rns OPTIMA.log	Configuration file Run Statistik File Log file
~:\Program Files\BMG\OPTIMA\Temp		Directory for temporary files
~:\Program Files\BMG\OPTIMA\User		Subdirectories for test protocol definitions and measurement data of standard user
~:\Program Files\BMG\OPTIMA\Admin		Subdirectories for test protocol definitions and measurement data of administrator
~:\Program Files\BMG\OPTIMA\ <UserName>		Subdirectories for test protocol definitions and measurement data of defined users
~:\Program Files\BMG_Flashtools		Used by Flash-EPROM Update Program

* This file is named 'FLUOstar OPTIMA.ini' instead of 'OPTIMA.ini' for compatibility reasons.

Borland Database Engine		
Directory	File	Usage
~:\Program Files\Borland\Common Files\BDE\	idapi32.cfg	BDE properties
	idapi32.bak	Backup of idapi32.cfg
~:\ You can use the BDE Administrator to change the location of the pdoxusr.net file (Configuration Drivers Native Paradox Net Dir).	pdoxusr.net	Paradox network control file

13.5.2 Registry Access

There are two places in the registry where the BMG LABTECH software stores information:

All user specific things, like program settings (e.g. the program window position or current state display options), are stored in the registry part `HKEY_CURRENT_USER`. There are no access problems to this part.

All settings which are important for all users, like the selected communication port or reading mode, will be stored in the `HKEY_LOCAL_MACHINE` part of the registry (as this part of the registry is intended for non-user specific information). For unintelligible reasons, Microsoft changed the default access permissions for this part of the registry beginning with Windows 2000. On all Windows versions before Windows 2000, every user has read and write access to this general information part of the local registry, beginning with version 2000 a standard user (non-power user), by default, only gets read access to newly generated keys in this part of the Windows registry.

This access limitation can cause the Error: '0509: The program has not been correctly installed or you are using W2000/XP without appropriate registry access rights.' at program start if you did not use the option '**Program should be usable also for non power users**' during installation (see chapter 1.3.2 Control Part - Installation).

Solution

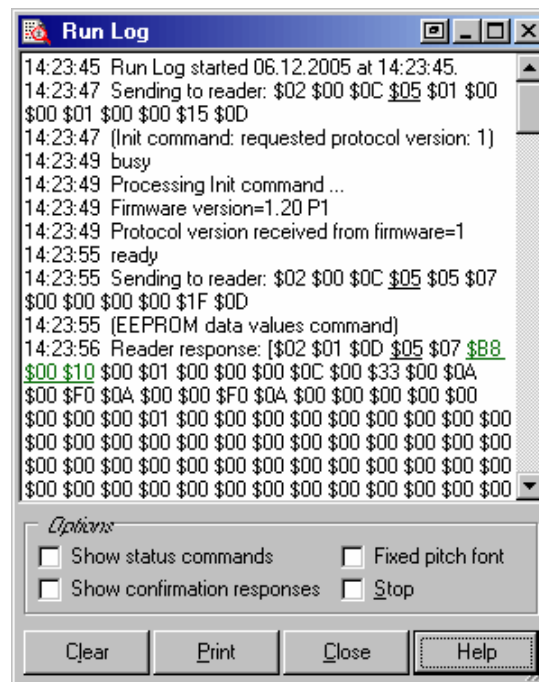
- Logon as Administrator (or a user with administrative rights).
- Run the program '**SetPermission**' from the OPTIMA CD-ROM (folder `~:\OPTIMA Vx.xx\`).

or

- Start the program 'Regedt32' (Windows XP: 'Regedit', use the 'Run' from windows start menu).
- Go to '`HKEY_LOCAL_MACHINE\Software\BMG Labtechnologies`'.
- Select 'Permissions'.
- Add read and write rights for everyone to this key and all sub keys.

13.6 Run Log Window

The Run Log window (control part) shows all commands sent to the reader and all responses. You can open the Run Log window using the key combination [Shift]+[Ctrl]+[L] from the program main window.



It is possible to stop the process of adding new entries to the run log by checking the **'Stop'** checkbox.

You can **print** the entire run log or a marked part of the run log.

13.6.1 Search Function

It is possible to search the log window forward or backward beginning from the cursor position or from the top / end of the text.

The following key combinations can be used to activate the search function:

1. Borland Style:

To open the search parameters window use [Ctrl]+[Q][F], to repeat the last search (in the defined search direction) use [Ctrl]+[L]. Use [Shift]+[Ctrl]+[L] to search against the defined direction, e.g. to go back to the previous occurrence.

2. Microsoft Style:

To open the search parameters window use [Ctrl]+[F], to search the next occurrence in forward direction use [F3], for backward direction use [Shift]+[F3].

If you select something before using the [Ctrl]+[Q][F] / [Ctrl]+[F] key combination this selection will be used as default search string, otherwise if the cursor is positioned onto a word (something containing letters, not just hexadecimal numbers) this word will be taken as default value.

Note: Searching something, which includes line breaks, is not possible. You might change the width of the Run Log window to remove soft line breaks.

13.6.2 Run Log File

The run log will automatically be saved into the file 'OPTIMA.log' in the OPTIMA main directory (usually '~\Program Files\BMG\OPTIMA\'). You can switch off the generation of the run log file by changing the line 'RunLog=true' in the configuration file '~\Program Files\BMG\OPTIMA\FLUOstar OPTIMA.ini' (section [Debug]) into 'RunLog=false'.

Note: At the next program start the existing content of this file will be erased. Whenever you need support from BMG LABTECH due to a software / firmware malfunction you should send us the log file together with a description of the nature of the problem.

14 Support

If you have any problems / questions regarding the software / the instruments, you should visit our web page (<http://www.bmglabtech.com>) and read the 'Frequently Asked Questions' (FAQ) on the Support page. If you can not find an answer there, please contact BMG LABTECH using the following email addresses:

- Problems / questions regarding software:
support@bmglabtech.com
- Problems / questions regarding the instruments:
tech.service@bmglabtech.com

You can also use our on-line bug report form:

<http://www.bmglabtech.com/html/support/bugreport.cfm>

APPENDIX °

STURGEON EARLY LIFE STAGE SEDIMENT TOXICITY REQUIREMENTS

These tables have been extracted from the
Draft Quality Assurance Project Plan for
the Assessment of Sediment Toxicity to
White Sturgeon (*Acipenser transmontanus*)

Table B-2. Sampling Design for the 2010 Water Toxicity Studies with White Sturgeon Early Life-Stages and ASTM (2005) Target Values for Conducting Early Life-Stage Fish Toxicity Studies.

Parameter	Sturgeon Sediment Study	ASTM 2005
Time of exposure initiation	≤ 8 hours	Salmonids: ≤ 96 hours; All other species: ≤ 48 hours
Exposure duration	66 days (> 40 days post-swim-up)	Salmonids: ≥ 30-day post-swim-up; Pike: 32 days; Fathead minnow: ≥ 28 days; White sucker: 32 days; Channel catfish: 32 days; Bluegill: 32 days
Loading density/rate	≤ 0.1 g/L/24 hours	≤ 0.5 g/L/24 hours
Number of true replicates per treatment/dose	3 x 3	≥ 2
Number of fish per treatment (controls) at end of study	≥ 60	≥ 40
Observations	≥ 2 times per day	≥ 1 time per day
Feeding	≥ 3 times per day	≥ 1 time per day

Table B-3. Test Conditions for Conducting a 66-Day Early Life-Stage Toxicity Test with White Sturgeon (*A. transmontanus*).

Parameter	Conditions
Test Type	Whole-sediment toxicity test with sediments collected at one site upstream of Teck's Trail facility (reference), at three locations between the U.S.-Canadian border and Kettle Falls, and an artificial sediment control
Temperature	15 ± 1°C
Light Quality	Wide spectrum fluorescent lights
Photoperiod	16 hours light; 8 hours dark
Test System	205 L recirculating flow-through system each with two 40 L exposure chambers.
Water Volume	205 L
Renewal Frequency of Water	Complete replacement of water (205 L) every 12 to 24 hours.
Age of Organisms	8-hour post-fertilization through ~60-day post-hatch
Number of Organisms per Replicate Group	Exposure experiment will be initiated with 1,000 eggs per replicate treatment group. Larvae will be thinned to 100 fish per replicate treatment group after they hatched, and then further thinned to 60 fish per group prior to initiation of self-feeding in accordance with sturgeon culturing recommendations based on the 2008 and 2009 surface water toxicity studies (Tompsett et al., submitted).
Number of Replicate Groups	Three replicate recirculating systems per replicate group
Feeding	Live diet, brine shrimp (<i>Artemia</i>), polychaetes, and blood worms 3 to 4 times per day <i>ad libidum</i> . Feeding will be initiated 2 to 3 days post-swim-up to acclimate fry to food.
Aeration	None; recirculation of water provides sufficient aeration
Water Source	Artificial river water (de-chlorinated lab water adjusted to river water for pH, DOC, and hardness)
Test System Cleaning	Gently scrape tanks and brush screens if these should become overgrown with organic matter; in-line coarse particle filter at exposure chamber outflow to reduce particle/organic matter build-up.
Water Quality	Daily: DO, pH, temperature, and conductivity One to three times per week: Ammonia, alkalinity, chlorine, hardness, nitrate + nitrite, and DOC. Frequency will be increased if marked changes in readings occur between measurements.
Test Duration	~66 days

Table B-3. Test Conditions for Conducting a 66-Day Early Life-Stage Toxicity Test with White Sturgeon (*A. transmontanus*) (continued).

Parameter	Conditions
Endpoints	Hatchability, survival, growth, gross morphology, development
Test Acceptability	<p>Minimum hatching rate in the controls/reference should be 60% or within 90% confidence interval of that observed at the hatchery for the same fish</p> <p>Minimum survival of fry until swim-up in the controls/reference (~12- to 16-day post-hatch) should be 80%, or within 90% confidence interval of that observed at the hatchery for the same fish</p> <p>Minimum survival of fish post-swim-up through the end of the experiment in the controls/reference should be within 90% confidence interval of that observed at the hatchery for the same fish</p>

Notes:

L = liter

DOC = dissolved organic carbon

DO = dissolved oxygen

Table B-4. General Activity Schedule for Conducting a 66-Day Early Life-Stage Toxicity Test with White Sturgeon (*A. transmontanus*).

Day	Conditions
-98 to -84	<ul style="list-style-type: none"> Collection of sediments at selected sampling sites in the UCR Transport of sediments to the U of S at 4°C Storage of sediments at U of S at 4°C
-62 to -15	<ul style="list-style-type: none"> Set up of re-circulating exposure systems in the Aquatic Exposure Facility at the University of Saskatchewan Homogenize sediments from each sampling site immediately prior to placing sediments into exposure systems Take sub-sample for SEM, AVS, TOC, grain size and TAL analyses from homogenized sediment Add sediments to each exposure chamber Adjust exposure system flow through and equilibrate exposure systems with sediments and test water (> 4 weeks) Measure water quality in porewater and overlying water at 0, 12, 24, 48, 96 every 48 hrs thereafter after filling systems with water and initiation of flow-through conditions
-14 to -1	<ul style="list-style-type: none"> Begin water quality monitoring
Hatchability Test\	
0	<ul style="list-style-type: none"> Test water quality in each exposure system (see Table B-6) Acclimate freshly fertilized eggs to water temperature in exposure systems Transfer eggs to egg hatching jars Adjust flow through egg hatching jars so that the top layer of eggs roles very gently but do not get pushed up in the water column
1 to 6	<ul style="list-style-type: none"> Observe systems with hatching jars 2- to 3-times per day Measure DO, Temperature, pH and conductivity daily
3	<ul style="list-style-type: none"> Ensure that neurolation has been completed Increase water flow to hatching jars such that eggs are vigorously circulated throughout the jar Test water quality in each exposure system
6 to 9	<ul style="list-style-type: none"> Observe and record hatching activities Count and transfer hatched larvae to main exposure chambers Count and remove dead eggs from hatching jars
Pre-Swim-Up Test	
7 to 20	<ul style="list-style-type: none"> Observe larvae 2- to 3-times per day Measure DO, Temperature, pH and conductivity daily and all other water quality parameters at least every 7 days Count, weigh and remove dead fry and preserve dead fish in formalin Observe and record any behavioral abnormalities Observe and record swim up and presence/rejection of black yolk sac plugs
11	<ul style="list-style-type: none"> Thin fish to 100 individuals per replicate system
18	<ul style="list-style-type: none"> Begin adding diet to the chambers to condition fry to food
Post-Swim-Up Test	
21 to 25	<ul style="list-style-type: none"> Record swim-up and feeding behavior of fry Record weight and length of fry removed for thinning purposes and fix these fish in formalin
21 to 65	<ul style="list-style-type: none"> Thin fish to 60 individuals per replicate system Feed fish 4-times per day Clean systems daily Record and remove mortalities daily, and measure, weigh and fix dead fish in formalin Test water quality in each exposure system (see Table B-6)

Table B-4. General Activity Schedule for Conducting a 66-Day Early Life-Stage Toxicity Test with White Sturgeon (*A. transmontanus*) (continued).

Day	Conditions
66	<ul style="list-style-type: none"> • Terminate study • Measure complete water quality suite including metals • Euthanize fish in MS222 • Measure and weigh fish • Fix 75% of fish in formalin and archive • Archive sediment sub-sample at 4°C

Notes:

AVS = acid volatile sulfides

COI = chemical of interest

DOC = dissolved organic carbon

DO = dissolved oxygen

TAL = target analyte list

TOC = total organic carbon

SEM = simultaneously extracted metals

Table B-5. Test Acceptability Requirements of a 66-Day Early Life-Stage Toxicity Test with White Sturgeon (*A. transmontanus*).

Acceptance Criteria	
1	Freshly fertilized eggs from at least 2 to 4 different females and males are to be used (time between hatching and initiation of study must not exceed 24 hours)
2	Average hatching rate of eggs in the lab water controls should not be less than 60%, or within 90% confidence interval of that observed at the hatchery for the same fish
3	Average survival of fry until swim-up in the lab water controls should be greater or equal to 80%, or within 90% confidence interval of that observed at the hatchery for the same fish
4	Average survival of fry post-swim-up in the lab water controls should be within the 90% confidence interval of that observed at the hatchery for the same fish
5	All water quality parameters with the exception of DO and temperature should not vary by more than 50% during the exposure
6	Dissolved oxygen should be maintained above 70% saturation
7	Average daily temperature should be maintained at $15 \pm 1^{\circ}\text{C}$; the instantaneous temperature must always be within $\pm 3^{\circ}\text{C}$ of 15°C
Additional Acceptance Criteria	
1	All organisms must be from the same source
2	Survival and hatchability in the lab water controls should be comparable to those observed at the Kootenay Trout Hatchery for the fish from the same fertilization event
3	All test systems and chambers should be identical and should be run under the same recirculating conditions for each study, except if otherwise stated in the protocols
4	Natural physico-chemical conditions of the control lab water should be within the tolerance limit for white sturgeon early life-stages
5	Food used for both studies should be obtained from the same source and should have been tested for possible comparability with white sturgeon early life-stages prior to initiation of the studies

Notes:

DO = dissolved oxygen

Table B-6. Parameters Measured in Sturgeon Exposure Chambers.

Analyte/Parameter	Sediment		Pore/overlying Water	
	CAS	UofS	CAS	UofS
Conventional Parameters				
Alkalinity			W	W
Hardness			W	D
TDS			W	
TOC	start			D
pH	start			D
DO				D
Temperature				D
Conductivity				D
Cations/Anions				
Calcium			W	
Chloride			W	
Fluoride			W	
Magnesium			W	
Potassium			W	
Sodium			W	
Sulfate			W	
Nutrients				
Ammonia				W
Nitrate+Nitrite				W
Common Metals and Metalloids				
Aluminum	start		W	
Antimony	start		W	
Arsenic	start		W	
Barium	start		W	
Beryllium	start		W	
Cadmium	start		W	
Chromium	start		W	
Cobalt	start		W	
Copper	start		W	
Iron	start		W	
Lead	start		W	
Manganese	start		W	
Mercury	start		W	
Molybdenum	start		W	
Nickel	start		W	
Selenium	start		W	
Silver	start		W	
Thallium	start		W	
Vanadium	start		W	
Zinc	start		W	

Table B-6. Parameters Measured in Sturgeon Exposure Chambers (continued).

Analyte/Parameter	Sediment		Pore/overlying Water	
	CAS	UofS	CAS	UofS
Biological Measurements				
				length
				weight
				survival
				AVS
				SEM
				Grain Size
				when dead
				when dead
				D
				start
				start
				start

Notes:

- D Daily measurements conducted by the U of S to control for appropriate water quality required for successful sturgeon culture
- W Weekly measurements will be conducted by the U of S to assess changes in exposure conditions that require immediate corrective action. During the beginning of the exposure these parameters will be measured more frequently (e.g. every 3 days).

APPENDIX C

CULTURAL RESOURCES COORDINATION PLAN

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Attachment 1. Protocols and Procedures for Inadvertent Discoveries

LIST OF MAPS

Map 3-1. Proposed On-site Sediment Sampling Locations and Alternative Sampling Locations

LIST OF TABLES

Table 3-1. Proposed Surface Sediment Sampling Locations

ACRONYMS AND ABBREVIATIONS

ACHP	Advisory Council on Historic Preservation
APE	area of potential effects
ARPA	Archeological Resources Protection Act of 1979
CCT	Confederated Tribes of the Colville Reservation
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CFR	Code of Federal Regulations
CRCP	cultural resources coordination plan
EPA	U.S. Environmental Protection Agency
FOIA	Freedom of Information Act
Lake Roosevelt	Franklin D. Roosevelt Lake
MOA	Memorandum of Agreement
NAGPRA	Native American Graves Protection and Repatriation Act
NEPA	National Environmental Policy Act
NHPA	National Historic Preservation Act
NPS	National Park Service
QAPP	quality assurance project plan
RCW	Revised Code of Washington
RI/FS	remedial investigation and feasibility study
RM	river mile
SHPO	State Historic Preservation Officer
Site	Upper Columbia River site
STI	Spokane Tribe of Indians
Teck	Teck American Incorporated
THPO	Tribal Historic Preservation Officer
UCR	Upper Columbia River
USBR	U.S. Bureau of Reclamation
WAC	Washington Administrative Code

1 INTRODUCTION

This document presents the cultural resources coordination plan (CRCP) for the Upper Columbia River (UCR) site (Site) remedial investigation and feasibility study (RI/FS) with emphasis placed for sampling activities associated with the methods development for white sturgeon/sediments program.

1.1 BACKGROUND

As specified in the Statement of Work associated with the June 2, 2006 Settlement Agreement (USEPA 2006), "For all RI/FS activities at the Site involving sediment collection or ground penetration/disturbance, the Company shall work with the potentially affected parties to assess the effects of the planned work and seek ways to avoid, minimize or mitigate any adverse effects on historic properties." The purpose of this CRCP is to describe known or likely physical impacts of proposed sediment sampling, provide relevant background information, define measures for protecting resources, and define procedures for consulting with the appropriate state, federal, and Tribal parties with interests in the cultural resources of the Site.

The Site is located wholly within Washington State and includes approximately 150 river miles of the Columbia River extending from the U.S.-Canadian border to the Grand Coulee Dam. The Colville Indian Reservation borders the UCR from approximately river mile (RM) 690 to the Grand Coulee Dam. The Spokane Indian Reservation borders the UCR to the east from approximately RM 650 to RM 640. Franklin D. Roosevelt Lake (Lake Roosevelt) and associated lands are administered by the U.S. Bureau of Reclamation (USBR) and the National Park Service (NPS) of the U.S. Department of the Interior.

The U.S. Environmental Protection Agency (EPA) has responsibilities under the National Historic Preservation Act (NHPA) to consider how its undertakings would affect historic properties. As defined in the NHPA, "historic properties" include archaeological resources, historic-period buildings and structures, and traditional cultural places listed in or determined eligible for listing in the National Register of Historic Places. To meet the NHPA requirements, EPA must ensure that sampling and other activities would avoid, minimize, or mitigate any adverse effects to any historic properties.

The CRCP is organized into six sections, as follows: 1) this introductory section, which includes summary information on the archaeology, prehistory, Native peoples, and Euroamerican historical development of the project area; 2) an overview of the relevant

federal, state, and tribal laws and regulations, and other appropriate procedures and requirements; 3) a description of the proposed sampling program and its potential physical effects; and 4) a plan for coordination and consultation with all affected parties to address known and likely impacts to cultural resources in implementing the proposed work.

1.2 CULTURAL SETTING

The broader context of the cultural development of the upper Columbia region¹ provides the critical framework for understanding the importance of the cultural resources in the area. Archaeological and historical resources reflect broad patterns of cultural use and development, just as ongoing traditional use of areas and natural resources represents cultural continuity that can be important to individual and social identities. This section of the CRCP serves as a brief introduction to the cultural history of the upper Columbia region.

Archaeological research contributes significantly to our understanding of the prehistoric past. In the upper Columbia region, systematic archaeological research began in the late 1930s and has continued to the present. Almost 500 archaeological resources have been recorded in and along Lake Roosevelt, representing prehistoric, protohistoric, ethnohistoric, and historic-period human use and occupation. Research at some of these resources has provided the outlines of prehistoric cultural development in the upper Columbia region. Human presence in the region extends back at least 11,000 years. These first humans lived in small groups and were mobile foragers, hunting and gathering plants. The presence of the Columbia River led to an early focus on the abundance of riverine resources. Beginning about 8,000 years ago, populations appear to have increased and led to a gradual trend to less mobility and more permanent settlements. The growing population also led to use of a greater diversity of resources and increasing reliance on fish.

Permanent settlements increased in size and became concentrated in the river valleys beginning about 6,000 years ago, probably in response to continued population growth. Use of resources in upland areas expanded to meet the needs of the burgeoning populations and settlements. These trends continued until about 1,000 years ago, when

¹ The phrase “upper Columbia region” herein refers to the drainage of the upper Columbia River from around Grand Coulee to the Arrow Lakes area in British Columbia. The upper Columbia region includes, but is not limited to the Site as defined in the Settlement Agreement. This distinction is important because general patterns of cultural development in the upper Columbia region as a whole provide the framework for addressing the significance of the cultural resources within the Site boundaries.

there is evidence for a decline in population size. There were fewer settlements, villages were smaller, and there was less use of upland areas.

Cultural patterns of the late prehistoric period were reflected in the lives of the Native peoples at the time of Euroamerican contact. At the time of contact, the UCR was the homeland of the Lakes, Colville, Spokane, and Sanpoil peoples. The Lakes people occupied the Columbia River valley from the vicinity of modern Northport, Washington, north into the Arrow Lakes area of modern British Columbia. The Colville lived along the river downstream of the Lakes as far as around the mouth of the Spokane River. Downriver of the Colville were the Spokane, in the Spokane River drainage, and the Sanpoil, who lived along the Columbia River from around the mouth of the Spokane River to the near the modern location of the Grand Coulee Dam.

All of these groups spoke Interior Salish languages and shared many cultural features. Their cultural differences largely reflected differences in the local environments in which they lived. The social, political, and economic foundation of these groups was historically the winter village. The villages were concentrated in the river valleys, and each village was politically independent. Residents of the villages relied on provisions gathered, dried, and stored during the summer to survive through the winter. With the coming of spring, families began moving out of the winter village and shifting among the warm-season camps near resource locations. Gathering of plants and hunting game in upland areas were important subsistence activities during this season, but salmon constituted the most important food staple. Kettle Falls was a major aboriginal fishery, attracting people from throughout the region.

Native life began to change with the introduction of elements of Euroamerican culture. Horses reached the region in the 1700s and significantly changed Native travel and transportation. European diseases such as smallpox appeared in the late 1700s and had disastrous consequences for Native groups. Populations may have declined as much as 80 percent between the 1780s and 1840s. Direct contact with Euroamericans came in the early 1800s, when fur-trade posts were established on the Spokane River and at Kettle Falls.

When American settlement began in the 1840s, it bypassed the upper Columbia region. The discovery of gold in the region in the 1850s led to a major influx of Americans and growing conflict between the new settlers and Indian groups. A series of treaties with Indian groups was signed in 1855 but did not include the peoples of the upper Columbia region. As American settlement continued, the federal government responded by creating the Colville Reservation in 1872 for the Colville and Spokane people. The separate Spokane Reservation was established in 1881. Both reservations have subsequently lost lands to the allotment process in the late 1800s and early 1900s and inundation from the

waters of Lake Roosevelt. The Colville Reservation is now the home of the Confederated Tribes of the Colville Reservation; the Spokane Reservation is the home of the Spokane Tribe of Indians.

As already noted, the direct Euroamerican presence in the upper Columbia region began with the establishment of fur-trade posts on the Spokane River and at Kettle Falls. These posts were constructed between 1810 and 1825. The fur traders were followed by Christian missionaries in the 1830s and 1840s. A more substantial Euroamerican presence in the region developed in the 1850s, with the discovery of gold near Fort Colville. Conflicts between miners and Indians led to a military campaign in the Spokane River valley in 1858 and the establishment of an army post (Fort Colville) near Kettle Falls in 1859.

American settlement in the upper Columbia River drainage accelerated in the 1860s, initially spurred by mining. Farmers eventually followed the miners, but agricultural activity was limited until the construction of the Spokane Falls and Northern Railway through the region in 1890. With improved access to markets, farming—especially orchard crops—developed as one of the economic mainstays of the area, although mining has continued to play an important role.

The growing demands for agriculture led to plans to construct a dam at Grand Coulee. The dam would provide water for irrigation and inexpensive hydroelectric power. Construction of the dam began in 1934 and was completed in 1942. More than 82,000 acres above the dam was flooded, resulting in the relocation of 11 towns and about 3,000 residents. Since its creation, Lake Roosevelt has provided a growing number of recreational and tourist activities, which have become increasingly important to local economies.

2 OVERVIEW OF LAWS AND REGULATIONS

Implementation of the RI/FS would occur primarily on federal and Tribal lands. Federal and Tribal laws and regulations addressing cultural resources will therefore provide the primary legal framework for this coordination plan. It is possible, however, that implementation of the RI/FS may require activities on private or non-federal, non-Tribal public lands. This overview therefore includes a brief description of relevant state laws and executive orders. Ferry, Lincoln, and Stevens counties, which border the UCR, do not appear to have any ordinances addressing cultural resources that would be relevant to the Site RI/FS.

Relevant federal, Tribal, and state laws and regulations directly addressing cultural resources are briefly outlined below, as well as pertinent executive orders issued by the President of the United States and the Governor of Washington.

2.1 FEDERAL LEGISLATION AND REGULATIONS

An overview of federal legislation and regulations is provided below. There are three key laws relevant to Site RI/FS activities. The NHPA guides all federal agency actions that could affect cultural resources. Implementation of the RI/FS constitutes an “undertaking” as defined in the NHPA and therefore complying with the NHPA requirements is the responsibility of EPA. The Archeological Resources Protection Act of 1979 (ARPA) and the Native American Graves Protection and Repatriation Act (NAGPRA) apply to activities that could affect archaeological resources and Indian burials on federal and Tribal lands. These laws and their implementing regulations would therefore apply to RI/FS activities conducted on federal and Tribal lands..

2.1.1 National Historic Preservation Act of 1966, as Amended through 1992 (16 USC 470-470w)

The NHPA is the centerpiece of federal legislation protecting cultural resources. In the Act, Congress states that the federal government will “provide leadership in the preservation of the prehistoric and historic resources of the United States,” including resources that are federally owned, administered, or controlled. For federal agencies, Sections 106 and 110 of the Act provide the foundation for how federal agencies are to manage cultural resources, but other sections provide further guidance. The implementing regulations for the NHPA are in 36 CFR Part 800. These regulations are summarized below.

2.1.1.1 Section 106

Similar to the National Environmental Policy Act of 1969 (NEPA), Section 106 of the NHPA requires federal agencies to take into account the effects of their actions or programs specifically on historic and archeological properties, prior to implementation. This is accomplished through consultation with the State Historic Preservation Officer (SHPO) and/or the Advisory Council on Historic Preservation (ACHP). On lands held by a Tribe with a Tribal Historic Preservation Officer (THPO), the THPO has the same duties and responsibilities as the SHPO. If an undertaking on federal lands may affect properties having historic value to a federally recognized Indian Tribe, such Tribe shall be afforded the opportunity to participate as interested persons during the consultation process defined in 36 CFR 800. Compliance can also be accomplished using agreed-upon streamlined methods and agreement documents such as Programmatic Agreements.

The Section 106 process is designed to identify possible conflicts between historic preservation objectives and the proposed activity, and to resolve those conflicts in the public's interest through consultation. Neither the NHPA nor the Advisory Council's regulations require that all historic properties be preserved. Rather, they only require the agency proposing the undertaking to consider the effects of the proposed undertaking prior to implementation.

Failure to take into account the effects of an undertaking on historic or cultural properties can result in formal notification from the ACHP to the head of the federal agency of foreclosure of the ACHP's opportunity to comment on the undertaking pursuant to NHPA. A notice of foreclosure can be used by litigants against the federal agency in a manner that can halt or delay critical activities or programs.

The process for compliance with Section 106 consists of the following steps.

1. **Identification of Historic Properties**—Identification of historic properties located within the area of potential effects (APE) is accomplished through review of existing documentation and/or field surveys.
2. **Property Evaluation**—Evaluation of the identified historic properties using National Register criteria (36 CFR Part 63) in consultation with the SHPO and, if necessary, the ACHP. Properties that meet the criteria will be considered "Eligible" for listing in the National Register, and will be subject to further review under Section 106. Properties that do not meet the criteria will be considered "Not Eligible" for listing in the National Register, and will not be subject to further Section 106 review.

3. **Determination of Effect**—An assessment is made of the effects of the proposed project on properties that were determined to meet the National Register criteria, in consultation with the SHPO and if necessary, the ACHP. One of the following effect findings will be made:
- **No Historic Properties Affected**—If no historic properties are found or no effects on historic properties are found, the agency official provides appropriate documentation to the SHPO/THPO and notifies consulting parties. However, the federal agency must proceed to the assessment of adverse effects when it finds that historic properties may be affected or the SHPO/THPO or Council objects to a “No Historic Properties Affected” finding. The agency must notify all consulting parties and invite their views.
 - **No Historic Properties Adversely Affected**—When the Criteria of Adverse Effect are applied (36 CFR 800.5(a)), and it is found that historic properties will not be adversely affected by the undertaking, the agency may make a finding of “No Historic Properties Adversely Affected.” This finding is submitted to the SHPO for concurrence. Typically, the Council will not review “No Adverse Effect” determinations. However, the Council will intervene and review “No Historic Properties Adversely Affected” determinations if it deems it appropriate, or if the SHPO/THPO or another consulting party and the federal agency disagree on the finding and the agency cannot resolve the disagreement. If Indian Tribes disagree with the finding, they can request the Council’s review directly, but this must be done within the 30-day review period. Agencies must retain records of their findings of “No Historic Properties Adversely Affected” and make them available to the public. The public should be given access to the information when they so request, subject to Freedom of Information Act (FOIA) and other statutory limits on disclosure, including the confidentiality provisions in Section 304 of the NHPA. Failure of the agency to carry out the undertaking in accordance with the finding requires the agency official to reopen the Section 106 process and determine whether the altered course of action constitutes an adverse effect.
 - **Historic Properties Adversely Affected**—Adverse effects occur when an undertaking may directly or indirectly alter characteristics of a historic property that qualify it for inclusion in the Register. Reasonably foreseeable effects caused by the undertaking that may occur later in time, be farther removed in distance, or be cumulative also need to be considered. The finding of “Historic Properties Adversely Affected” is submitted to the SHPO for concurrence. The SHPO/THPO may suggest

changes in a project or impose conditions so that adverse effects can be avoided and thus result in a “No Historic Properties Adversely Affected” determination

4. **Resolution of Adverse Effects/Mitigation**—When adverse effects are found, the consultation must continue among the federal agency, SHPO/THPO, and consulting parties to attempt to resolve them. The agency official must notify the Council when adverse effects are found and should invite the Council to participate in the consultation when circumstances as outlined within 36 CFR 800.6(a)(1)(i)(A)-(C) exist. A consulting party may also request the Council to join the consultation.

When resolving adverse effects without the Council, the agency official consults with the SHPO/THPO and other consulting parties to develop a Memorandum of Agreement (MOA). The MOA will outline the steps or actions to be taken prior to implementation of the project, in order to mitigate the adverse effects on the historic property. Stipulations included in an MOA may include (but are not limited to) documentation, modification of the project to lessen the adverse effects on the property, efforts to sell or relocate the resource, or step-by-step consultation with interested parties throughout the process to ensure it is carried out according to plan.

The MOA is executed between the agency official and the SHPO/THPO and filed with required documentation with the Council. This filing is the formal conclusion of the Section 106 process and must occur before the undertaking is approved.

In some cases, streamlining of the Section 106 process can be accomplished through the use of Programmatic Agreements. The Advisory Council and the agency official may negotiate a Programmatic Agreement to govern the implementation of a particular program or the resolution of effects from complex projects or multiple undertakings. Programmatic Agreements are particularly useful when programs or projects affecting historic properties are similar and repetitive, and have known effects, such as routine maintenance or a series of similar rehabilitation projects.

2.1.1.2 Section 101(d)(2)

This section of the NHPA provides for the assumption by federally recognized Indian Tribes of all or any part of the functions of a SHPO with respect to Tribal lands (e.g., all lands within the exterior boundaries of any Indian reservation and all dependent Indian communities). Section 101(d)(2) requires federal agencies, in carrying out their Section 106

responsibilities, to consult with federally recognized Indian Tribes that attach religious or cultural significance to a historic property. The agency will consult with federally recognized Indian Tribes in the Section 106 process to identify, evaluate, and treat historic properties that have religious or cultural importance to those groups.

2.1.1.3 Section 110

Section 110 of the NHPA is intended to ensure that historic preservation is integrated into the ongoing programs of Federal agencies. This section of the Act requires agencies to identify, evaluate, and nominate for listing in the National Register, historic properties owned or controlled by the agency; use historic properties to the maximum extent feasible; ensure documentation of historic properties that are to be altered or damaged; carry out programs and projects that further the purpose of the Act; and undertake such planning and actions as may be necessary to minimize harm to any formally designated National Historic Landmark properties.

2.1.1.4 Section 111

Section 111 of the NHPA requires agency officials, to the extent practicable, to establish and implement alternatives for historic properties, including adaptive use, that are not needed for current or projected agency uses or requirements. Further, Section 111 allows the proceeds from any lease to be retained by the agency to defray the cost of administration, maintenance, repair, and related expenses of historic properties.

2.1.1.5 Section 112

Section 112 of the NHPA requires that agency officials who are responsible for protection of historic properties pursuant to the NHPA ensure that all actions taken by employees or contractors meet professional historic preservation standards established by the Secretary of the Interior (Professional Qualifications Standards of the *Secretary of the Interior's Standards and Guidelines in Archaeology and Historic Preservation*).

2.1.1.6 Section 304

Section 304 of the NHPA requires that information about the location, character, or ownership of a historic property be withheld from public disclosure when the federal agency head or other public official determines that disclosure may cause a significant invasion of privacy, risk and/or harm to the historic property, or impede the use of a traditional religious site by practitioners.

2.1.1.7 CERCLA and the NHPA

EPA's *CERCLA Compliance with Other Laws Manual: Part II. Clean Air Act and Other Environmental Statutes and State Requirements* (USEPA 1989) outlines how "substantive compliance" with the NHPA is to be achieved in Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) actions.² The initial step is determining if cultural resources are known or are likely to be present "in or near the area under study in the RI." This step may require conducting a survey of both the location of the proposed remedial action and any associated actions that would occur off-site. The CERCLA manual referenced above defines three stages of a survey: Stage IA, literature search and sensitivity study; Stage IB, field investigation; and Stage II, site definition and evaluation. All studies should include Stage IA but implementation of Stage IB is contingent on the results of Stage IA, and the need for Stage II is contingent on the results of Stage IB. If results of the survey identify significant cultural resources (i.e., resources listed or considered eligible for listing on the National Register), effects of the proposed remedial action and associated actions to the significant resources must be evaluated. Adverse effects to significant resources must be either avoided or mitigated. Any proposed mitigation measures must be incorporated into the remedial design process.

2.1.2 Archeological Resources Protection Act of 1979 (16 USC 470aa-470ll)

ARPA is essentially an update to the 1906 Antiquities Act. It expands and strengthens the activities prohibited under the Antiquities Act, increases the criminal penalties for violation, establishes civil penalties, and provides further guidelines for the issuance of permits. This Act continues to apply only to federal and Indian lands (the definition of "Indian lands" in ARPA differs very slightly from the definition of "Tribal lands" in the NHPA). Most archaeological excavations and collection of artifacts on these lands are allowed only with an ARPA permit. Trafficking in illegally obtained archeological resources from federal and Indian lands is also prohibited. Individuals convicted of violating the Act are liable for the value of the archaeological resource itself, and the cost of restoration or repair of the damage caused by illegal excavation or collection.

The implementing regulations are 43 CFR Part 7 (Department of the Interior), which applies to Federal lands that are not within military reservations or national forests. The regulations include detailed definitions of "archaeological resource" and "Indian lands"

² As stated in the Agreement, "The Parties intend that this RI/FS, while not being carried out under an administrative order or judicial order issued pursuant to the provisions of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), will be consistent with the National Contingency Plan ('NCP'), 40 CFR Part 300."

(lands held in trust by the United States on behalf of a federally recognized Tribe or individual members of a federally recognized Tribe).

2.1.3 Native American Graves Protection and Repatriation Act (25 USC 3001-3013)

NAGPRA establishes that Native American human remains and associated funerary objects found on federal or Tribal lands belong to the lineal descendants of the Native American. When the lineal descendants cannot be determined, the remains belong to the Tribe on whose land the remains were found (when found on Tribal lands), or to the Indian Tribe with the “closest cultural affiliation.”³ This latter rule also applies to unassociated funerary objects, sacred objects, and objects of cultural patrimony (all defined in the Act) NAGPRA applies to both human remains intentionally excavated (which would require an ARPA permit) and those accidentally discovered.

NAGPRA also requires all federal agencies and museums to inventory their holdings of Native American human remains and funerary objects. Once the inventories are completed, the agencies and museums are to notify the appropriate Tribes of the remains and other objects in their collections. The remains and associated funerary objects are to be returned (repatriated) at the request of the lineal descendant(s) or Tribe. The same requirement applies to unassociated funerary objects, sacred objects, and objects of cultural patrimony for which a cultural affiliation can be demonstrated. Exceptions to the repatriation requirement are objects that are “indispensable for completion of a specific scientific study, the outcome of which would be of major benefit to the United States.”

The implementing regulations are 43 CFR Part 10, which largely expand on the elements of the statute. The regulations detail 1) the process of consultation with Indian Tribes to address either intentional excavation of human remains or inadvertent discovery of human remains; 2) how agencies and museums are to inventory their collections; and 3) the repatriation process. When human remains, funerary objects, sacred objects, and objects of cultural patrimony are inadvertently discovered on federal lands the following steps are to be followed: 1) ongoing activity in the area of the find must cease and a reasonable effort made to protect the find; and 2) the federal land agency (i.e., the federal agency on whose lands the remains or objects have been found) must be immediately notified by telephone, with written confirmation. The federal land agency must then notify the appropriate Tribe(s) and further secure and protect the discovery. The activity

³ Cultural affiliation is defined in the implementing regulations [43 CFR 10.2(e)] and refers to a relationship of shared group identity, which can be reasonably traced historically or prehistorically between a present day Indian tribe or Native Hawaiian organization and an identifiable earlier group.

may be halted for up to 30 days while an appropriate response to the find is negotiated by the federal agency and the appropriate Tribe(s).

2.1.4 American Indian Religious Freedom Act (42 USC 1996)

This Act states that it is the policy of the United States to protect and preserve the rights of American Indians to practice traditional religions. That policy includes rights of access to sacred sites and to the use and possession of sacred objects. There are no implementing regulations.

2.2 PRESIDENTIAL EXECUTIVE ORDERS

Presidential Executive Orders define policies and procedures for federal agencies to facilitate their execution of laws passed by the U.S. Congress or clarify how specific laws are to be implemented. Presidential executive orders can be considered instructions or directives from the President to federal agencies on how to carry out specific laws. The executive orders listed below are either directly related to cultural resources or define relationships between federal agencies and tribes.

2.2.1 Executive Order 11593. Protection and Enhancement of the Cultural Environment

Issued in 1971, this executive order stated that the federal government would provide leadership in “preserving, restoring, and maintaining the historic and cultural environment of the Nation.” Federal agencies were directed to inventory cultural resources under their jurisdiction and nominate National Register-eligible properties to the National Register. Properties that have been determined eligible are not to be transferred, sold, demolished, or altered without providing the Advisory Council on Historic Preservation with an opportunity to comment. Properties to be demolished or substantially altered were to be documented prior to demolition or alteration. National Register properties or National Register-eligible properties under federal control were to be maintained following standards set by the Secretary of the Interior. Executive Order 11593 also assigns specific responsibilities to the Secretary of the Interior, including managing the National Register of Historic Places and assisting and advising other federal agencies in the management of cultural resources.

2.2.2 Executive Order 13007. Indian Sacred Sites

Issued in 1996, Executive Order 13007 directs federal agencies to provide access and ceremonial use of Indian sacred sites, where practicable, legal, and not inconsistent with essential agency functions. Agencies are also directed to avoid adversely impacting sacred sites and maintain the confidentiality of such sites. A “sacred site” as defined by this executive order is a specific location that is sacred because of its religious significance to or ceremonial use in an Indian religion.

2.2.3 Executive Order 13175. Consultation and Coordination with Indian Tribal Governments

Issued in 2000, this Executive Order directs federal agencies to consult with Tribal officials in the development of policies and regulations that have “tribal implications” or that preempt Tribal law. Executive Order 13175 also emphasizes the importance of government-to-government relationships between the U.S. government and Tribes. Agencies must designate an official responsible for implementing the Executive Order and must document Tribal consultation in the development of the relevant policies and regulations.

2.3 TRIBAL LEGISLATION AND REGULATIONS

Tribal laws and regulations addressing cultural resources would apply to lands on the reservations and off-reservation trust lands. The Confederated Tribes of the Colville Reservation (CCT) and the Spokane Tribe of Indians (STI) are the two Tribes whose laws and regulations would be potentially applicable to the Site. The legal code of the CCT addresses cultural resources, as summarized below. This code applies to both on-reservation actions and off-reservation actions by federal agencies that could affect cultural resources. STI does not currently have laws that specifically address cultural resources. Both Tribes have THPOs, who have the same authority and responsibilities as the SHPO on their respective reservations and on off-reservation trust lands.

2.3.1 Confederated Tribes of the Colville Reservation. Colville Tribal Law and Order Code Chapter 4-4, Cultural Resources Protection

This Colville Tribal Code establishes the Colville Cultural Resources Board, which has the responsibility of developing policies and procedures to protect cultural resources of interest and concern to the Colville Tribes, both on and off the Colville Reservation. The

Board reviews proposed federal agency actions off the reservation and is responsible for reviewing all proposed on-reservation actions that could affect significant cultural resources. The code also establishes a Colville Register of Historic and Archaeological Properties for listing of historic properties on the Colville Reservation.

This code defines the roles and responsibilities of the Colville History and Archaeology Department, which include identifying significant cultural resources on the reservation, nominating properties to the National Register and the Colville Register, and promoting efforts to protect cultural resources on the reservation.

Chapter 4-4 of Colville Tribal Code prohibits the excavation, disturbance, or other adverse effects to archaeological resources and historic properties on the reservation without a permit issued by the History and Archaeology Department. The code defines the procedure for the issuance of permits and the duties of permittees.

2.4 STATE LEGISLATION AND REGULATIONS

Washington State laws and regulations regarding archaeological and historical resources, as well as the law protecting Indian graves, are not applicable on federal lands or on Tribal trust lands. These laws would apply, however, to any RI/FS-related activities that would affect private lands or non-federal or non-Tribal public lands.

2.4.1 Revised Code of Washington (RCW) Chapter 27.44, Indian Graves and Records

This legislation prohibits the removal or other disturbance of Indian burials, cairns, and “glyphic or painted records.” “Burials” and “graves” are not defined in the statute. Excavation or removal of burials is permitted only under provisions of a permit issued by the Washington Department of Archaeology and Historic Preservation. Procedures for obtaining permits are defined in WAC Chapter 25-48.

2.4.2 RCW Chapter 27.53, Archaeological Sites and Resources

This legislation prohibits the excavation or disturbance of archaeological sites on public and private lands in Washington except under provisions of a permit issued by the Washington Department of Archaeology and Historic Preservation. Procedures for obtaining permits are defined in WAC Chapter 25-48.

2.4.3 RCW Chapter 68.60, Abandoned and Historic Cemeteries and Historic Graves

This legislation prohibits the destruction, alteration, or other disturbance of historical and abandoned cemeteries and historic graves (Indian graves and burials are protected in RCW Chapter 27.44). A historic cemetery is defined in the statute as one established before November 1889. A historic grave is a grave or graves outside of a cemetery placed prior to June 1990.

2.4.4 RCW Chapter 43.21C, State Environmental Policy Act

This legislation directs state and local agencies in Washington to address environmental impacts of proposed projects. The implementing rules (WAC Chapter 197-11) require that impacts to historic and cultural resources are to be addressed in the State Environmental Policy Act process.

3 PROPOSED SAMPLING PROGRAM

A summary of the proposed sampling locations (coordinates) are provided within Table 3-1; with a detailed description of sampling techniques provided within the Quality Assurance Project Plan (QAPP). As indicated within the QAPP, sediment sampling activities will be completed to depths no greater than 12 inches below ground surface using hand-held tools such as shovel.

During this work, an area on the gravel of Deadman's Eddy (Map 3-1), with sampling station coordinates provided within Table 3-1 will be targeted for surface sediment collection. Surface sediment samples will be collected using a shovel and will not extend below 12 inches of the original ground surface. A total of approximately 50 gallons of sediment will be retrieved from the area and placed in 5-gallon buckets.

Photographs will be taken of the sampling area before and after sample collection. All photographs will be labeled with station location, date, and time.

4 COORDINATION PLAN

The objective of the CRCP is to ensure that implementation of the RI/FS and associated sampling activities does not adversely affect any cultural resources. The plan therefore defines a general process and more specific procedures to meet this objective.

The two chief challenges in meeting this objective are 1) the iterative process of remedial investigations; and 2) the high density of cultural resources in the study area. The iterative process is a challenge because there are likely to be several rounds of sampling (and associated actions) that extend over several years. Coordination and consultation must therefore also be an iterative process as methods and locations are defined for each round of sampling.

The high density of cultural resources is a challenge because it is highly likely that every round of intrusive sampling will occur at the identified location of one or more cultural resource(s). At the same time, the high density is potentially misleading by suggesting that all cultural resources in the UCR have been identified. Most—if not all—of the Lake Roosevelt lands have been surveyed for cultural resources in the past. Few of the surveys conducted prior to about 1975 are likely to have met current regulatory and professional standards. In addition, many of the previous surveys focused on archaeological resources to the exclusion of other types of cultural resources (and older archaeological surveys documented only evidence of prehistoric use or occupation). Finally, it is likely that there are some locations previously surveyed at which burials or buried archaeological resources are present but not evident and therefore not recorded at the time of the survey (many surveys both in the past and in the present rely entirely or primarily on surface evidence of archaeological resources or burials).

This plan therefore defines procedures that address sampling at both known locations of cultural resources and locations where no cultural resources are presently recorded.

4.1 GENERAL CONSULTATION FRAMEWORK

Successful implementation of the RI/FS and of this CRCP, given the issues defined above, will require ongoing consultation and coordination with the NPS, the USBR, the CCT, the STI, and the Washington SHPO (i.e., the consulting parties). Other consulting parties (as defined in 36 CFR 800.2(c)) may be recognized in the future whose participation would be important for general consultation or coordination in the RI/FS process or for specific sampling locations. For the purposes of cultural resources coordination activities, the

“consulting parties” referred to in this plan are distinguished from other “participating parties” to the RI/FS process.

4.2 CULTURAL RESOURCE PROCEDURES IN THE SAMPLING PROCESS

This section defines general procedures to be followed in the sampling process to minimize the potential for inadvertent disturbance of cultural resources. More specific protocols to respond to discoveries are defined in the following sections.

A Tribal cultural resources specialist or a professional archaeologist will be present on-site to monitor sediment sampling conducted at a known cultural resource or within 100 m (330 ft) of a known resource. The protocol for this monitoring is defined below.

4.2.1 Archaeological Monitoring in the Sampling Program

To assure compliance with the NHPA and the applicable requirements, procedures, and standards of the NPS, USBR, CCT, and STI, the following procedures have been developed to address potential discoveries, including inadvertent discoveries, of cultural materials and deposits (including sacred objects, funerary objects, and objects of cultural patrimony as defined in NAGPRA) and Indian burials and human remains (as defined in NAGPRA) during sediment and soil sampling and associated activity that could result in ground disturbance.

4.2.1.1 Notification of Planned Sediment and Soil Sampling

Teck American Incorporated (Teck) shall notify EPA at least 15 days in advance of any sample collection activity, unless shorter notice is agreed to by EPA. Notification to EPA may be provided by e-mail or by letter. As for all RI/FS activities at the Site involving sediment collection or ground penetration/disturbance, Teck shall work with potentially affected parties to assess the effects of the planned work and seek ways to avoid, minimize, or mitigate any adverse effects on historic properties. Further, sediment sampling cannot be performed at the Site without 1) clearance of proposed sediment sample locations by tribal and federal/state cultural resources coordinators, and; 2) approval by EPA.

The names and contact information for potentially affected parties (i.e., representatives of the federal land-managing agencies and Tribes) are provided in Attachment 1 of this plan. Teck will work with EPA to establish a procedure for timely notification of these parties.

4.2.1.2 Professional Archaeologist and Tribal Representative On-Site

An archeological monitor and/or Tribal representative will be present on-site when sampling or sampling-related activity occurs. The archaeological monitor and/or Tribal representative will visually examine all samples to determine if evident or likely artifacts are present or if other deposits are present that are likely to be cultural in origin. The archaeological monitor and/or Tribal representative will not make physical contact with the sample unless artifacts or other cultural deposits are present. If artifacts or likely archaeological deposits are present, the archaeologist or Tribal representative will record the location of the materials and photograph the materials in place in such a manner to provide information on provenience. The artifacts and other archaeological materials will then be re-deposited at their original location.

The archaeological monitor and/or Tribal representative will document their observations on a daily basis, including field notes and photographs that record the location, character of the sampling or other ground-disturbing activity, any archaeological discoveries made, and any decisions made within the provisions of this plan by the archaeological monitor and Tribal representative in response to any archaeological discoveries. A standardized archaeological monitoring form may be substituted for the field notes referenced above.

All archaeological monitors and Tribal representatives will be required to have read the applicable health and safety plan and to have complete understanding of the archaeological monitoring provisions of this plan. The archaeological monitors will also be required to meet requirements for personal protective equipment. In addition, all on-site personnel are subject to the directions of the task field supervisor at all times.

4.2.1.3 Discoveries: Archaeological Monitors Present

At the discretion of the archaeological monitor or Tribal representative, ground-disturbing sampling or associated activity may be slowed or halted at any time that a suspected archaeological object or archaeological resource is encountered. The objective of this slowing or halting of ground-disturbing cleanup activity is to allow the archaeologist to confirm and/or make a preliminary assessment of the discovery. At the discretion of the archaeological monitor or Tribal representative, a specific sample may be relocated from the location of the discovery but at the sampling location. Such relocation will be coordinated with the on-site sampling manager or supervisor.

At the request of the archaeological monitor or Tribal representative, the sampling personnel will either:

- Assist in securing access to the location of the discovery and take appropriate measures to protect the location of the discovery from rain, stormwater, and other possible disturbances, or
- Assist in moving the artifacts to a protected and secure area of the site away from the immediate sampling area. Removal of artifacts from the discovery location will be undertaken only if leaving the artifacts in place would jeopardize their integrity due to erosion or collection by unauthorized individuals.

The archaeological monitor, Tribal representative, or a member of the Teck Technical Team will remain on-site to ensure the security of the find until more extensive efforts can be made to secure the site from further disturbance or a more extensive evaluation and documentation of the discovery can be made.

Notification of any archaeological discoveries must be provided to EPA for further coordination with consulting parties within 24 hours of the discovery. All telephone notification of discoveries must be promptly followed by notification in writing (via e-mail or conventional mail).

4.2.1.4 Discovery of Human Remains

Native peoples in the study area consider the graves of their ancestors to be important in both their cultural identity and in defining their relationship with the land. These graves are therefore considered sacred and should be left undisturbed. Should inadvertent disturbance occur, the remains and associated materials (“funerary objects”) must be treated with respect and honor. All appropriate federal, Tribal, and state laws, regulations, and procedures regarding burials should be rigorously enforced.

In the event that likely or confirmed human remains are encountered, all further sampling or other ground-disturbing activity will cease immediately. To comply with 43 CFR 10.4(b), any discoveries of human remains must be reported to the NPS and USBR immediately by telephone, followed by written notification. Any discoveries within the boundaries of the Colville or the Spokane reservations must also be reported immediately to the respective Tribe.

Teck will notify EPA for further coordination with consulting parties (consisting minimally of the NPS, USBR, CCT, STI, and the Washington State Historic Preservation Officer). The Teck Technical Team will assist the archaeological monitor and Tribal representative in securing the location of the discovery.

If no archaeological monitor or Tribal representative is present, the Teck Technical Team will secure the location of the discovery in such a manner that both maintains the physical integrity of the remains and any associated objects and precludes further disturbance, or a

member of the Teck Technical Team will remain on-site until an archaeologist or Tribal representatives can arrive to assess the find.

Other conditions for responses to discoveries of archaeological materials may be defined in the permit(s) issued for the sampling program. Responses to any discoveries of burials must comply with provisions of NAGPRA and its implementing regulations (in addition to those referenced above), as well as the existing protocols of the NPS, USBR, CCT, and STI (copies of these protocols are provided in Attachment 1).

4.2.1.5 Discoveries: Archaeological Monitors Not Present

Should suspected or evident artifacts or other archaeological deposits be encountered during sampling or associated ground-disturbing activity that is not being monitored by a professional archaeologist or Tribal representative, further sampling and other ground-disturbing activity will be halted in the immediate vicinity of the discovery and the location secured from further disturbance. The discovery will be mapped and photographed in place but will be otherwise left as found (other than appropriate measures to secure the find and maintain this security). In consultation with the land-managing agency or appropriate Tribe, as well as other interested parties, Teck will arrange for the location of the discovery to be examined by a professional archaeologist and/or Tribal representative in a timely manner. If the archaeologist confirms the presence of artifacts or other archaeological deposits, the procedures defined above for discoveries made during ground-disturbing activity monitored by an archaeologist will be implemented. The archaeologist will prepare appropriate State of Washington archaeological forms to document the find.

To ensure proper recognition of artifacts and other cultural items or deposits, a minimum of one Teck field-staff member per sampling boat or shoreline sampling group will be provided with training in recognizing these materials by a professional archaeologist prior to the initiation of any sediment and soil sampling.

4.2.2 Curation

Artifacts and other cultural materials that may be recovered during the sampling program (with the exception of human remains and associated items subject to NAGPRA) will be curated at a facility that meets the standards of 36 CFR 79. The appropriate facility or facilities will be designated by the NPS and USBR in consultation with the Tribes for items recovered from federal lands. The appropriate Tribe will designate the curation facility for cultural materials recovered from Tribal lands.

4.2.3 Reporting

Within 150 days of completion of each sampling activity that is covered under this plan,⁴ a professional archaeologist will prepare a confidential⁵ written report and presents the results of the archaeological monitoring and responses to any discoveries of archaeological resources or burials. The report will include 1) copies of field notes, descriptions and maps of all locations at which sampling-related archaeological monitoring was conducted; 2) descriptions of any discoveries made during such monitoring and the outcome of the discoveries (including the rationale for the decisions for the disposition of any finds); 3) descriptions and maps of all non-monitored locations at which inadvertent discoveries were made and the outcome of those discoveries; and 4) recommendations for any changes in the monitoring protocol or coordination plan that may be appropriate to address results of the monitoring or how well existing coordination procedures worked.

The draft report will be provided to EPA for review and dissemination to the consulting parties for review and comment.

4.3 CONFIDENTIALITY

Teck shall make its best efforts, in accordance with state and federal law, to ensure that its employees and contractors keep the discovery of any found or suspected human remains, other cultural items, and potential historic properties confidential. Pertinent Teck employees and contractors will be required to read and sign a confidentiality statement that specifies procedures to be followed in response to media and public contacts regarding archaeological and other cultural resources. To the extent permitted by law, prior to any release of information, EPA, Teck, and the other consulting parties shall concur on the amount of information, if any, to be released to the public, any third party, and the media and the procedures for such a release.

⁴ Sampling or other RI/FS activities that do not require coordination under this plan will not result in generation of this reporting requirement.

⁵ Refer to Section 5.3, "Confidentiality."

5 REFERENCES

- USEPA (United States Environmental Protection Agency). 1989. CERCLA compliance with other laws manual: Part II. Clean act and other environmental statutes and state requirements. U.S. Environmental Protection Agency, Region 10, Seattle, WA.
- USEPA. 2006. Settlement agreement for implementation of remedial investigation and feasibility study at the Upper Columbia River Site. June 2, 2006. U.S. Environmental Protection Agency, Region 10, Seattle, WA.

6 GLOSSARY OF TERMS

Burial—A burial is defined in NAGPRA as “[a]ny natural or prepared physical location, whether originally below, on, or above the surface of the earth, into which as part of the death rite or ceremony of a culture, individual human remains are deposited.”

Curation—Long-term storage and preservation of archaeological collections. Archaeological collections from federal lands must be curated at facilities that meet the standards of 36 CFR 79.

Ethnohistoric—Information on Native peoples gathered from historical accounts.

Historic, historic-period, historical—The NHPA uses the term “historic” to refer to properties that are listed or have been determined eligible for listing on the National Register of Historic Places. To avoid confusion with this definition of “historic,” “historic-period” or “historical” are used to reference resources, places, events, and people associated with the period since the appearance of Euroamericans and the beginning of written accounts (ca. 1780–1810 in the Pacific Northwest).

Protohistoric—The period of time transitional from prehistory to history. In the Pacific Northwest, the protohistoric can be generally defined as from the late 1600s until late 1700s.

MAP



Entrix Parametrix
Exponent



Map 3-1 Proposed On-site Sediment Sampling Locations

Upper Columbia River, WA

TABLE

Table 3-1. Proposed Surface Sediment Sampling Locations

Sampling Area Description	UTM Coordinates	
	Easting	Northing
Deadman's Eddy Gravel Bar		
Northeast Corner	447158	5421097
Northwest Corner	447026	5421144
Southwest Corner	447023	5421127
Southeast Corner	447077	5421068

ATTACHMENT 1

PROTOCOLS AND PROCEDURES FOR INADVERTENT DISCOVERIES AND/OR INTENTIONAL EXCAVATIONS

Attachment – Inadvertent Discoveries on Interior lands

Draft Lake Roosevelt Protocols for Native American Graves Protection and Repatriation Act (NAGPRA) Inadvertent Discoveries or Intentional Excavations: National Park Service and the Bureau of Reclamation

This protocol is intended to cover NAGPRA items exposed by inadvertent discoveries or intentional excavations within the boundaries of lands managed by the National Park Service/Lake Roosevelt National Recreation Area and the Bureau of Reclamation/Grand Coulee Dam Project. The term "NAGPRA items" in this document refers to human remains associated funerary objects, and objects of cultural patrimony as they are defined in 25 USC 3001 and its implementing regulations (43 CFR Part 10). Funding of actions is not covered under this protocol.

1. If potentially human remains are encountered, any activity in the vicinity of the discovery shall cease and all appropriate effort shall be made to determine if the remains are human. Secure the area and take protective measures to assure that the remains are not in danger of further depredation or disturbance.
2. A National Park Service law enforcement officer will be immediately notified. Law Enforcement, in consultation with the Park Archaeologist (if needed), will determine if the find is human, whether it is of recent origin, and if it is part of a crime scene.

If Law Enforcement has determined that the find is human and not of Law Enforcement concern, they will notify the Park Archaeologist and the Chief of Cultural Resources, who will contact the appropriate Tribal representatives and the Bureau of Reclamation archaeologist about the Inadvertent Discovery. Live phone contact is required; backup staff are identified if the primary contacts are unavailable. Phone contact will be followed up by written confirmation. Contact Phone Numbers are provided at the end of the Protocols.

3. As soon as the items have been determined to be human, then all effort shall be made in the field to determine whether the remains are Native American.
4. If the items are determined not to be Native American, then Washington State burial laws apply and shall be followed (Title 68, Chapter 68.50 RCW HUMAN NAGPRA ITEMS).
5. If the ethnicity of the human remains cannot be determined in the field, further analysis of the bones and/or associated funerary objects or other archeological materials may be required. The, NPS and Reclamation shall consult with the Tribal representatives regarding the types of analysis to be conducted.

6. If it is determined that the human remains are Native American, a Written Plan of Action will be prepared that will outline the procedures that will be taken to address the NAGPRA regulations on Inadvertent Discoveries.
7. The manner of treatment will be decided in consultation between the NPS, BOR and the Tribal representatives.
8. If it is possible to rebury or cap the NAGPRA items in place, then that decision shall be documented in the Written Plan of Action (see below).
9. Should excavation or removal be the preferred option, NPS will review the excavation proposal and issue an ARPA permit following the NPS guidelines on ARPA permit requirements. The Reclamation contract language for burials recovered in the shoreline of the NRA will also apply and should agree with the Written Plan of Action and these protocols.
10. Provenience information will be collected as specified by the Written Plan of Action. The Reclamation contract language for burials recovered from the shoreline of the National Recreation Area will also apply and should agree with the Written Plan of Action and these protocols.
11. Recording of provenience may include any or all of the following: documenting the location of the burial or scattered NAGPRA items and general site conditions on a site form or on an addendum to an existing form; describing the surface visible NAGPRA items to the degree that can be accomplished without causing additional disturbance to the grave; and documenting the location of the burial on a USGS 7.5' topographic sheet and with a GPS unit.
12. NAGPRA items will be removed using standard professional archaeological practices. Such practices may include collection of horizontal provenience data referenced to a site datum point. If excavation is required, vertical provenience data shall be tracked through the use of stratigraphic or controlled 10-cm levels within a standard grid unit, screening of all excavated fill through 1/8-inch screen mesh, and photographic and to-scale plan map documentation of excavated features. All recovered items shall be listed in the field during collection to minimize handling after recovery.
13. Inadvertent discoveries that result from activities requiring easements or other non- ARPA permits (such as access, construction, etc.) shall be dealt with by the permitting agencies, which may be Reclamation or the NPS. This protocol document will be included with documents issued to permittees.
14. The Written Plan of Action for individual discoveries will detail exact procedures for further implementation of NAGPRA. A sample Written Plan of Action is included below.

15. Contact Names and Numbers.

- Camille Pleasants, CCT THPO (509) 634-2654, FAX 634-2694, John Pouley, Project Director Archaeologist, 509-634-2699. After work hours Mr. Pouley can be reached at 633-0498. In the event that neither Ms. Pleasants or Mr. Pouley can be contacted, then Guy Moura, TCP Coordinator, will be contacted at (509) 634-2695 (office), 633-8361 (home), 631-1705 (cell), who shall participate in the NAGPRA consultation process on Ms. Pleasants' behalf until her return.

Ray DePuydt, Park Archeologist or Frank Andrews Jr. are the primary contacts for the Lake Roosevelt National Recreation Area. Mr. DePuydt's phone number is (509) 738-6266, ext 101, and internet address is ray_depuydt@nps.gov. Frank Andrews Jr. phone number is (509) 633-9441 ext. 132 and his internet address is frank_andrews@nps.gov.

- Sean Hess, Power Office Archaeologist, is Reclamation's primary contact for NAGPRA on Lake Roosevelt. His phone number is (509) 633-9233, cell 631-0213, FAX (509) 633-9138, and internet address is "shess@pn.usbr.gov." he may also be reached in cases of emergency at 633-7158. If Mr. Hess is not available, then Lynne MacDonald, Regional Archaeologist, is Reclamation's alternate contact. Her phone number is (208) 378-5316, FAX 378-5305, and internet address is "lmacdonald@pn.usbr.gov." In the event neither Mr. Hess nor Ms. MacDonald is available, Reclamation's Contracting Officer will be contacted directly at (208) 378-5364.
- Randy Abrahamson, THPO, and John Matt, Cultural Program Director, are the primary contacts for the Spokane Tribe of Indians. Mr. Abrahamson's number is (509) 258-4315 randva@spokanetribe.com and John Matt's is (509) 258-4060 johnm@spokanetribe.com.

Template NAGPRA Plan of Action for Lake Roosevelt

This plan of action shall comply with the requirements of the Native American Graves Protection and Repatriation Act (NAGPRA) (25 USC 3001 et seq.), its implementing regulations (43 CFR Part 10) and the Archaeological Resources Protection Act (ARPA) (16 USC 470 et seq.) with its implementing regulations (43 CFR Part 7).

1. The kinds of objects to be considered as cultural items as defined in Sec. 10.2 (b):

- ✓ Human remains
- ✓ Associated funerary objects
- ✓ Unassociated funerary objects
- ✓ Objects of cultural patrimony
- ✓ Sacred objects

These objects are cultural objects as defined under NAGPRA 43CFR Part 10.2 (d).

2. The specific information used to determine custody pursuant to Sec. 10.6:
 - ✓ Traditional association (this is where tribe's area of interest is cited with reference to Lake Roosevelt)
 - ✓ Cultural affiliation
 - ✓ Evidence: Geographical, archaeological, linguistic, folklore, oral tradition, historical
3. The planned treatment, care, and handling of human remains and other objects as defined in NAGPRA
4. The planned archaeological recording of the human remains and other objects as defined in NAGPRA
5. The kinds of analysis planned for each kind of object
6. Any steps to be followed to contact Indian tribe officials at the time of intentional excavation or inadvertent discovery of specific human remains and other objects as defined in NAGPRA
7. The kind of traditional treatment, if any, to be afforded the human remains and other objects as defined in NAGPRA by members of the Indian tribe
8. The nature of reports to be prepared
9. The planned disposition of human remains, and other objects as defined in NAGPRA.

**Spokane Tribe of Indians
P.O. Box 100-Wellpinit, WA 99040
Tel 509-458-6500, Fax 509-458-6575**

**Century of Survival
1881-1981:
Procedure for the Inadvertent Disturbance or
Discovery of Spokane Human Remains and
Cultural Resources**

Introduction

Because many ground-disturbing processes, both natural and cultural, have the effect of prompting the destruction of evidence of Spokane Tribal heritage, it is the policy of the Spokane Tribe of Indians (hereafter "Spokane Tribe") to leave Spokane human remains and cultural resources in place and undisturbed. Purposeful disturbance of these resources without proper permit and consultation and/or approval of the Spokane Tribe is a violation of federal, Tribal, State, and/or local law. The National Historic Preservation Act (NHPA) and the Native American Graves Protection and Repatriation Act (NAGPRA) require that federal agencies take responsibility for damage to or loss of human burials caused by the project actions or that occur on off-reservation lands under the management jurisdiction. The Spokane Tribe has been delegated the federal authority as a Tribal Historic Preservation Office for Reservation lands pursuant to Section 101 (d)(2) of the National Historic Preservation Act.

Geographic Area of Applicability

This procedure for the inadvertent disturbance or discovery of Spokane human remains and cultural resources applies to all lands within the boundaries of the Spokane Indian Reservation and is advisory for all lands within the Spokane Tribe's aboriginal territory, as determined in proceedings before the Indian Claims Commission. For the purposes of cultural resource management, the ceded territory is bounded by and includes the Columbia River on the west, the Canadian border to the north, the Idaho border to the east, Rosalia to the southeast, Rosalia to the southeast, and Ritzville to the southwest.

Procedure

In cases of inadvertent disturbance or discovery of Spokane human burials or cultural resources, the following procedure is to be followed:

1. Upon inadvertent disturbance or discovery of human burials or cultural resources, any action(s) affecting the burials or resources shall immediately be halted.
2. The person(s) making the discovery shall immediately notify the appropriate office of the coroner or police. Upon a determination of the appropriate death investigation authority that the location of the remains is not the result of a crime, the following procedures shall apply:
 - a) The entity making such disturbance or discovery shall notify the landowner, occupant, or manager. If the land occupant or manager is notified in lieu of the landowner, the occupant or manager will immediately notify the landowner. The entity making the disturbance or discovery will immediately notify the Spokane Tribal Historic Preservation Office, Wellpinit, Washington, in person or by telephone (at 509-258-4315), or by fax (at 509-248-6965), of the disturbance or discovery. The entity is advised to keep written documentation of such contact.
 - b) The entity making the disturbance, or discovery will exert its best effort to protect such remains and/or objects until the landowner and/or land occupant or manager arrives to protect these remains and/or objects. Within 24 hours of notification, the

landowner shall supply protection for such remains and/or objects, until disposition or control of such remains and objects has been implemented.

3. The Spokane Tribal Historic Preservation Officer or designated representative(s) shall inspect in person the affected site, human remains, or cultural resources, and shall determine, if possible evidence at the site, oral history, and/or existing records, the cultural affiliation of such site, human remains, and/or objects, until disposition or control of such remains and objects has been implemented.
 - a. If the exposed human remains or cultural resources are clearly Native American and have known lineal descendants or owners, the Spokane Tribal Historic Preservation Officer shall then have the opportunity to make disposition or to take control of such human remains and/or associated funerary objects.
 - b. If the exposed human remains and /or associated funerary objects are clearly prehistoric or non-modern Native American in origin and have no known lineal descendants, or if the lineal descendants decline the disposition or control, the Spokane Tribe, as the Indian Tribe which has the closest cultural affiliation and aboriginally occupying the area, claims ownership of such human remains and associated funerary objects, as they choose.¹ The Tribe's ownership and right to disposition and control of the human remains and/or associated funerary objects refers to the entire burial, to the extent it can be recovered, and does not allow in any case for separation of part of an individual's remains from other parts or from their associated funerary objects.
 - c. If the exposed human remains and/or associated funerary objects are historic and non-Native American in origin, the Spokane Tribal Historic Preservation Officer will notify the Washington State Historic Preservation Officer (SHPO), Disposition and control over such burials will be determined the SHPO.
 - d. If the exposed human remains and/or associated funerary objects are of uncertain or unidentifiable cultural identity, but clearly non-modern in origin, the Spokane Tribal Historic Preservation Officer will use reasonable means, such as professional consultation, to obtain a determination of the responsibility of the entity disturbing such remains. After cultural identity has been satisfactorily determined, the disposition or control of such remains and /or objects shall follow as otherwise provided in this procedure.
 - e. If the exposed human remains and/or associated funerary objects are modern or possibly modern in origin, regardless of cultural affiliation, the Spokane Tribal Historic Preservation Officer will notify the local law enforcement authorities. Disposition and control over such burials will be determined by the law enforcement authorities.

¹ For the purposes of this procedure, modern is here defined as less than 50 years old; non-modern is defined as 50 years of age or older. For human remains, the age of such remains is defined as beginning at the death of the individual, to the present.

4. Within 48 hours of notification, the entity with right of disposition and control shall notify the landowner concerning plans for disposition and control over such objects. Actual disposition and control shall be implemented as soon as possible, although may be delayed is so agreed by the landowner and the entity with right of disposition and control, or is the extent of the damage or other circumstances require delay in disposition and control.

The entity performing any action which inadvertently disturbs or damages Spokane human remains or cultural resources shall be responsible for costs of inspection of the damage or disruption by Tribal staff; removal, reburial, and/or restoration of the site; identification of resources. Costs may include but are not limited to staff, equipment, supplies, laboratory costs, and travel. If the entity performing the action which inadvertently disturbs or damages such resource is not also the land owner, such entity is responsible for reimbursing the land owner for costs incurred by the land owner as a direct result of this procedure. In no case shall the required associated with the action or resources involved.

The Spokane Tribal Historic Preservation Office shall make best effort to minimize the costs associated with Inadvertent Disturbance or Discovery, especially when the entity involved fully cooperates with preservation and protection efforts; however, appropriate project undertaking funding shall ensure that sufficient measures are taken to complete the activities described in these procedures.

An entity solely reporting human remains or cultural resources to the Spokane Tribe, provided they have not damaged or disturbed such resources, or caused or been responsible for damage or disturbance of such resources, shall not be responsible for any additional costs under this section.

Relationship to Other Applicable Laws

Full compliance with all aspects of this procedure shall be considered by the Spokane Tribe as full and complete consultation and cooperation with the Spokane Tribe, as required by law, for the purposes of Inadvertent Disturbance and Discovery of human remains and cultural resources.

Limitations

Compliance with this procedure for a particular disturbance or discovery does not constitute consultation and cooperation with the Spokane Tribe on other disturbances or discoveries.

Notification of the Spokane Tribe under this procedure does not release the entity from responsibility for violations of federal, Tribal, state or local law.

Violations

Any entity discovering or disturbing any Spokane human remains or cultural resources who does not follow the procedure described here, shall be considered in violation of this procedure. Such action shall be considered deliberate and causing unauthorized damage to the affected resource; this action is subject to prosecution under applicable federal, Tribal, state and/or local laws.

Recovery of Eroding Human Remains

When approval from the appropriate authorities is given for the collection of scattered human remains or recovery of exposed and immediately endangered remains, standard professional practices will be used to ensure that all associated remains and grave goods are recovered, and that the location is documented to assist future monitoring or management practices. However, those making the recovery shall not open up areas around the burial or discovery with the intention of discovering additional burials and materials or to learn more about the site context. Excavations of this sort are strictly for the salvage of eroding or disturbed burials.

The methods for documentation are to be consistent with practices employed by the Spokane Tribe, including collection of locational data, controlled excavation of the burial pit, and screening of the pit fill.

A professional archaeologist shall be in the field with the burial recovery crew at all times, and shall participate in the documentation of burials in all aspects where their involvement does not violate traditional custom or practices. If permitted by the Spokane, to scale map documentation of excavated features (i.e., distribution of remains and grave goods in the burial pit) is recommended.

The project entity is responsible for the preparation of a site plan map that shows the locations of surface-visible cultural features, significant topographic features, and other information needed to relocate the site in subsequent years for management purposes.

Photographs shall be taken that show the location of excavated burials in relation to identifiable landmarks. Human remains will not be visible in the photograph if not approved by the Spokane Tribe; this authorization will be decided on a case by case basis. The location of un-recovered remains or each excavated grave will be documented on a 7.5' USGS quadrangle topographic map. GPS measurement of location is required.

Associated artifacts and grave goods may be subjected to examination and documentation if that is approved by the Spokane Tribe. Permission from the Spokane Tribal Business Council for examination and documentation of Native American burials and grave good, beyond that required to determine if the remains are Native American in origin, shall be gained in writing and a copy of the written approval shall be provided to the contracting professional investigation of the burial(s).

If the remains are Euro-American in ancestry, standard non-destructive analysis shall be completed of remains and any associated grave goods or mortuary materials.

All grave goods shall be stored with the appropriate skeletal remains.

Any recovered remains will be boxed according to Tribal standards (appropriate size and material to be decided by Tribal Elder in consultation); the contracted investigator will retain and protect the burials in their custody until repatriation occurs or, if such would prove necessary after completion of NAGPRA consultations, the Tribe notifies them to deliver the burial(s) to another location. We anticipate that, after completion of notification processes defined in NAGPRA, Native American remains would be repatriated to Spokane Tribe in Wellpinit, Washington.

Coordination

The Tribal Historic Preservation Officer, is the primary contact for the Spokane Tribe for notification purposes as well as consultation on matters of cultural patrimony. The phone number is (509) 258-4315, or FAX (509) 258-6965. The THPO shall be immediately notified whenever a human burial or scattered human remains are found on any Reservation or ceded land location.

Definitions

Cultural Resources

Cultural resources include (but not by way of limitation): archeological, historic, traditional, and ethnographic resources older than 50 years or originating more than 50 years ago. These include artifacts, features, and sites; pictographs and petroglyphs; traditional cultural properties; sacred sites and continuing practices; traditional gathering areas and resources; the Spokane and Columbia rivers; oral histories, myths, and stories; traditional ceremonies (separate from those practiced at historic sites), gatherings, and activities; and recordings of these in various formats. Those cultural resources specifically excluded from this definition are burial sites, human remains, and associated funerary objects, which possess certain qualities for the Spokane People that are not to be disclosed or discussed in this context.

To further expand this operational definition of cultural resources, three categories of property types should be noted; ancestral lifeways, property is usually an archaeological resource that contains material remains or physical evidence of past human life or activities, including the record of the effects of human activities on the environment. They are capable of revealing scientific and/or humanistic information through archaeological research. For the purposes of the Spokane Tribe, these sites are those that can be dated as originating prior to contact, that is, A.D. 1730.

An historic property may also be archeological in nature, but is better delimited by the time period of contact between the Spokane(e) Peoples and Euro-Americans, that is, between 1730 and 1950. This transitional period and the material culture generated may provide useful insights on assimilation and cultural resistance. In the long run, these contrasts will offer broader cultural and chronological reconstructions, documenting significant events, occupations or activities, and/or structures or landscapes whether extant or vanished, apart from the value of any existing structure or landscape.

Additional cultural properties are those associated with cultural practices or beliefs of a living community that are rooted in that community's history or are important in maintaining its cultural identity. These may also include traditional resource areas, those which traditionally support subsistence or other consumptive or ceremonial use of natural resources. Use can be on-site and visible, inferred from effects, or off-site and referenced in traditional narratives. Traditional ceremonial use may also involve sites, structures, each with their own special local names; as such they are eligible for listing in the National Register Historic Places.

Damage to Cultural Resources

Any intentional or unintentional disturbance to any cultural resource which has not been authorized by the Spokane Tribal Council as appropriate for that resource is considered damage. Damage to cultural resources includes (but not by way of limitation) looting, vandalism, disturbance, or displacement of any artifact, human remains or associated cultural objects, cultural features or sites, sacred sites, or burial sites; collection of non-modern artifacts (older than 50 years) from the surface of the ground; painting, drawing, carving, or other defacement of pictographs or petroglyphs; digging or disturbance in cultural sites; disturbance, clearing, or spraying pesticides in traditional gathering areas; handling of Spokane burial remains or associated objects by non-Tribal members; and desecration of burial grounds.

Entity or Person

For the purposes of the procedure "entity or person" shall mean an individual, corporation, partnership, trust, institution, association, or any other private entity or any officer, employee, agent, department, or instrumentality of the United States, of any Native American Tribe, and/or of any State or political subdivision thereof.

Objects of Cultural Patrimony

For the Spokane Tribe these objects include (not by way of limitation) Spokane Elders' oral histories, myths, stories; burial remains and associated objects of individuals without known descendants; objects associated with cemeteries and sacred sites; and the recordings in any and all media of these classes of objects.

APPENDIX D

U OF S ETL

LABORTORY SAFETY GUIDE



Laboratory Safety Guide

January 2008

Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

LABORATORY SAFETY GUIDE

Version 1, Jan 2008

John P. Giesy, Ph.D., Jong Seong Khim, Ph.D., and Paul D. Jones, Ph.D.

Supported through:
Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
Environmental Toxicology Laboratory
Toxicology Centre
44 Campus Drive,
Saskatoon, Saskatchewan, S7N 5B3
Canada

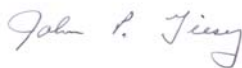
Phone: (306) 966-2096; 5206; 5062
Facsimile: (306) 966-4796

APPROVAL PAGE

Revisions to an existing SOP, addition of a SOP change form, or preparation of a new SOP must be reviewed, approved, and signed by the following:

Authored By: John P. Giesy
Jong Seong Khim
Paul D. Jones

Date: February 19, 2008



Supervisor Review By: John P. Giesy, Ph.D.

Date: February 20, 2008

DEFINITIONS AND ACRONYMS

ATRF	Aquatic Toxicology Research Facility
DHSE	Department of Health Safety and Environment
ERP	Emergency Response Plan
ETL	Environmental Toxicology Laboratory
PPE	Personal Protective Equipment
SOP	Standard Operating Procedure
U of S	University of Saskatchewan

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1 LABORATORY SAFETY PLAN

1.1 Laboratory Safety Policy

Work conducted in the laboratory and field can be hazardous, but if all of the safety protocols are followed a person can minimize the potential for injury to themselves and others. None of the work that is conducted by the Environmental Toxicology Laboratory (ETL) is more important than the health and safety of our people. While the laboratory directors, Prof. John Giesy and Prof. Paul Jones, have the ultimate responsibility for providing a safe and comfortable work environment, the daily overall supervision and assurance of safety in the laboratory and field is delegated to the laboratory manager and individual team leaders in charge of the individual studies.

Safety is the responsibility of both the management and workers. We have put procedures in place to insure the safety of all workers. These procedures are outlined in the University of Saskatchewan (U of S) safety manuals for general, biological, chemical and radiation safety and waste management and sample transportation, shipping and receiving. More specific information on safety procedures can be obtained in the individual ETL-standard operating procedures (SOPs) provided for each procedure and/or in the Health and Safety Plan (HASP) for each project.

This safety manual is not meant to be a complete compendium of all policies and procedures and does not replace or supplant any of the policies and procedures put in place by the U of S. In fact, this document builds upon and augments those policies and procedures. The relevant manuals and policy statements of the U of S are appended to this Safety Plan. In addition, more complete guidance can be obtained from the Department of Safety Health and Environment (DHSE) web site: www.usask.ca/DHSE. Furthermore, there are national and provincial laws in place that govern the workplace and these policies and procedures must be followed at all times. It is the responsibility of each employee to know the rules that apply to their situation and to follow the proscribed procedures at all times. Failure to do so will result in a warning and if the policies and procedures set forth in this document are not followed, it can lead to dismissal from the ETL.

Furthermore, each project conducted through the auspices of the ETL of the University of Saskatchewan Toxicology Centre is required to have a safety plan. In addition, each standard operating procedure (SOP) developed for ETL projects is required to have a project and procedure specific identification of potential hazards and a section on safety procedures.

1.2 Laboratory Safety Training

All laboratory personnel, including principle investigators, technicians, post doctoral fellows, graduate students, undergraduate assistants and visiting scientists are required to have the necessary and appropriate safety training. This training can be obtained in several ways. In addition to the laboratory and project specific training, each member of the ETL is required to take all of the appropriate safety classes listed below (provided from U of S, DHSE, <http://www.usask.ca/dhse/trainingcourses>) and in the appropriate HASPs and SOPs for the laboratories in which they work or the projects on which they work. When a class is taken, a certificate of training will be issued by DHSE. The employee is to retain the original and provide a copy to the ETL management team to be kept on file in the ETL. It is also the responsibility of all personnel to ensure appropriate refresher courses are taken to keep their safety training current.

1.2.1 Basic Required Safety Training

1) Safety Orientation for New Employees: This course is offered to new employees for the purpose of helping prevent job related injury and illness and to provide general training required under Saskatchewan Occupational Health and Safety Regulations (mandatory as per Sec. 18 & 19). The course provides generic health and safety information and will introduce attendees to safety processes on campus. Site-specific training is the responsibility of the supervisor.

2) Safety Orientation for Supervisors: Individuals who have employees report to them are supervisors. Incumbents with supervisory roles or functions are responsible for the health and safety of workers and for workplaces in their areas of jurisdiction. This course covers legal aspects including Bill C-45, University policy on Health, Safety and Environmental Protection and supervisor responsibilities. Supervisory faculty and staff will be provided with some tools to help them integrate and manage health, safety and environment business into their planning and operations processes.

3) Laboratory Safety: This course is mandatory for all employees, including faculty and graduate students, who work in a laboratory and handle chemicals and/or other hazardous substances. (Employees working with other hazards such as electricity, heavy equipment, etc. should contact DHSE for guidance). The course will include training in: WHMIS, accident response procedures, emergency response plans, the safe use, storage and disposal of a range of hazardous chemicals, the proper use of personal protective equipment, and laboratory safety requirements. This course is supplementary to the Biosafety and Radiation Safety Courses. Participants must attend both days.

4) Biosafety: This course is mandatory for all employees, including faculty and graduate students, who work with biological materials. This course provides training in: Biosafety regulations, University policies and procedures pertaining to Biosafety, exposure routes, selection and use of PPE, biosafety cabinets, laboratory acquired infections and prevention,

biosafety considerations when working with animals or plants, importation of pathogens or animal by-products, and the safe use, storage and disposal of biological materials.

5) *Radiation Safety:* This course is mandatory for all employees, including faculty and graduate students who work with radioactive nuclear substances. This course will include training in: University policies and procedures, handling procedures, physics of radiation, contamination control, monitoring, disposal, local emergency procedures pertaining to nuclear substances, and the responsibilities and duties of permit holders and nuclear energy workers. Participants must attend all 3 days.

1.2.2 Optional Safety Training

6) *Hazardous Waste Management:* Hazardous Waste Management is an essential supporting element of the biological, chemical, radiological and environmental safety programs. Centrally-funded hazardous waste disposal helps our University community meet its legal and ethical obligations by providing a comprehensive and convenient waste disposal system to support research and teaching. Please refer to the Hazardous Waste Disposal Manual for essential technical and contact information.

7) *Transportation of Dangerous Goods:* DHSE offers training in the safe transport and receipt of dangerous goods. Anyone who ships, transports or receives dangerous goods must be trained and certified to do so. Different courses are offered to suit the needs of the campus community.

8) *Fire Safety:* This course is designed to instruct employees on the BASICS of Fire Science, the different classes of fires and fire extinguishing methods. Employees will be instructed in the proper use of a fire extinguisher and the topics of Flight or Fight and Life Safety.

1.2.3 Safety Information and ETL-SOP

In addition to the basic safety training courses offered by the U of S-DHSE, there is additional safety information provided in any and all HASPs and SOPs appropriate to your particular studies. It is required that all workers obtain and read all appropriate safety materials. In addition to reading the written materials, each worker will receive personal instruction before being asked to undertake any task. If the worker does not understand the instruction, or has concerns about the safety of any procedure, it is the joint responsibility of the supervisor and the employee to assure that the procedures and appropriate safety procedures are understood.

ETL-SOPs are written in response to a requirement to document and standardize routine activities, procedures, or schemes used to accomplish the task. The objective of an SOP is to provide a systematic, usable, and efficient method of accomplishing a routine laboratory task and individual SOP always includes 'Safety Considerations' section to

provide all safety information required when applying procedures. Appropriate SOPs will be provided to all individuals working in the ETL. All laboratory and field workers need to read and understand the SOPs given and/or related with his/her task before and during their work. All individuals in the ETL need to strictly follow the procedures, particularly for safety guide, to protect their and others' health and safety. Refer to ETL-SOP #1000, entitled 'Requirements for the Preparation, Review, Approval, and Implementation of Standard Operating Procedures (SOPs)', for the detailed guideline for SOPs

1.2.4 Certificates of Training

When an employee completes the training courses, they will be issued certificate of completion. They should keep the original for their records and provide a copy to be maintained by the ETL management. These records will be available to anyone who wishes to determine whom has had what formal training. In some cases ETL employees will take additional training not offered by the U of S. In such cases, this training will not substitute for or replace the required U of S training, but the employee should provide a record of all such training to the ETL management.

1.3 Laboratory Safety Responsibilities

Safety is the responsibility of all members of the ETL team. Regulations and guidelines, however well conceived or communicated are not sufficient to assure a completely safe working environment. It is the skill, knowledge, vigilance, diligence, communication and basic common sense of the individual workers that are essential to maintaining a safe working environment. Do not undertake any operations that you have not been trained for or if you are uncertain of how to conduct the operation. When in doubt ask. Furthermore, if you observe any of your colleagues conducting an unsafe operation bring it to their attention immediately. If you see an unsafe situation in the laboratory, correct it immediately and inform appropriate supervisory personnel. If you can not do so bring the situation to the attention of laboratory management immediately. No issue is too small to bring to the attention of others. If information on safety is lacking in HASPs or SOPs, bring it to the attention of the laboratory management so that the necessary safety information can be added to the appropriate documents.

If you are not satisfied with the response received from laboratory management to any safety issue it can be raised with the U of S Local Safety Committee #21 (LSC #21). This committee is responsible for all safety matters relating to the ETL and the Toxicology Centre as a whole. ETL management supports and encourages the use of LSC #21 to resolve any contentious safety issues after all possible internal avenues to resolution have been exhausted.

1.3.1 Safety Management in ETL

The laboratory management will do its part to assure the safety of the working condition of the laboratory and field campaigns. **It is the responsibility of the workers to:**

- 1) Attend safety training courses and to read all appropriate safety materials issued to him/her (such as instrument manuals, hazard alerts etc.).
- 2) If new hazards come to his/her attention, these should be corrected immediately and the issue and corrective action taken communicated immediately to the principal investigator (Prof. John Giesy or Prof. Paul Jones) and or the unity Safety Officer (Dr. Jong Seong Khim).
- 3) Be aware of all of the policies, regulations and practices of the Federal Government, Provincial government, U of S and the ETL.
- 4) Comply fully with all of the established safety regulations and practices and to consult the principle investigator or designated representative for advice in circumstances where safe practices or the appropriate measures are in doubt.
- 5) Limit laboratory and field work to projects authorized by the principal investigator.

- 6) Advise coworkers if unsafe activities are observed and to report such instances to the principal investigator so that corrective actions can be put into place.
- 7) Advise visitors to the existing hazards and when necessary (when others are using equipment in the laboratory or field) to inform them of the ETL, U of S and any other pertinent regulations or safety procedures. Assure that others making use of equipment have the necessary safety training.
- 8) Be sure that all required monitoring is conducted and reports generated.
- 9) Assure that laboratories are secured by locking and monitoring who comes and goes so that unauthorized persons are not allowed access.

**Individual workers need to understand and agree his/her responsibilities
before they start working in the ETL**

1.3.2 Work Approval in ETL

To work in the ETL laboratories or take part in field studies, requires the permission of the laboratory director (Prof. Giesy). This permission is granted by the employee signing the acknowledgement and approval form stating that they have taken all of the required training and received all of the supplemental training required to do their tasks in a safe manner both to themselves and those around them. Unauthorized personnel (those not explicitly approved by Prof. Giesy) are not allowed to enter the laboratory portions of the ETL or take part in field expeditions. Signed, approval authorization forms will be maintained for all employees in the ETL main office. An example of the authorization form is attached as an appendix to this document (see appendix, **LABORATORY WORKER SAFETY AGREEMENT**).

2 WORK ENVIRONMENT

The **Environmental Toxicology Laboratory (ETL)** is part of the ca. 38,600 sq-ft Toxicology Centre at the University of Saskatchewan (U of S) in Saskatoon, SK, Canada. The 15,600 sq-ft ETL consists of a 2,260 sq-ft biochemistry/molecular biology lab, a 2,720 sq-ft wet chemistry and analytical lab (a separate 320 sq-ft high resolution (HR) GC-MS for dioxin analysis), and 3,560 sq-ft office and lounge spaces to accommodate room for up to 50 graduate students, post-docs, visiting scientists and professors. ETL also manages the 7,100 sq-ft Aquatic Toxicology Research Facility (ATRF) for the maintenance, culture, and exposure of aquatic organisms. The ATRF is a facility for the use of all investigators in the Toxicology Centre as well as for investigators from outside the U of S.

The facility allows U of S researchers to conduct long-term studies of the accumulation, metabolism, and effects of toxic substances and therapeutic agents on aquatic organisms under controlled laboratory conditions. Aquaculture drug studies can also be conducted. The facility was built to assist researchers from academic institutions, provincial and federal agencies and businesses and industries as well as non-government institutions in conducting research into the fates and effects of trace substances in aquatic environments. The facility is specifically designed to assist the aquaculture, commercial and sport-commercial fishing and chemical industries by providing a safe and efficient facility in which to conduct studies without danger to researchers or releases to the ambient environment. The containment laboratories also minimize contamination from normal urban and agricultural environments so that controlled studies can be conducted. In addition to the culturing and rearing capabilities and exposure capabilities, the facility contains the following specialized areas: storage areas rearing and exposure tanks and coolers, food, chemicals, solvents and drugs. There are also freezers and walk-in chambers to permit experiments to be run in a constant temperature controlled environment.

The ETL laboratories were designed to allow researchers to conduct all of the steps in a study from rearing organisms, preparing chemicals, diet preparation, exposure, sample collection and both biochemical and instrumental residue analyses of compounds and their metabolites in a single completely contained and regulated facility. The specific types of studies that can be conducted include setting water quality standards for the protection of aquatic life and determining the kinetics of accumulation of trace contaminants from water, food and sediments by aquatic biota. This information can then be used to assist regulators in setting safe environmental concentrations of these compounds or determining the degree of remediation required at contaminated sites. The flexible facility can also be used to determine the accumulation, metabolism, and safety of veterinary pharmaceuticals used in fish culture and of industrial chemicals. In addition to the applied aspects, the facility can also be used to study the basic mechanisms of effects of contaminants such as mutagens and carcinogens, in animal models.

More information can be found at our ETL web-site: <http://www.usask.ca/toxicology/jgiesy>

2.1 Occupational and Environmental Safety

The ETL was designed so that all aspects of research into toxic substances can be conducted under one roof without the need to transport toxic or radio-active materials from one building to another. The building has special treatment facilities for water entering and leaving the building to guard against releases, to maintain the integrity of experiments and to protect the health and safety of its workers.

2.2 Work Area and Laboratory Facilities

The facility in the ETL is designed so that all phases of studies can be conducted under one roof, without the need to transport samples among buildings. These specialty areas include the Aquatics lab (Fig. 1), Chemistry lab (Fig. 2), and Biochemistry and Molecular Biology lab (Fig. 3), respectively.

	<u>Room</u>	<u>Ext.</u>
• Aquatics lab (ATRF Culture room)	55	4169
• Aquatics lab (ATRF Exposure room)	57	4942
• Chemistry lab (Wet & Instrumental lab)	158-159	4935
• Biochemistry and Molecular Biology lab	261	4940

2.2.1 Aquatic Toxicology Research Facility (ATRF)

The 4,600 sq-ft rearing laboratory is divided by moveable plastic curtains so that research areas can be configured in a variety of ways to accommodate and separate various experiments. The area can be set up as one large area or divided into several separate research areas. Lighting in each region of the laboratory is controlled by individual timers so that the duration of lighting can be regulated in each area separately. The culture room is equipped with a range of containers for rearing aquatic organisms including aquariums, large round and rectangular fiberglass tanks speciality rearing systems for small tropical fish. The facilities are designed and equipped to allow both static- and continuous flow through exposures with organisms ranging from phytoplankton, and zooplankton to benthic invertebrates, including insects and clams, fish and amphibians. Adult salmon as large as 10 kg can be accommodated.

The exposure area is similar in most respects to the culture area. The exposure rooms are equipped with HEPA filters in the influent and effluent air to maintain clean air in the laboratory while minimizing the chance that any toxicants would escape the containment room. In addition, the biochemistry/microbiology and chemistry labs contain laminar flow hoods and fume hoods so that studies can be conducted with toxic chemicals, radioisotopes and genetically engineered organisms.

Influent water treatment: The source of water for the facility is Saskatoon city water with a maximum potential flow rate of 100,000 L per day. The incoming city water is treated with activated carbon and biological filtration before it is stored in 2 50,000 L cement storage tanks. The treated fresh water (FW) then passes through ultraviolet light banks to kill any possible pathogens, including spores. Portions of the water can be diverted to a reverse osmosis (RO) system to decrease hardness and to a chiller/heater system to provide temperatures ranging from 4 to 45 °C. Each tank or research area is plumbed with three water lines that deliver normal water, heated/cooled water and RO water. In addition to these three types of water, there are several additional larger capacity lines carrying treated freshwater. The water can be mixed at the tank to make any combination of hardness from 0 to 235 mg CaCO₃/L and temperature from 6 to 45 °C. The rearing and containment rooms each contain a 108 sq-ft controlled temperature room that researchers can use to maintain temperatures from 4 to 30 °C within 0.2 °C.

Effluent water treatment: The effluent water can be sent directly to the sanitary sewer if it does not require any special treatment. Inorganic and organic contaminants can be removed from water before it is released to the sewer. The treatment system is a series of closed cartridge filters that can be filled with different materials, depending on what contaminant needs to be removed from the system. These are closed systems so that workers are never exposed to the contaminants. In addition to the sanitary line, there are two separate lines to receive effluent water so that two different types of treatment can be used simultaneously. All of the effluents are removed from the tanks by gravity flow through piping enclosed in trenches in the floor. Before treatment effluent water is stored in two 50,000 L storage tanks.

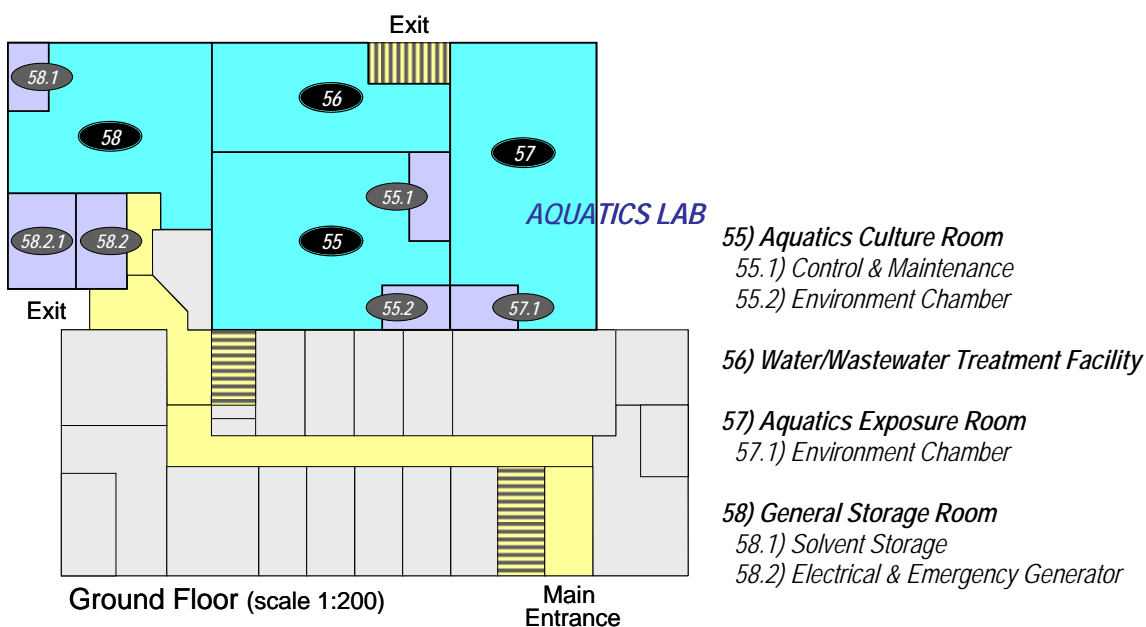


Fig. 1. Map of Aquatics Lab in ETL, Toxicology Centre, U of S

2.2.2 Chemistry Facilities

The chemistry lab at the ETL is designed to handle all aspects of sample processing from extraction to cleanup. The lab has a glassware washing station with nano-pure water, 4 chemical hoods, 6 lab benches, and a gas cylinder rack for safe and convenient storage of gas cylinders. The lab is set up so that everything from glassware washing to sample cleanup can take place in one room to maximize efficiency and minimize possible contamination to other parts of the facility.

The analytical lab is connected to the wet chemistry lab to maximize efficiency and communication between all aspects of the research. The analytical lab contains instruments including a Agilent 5975 GS-MSD including G1942N 6890 GC and 5973N MSD, an Applied Biosystems/SCIEX API 3000 Triple Quadrupole Mass Spectrometer, a Micromass Q-ToF II/Ultima (housed at Environment Canada), 2 Agilent 5975 GC/MSD systems, 2 Micromass Platform II LC/MS systems, a Mitsubishi AQF-100 for combustion ion chromatography, and two Dionex DX-500 Ion Chromatography system. There is also a separate room which houses a Micromass AutoSpec-S High Resolution Mass spectrometer used for the analysis of dioxins and similar semi-volatile organic contaminants.

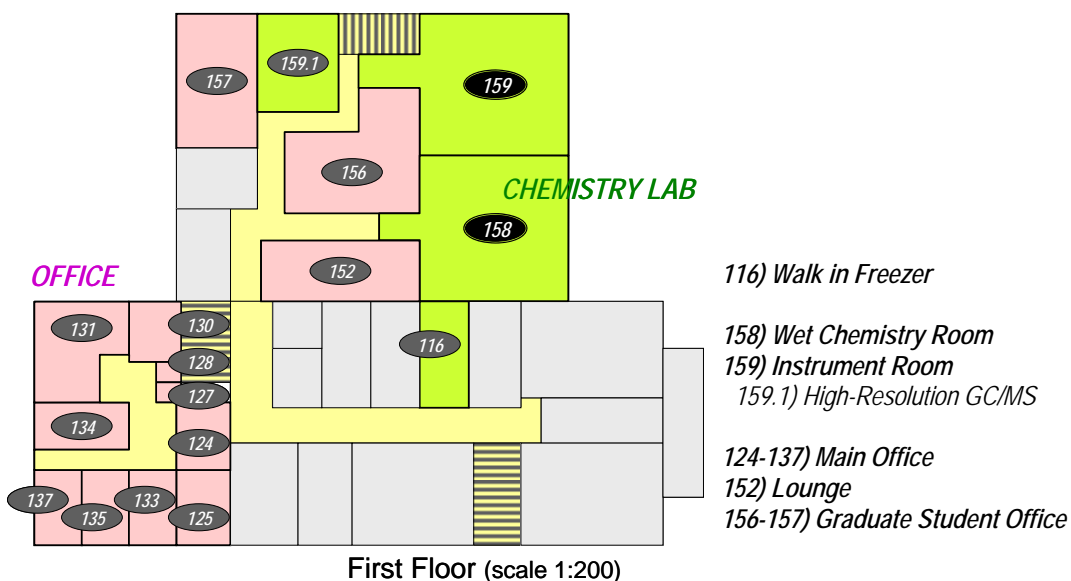


Fig. 2. Map of Chemistry Lab in ETL, Toxicology Centre, U of S

2.2.3 Biochemistry and Molecular Facilities

This facility is capable of doing just about any type of biochemistry and molecular work used on the toxicology field. New equipment includes Polarstar Optima which can read fluorescence intensity, fluorescence polarization, time resolved fluorescence, luminescence and absorbance. The 7300 Real time PCR system, which runs 96 well plates, for the analysis of gene expression. Also, a new micro injector, Injectman NI 2, that can micro inject small specimens including fish eggs, cells, and bird eggs. These facilities have the equipment (centrifuges, water baths, incubators, etc.) and capacity to run anything from gel electrophoresis and western blotting to ELISA's and enzyme activity (EROD, MROD and Aromatase). Three rooms adjacent to this facility are a dish washing room with an autoclave and nanopure system for washing and sterilizing dishware. ETL also includes a chemical storage room for all the chemicals used in the facilities and a separate room for micro and analytical balances. Within the biochemical and molecular rooms are a -80 degree freezer, liquid nitrogen dewars and many -20 and 4 degree freezers and fridges for preserving all samples and reagents. Worker safety is a big concern and will be the number one priority for these labs. There are eye wash and emergency shower stations throughout the facilities for anyone accidentally exposed to any toxic chemicals, All experiments using volatile compounds or solvents are carried out in fume hoods.

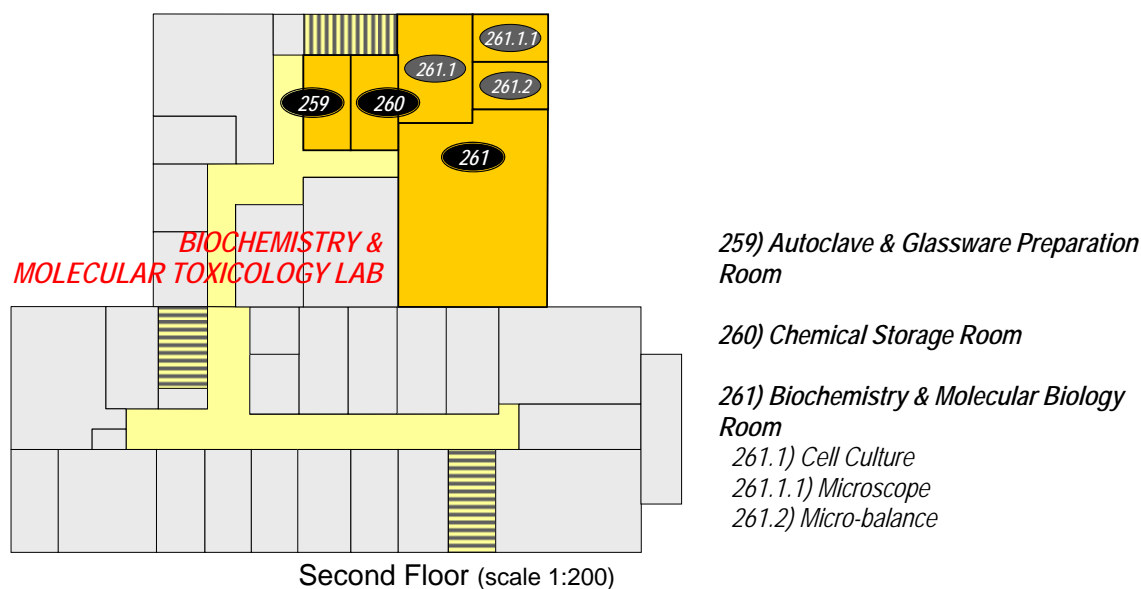


Fig. 3. Map of Biochemistry and Molecular Toxicology Lab in ETL, Toxicology Centre, U of S

Cell Culture Facilities: The cell culture facility in the biochemistry lab contains two large Microzone biosafety cabinets used in the safe cell culturing of many environmentally relevant cell lines (H295R, MVLN, H4IIE, MDA). The lab has two water jacketed CO₂ incubators and 2 other Hotpack incubators for more than one cell line can be in culture at any one time. The cell culture facilities are attached to the biochemistry main room for easy analysis of all the cell samples. Also attached to the cell culture lab is a microscope room equipped with an Olympus fluorescence microscope and many other monocular and stereo light microscopes.

2.3 Support Facilities

- Autoclave and dishwasher room
- Equipment and tank storage
- Field equipment storage and staging area
- Food storage
- Drug storage
- Chemical storage (special explosion proof room)
- Chemical storage
- Controlled Environment Chambers (4-30 °C)
- Walk-in freezer (-20 °C)
- Lunch and Conference Room
- Auxiliary power generator to supply 65% of peak power for critical areas

3 EMERGENCY RESPONSE PLAN (ERP)

3.1 Fire Evacuation Procedures

The fire alarm is tested for periods of less than ten seconds on the first Monday of each month in the Toxicology Centre.

On any occasion, the sounding of the alarm for more than ten seconds means immediate evacuation of the building is mandatory.

The following procedures must be followed in case of emergency evacuation of the ETL and/or Toxicology Centre.

• **When the Fire Alarm Sounds:**

- Step 1:** Everyone in the building must immediately evacuate the building in as orderly a fashion as possible using the pre-determined exit route.
- Step 2:** Each floor will have wardens and door guards who will direct the evacuation as quickly as possible in a safe and controlled manner.
 - a) Duties of the fire wardens and door guards are listed in Appendix I.
 - b) Exit routes, fire wardens and door guards are shown in Appendix II.
- Step 3:** Every instructor is responsible for his or her own classroom. Students must exit the building from the predetermined exit route. (An overhead of the predetermined exit route should be prepared by the instructor and available in all classrooms.)
- Step 4:** If a disabled person cannot easily be guided out of the building, 1 or 2 people (preferably 2) should be assigned to stay with them in a safe place until the Fire Department arrives to evacuate them. Someone must be sent to notify the Chief Fire Warden or the Fire Department of the situation and location. They should never be left to wait alone. If it developed that the situation could not wait, or if the person refuses to wait, the monitor (s) would assist her/him to evacuate the building (carry if necessary).
- Step 5:** The elevator must not be used during the evacuation of the building.
- Step 6:** In the event that one of the exit routes cannot be used due to the emergency, fire wardens will direct evacuees to the nearest clear exit route, and filter them into the stream of evacuees allotted those exit routes.
- Step 7:** All building personnel and visitors will follow the instructions of the fire wardens when asked to evacuate the building.
- Step 8:** No one shall re-enter the building following a fire or fire drill until permission has been given by the Fire Department in attendance, the Chief Fire Warden, the Assistant Chief Fire Warden or a representative from the campus DHSE.

- **After hours**

The senior person (supervisor) working after hours is responsible for all evacuation procedures.

Contact DSS (5555) for assistance if building alarm has not been activated.

APPENDIX I

NOTE: ALL WARDENS MUST MAKE THEMSELVES AWARE OF THEIR DUTIES

Duties of Chief Fire Warden:

- Will go immediately to the annunciator panel to determine the location of the emergency.
- Will go to the area, assess the situation, assist in first aid if required and notify fire fighter of special hazards in the area.
- After the emergency is over will notify wardens and door guards that it is safe to re-enter the building. The campus DHSE representative will arrive and will have a radio to assist in re-entry by calling Security and announcing an all clear when given that information by the Fire Department.

Duties of Deputy Fire Warden:

- Will go immediately to the annunciator panel to determine the location of the emergency.
- Will remain at the Service Area Door, meet with firefighters and direct them to the location of the emergency.
- Will act as Chief Fire Warden in his/her absence.

Duties of Floor Wardens:

- To see the complete and immediate withdrawal of everyone from his or her area through a pre-determined route to the nearest exit.
- If the emergency has occurred in the Warden's area, then he/she should assess the situation and take appropriate action to ensure everyone's safety. They should notify the Chief Warden (or firefighter) of any special hazards in the immediate area of the emergency or about anyone who did not or could not leave the area.

Duties of Door Guards:

- Will go directly to the exit door to be guarded.
- Insist that everyone move a safe distance away from the exit door so that others can get out.
- Guard exit door to prevent premature re-entry during an emergency.
- Ensure that everyone stay a safe distance away from the building during the emergency.
- Notify everyone that they may re-enter the building when permission has been given by the Fire Department, the Chief Fire Warden or the Deputy Fire Warden or Security.
- Inform people evacuating to the assigned exit.

APPENDIX II

Chief Fire Warden: (1)	Adriana Brown
Deputy Fire Warden: (1)	Mark Wickstrom
Ground Floor Wardens: (3)	Fiona Price = south front door Michael Pollock = west door (side)
1st Floor Wardens: (2)	Shanda Sedgwick = south door (front) Paul Jones = west door (side)
2nd Floor Wardens: (2)	Brian Saraurer = south door (front) Steve Wiseman = west door (side)
Front Entrance Door Guard:	Adriana Brown = south door (front)
Back Entrance Door Guard:	Michael Pollock = west door (side)

3.2 Chemical Spills

- Chemical spill procedures are dependent on the type chemicals involved.
- Ensure that the procedures are specific for the type of chemicals.

The following procedures must be followed in case of chemical spills in the ETL.

• Minor Spill

Step 1: *Assessment*

Are you capable of dealing with this on your own based on training, equipment and circumstances?

Step 2: *Containment*

Alert all people in the area that there has been a spill. Keep the material from contaminating a other areas.

Step 3: *Cleaning*

Gather the spilled material and absorbent and place it in an appropriate container.

Step 4: *Decontamination*

Complete the final cleanup of the area and yourself.

Step 5: *Reporting*

Report the incident to your supervisor and fill out an Incident Report Form.

• Major Spill (larger than you or your colleagues can handle)

Step 1: Cordon off area with caution tape from the chemical spill kit and/or use appropriate signage.

Step 2: Alert everyone in the area that there has been a chemical spill.

Step 3: Call the Waste Management Facility (WMF) @ 8497 (after hours 5555).

Step 4: Report spill to supervisor or lab manager.

Step 5: Fill out an Incident Report Form.

***Note: Chemical Use:** Chemicals purchased or brought to the laboratory will be covered under a specific chemical use SOP. All chemicals brought into the ETL will be logged into the chemical registry and a materials safety data sheet (MSD) procured. The MSD will be provided to ETL management and kept electronically on the ETL server. A hard copy of the MSD will be placed into the MSD binder that will be kept in the chemical storage area. Each chemical will be stored appropriately based on the MSD. Specifically, when not being used, all chemicals will be stored in the chemical storage room and the room will be locked at all times. Access to the room will be to only authorized ETL workers. When chemicals are used the use and amount used will be logged into the chemical use log which is kept in the chemical storage room.

3.3 Biological Spills

- Biological spill procedures are dependent on the type biological agent involved.
- Ensure that the procedures are specific for the type of agent.

The following procedures must be followed in case of biological spills in the ETL.

• Minor Spill

- Step 1:** Wear protective and appropriate PPEs, i.e. lab coats, gloves, and masks etc.
- Step 2:** Allow aerosols to settle.
- Step 3:** Alert everyone in the area that there has been a biological spill.
- Step 4:** Gently cover spill with paper towels.
- Step 5:** Apply the appropriate disinfectant to the paper towels starting at perimeter and working saturation towards centre of spill.
- Step 6:** Allow contact time appropriately before cleaning up.
- Step 7:** Seal waste in appropriate Biohazard autoclave bag or container.
- Step 8:** Decontaminate area appropriately.
- Step 9:** Report spill to supervisor or lab manager.
- Step 10:** Fill out an Incident Report Form.

• Major Spill (Larger than you or your colleagues can handle)

- Step 1:** Cordon off area with caution tape out from a chemical spill kit and/or use appropriate signage.
- Step 2:** Alert everyone in the area that there has been a biological spill.
- Step 3:** Call the Biosafety Office @ 8496 and WMF @ 8497 (after hours 5555).
- Step 4:** Report spill to supervisor or lab manager.
- Step 5:** Fill out an Incident Report Form.

3.4 Radiation Spills

- All clean ups should be carried out according to a prearranged plan.
- Only persons properly trained shall clean a radiation spill.
- Detailed steps can be found in the Radiation Safety training manual.

The following procedures must be followed in case of biological spills in the ETL.

• Minor Spill

Step 1: *Assess the situation*

Take your time to assess the situation for any potential hazards and then determine your plan of action. The protection of personnel and the containment of the nuclear substance should be given primary consideration.

Step 2: *Alert everyone in the area*

Ensure that everyone in the vicinity of the accident has been alerted. Be sure to make an effective warning, especially in large laboratories or areas.

Step 3: *Confine the spill*

Restrict access to the area. Confine the problem (contain the nuclear substance) to minimize the exposure to personnel or release to the environment.

Step 4: *Clear the area*

Evacuate all persons from the immediate vicinity of the contaminated area. Ensure a sufficient separation such that persons near the spill cannot become exposed to the hazard.

Step 5: *Summon aid*

In any emergency situation, it is vital to notify the appropriate personnel (Security, Ambulance, and/or Fire Department). Do not delay medical attention for seriously injured people due to concerns relating to radiation contamination.

Step 6: *Cleaning*

Wear a lab coat and two pair of gloves; outline the location of the spill and probable extent of the contamination. Collect the spilled substance by blotting the area.

Step 7: *Decontamination*

Apply appropriate cleansing agent to contaminated area. Working from the outside of the spill area towards the centre wipe the spilled area. Repeat 3-4 times. Dispose of all cleaning material into an appropriate container.

Step 8: *Monitoring*

Monitor for contamination. Ensure no access to area while results are pending.

Step 9: *Reporting*

Report the incident to your Permit Holder and the Radiation Safety Manager. Fill out an Incident Report form.

• **Major Spill** (Activity spilled is greater than 100 uCi)

- Step 1:** Cordon off and clear the area.
Step 2: Alert everyone in the area.
Step 3: Confine the spill, if required.
Step 4: Call the Radiation Safety Manager @ 8494 (after hours 5555).
Step 5: Fill out an Incident Report form.

3.5 Waste Disposal

During the normal course of conducting studies, waste will be generated. This will include normal paper waste, chemical wastes and biological wastes and radiological wastes. It is important that wastes be stored, labeled, transported, and disposed of properly. Detailed instructions for all of these are given in the U of S DHSE manual on waste management. A copy of this manual is attached to this safety manual. Please consult this manual for specifics for handling wastes safely and legally.

3.6 Medical Emergencies

The following procedures must be followed in case of medial emergencies in the ETL.

- Step 1:** **Dial 9-911** for all medical emergencies. Be prepared to provide the nature of the emergency, exact location (including building, room number and closest entrance), your name and the phone number at which you can be reached. Follow the 911 operator's instructions.
- Step 2:** Dial 5555 for Security Services.
- Step 3:** When responding directly to a first aid emergency, first ensure that there is no further danger to yourself or the victim before commencing first aid techniques.
- Step 4:** Provide first aid for any life-threatening conditions. **DO NOT** transport victim yourself.
- Step 5:** Fill out an Incident Report Form.

3.7 Incident reporting

It is important that incidents be reported immediately to your supervisor and ETL management. There are several reasons for this. The first and most important reason is so that the laboratory management is aware of the situation, and so that it can be managed properly in a timely and efficient manner. Second, because it is important to identify these situations so that corrective actions can be put into place to avoid the situation in the future. It is thus, important to report situations that you feel are impending issues and those that were “near misses”. Even in these cases where injury was avoided, it is important to report the situation immediately.

Safety is everyone’s responsibility. We are responsible for the safety, health, well being and security of ourselves as well as those around us. So if you see an unsafe act, bring it to the attention of the offending person immediately and also report it to the ETL management. We are all busy so it is important to make a written record of the situation and then follow up so that everyone knows about it and it can be avoided in the future. Be proactive. If you see an unsafe situation, and you are properly trained to do so, remedy it immediately. This includes things so minor as a wet spot on the floor or a paper clip on which someone could slip. There is a formal U of S process for incident reporting. Please see the detailed information below and in the attached appendices for the policies and the necessary forms to be filed.

4 EMERGENCY CONTACT

**Please contact person immediately and appropriately among listed
for the safety issue(s) and/or emergency in the ETL**

4.1 ETL-Emergency Contact

	<u>Home</u>	<u>Cell</u>	<u>Office (Ext.)</u>
Prof. John Giesy	955-3819	517-614-6123	2096
Prof. Paul Jones	242-8957	517-281-5666	5062
Dr. Jong Seong Khim	955-0405	306-281-6204	5206

4.2 University-Emergency Contact

	<u>Extension</u>
SECURITY SERVICES (24 hours)	5555
Ambulance (if using a pay phone)	9 911 911
Fire (Pull an alarm and call) (if using a pay phone)	9 911 911
Safety Related Incidents (Mon – Fri: 8:30 to 4:30)	8493
Spills (Mon – Fri: 8:30 to 4:30)	8497

4.3 DHSE-General Contact

	<u>Ext. (Fax)</u>
General Inquiries	8493 (8394)
Hazardous Waste Disposal	8497 (6146)
Safety Training Inquiries	8492
Biosafety	8496
Biosafety Cabinet Testing Inquiries	8510
Chemical/Environmental Safety	8512
Safety Inspections Inquiries	8738
Environmental Program Inquiries	2379
Community Safety	1957
Fire Safety	8838
Contractor Safety Inquiries	6076
Occupational Hygiene / Injury Prevention	8511
Radiation Safety	8494
Radiological Inquiries	8491
Health, Safety and Environment Management System Coordinator	2370
Director	8463

REFERENCES

1. *LabSafety Manual, DHSE, University of Saskatchewan, February 2006*
2. *BioSafety Manual, DHSE, University of Saskatchewan, January 2005*
3. *Radiation Safety Manual, DHSE, University of Saskatchewan, June 2004*
4. *Hazardous Waste Disposal Manual, DHSE, University of Saskatchewan, Aug 2007*
5. *DHSE, University of Saskatchewan: <http://www.usask.ca/dhse/>*

APPENDICIES

- Laboratory Worker Safety Agreement, ETL, University of Saskatchewan, Sep 2007
- University Safety Manual, DHSE, University of Saskatchewan, 2004-2006
 - *LabSafety, Feb 2006*
 - *BioSafety, Jan 2005*
 - *Radiation Safety, Jun 2004*
 - *Hazardous Waste Disposal, Aug 2007*

**ENVIRONMENTAL TOXICOLOGY LABORATORY
TOXICOLOGY CENTRE
UNIVERSITY OF SASKATCHEWAN
LABORATORY WORKER SAFETY AGREEMENT**

Work conducted in the laboratory and field can be hazardous, but if all of the safety protocols are followed a person can minimize the potential for injury to themselves and others. None of the work that is conducted by the Environmental Toxicology Laboratory (ETL) is more important than the health and safety of our people. While the laboratory directors, Prof. Giesy and Prof. Paul Jones, have the ultimate responsibility for providing a safe and comfortable work environment, the daily overall supervision and assurance of safety in the laboratory and field is delegated to the laboratory manager and individual team leaders in charge of the individual studies. Safety is the responsibility of both the management and workers. We have put procedures in place to insure the safety of all workers. These procedures are outlined in the University of Saskatchewan (U of S) safety manuals for general, biological, chemical and radiation safety and waste management and sample transportation, shipping and receiving. In addition, specific safety instructions are contained in the ETL safety manual, or Toxicology Centre (TC) manuals or directives, which give specific safety procedures for individual laboratories and procedures. Also, more specific information on safety procedures can be obtained in the standard operating procedures (SOPs) provided for each procedure and in the Health and Safety Plan (HASP) for each project. All laboratory personnel, including principle investigators, technicians, post doctoral fellows, graduate students, undergraduate assistants and visiting scientists are required to have taken all of the appropriate safety classes listed below and in the appropriate HASPs and SOPs for the laboratories in which they work or the projects on which they work.

The basic required safety training includes the following U of S courses:

- 1) Basic safety orientation for employees
- 2) Safety training for supervisors
(for anyone who has supervisory responsibilities, this can include anyone in the laboratory)
- 3) Basic Laboratory safety
- 3) Biological Safety
- 4) Chemical Safety
- 5) Radiation Safety
(required even if you will not be using isotopes because others in the laboratory do use these materials and workers need to be aware of the issues and safety precautions)

Optional training includes:

- 1) Waste management
- 2) Transportation and shipping of hazardous materials
- 3) Fire safety

In addition to these courses offered by the U of S there is additional safety information provided in the Laboratory Safety plan and any and all HASPs and SOPs appropriate to your particular studies. It is required that all workers obtain and read all appropriate safety materials. In addition to reading the written materials, each worker will receive personal instruction before asked to undertake any task. If the worker does not understand the instruction, or has concerns about the safety of any procedure, it is the joint responsibility of the supervisor and the employee to assure that the procedures and appropriate safety procedures are understood.

Safety is the responsibility of all members of the ETL team. Regulations and guidelines, however well conceived or communicated are not sufficient to assure a completely safe working environment. It is the skill, knowledge, vigilance, diligence, communication and basic common sense of the individual workers that are essential to maintaining a safe working environment. Do not undertake any operations that you have not been trained for or if you are uncertain of how to conduct the operation. When in doubt ask. Furthermore, if you observe any of your colleagues conducting an unsafe operation bring it to their attention immediately. If you see an unsafe situation in the laboratory, correct it immediately and inform appropriate supervisory personnel.

If you can not do so bring the situation to the attention of laboratory management immediately. No issue is too small to bring to the attention of others. If information on safety is lacking in HASPs or SOPs, bring it to the attention of the laboratory management so that the necessary safety information can be added to the appropriate documents.

The laboratory management will do its part to assure the safety of the working condition of the laboratory and field campaigns. It is the responsibility of the workers to:

- 1) Attend safety training courses and to read all appropriate safety materials issued to him/her (such as instrument manuals, hazard alerts etc.).
- 2) If new hazards come to his/her attention, these should be corrected immediately and the issue and corrective action taken communicated immediately to the principal investigator (Prof. Giesy or Prof. Jones) and or the unity Safety Officer (Dr. Khim).
- 3) Be aware of all of the policies, regulations and practices of the Federal Government, Provincial government, U of S and the ETL.
- 4) Comply fully with all of the established safety regulations and practices and to consult the principle investigator or designated representative for advice in circumstances where safe practices or the appropriate measures are in doubt.
- 5) Limit laboratory and field work to projects authorized by the principal investigator.
- 6) Advise coworkers if unsafe activities are observed and to report such instances to the principal investigator so that corrective actions can be put into place.
- 7) Advise visitors to the existing hazards and when necessary (when others are using equipment in the laboratory or field) to inform them of the ETL, U of S and any other pertinent regulations or safety procedures. Assure that others making use of equipment have the necessary safety training.
- 8) Be sure that all required monitoring is conducted and reports generated.
- 9) Assure that laboratories are secured by locking and monitoring who comes and goes so that unauthorized persons are not allowed access.

I have read and understand the above responsibilities and agree to observe them for all of my laboratory and field work with the ETL. I recognize that I may be conducting procedures or using materials that may be hazardous and will make every effort to conduct these operations such that I maintain my personal safety and those around me. I consent to work with these potentially hazardous procedures and materials. If I am not confident in my training or understanding of the regulations and or safety precautions, I will inform my supervisor of any deficiencies I perceive in my training.

Signing of this Worker Safety Agreement is not a waiver of individual rights or redress in case of injury.

Signature Worker

Date

Printed Name

Permission is hereby granted to _____ to participate in studies of the ETL under our supervision. We have discussed the identified hazards of the proposed work with the above-signed worker and he/she has been given copies of all of the relevant unit safety regulations and procedures.

John P. Giesy, PI

Date

Paul D. Jones, co-PI

Date



DHSE

DEPARTMENT OF HEALTH
SAFETY & ENVIRONMENT

LabSafety Manual



University of Saskatchewan

February 2006

Emergency Numbers

SECURITY SERVICES (24 hours)	5555
Ambulance	9 911
(if using a pay phone)	911
Fire (Pull an alarm and call)	9 911
(if using a pay phone)	911
Safety Related Incidents (Mon – Fri: 8:30 to 4:30)	8493
Spills (Mon – Fri: 8:30 to 4:30)	8497

Department of Health, Safety and Environment

General Inquiries	8493
Fax	8394
Hazardous Waste Disposal	8497
Fax	6146
Safety Training Inquiries	8492
Biosafety	8496
Biosafety Cabinet Testing Inquiries	8510
Chemical/Environmental Safety	8512
Safety Inspections Inquiries	8738
Environmental Program Inquiries	2379
Community Safety	1957
Fire Safety	8838
Contractor Safety Inquiries	6076
Occupational Hygiene / Injury Prevention	8511
Radiation Safety	8494
Radiological Inquiries	8491
Health, Safety and Environment Management System Coordinator	2370
Director	8463

Other Essential Services

Bloodborne Pathogen Exposures (Sharps Puncture)	
Department of Immunology and Infectious Disease	655-1777
Public Health Consultation	655-4620
Employee Assistance Program	4300
Facilities Management Work Control (Mon – Fri: 8:00 to 4:30)	4496

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Disclaimer

The information included in this manual has come from a variety of reliable sources and is believed to be accurate. It is intended for the use of persons at the University of Saskatchewan and those who have taken the LabSafety Course. While every effort has been made to ensure its accuracy, there is no guarantee that it is free of errors and omissions. If, through your use or review of this document, you discover errors or omissions, please report them to us at 966-8493 or hse.dept@usask.ca

1. Introduction

Significant legislation exists that governs activities in work places across Canada. Through the Saskatchewan Occupational Health & Safety Act and applicable regulations and standards, every employer has both a moral and legal responsibility to ensure that all the necessary information, instruction, training and supervision are provided to protect the health and safety of employees, students and the campus public. It is the duty of workers to conduct themselves in a safe and responsible manner by incorporating safety as an integral part of their training and laboratory work. Information is provided here on health and safety policies, procedures and guidelines to be used by University employees and students so that risks will be reduced to as low as is reasonably achievable (**ALARA**) and that accidents can be avoided.

Regulations regarding the storage, use and disposal of hazardous materials are not so prevalent. The Saskatchewan Occupational Health and Safety Regulations (encompassing WHMIS) and the National Fire Code are the two significant pieces of legislation that specify the rules for us to follow with respect to laboratory work with hazardous materials. In this sparse legislative environment, the University has developed and implemented its own policies, procedures and guidelines with respect to hazardous materials management. Thus, the requirements presented in this manual have been adapted from many sources in an effort to achieve a safe environment for all, while allowing each person as much freedom as possible to conduct their work. A combination of published best practices, University history, and experiences of the members of DHSE and research and teaching persons on campus have gone into the development of this manual.

Through the **Health, Safety and Environment Protection Policy**, DHSE is empowered and directed to work to establish exemplary and proactive risk reduction practices. These are the objectives of this manual and the LabSafety training course which it accompanies.

It is not the intent of this manual, nor the Department of Health, Safety and Environment (DHSE), to in any way impede any work activities at this University, but rather to remain sensitive to the needs of all of the members of the University community and to assist individuals in meeting health, safety and environmental compliance to set standards wherever applicable.

2. Objectives

The objectives of this manual and the accompanying training are to:

- 1) Provide a guideline for setting safety procedures in all laboratories.
- 2) Promote compliance with the Saskatchewan Occupational Health & Safety Act and Regulations and other applicable Provincial and Federal legislation.
- 3) Identify the responsibility of persons who acquire, use or store potentially hazardous substances and assist them to meet compliance and implement good laboratory safety practices.
- 4) Promote the University's safety policies, codes and safety procedures, as well as those associated with the research granting agency's safety conditions for working with hazardous substances.
- 5) Assist the campus community in the area of health, safety and environmental issues that involve hazardous substance use, transport, storage, release or disposal.
- 6) Ensure that all workers and students know the safety and compliance requirements of the legislation and are aware of their rights under Canadian and Saskatchewan Health & Safety legislation.

- 7) Ensure that all workers and students have access to hazard information on the substances they use, transport, store or dispose of.
- 8) Ensure that all workers and students have the proper engineered and procedural safety controls and personal protective equipment (PPE) in place in order to work safely with their hazardous substances and to also ensure that they know how to use and maintain them.
- 9) Ensure that all substances used, transported or stored are known and are WHMIS labeled.
- 10) Ensure that all areas are inspected for safety and compliance on a routine basis.
- 11) Ensure that **ALL** accidents and incidents are investigated and recorded with DHSE (See Appendix).

3. General Duties and Responsibilities

3.1. President's Safety Committee

The President, through the Vice President of Research, appoints committee members to serve on the President's Chemical, Biosafety or Radiation Safety Advisory Committees. The membership is made up of researchers and technical support employees from campus that are knowledgeable in health, safety and environmental aspects and can assist in setting or reviewing campus safety policies and procedures.

Their Duties and Responsibilities are to:

- 1) Advise the President of the University of Saskatchewan, through the Vice-President of Research, on compliance with health, safety and environmental regulations that are related to the safe acquisition, use, storage, handling, and disposal of hazardous substances on the campus.
- 2) Act as a liaison between researchers or units utilizing hazardous substances and the applicable DHSE Managers if and when there is a difference of opinion on matters related to health, safety and environmental compliance, safety policy, and/or hazardous substance use.
- 3) Review proposals and make recommendations on safety policies and procedures developed by DHSE.
- 4) Provide technical advice and review the operations of the applicable DHSE program.
- 5) Review and approve Operating Permits for research activities involving the use of hazardous materials, agents in Risk Group Level 2 and greater, Notifiable Substances, genetically modified microorganisms (GMMOs) and nuclear substances.

3.2. DHSE Managers

DHSE Managers have the rights and responsibilities to conduct the following activities:

- 1) Facilitate the implementation of the policies and procedures represented by University Codes and Statutes.
- 2) Conduct and promote safety training courses.
- 3) Investigate and assist in the prevention of accidents, incidents, spills, and exposures which involve hazardous substances.

- 4) Assist in the resolution of any employee's unresolved health and safety concerns by reporting them to the Statutory Occupational Health Committee.
- 5) Ensure that regular safety and compliance inspections are carried out in all areas using or storing hazardous substances. Consult with individuals who work with hazardous substances on health, safety and environmental or compliance related issues.
- 6) Act in an advisory capacity to supervisors to assist them in developing their written procedures related to; use and maintenance of PPE, immunization, substance storage, waste disposal, safety policies, Standard Operating Procedures (SOPs) and others.
- 7) Ensure that areas which have been issued Permits or have been registered by the Supervisor or Principal Investigator as High Risk Areas comply with all health, safety and environmental regulations and granting agency's requirements.
- 8) Suspend any operation that is considered to be unsafe until corrective action is taken. The applicable President's Safety Committee may be asked to review the matter if necessary. The **Non-Compliance Enforcement Policy** can be initiated when life safety issues have not been addressed in a timely manner.
- 9) Submit reports to the chair of the applicable President's Safety Committee.

3.3. Statutory Occupational Health Committee

Provides a mechanism for employees to address unresolved health and safety concerns if they have not been resolved by i) the Supervisor, ii) the Department Head, or iii) DHSE.

- 1) Cooperates with DHSE to ensure that workers' unresolved health and safety concerns are addressed.
- 2) Promotes and reviews health and safety training courses given by DHSE.
- 3) Maintains and posts the minutes of their meetings and sends a copy to the Department of Labour and DHSE.
- 4) Investigates any work refusals and maintains the applicable health and safety records.
- 5) Conducts or directs workplace inspections.
- 6) Reviews unsafe conditions, accidents, incidents and spills.

3.4. Supervisors and Principal Investigators

The Principal Investigator or Supervisor (Professors, Scientists, and Laboratory Supervisors) must ensure that their work areas are in safety compliance with both Federal and Provincial health, safety and environmental regulations and also with any requirements of their respective granting agencies, where applicable. DHSE will inspect areas on campus to assist in identification of any deficiencies so that they may be rectified.

The Supervisor must ensure that their workers are aware of their rights under the SOHS Act and Regulations by ensuring the worker(s):

- 1) have received safety orientation courses offered by DHSE and site specific safety training for the jobs assigned along with written Standard Operating Procedures (SOP's) where applicable.

- 2) are competently supervised and have reviewed their Department's safety policies and procedures.
- 3) are informed of the potential risks associated with their work and have access to any hazard information on the substances used, transported, stored or disposed of (Right to know & WHMIS).
- 4) are provided with the appropriate safety equipment, engineering safety controls, and PPE necessary to protect their health and safety and that the worker knows how to use and maintain this equipment.
- 5) are provided with the proper supervision, guidance and written SOPs which will help prevent hazardous exposures to them or to any people peripheral to the work area.
- 6) register with or obtain required Permits from DHSE for any activities that use high risk substances, Biohazardous Materials, Notifiable Substances or Nuclear Substances.

Note: Basic Safety and Orientation Courses are provided by the DHSE, while site or task specific training is provided by the Supervisor or Principal Investigator of the projects or their qualified designate. Records must be kept of this training. DHSE will keep the worker's basic orientation and safety training records.

3.5. Waste Generators and Waste Contact Person

Waste Generators are any persons who generate hazardous waste as a result of work conducted. For example, a supervisor, a faculty member, a student, a technician, a research fellow, a postdoctoral fellow, etc. may be waste generators. Each is responsible for:

- 1) ensuring that waste is properly segregated, identified, and labeled for disposal,
- 2) conducting experiment in a safe manner,
- 3) ensuring that processes that generate unusual or novel waste have been identified and that a waste disposal plan has been developed and approved by DHSE prior to work starting,
- 4) collecting the waste in accordance with the University's waste procedures, and
- 5) keeping an inventory of the waste deposited into each waste container.

A Waste Contact Person is a person designated by his/her supervisor. The names, department(s), and phone number(s) of the supervisor and the contact person should be registered with the Waste Management Facility (WMF).

A Waste Contact Person is responsible for:

- 1) maintaining a liaison with the WMF staff,
- 2) ensuring that hazardous waste is securely packaged in appropriate containers,
- 3) ensuring that waste is packaged in compatible groups,
- 4) labeling the containers in accordance with the University's waste procedures, and
- 5) correctly completing the appropriated waste disposal authorization forms.

3.6. Students or Employees

Students and employees are required to:

- 1) Know their duties and responsibilities and conduct work in a safe and responsible manner so as to protect their health and safety as well as that of others that may be affected by their acts or lack of action.
- 2) Comply with the appropriate standards set forth by regulatory agencies and University policies, codes, terms and conditions.
- 3) Co-operate with their Supervisor or Principal Investigator and DHSE personnel in discharging their responsibilities as well as with any other person exercising a duty entrusted by a regulatory agency or agencies to promote safety.
- 4) Ensure they have received and understood the safety training, SOP and any other relevant safety information received from their Supervisor or Principal Investigator before commencing work that involves hazardous substances. This training must include the proper use and maintenance of safeguards, safety devices and PPE, so that they may carry out their work in a safe manner.
- 5) Notify their Supervisor or Principal Investigator when they become aware of any accident, incident, spill or unsafe act or condition.

4. Permits and Registration

The Supervisor or Principal Investigator of the laboratory or area should register every laboratory that uses or stores hazardous substances with DHSE. All laboratories should have the *Biological and Chemical Safety Poster* posted just inside the main door of the laboratory. In special cases when the hazards are high, the laboratory may have to have the doors into the lab appropriately labeled with warning signs.

The Supervisor/Principal Investigator of a project that uses Notifiable Substances must register these areas with DHSE. In turn, DHSE will notify the Occupational Health and Safety Branch of the Saskatchewan Department of Labour to receive any other requirements that are to be specified.

4.1 Chemical Substances

Contact the Chemical Safety Manager for more information about or to notify of the intended use of a Notifiable Chemical Substances as defined in Table 19 of the OHS Regulations.

4.2 Biological Substances

Principal Investigators in research and teaching laboratories using or storing biological substances in Risk Groups Level 2 or higher, using Notifiable Biological Substances (GMMOs) or biological materials are required to obtain a Biosafety Operating Permit or Registration. Submit a completed Permit Application Form to the Biosafety Office. DHSE will contact you if more information is needed. If the function of the laboratory or work changes, the onus is on the Supervisor or Principal Investigator to inform DHSE of this change.

4.3 Radioactive Substances

Contact the Radiation Safety Manager in DHSE.

5. General Safety and Compliance Requirements

Each person has an obligation to follow these instructions.

- 1) Comply with the SOHS Act and Regulations.
- 2) Follow all University safety policies, codes and procedures.
- 3) Follow the Research Granting Agency's Condition and the SOHS Act and Regulations for working with Notifiable Chemical or Biological Substances.
- 4) Keep unauthorized persons out of the laboratory and keep the laboratory locked when unattended.
- 5) Use Good Laboratory Safety Practices to keep all individuals as safe as possible.
- 6) Do not eat, drink, store food or smoke in a laboratory. Take the time to keep your laboratory neat and tidy.
- 7) Do not pipette solutions by mouth.
- 8) Know how to maintain and use appropriate PPE to limit exposures.
- 9) Wear gloves where appropriate and a lab coat in the laboratory and remove them when leaving the laboratory area as defined by internal department policies.
- 10) Wash hands thoroughly before leaving any laboratory area.
- 11) Minimize your risk of exposures by reviewing the appropriate sections of the MSDS on hazardous substances before they are used, transported, or stored. Know the location of the MSDS and know how to interpret the safety information on it.
- 12) Periodically review and monitor your procedures for safety and improve procedures wherever possible.
- 13) Substitute less hazardous substances when possible.
- 14) Equipment or areas which may contain or have the potential to become contaminated with any High Risk Substances must be kept as clean as possible. These areas should be labeled to identify the hazards. The NFPA *Safety Diamond* or the *WHMIS labeling System* may be used to identify the primary health hazards.
- 15) Use a fume hood for any work with volatile chemical substances and a Biosafety Cabinet for infectious substance containment and product sterility. Ensure that these units are included in your quality assurance program by having them tested yearly. Wherever possible, any equipment or area that may contain a hazardous residue must be appropriately labeled with warning signs identifying the substance(s) of concern.
- 16) Wherever appropriate use disposable absorbent liners on trays or surfaces to contain splashes or any spills of hazardous substances and replace when activity is complete.
- 17) Laboratory equipment used for hazardous substances must not be transferred to lower level labs, sold to the public, or passed to service employees without decontamination / inactivation or neutralization. An "**Equipment & Area Release Form**" must be filled out and attached to any laboratory equipment which is to be serviced, sold or relocated (see Appendix).
- 18) Written Emergency Response Plans (ERP) should be developed for high possibility emergency situations (eg spill response, fire evacuation, power outage, etc). Employees must have received training, know the location of response materials, and know how to interpret the information on a MSDS so as to respond safely and effectively.

6. Non-Compliance Enforcement Policy

This policy sets forth the actions the University of Saskatchewan is prepared to take in order to enforce compliance with terms and conditions of various licenses issued to the University and with the applicable federal and provincial statutes pertaining to the use, handling, storage, and disposal of hazardous substances¹.

Non-Compliance

- Step 1. On the first occurrence of non-compliance; the Department of Health, Safety and Environment (DHSE) will send a written notification to the Supervisor or Principal Investigator with copies to the Dean and Head of the Department outlining the nature of the infraction. Immediate response to and correction of the violation is required within the time frame specified on the notification.
- Step 2. On a second occurrence of non-compliance within a twelve-month period or when there is no response to the first infraction within the specified time, DHSE will suspend privileges to obtain and use hazardous substances. The Supervisor or Principal Investigator may have this privilege restored upon written verification from the Dean indicating rectification of the infraction. A copy will be forward to the appropriate Department Head and respective President's Advisory Committee².
- Step 3. On a third occurrence of non-compliance within a twelve-month period, the permit will be revoked and research activity suspended by DHSE. The Supervisor or Principal Investigator may appeal by requesting a meeting with the representatives of DHSE and the executive members of the appropriate President's Advisory Committee. Written notification of the above actions will be sent to the Dean and the Department Head.

Unacceptable Risk

DHSE shall take appropriate action when determined by the Safety Manager that there is an unacceptable risk to employees, the public, the environment, university property, security, and/or gross disregard to health and safety standards involving the handling of hazardous substances. Action may include the immediate suspension of research activity, prohibited entry to the laboratory, and/or removal of hazardous material from the premises.

¹ Hazardous substance means any substance regulated by the federal or provincial statutes, e.g. radioactive, chemical, and infectious substance, radiation producing equipment.

² Radiation Safety Advisory Committee, Biosafety Advisory Committee, Chemical Safety Advisory Committee and Environmental Safety Advisory Committee.

7. Substance Acquisition, Storage, Inventory, Transportation, Waste Disposal and Minimization

Also see Fire Safety Code, National Fire Code, Hazardous Waste Disposal Manual and the Transportation of Dangerous Goods Regulations.

7.1. Acquisition

- 1) All chemical and other hazardous materials purchases must be made through the University purchasing system. No P-Card purchases of hazardous materials are allowed.
- 2) Effective May 1, 2005 the purchasing system will require that chemicals be purchased through the use of one of a number of predetermined commodity codes. Some of these codes are associated with special groups of chemicals which we are required to gather statistics about. Statistics such as amount purchased or the mere presence of certain materials on campus must be reported to various branches of the government under international treaty obligations, such as the Chemical Weapons Convention and the Precursor Control Regulations. Purchasing information will only be reported as campus wide statistical data – it is not our intention to identify individual purchasers. The detailed lists of compounds will be kept current on the DHSE website. The website may be accessed at:
<http://www.usask.ca/dhse/chemicalsafety/index.html> . Users are expected to keep themselves abreast of updates by periodically revisiting these data to ensure they are complying with the most recent lists. DHSE will endeavor to keep the University community apprised of changes in information.

7.2. Storage

- 1) Ensure that all containers of hazardous substances are WHMIS compliance labeled, dated, and are on the required inventory list.
- 2) Place WHMIS (can also use Transportation of Dangerous Goods) warning symbols or signs on all hazardous bulk storage areas.
- 3) Label all peroxide forming substances (e.g. ethers) on the outside of the container with the date received, date opened, and the dates and results of every periodic peroxide test. See section on unstable substances for testing frequency.
- 4) Ensure that procedures are in place to prevent unnecessary storage (accumulation) of substances in the laboratory, especially if the substances become unstable with time (e.g. review inventory yearly).

Store hazardous substances in compatible groups in secure areas. DHSE is available to assist you in developing a storage plan. Follow these guidelines:

- Keep flammable and combustible material away from inorganic acids, oxidizers and reactive substances.
- Keep acids away from alkali materials.
- Keep inorganic acids away from flammable and alkali materials. Separate from inorganic and organic acids (e.g. nitric acid separate from glacial acetic acid).
- Keep oxidizers and organic peroxides away from flammable material, inorganic acids and organic material including wooden bench components.

- Keep health toxins and other especially dangerous items under added security.
- Keep pressurized gases securely strapped to wall, bench or proper cart at all times and their safety caps on while not in use. Toxic and flammable gases have special storage requirements, see DHSE for further information if you are storing or using flammable or toxic gases in any amount.

7.3. Inventory

The SOHS Regulations require that an employer develop and maintain a list of all chemical substances that may be hazardous to the health and safety of worker at the place of employment. This means that each lab must maintain a list available for inspection and use during and emergency of all hazardous materials (ie anything governed under WHMIS).

7.4. Transportation of Dangerous Goods

OFF CAMPUS

Individuals who package and/or complete shippers declarations for TDG on or off campus must have received training and hold a valid training certificate. Training and certificates are obtained through DHSE. Training must be updated every three years with records kept by the individuals' department. With only a few exceptions, all hazardous material transported by motorized carrier must comply with the TDG packaging, training and manifesting requirements. See DHSE for possible exclusions.

ON CAMPUS

All substances being transported out of a laboratory must be in a secondary container that shall be capable of containing all of the substance in the event it is dropped or the primary container leaks for any reason. The container must be WHMIS labeled and the secondary container must have the contact person's name and telephone number printed on the outside. For transportation outside of buildings, we also recommend an Emergency Response Plan be included with the package in case the carrier is unable to respond in the event of a spill.

7.5. Waste Disposal

See the Hazardous Waste Disposal Manual for details on collection, packaging and disposal of hazardous waste.

Chemical waste removed from laboratories is typically bulked prior to shipment for disposal. For the safety of the WMF staff, it is important that your waste information is accurate. If special handling procedures are required for specific items then this information should also be included. The WMF staff often decide the classification and compatibility solely relying on your information. The consequence of misinformation may lead to a major accident. Any extra processing and/or rectification created due to misinformation provided could be dangerous or very expensive.

7.6. Waste Minimization and Pre-planning

Waste generation and disposal should be an integral part of your experiment planning. The following items should be considered at the design stage of any experiment.

- 1) Minimize the amount of reagents used. Purchase just the enough of the reagent that you would use within a reasonable time frame. Pay close attention to the expiry dates of the reagents you are utilizing. Disposal of 'leftover' material and expired reagents are usually much more expensive than purchasing in smaller batches.
- 2) It is expensive to dispose of pesticides, cyano compounds and reactive and shock sensitive chemicals. Whenever feasible, use alternative reagents or procedures to avoid or minimize generating costly waste streams. If there are no alternative reagents or procedures available, then the following ideas should be given some consideration:
 - use smaller quantity of reagents at the beginning of the experiment until the procedure is operating,
 - try to reclaim reagents whenever possible for reuse in the experiment,
 - when it is possible and safe, convert very hazardous by-products and reagent waste to less hazardous material before sending them to the WMF for disposal; and
 - know all the by-products of the waste generated during the experiment and their toxicity.
- 3) Investigate disposal options with DHSE prior to generating any new waste. New types or combinations of waste types may require additional time or resources to find a suitable disposal method. DHSE will work collaboratively with waste producers to meet their needs.

8. Emergencies: Accidents, Incidents, Spills and Exposures

Comply with your written ERP and ensure that you have been properly trained to handle an emergency. Emergency contact information is contained inside the front cover of the LabSafety Manual. Emergency contact is through 9 911 and campus security at 5555.

Accidents

Seek medical aid for all accidents where bodily injury or exposure occurs. Fill out an Incident Report Form and return it to DHSE (see Appendix).

If an employee loses work time or seeks medical aid because of an accident, injury or exposure, contact Human Resources as soon as possible to complete a Workers' Compensation Board (WCB) form.

Incidents (Near Miss)

A near miss incident is an unplanned or unanticipated event where nobody was injured and nothing was spilled or damaged but only by good fortune. These must be investigated to prevent the set of circumstances leading to the incident from arising again. Take immediate steps to contain any further possible danger, inform you supervisor, complete and Incident Report Form and return it to DHSE.

Spills

- 1) Protect yourself first by leaving the immediate area of a spill or release especially if hazardous materials are volatile and/or toxic. Inform individuals in the affected area(s).
- 2) Put on PPE and re-enter the area to do the spill inactivation and clean up with the appropriate spill media only if it is safe.

- 3) Take immediate steps to contain the spill or release of the hazardous substances.
- 4) If the spill or release gets out of control, is too large or is life or health threatening to you or anyone in the area, consider evacuation and call the spill response team at the WMF for assistance (8497 or 5555).
- 5) Restrict entry to the area by posting the area with warning signs. Do not allow custodial employees to clean up hazardous substances.
- 6) Do not allow persons who may be contaminated to leave the area until cleared by DHSE. They must be decontaminated and may need to be monitored for possible effects of the exposure.
- 7) Complete an Incident Report Form and return it to DHSE.

Exposures

Exposures by individuals or groups of people to chemical, radioactive or biological material that has or could lead to sickness or injury must be reported to DHSE. Contact DHSE immediately and contain the spill and people as well as possible.

Complete an Incident Report Form and return it to DHSE.

9. Laboratory Spill Response Materials

Spill response assistance is required whenever a spill is;

- 1) Health or life threatening to you or anyone else,
- 2) There is a lack of proper cleanup equipment or spill absorbent, or
- 3) You have insufficient training or experience.

It is therefore obvious that equipment availability and spill training are important. Spill kits and suitable sorbent must be available and individuals should be trained. Spill response training is available in the LabSafety Course.

The minimum materials needed are:

- 1) Hazard information on the substance spilled; MSDS, container label and additional information.
- 2) Warning signs to ensure others don't inadvertently wander into a contaminated area.
- 3) Proper PPE to protect hands, feet, eyes, body, and (possibly) respiratory system.
- 4) Compatible adsorbent or absorbent material; bentonite, diapers, charcoal or vermiculite.
- 5) Spill clean-up tools; brooms, dust pan, shovel or scoop.
- 6) Leak-proof waste container with closure; heavy duty plastic bags or 20L pail with a snap lid.
- 7) Accessories; pH paper, duct tape, long tweezers and plastic scoop.
- 8) Reporting Forms to DHSE.

10. Your Right-to-Know, Right-to-Refuse and Unresolved Safety Concerns

Right to Know

It is your right under the SOHS Act and Regulations to know about the presence of hazards in the workplace, to know the details of those hazards and how to protect yourself against them. This information is usually supplied to you via a local orientation, access to WHMIS compliance information such as MSDS's and access to proper safety equipment in the form of engineered and procedural safety controls and PPE.

Right to Refuse

You have the right to refuse to do any particular act or series of acts at work that you have reasonable grounds to believe are unusually dangerous to your health or safety or to the health or safety of any other person at the place of employment. The regulation states that you cannot be discriminated against because you have exercised this right conferred to you under the law. You may stop the unsafe work immediately and bring your health and safety concerns to the attention of your Supervisor/Principal Investigator. He/she is obliged to investigate the matter and inform you of his/her decision. If another person is assigned the task, that person must be notified, in writing, that you had previously refused the work, the reason(s) why you refused and the reasons why he/she believes the work is not unusually dangerous.

Unresolved Safety Concerns

If unresolved, you should talk to your Department Head and/or the applicable Local Safety Committee. A Committee representative must be informed of all stop work or unresolved safety concerns. Call DHSE to find out who is the representative, check our website or see your OH&S bulletin board in your building for this information. DHSE Managers are available to consult with anyone who wishes to review their safety procedures in order to minimize their risk of exposure. For work refusals, the Statutory Occupational Health Committee must be notified. For workplace harassment, contact Discrimination and Harassment Prevention Services.

11. Quick Guide to Risk Assessment for Hazardous Chemicals

1. **Identify chemicals to be used and circumstances of use.** Identify the chemicals involved in the proposed experiment and determine the amounts that will be used. Is the experiment to be done once, or will the chemicals be handled repeatedly? Will the experiment be conducted in an open laboratory, in an enclosed apparatus, or in a fume hood? Is it possible that new or unknown substances will be generated? Are any of the workers involved in the experiment pregnant or likely to become pregnant? Do they have any known sensitivities to specific chemicals?
2. **Consult sources of information.** Consult an up-to-date MSDS for each chemical involved in the planned experiment. In cases where substances with significant or unusual potential hazards are involved, it may also be advisable to consult more detailed references. Depending on the worker's level of experience and the degree of potential hazard associated with the proposed experiment, it may also be necessary to obtain the assistance of experts in the field, supervisors and safety professionals before proceeding with risk assessment.
3. **Evaluate the types of toxicity and other dangers.** Are any of the chemicals to be used toxic, corrosive, irritants or sensitizers? Will any carcinogens or possible carcinogens be encountered? How about flammability and environmental toxicity? Are any chemicals involved in the proposed experiment suspected to be reproductive or developmental toxins or neurotoxins? Examine all possible types of toxicity and other danger.
4. **Consider possible routes of exposure.** Determine the potential routes of exposure for each chemical. Are the chemicals gases, or are they volatile enough to present a significant risk of exposure through inhalation? If liquid, can the substances be absorbed through the skin? Is it possible that dusts or aerosols will be formed in the experiment? Does the experiment involve a significant risk of inadvertent ingestion or injection of chemicals? What about environmental impacts.
5. **Evaluate quantitative information on toxicity.** Consult the information sources to determine the LD50 for each chemical via the relevant routes of exposure. Determine the acute toxicity hazard level for each substance, classifying each chemical as highly toxic, moderately toxic, slightly toxic and so forth. For substances that pose inhalation hazards, take note of the threshold limit value time-weighted average (TLV-TWA), short-term exposure limit (STEL) and odour threshold.
6. **Select appropriate procedures to minimize exposure.** Use Good Laboratory Practices for all work with chemical in the laboratory. In addition, determine whether any of the chemicals to be handled in the planned experiment are extremely hazardous. I.e. Deserve a '3' or '4' rating under any of the NFPA categories. If so, pay special attention to the control of these compounds. Consider the total amount of the substance that will be used, the expected frequency of use, the chemical's routes of entry, and the circumstances of its use in the proposed experiment.
7. **Prepare for contingencies.** Note the signs and symptoms of exposure to the chemicals to be used in the proposed experiment. Note appropriate measures to be taken in the event of exposure, accidental release or other unplanned situation involving any of the chemicals.

Adapted from:

Committee on Prudent Practices for Handling, Storage and Disposal of Chemicals in Laboratories. Prudent Practices in the Laboratory: Handling and Disposal of Chemicals. Washington: NATIONAL ACADEMY PRESS, 1995. Page 47

Appendices

A. Notifiable & Designated Substances

Preamble

The Supervisor or Principal Investigator shall ensure that adequate engineering and procedural controls and suitable PPE are provided where workers are required or allowed to handle, use or produce:

1. any substance listed in **Table 19** or **Table 20** of the Regulations, or
2. any substances listed in **Table 21** of the Regulations. These substances must have the safety controls in place to ensure that any exposures are kept below the *contamination limits* as set in the regulations.

A.1 Notifiable Chemical Substances

Identified in Section 305 and 311 and in **Table 19** in the Saskatchewan Occupational Health and Safety Regulations.

Supervisors of projects shall provide written notice to the Department of Health, Safety and Environment of their activity or intention to procure, handle, use, store, produce, distribute or dispose of any of the following chemical substances or any mixture containing more than 1% of any of them. DHSE will then request permission from the Saskatchewan Director of Occupational Health and Safety. The director may issue permits subject to any conditions which, in his/her opinion, are warranted in the particular situation. Please note that there are many other chemical substances that have hazards associated with them and therefore this list is only the minimum of the reportable substances.

To Register

Contact the Department of Health, Safety and Environment if you have any Notifiable Chemical Substances in your area. These compounds are listed below or can be found in **Table 19** of the regulations. No exposure or contact by any route to any of these substances is regarded as acceptable; therefore no contamination limits are stipulated.

4-Aminobiphenyl	Auramine	Benzidine
bis(Chloromethyl) ether	o-Dianisidine	3,3'-Dichlorobenzidine
Methyl chloromethyl ether	Mustard gas	2-Naphthylamine
4-Nitrobiphenyl	Vinyl chloride	

A.2 Biological Substances (Permit or Registration Required)

A Biosafety Permit or Registration may be required for:

- a) any research or teaching involving Genetically Modified Microorganisms or
- b) any research or teaching involves biological material in Risk Groups 1 (registration) or 2, 3 and 4 (permit).

Contact the Biosafety Office for further information.

A.3 Designated Chemical Substances

Substances listed in **Table 20** of the Saskatchewan Occupation Health and Safety Regulations as well as those containing 1% or less of any substance listed in **Table 19** are classes as Designated Substances. The Supervisor/Principal Investigator shall ensure that adequate engineering and procedural controls and suitable PPE are provided to prevent any significant risk to workers caused by exposure to any of

these substances where workers are required or allowed to produce, handle or use them.

A.4 Contamination Limits

Substances listed in **Table 21** of the Saskatchewan Occupation Health and Safety Regulations have specified occupational exposure limits, commonly referred to as Threshold Limit Values (TLVs). TLVs are maximum concentrations in air in which employees may work for specified periods of time. If your workplace uses or produces any of these substances, ensure that employees are protected.

A.5 Radioactive Substances

All radioisotope users must have an Operating Permit from DHSE.

A.6 Requirements for Use

The use of any Notifiable or Designated chemical or biological substance requires an elevated level of safety awareness and preparedness. The following items are the minimum requirements of areas involved with these substances:

- Written ERP's are in place for a spill or release of any hazardous substances.
- Activities are performed in the appropriately designed containment work areas. Containment level of work area is commensurate with the RGL of the agent. Likewise, fume hoods must be used where appropriate for chemical substances.
- Safety equipment (engineered safety controls) are used properly and maintained. (e.g. fume hoods and Biosafety Cabinets tested yearly and checked daily).
- Procedural controls being used will comply with the safety precautions necessary for the level of containment required for this activity.
- The applicable University Safety Committee has endorsed the work activities where applicable.
- The Supervisor or Principal Investigator is aware that any significant changes in the activities must be reported to the applicable University Safety Committee and to the research granting agency when registration or permitting is in place.
- Authorized workers are aware of any and all hazards (physical, chemical or other) that may be encountered in the course of the work activities.
- Personnel are adequately trained and any safety procedures are enforced, in compliance with the SOHS Act and Regulations.
- The Supervisor or Principal Investigator adheres to both the Federal and Provincial statutes pertaining to the protection of the environment. In addition, projects that may generate significant amounts of waste or waste of an unusual or novel nature will have their waste disposal process reviewed and approved by DHSE prior to generating any waste.

Penalty (as stated in the granting guide): Deliberate failure to comply with the guidelines of a granting agency could lead to termination of support to the investigator, the department or the University. Ignorance will not be an excuse for non-compliance.

B. Standard Operating Procedures

Laboratory supervisors should review their written Standard Operating Procedures (SOPs) with their workers once a year.

Goals:

- ensure consistency of work procedures
- provide training material on experimental protocols and explain the inherent risks associated with the work
- establish engineered and procedural safety controls to reduce the risk or exposure. These safety controls and identification of risks are one of the main parts of the SOP.
- comply with legislative aspects of the Occupational Health & Safety Act and Regulations. It is recommended that an ECP, SOP or equivalent documentation be developed in each laboratory where employees work with or near hazardous biological, chemical or physical substances.

The following topics should be considered when writing an SOP or a Departmental Safety Policies and Procedures manual.

- Identify the hazards, risks and symptoms of exposure associated with the work to the employees.
- Provide instructions on the safe use, handling, storage, transport and disposal of all materials.
- Ensure that ERP's are available to the employees.
- Provide procedures for the corrective action for unsafe acts and unsafe conditions.
- Provide procedure and frequency for routine housekeeping and inspections of all work areas.
- Ensure WHMIS hazard identification labeling and MSDSs are available as required.
- Use "Equipment and Area Release Forms" where applicable.
- Identify and ensure that equipment has the proper engineered safety controls (guards and shields are in place, biosafety cabinets and fume hoods are used and maintained).
- Develop policies for working alone and for unattended operations.
- Ensure that no food and drink is consumed or stored in an area where there are hazardous substances.
- Ensure that the mechanisms to maintain personal hygiene are in place.
- Provide PPE and ensure that employees know how to select, maintain and use it properly.
- Develop policies and procedures to restrict, limit and control access to high risk areas or substances.
- Maintain records on all employee orientation and training and all equipment maintenance.
- Ensure that Incident investigating and reporting takes place.
- Remember to control exposures first by engineering control, then by procedural and use PPE only as a last defense.

C. Handling Procedures for Unstable Substances

All hazardous compounds must be treated with respect. Certain compounds have the ability to become even more dangerous through neglect (dehydration or contamination) or chemical reaction (oxidation). Ethers, picric acid and some other common examples are listed here. There are others – know your chemicals!

Ethers

Ether functional groups can oxidize to unstable peroxide functional groups when exposed to a source of oxygen and given time. Containers of ether which have been opened should be checked for peroxide formation periodically, according to the table below. Unopened containers are not as susceptible, but should still be treated carefully if old. Test strips are the easiest method of measuring peroxide concentration. Any ethers which have developed more than 5 ppm peroxides should be disposed of through the Waste Management Facility. Do not evaporate or distil this ether since this will concentrate the peroxides. Chemical removal methods are available but should be only be attempted by experienced, knowledgeable people.

Do not try to open the cap of old ether containers which have not been opened for a long time in order to test for peroxides. If crystals are visible at the neck of the bottle or if crystals or sludge are noted inside the container, be especially careful. DO NOT OPEN the container and contact DHSE for advice. Friction on the dried peroxides in the cap threads could cause an explosion.

The following table provides some information about the more common compounds that are susceptible to peroxide formation. This list is not exhaustive, but is more representative of the structural groups that are prone to unstable compound formation. You are responsible to ensure your own safety – check first!

Class 1(C)

Acrylic acid
Acrylonitrile
Butadiene
Chlorobutadiene
Chlorotrifluoroethylene
Methyl methacrylate
Styrene
Tetrafluoroethylene
Vinyl acetate
Vinyl acetylene
Vinyl chloride
Vinyl pyridine
Vinylidene chloride

Class 2(B)

Acetal
Cumene
Cyclopentene
Cyclooctene
Diethyl ether
Furan
Methyl
acetylene
Tetrahydro
naphthalene
Vinyl Ethers
Cyclohexene
Dicyclopentadiene
Methyl cyclopentane
Diacetylene
diozane (p-dioxane)
Tetrahydrofuran
Ethylene glycol
dimethyl ether
Diethylene glycol
dimethyl ether

Class 3 (A)

Divinyl ether
Divinyl acetylene
Isopropyl ether
Vinylidene chloride
Potassium Metal
Potassium amide
Sodium amide

Class I (C) – Testing at 3 month intervals, with those showing peroxides discarded.

Class II (B) – Test stocks at 6 month intervals; discarded or purify those showing peroxides.

Class III (A) – Contain peroxidizable monomers and the presence of peroxides may initiate exothermic polymerisation. May explode without concentration. Test every 3 months.

The time frames and amounts of peroxide formed are greatly influenced by the amount of air space in the container (therefore the oxygen present), storage temperature and whether inhibitors are present or not.

Picric Acid, Perchloric Acid , 2, 4 DNP and others

Picric acid is stable when properly hydrated, but if allowed to dry out can become unstable and has the potential to explode like TNT when shocked. 2,4 Dinitrophenylhydrazine, perchloric acid and others have similar characteristics. Treat them similarly. Contamination with metals lowers stability further. Experienced persons have re-hydrated these materials by submerging the container in cold water for a number of days until the dry crystals are rewetted. DHSE does **not** recommend this procedure, but rather that you contact us to remove and disposal of this material.

D. WHMIS

The Federal Hazardous Products Act and Controlled Products Regulations have created the Workplace Hazardous Materials Information System (WHMIS). This Federal legislation is also enforced in Saskatchewan through Part XXII of the OHS Regulation. The WHMIS system is composed of three elements: training, labeling and Material Safety Data Sheets (MSDS's).

Training

Employers are required to train workers who work with or in proximity to hazardous substances. This training must include:

- the content required on a supplier label, a workplace label and an MSDS,
- all necessary procedures for the safe use, storage, handling and disposal of the material, and
- information on how to handle fugitive emissions and how to respond to an emergency involving a controlled product.

Employees are required to attend training when offered and to apply knowledge gained during the training. DHSE offers generic WHMIS training for all University persons required to take it. This training addresses general information regarding the above aspects, but must always be followed up by site specific training at the work area about the specific materials used in that area.

Labeling

Containers holding hazardous materials (controlled products) must be labeled according to the requirements of the legislation. There are two primary labeling situations and one additional one that laboratory employees need to be aware of.

Supplier Labels

By law, the supplier of a hazardous chemical or biological substance must label their product in a specific manner. Any container obtained from a supplier that does not meet the WHMIS labeling requirements should be reported to DHSE. Supplier labels have the following nine components:

1. Hatched border
2. Material identifier
3. Risk phrases
4. Precautionary phrase(s)
5. First Aid measures
6. Supplier identification
7. Symbols
8. Reference to the MSDS
9. Bilingual

Laboratory Supplier Labels

Packages produced and supplied specifically to laboratories have relaxed requirements for labeling. You will likely see many of these types of labels in your work area. They have only 6 requirements:

1. Product identifier
2. Risk phrases
3. Precautionary phrase(s)
4. First Aid measures
5. Reference to MSDS
6. Bilingual

Workplace Labels

For occasions where a material has been transferred to another container, the original label has been defaced or the material has been produced in the work area (and therefore there is no supplier label) a workplace label shall be used. Workplace labels are generated by knowledgeable people using the substance based on information from the supplier label and/or the MSDS. These labels have three requirements:

1. Product identifier
2. Precautionary phrase(s)
3. Reference to MSDS

Assistance in generating workplace labels can be obtained from DHSE.

WHMIS Symbols

As is clear from the above lists, symbols form an important element of some labels. These symbols assist in the identification of hazardous substances. A table of classes, divisions and symbols appears at the end of this section.

The Class of a WHMIS substance is dictated by the nature of its hazard. There are six WHMIS Classes: A through F. Two of these classes have differentiation within them referred to as Divisions:

Class B has six divisions further detailing the nature of the flammability danger. All six divisions within class B have the same symbol.

Class D has three divisions within it:

Division 1 is the familiar skull and cross bones indicating a material with immediate and serious effects.

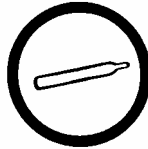



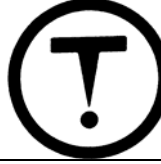



Division 2 is the chronic toxic category indicating a material which may require multiple exposures or may have serious effects that may not appear for a significant time.

Division 3 is biohazardous or infectious material which has different protection and decontamination requirements and can have very serious health consequences.

Each gets its own symbol.

MSDS

MSDS's for all the hazardous products used or stored in a work area must be available to all workers. Suppliers must provide MSDSs with the first shipment of a compound and periodically after that with new orders. Contact the supplier of the product to obtain updates since these documents must be renewed every three years. Visit our web page at <http://www.usask.ca/dhse> for access to MSDS information.

WHMIS Class	Division	Symbol
A Compressed Gas	(Often requires another symbol if not inert) - Toxic - Oxidizer - Corrosive - Inert - Flammable - Reactive	
B Flammable and Combustible	Division 1: Flammable Gases Division 2: Flammable Liquids (Fp < 37C) Division 3 Combustible Liquids (Fp > 37C) Division 4: Flammable Solids Division 5: Flammable Aerosols Division 6: Flammable Reactive Materials	
C Oxidizing Material		
D Poisonous or Infectious Material	DIVISION 1: Material Causing Immediate and Serious Toxic Effects	
	DIVISION 2: Material Causing Other Toxic Effects	
	DIVISION 3: Biohazardous Infectious Material	
E Corrosive Material		
F Reactive Material		

E. MSDS Information for 9 Section (Canadian) Form

Category/Section	Information	Item
1. Product Information	<ul style="list-style-type: none"> Product identifier Manufacturer's name, street address, city, province/state, postal/zip code, emergency phone number 	<ul style="list-style-type: none"> Product use Product identification number (PIN) Supplier identifier, street address, city, province/state, postal/zip code, emergency phone number
2. Hazardous Ingredients	<ul style="list-style-type: none"> Information required by the Hazardous Products Act 	<ul style="list-style-type: none"> CAS number LD₅₀ (species and route) LC₅₀ (species and route)
3. Physical Data	<ul style="list-style-type: none"> Physical state (gas, liquid or solid) Odour and appearance Vapour pressure Evaporation rate Specific gravity 	<ul style="list-style-type: none"> Odour threshold Vapour density Boiling point Freezing point PH Coefficient of water/oil distribution
4. Fire or Explosion Hazard	<ul style="list-style-type: none"> Conditions of flammability Flashpoint and method of determination Auto-ignition temperature Hazardous combustion products 	<ul style="list-style-type: none"> Means of extinction Upper flammable limit (% by volume) Lower flammable limit (% by volume) Explosion data - sensitivity to impact Explosion data sensitivity to static
5. Reactivity Data	<ul style="list-style-type: none"> Conditions under which the product is chemically unstable Name of any substance or class of substance with which the product is incompatible 	<ul style="list-style-type: none"> Conditions of reactivity Hazardous decomposition products
6. Toxicological Properties	<ul style="list-style-type: none"> Route of entry, including: skin contact, skin absorption, eye contact, inhalation and ingestion Effects of acute exposure to product Effects of chronic exposure to product 	<ul style="list-style-type: none"> Irritancy of product Sensitization to product Carcinogenicity Teratogenicity Reproductive toxicity Mutagenicity Name of toxicologically synergistic products
7. Preventive Measures	<ul style="list-style-type: none"> Personal protective equipment to be used Procedures to be followed in case of leak or spill Waste disposal 	<ul style="list-style-type: none"> Specific engineering controls to be used Handling procedures and equipment Storage requirements Special shipping information
8. First Aid Measures	<ul style="list-style-type: none"> Specific first aid measures 	
9. Preparation Information	<ul style="list-style-type: none"> Name and phone number of group department or party responsible for MSDS preparation. 	<ul style="list-style-type: none"> Date of preparation

F. MSDS Sample for 16 Section (ILO version) Form

MATERIAL SAFETY DATA SHEET

SECTION 1 CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

- a. Product identifier: Chemical class, Synonym
- b. Name of supplier, address and emergency telephone number:
- c. Name of manufacturer, address and emergency telephone number:
- d. Product use:
- e. Date of MSDS preparation:
- f. Name and telephone number of party responsible for MSDS preparation:

SECTION 2 COMPOSITION/INFORMATION ON INGREDIENTS

- a. Chemical identity and CAS registry no. of hazardous ingredients (WHMIS Controlled Products) present at 1.0%, or 0.1% as appropriate, by weight.
- b. Ingredients present which are on the WHMIS Ingredient Disclosure List at, or above, the minimum concentration specified on the List.
- c. Ingredients with unknown toxicological properties:
- d. Ingredients the supplier believes may be harmful:
- e. Generic chemical identity, registry number, and date of claim for trade secret ingredients:
- f. Ingredient concentration in units of wt./wt., vol./vol. or wt./vol. expressed as: i) actual concentration, or ii) a range as specified in the Controlled Product Regulations.
- g. LD50s and/or LC50s for ingredients.
- h. Exposure limits for ingredients:

SECTION 3 HAZARDS IDENTIFICATION

Potential Health Effects:

- a. Relevant routes of exposure:
- b. Adverse health effects from exposure to product or ingredients of product:
 - i) Length of exposure
 - ii) Severity of effect
 - iii) Target organ
 - iv) Type of effect
 - v) Signs and symptoms

SECTION 4 FIRST AID MEASURES

If accidental overexposure is suspected

Eyes:

Skin:

Inhalation:

Ingestion:

Note to physician:

Medical conditions likely to be aggravated by exposure:

SECTION 5 FIRE FIGHTING MEASURES

- 1. Flash point and method:
- 2. Upper and lower flammable (explosive) limits in air:
- 3. Autoignition temperature:
- 4. Hazardous combustion products:
- 5. Conditions under which flammability could occur:
- 6. Extinguishing media:
- 7. Sensitivity to explosion by mechanical impact or static discharge:
- 8. Fire fighting procedures:

SECTION 6 ACCIDENTAL RELEASE MEASURES

- a. Procedures for dealing with a release or spill:

SECTION 7 HANDLING AND STORAGE

- a. Handling practices and equipment:
- b. Appropriate storage practices/requirements:

SECTION 8 EXPOSURE CONTROLS/PERSONAL PROTECTION

- a. Applicable control measures, including engineering controls:
- b. Personal protective equipment for each exposure route:

SECTION 9 PHYSICAL AND CHEMICAL PROPERTIES

- a. Appearance:
- b. Odour:
- c. Physical state:
- d. pH:
- e. Vapour pressure and reference temperature:
- f. Vapour density:
- g. Boiling point:
- h. Freezing/melting point:
- i. Specific gravity or density:
- j. Evaporation rate:
- k. Partition coefficient:
- l. Odour threshold:
- m. Viscosity:
- n. Solubility in water:

SECTION 10 STABILITY AND REACTIVITY

- a. Chemical stability:
- b. Conditions to avoid:
- c. Incompatibility with other materials:
- d. Hazardous decomposition products:
- e. Hazardous polymerization:

SECTION 11 TOXICOLOGICAL INFORMATION

- a. Effects of short-term exposure:
- b. Effects of long-term exposure:
- c. Irritancy:
- d. Sensitization:
- e. Carcinogenicity:
- f. Reproductive toxicity:
- g. Teratogenicity:
- h. Mutagenicity:
- i. Name of toxicologically synergistic products:

SECTION 12 ECOLOGICAL INFORMATION

SECTION 13 DISPOSAL CONSIDERATIONS

- a. Waste disposal information:

SECTION 14 TRANSPORT INFORMATION

- a. Shipping information such as shipping classification:
PIN (Transport Canada - Product Identification Number) for the entire product.

SECTION 15 REGULATORY INFORMATION

- a. WHMIS classification for product:
- b. A statement that the MSDS has been prepared to meet the requirements of the Canadian Controlled Products Regulation:

SECTION 16 OTHER INFORMATION

G. MSDS Definitions

Reproduced from CCOHS Website, January 3, 2003 (<http://ccinfoweb.ccohs.ca/help/msds/msdstermse.html>)

ACGIH

ACGIH stands for American Conference of Governmental Industrial Hygienists. The ACGIH is an association of occupational health professionals employed by government and educational institutions. The Threshold Limit Value (TLV) Committee and Ventilation Committee of the ACGIH publish guidelines which are used worldwide.

ACID, ACIDIC

See pH.

ACTIVE INGREDIENT

An active ingredient is the part of a product which actually does what the product is designed to do. It is not necessarily the largest or most hazardous part of the product. For example, an insecticidal spray may contain less than 1% pyrethrin, the ingredient which actually kills insects. The remaining ingredients are often called inert ingredients.

ACUTE

Acute means sudden or brief. Acute can be used to describe either an exposure or a health effect. An acute exposure is a short-term exposure. Short-term means lasting for minutes, hours or days. An acute health effect is an effect that develops either immediately or a short time after an exposure. Acute health effects may appear minutes, hours or even days after an exposure. (See also Chronic.)

AEROSOL

An aerosol is a collection of very small particles suspended in air. The particles can be liquid (mist) or solid (dust or fume). The term aerosol is also commonly used for a pressurized container (aerosol can) which is designed to release a fine spray of a material such as paint.

Inhalation of aerosols is a common route of exposure to many chemicals. Also, aerosols may be fire hazards.

AIHA

AIHA stands for American Industrial Hygiene Association.

ALKALI, ALKALINE

See pH.

ANSI

ANSI stands for the American National Standards Institute.

AUTO-IGNITION TEMPERATURE

The auto-ignition temperature is the lowest temperature at which a material begins to burn in air in the absence of a spark or flame. Many chemicals will decompose (break down) when heated. The autoignition temperature is the temperature at which the chemicals formed by decomposition begin to burn. Auto-ignition temperatures for a specific material can vary by one hundred degrees Celsius or more, depending on the test method used. Therefore, values listed on the MSDS may be rough estimates. To avoid the risk of fire or explosion, materials must be stored and handled at temperatures well below the auto-ignition temperature.

BASE, BASIC

See pH.

BIOHAZARDOUS INFECTIOUS MATERIAL

Under the Canadian Controlled Products Regulations, a biohazardous infectious material is a material that contains organisms which can cause disease in humans or animals. For example, a person exposed to a blood sample from someone with hepatitis B may contract the disease. Some jurisdictions require MSDSs for products which contain biohazardous infectious materials.

BOD

BOD stands for biological oxygen demand.

BOILING POINT

The boiling point is the temperature at which the material changes from a liquid to a gas. Below the boiling point, the liquid can evaporate to form a vapour. As the material approaches the boiling point, the change from liquid to vapour is rapid and vapour concentrations in the air can be extremely high. Airborne gases and vapours may pose fire, explosion and health hazards. Sometimes, the boiling point of a mixture is given as a range of temperatures. This is because the different ingredients in a mixture can boil at different temperatures.

If the material decomposes (breaks down) without boiling, the temperature at which it decomposes may be given with the abbreviation "dec." Some of the decomposition chemicals may be hazardous. (See also Thermal Decomposition Products.)

CANUTEC

CANUTEC stands for Canadian Transport Emergency Centre, which is part of the Transport Dangerous Goods Directorate of Transport Canada. CANUTEC provides information and communications assistance in case of transportation emergencies involving dangerous goods. It is accessible in Canada by telephone, 24 hours a day, year round at (613) 996-6666 (collect).

CARCINOGEN, CARCINOGENIC, CARCINOGENICITY

A carcinogen is a substance which can cause cancer. Carcinogenic means able to cause cancer. Carcinogenicity is the ability of a substance to cause cancer.

Under the Canadian Controlled Products Regulations, materials are identified as carcinogens if they are recognized as carcinogens by the American Conference of Governmental Industrial Hygienists (ACGIH), or the International Agency for Research on Cancer (IARC).

Under the US OSHA Hazard Communication (Hazcom) Standard, materials are identified as carcinogens on MSDSs if they are listed as either carcinogens or potential carcinogens by IARC or the US National Toxicology Program (NTP), if they are regulated as carcinogens by OSHA, or if there is valid scientific evidence in man or animals demonstrating a cancer causing potential.

The lists of carcinogens published by the IARC, ACGIH and NTP include known human carcinogens and some materials which cause cancer in animal experiments. Certain chemicals may be listed as suspect or possible carcinogens if the evidence is limited or so variable that a definite conclusion cannot be made.

CAS REGISTRY NUMBER

The CAS Registry Number is a number assigned to a material by the Chemical Abstracts Service (CAS) of the American Chemical Society (ACS). The CAS number provides a single unique identifier. A unique identifier is necessary because the same material can have many different names. For example, the name given to a specific chemical may vary from one language or country to another. The CAS Registry Number is similar to a telephone number and has no significance in terms of the chemical nature or hazards of the material. The CAS Registry Number can be used to locate additional information on the material.

CC

Depending on the context, CC can stand for closed cup, cubic centimeters or ceiling concentration.

CCC

CCC stands for Cleveland closed cup, a standard method of determining flash points.

CCOHS

CCOHS stands for the Canadian Centre for Occupational Health and Safety. CCOHS provides an occupational health and safety information service through answers to inquiries, publications and a computerized information service. The computerized information is available both online (CCINFOWeb) and on CD-ROM (CCINFODisc).

CEILING (C)

See Exposure Limits for a general explanation.

CHEMICAL FAMILY

The chemical family describes the general nature of the chemical. Chemicals belonging to the same family often share certain physical and chemical properties and toxic effects. However, there may also be important differences. For example, toluene and benzene both belong to the aromatic hydrocarbon family. However, benzene is a carcinogen, but toluene is not.

CHEMICAL FORMULA

The chemical formula, sometimes called the molecular formula, tells which elements (carbon, hydrogen, oxygen, and so on) make up a chemical. It also gives the number of atoms of each element in one unit or molecule of the chemical. The chemical formula can be used to confirm the identity of ingredients or to indicate the presence of a potentially hazardous element.

For example, zinc yellow has the chemical formula ZnCrO_4 , which shows that it contains not only zinc (Zn) but also chromium (Cr).

CHEMICAL NAME

The chemical name is a proper scientific name for an ingredient of a product. For example, the chemical name of the herbicide 2,4-D is 2,4-dichlorophenoxyacetic acid. The chemical name can be used to obtain additional information.

CHEMICAL REACTIVITY

Chemical reactivity is the ability of a material to undergo a chemical change. A chemical reaction may occur under conditions such as heating, burning, contact with other chemicals, or exposure to light. Undesirable effects such as pressure buildup, temperature increase or formation of other hazardous chemicals may result. (See also Dangerously Reactive Material and Reactive Flammable Material.)

CHEMTREC

CHEMTREC stands for the Chemical Transportation Emergency Centre. It is a U. S. national center established by the Chemical Manufacturers Association (CMA) to relay pertinent emergency information concerning specific chemicals on requests from individuals. CHEMTREC has a 24-hour toll-free telephone number to help respond to chemical transportation emergencies for companies who have registered with them for this service.

CHRONIC

Chronic means long-term or prolonged. It can describe either an exposure or a health effect. A chronic exposure is a long-term exposure. Long-term means lasting for months or years. A chronic health effect is an adverse health effect resulting from long-term exposure or a persistent adverse health effect resulting from a short-term exposure. The Canadian Controlled Products Regulations describe technical criteria for identifying materials which cause chronic health effects. (See also Acute.)

CNS

CNS stands for central nervous system.

COC

COC stands for Cleveland open cup, a standard method of determining flash points.

COD

COD stands for chemical oxygen demand.

COEFFICIENT OF OIL/WATER DISTRIBUTION

The coefficient of oil/water distribution, also called the partition coefficient (abbreviated as P), is the ratio of the solubility of a chemical in an oil to its solubility in water. The P value is typically presented as a logarithm of P (log P). It indicates how easily a chemical can be absorbed into or stored in the body. The P value is also used to help determine the effects of the chemical on the environment.

COMBUSTIBLE

Combustible means able to burn. Broadly speaking, a material is combustible if it can catch fire and burn. However, in many jurisdictions, the term combustible is given a specific regulatory meaning. (See Combustible Liquid.)

The terms combustible and flammable both describe the ability of a material to burn. Commonly, combustible materials are less easily ignited than flammable materials.

COMBUSTIBLE LIQUID

Under the Canadian Controlled Products Regulations, a combustible liquid has a flash point from 37.8 to 93.3 degrees C (100 to 200 degrees F) using a closed cup test. The US OSHA Hazcom Standard uses a similar definition.

This range of flash points is well above normal room temperature. Combustible liquids are, therefore, less of a fire hazard than flammable liquids. If there is a possibility that a combustible liquid will be heated to a temperature near its flash point, appropriate precautions must be taken to prevent a fire or explosion.

COMPRESSED GAS

A compressed gas is a material which is a gas at normal room temperature and pressure but is packaged as a pressurized gas, pressurized liquid or refrigerated liquid. The Canadian Controlled Products Regulations and the U.S. Hazcom standard describe technical criteria for identifying materials which are classified as compressed gases.

Regardless of whether a compressed gas is packaged in an aerosol can, a pressurized cylinder or a refrigerated container, it must be stored and handled very carefully. Puncturing or damaging the container or allowing the container to become hot may result in an explosion.

CONTROLLED PRODUCTS

Under the Canadian Controlled Products Regulations, a controlled product is defined as a material, product or substance which is imported or sold in Canada and meets the criteria for one or more of the following classes:

Class A - Compressed Gas

Class B - Flammable and Combustible Material:

Class C - Oxidizing Material

Class D - Poisonous and Infectious Material:

Division 1 - Material Causing Immediate and Serious Toxic Effects:

Subdivision A - Very Toxic Material

Subdivision B - Toxic Material

Division 2 - Material Causing Other Toxic Effects:

Subdivision A - Very Toxic Material

Subdivision B - Toxic Material

Division 3 - Biohazardous Infectious Material

Class E - Corrosive Material

Class F - Dangerously Reactive Material

CONTROLLED PRODUCTS REGULATIONS (CPR)

The Controlled Products Regulations are Canadian federal regulations developed under the Hazardous Products Act. They are part of the national Workplace Hazardous Materials Information System (WHMIS).

The regulations apply to all suppliers (importers or sellers) in Canada of controlled products intended for use in Canadian workplaces. The regulations specify the criteria for identification of controlled products. They also specify what information must be included on labels and MSDSs.

CORROSIVE MATERIAL

A corrosive material can attack (corrode) metals or human tissues such as the skin or eyes. Corrosive materials may cause metal containers or structural materials to become weak and eventually to leak or collapse. Corrosive materials can burn or destroy human tissues on contact and can cause effects such as permanent scarring or blindness.

The Canadian Controlled Products Regulations and the US OSHA Hazcom Standard, specify technical criteria for identifying materials which are classified as corrosive materials for the purposes of each regulation. (See also pH.)

CU M or CU.M

This stands for cubic meter

DANGEROUSLY REACTIVE MATERIAL

The Canadian Controlled Products Regulations describes technical criteria for identifying materials which are classified as dangerously reactive. A dangerously reactive material can react vigorously:

- with water to produce a very toxic gas;
- on its own by polymerization or decomposition; or
- under conditions of shock, or an increase in pressure or temperature.

ANSI defines a dangerously reactive material as one that is able to undergo a violent self-accelerating exothermic chemical reaction with common materials, or by itself.

A dangerously reactive material may cause a fire, explosion or other hazardous condition. It is very important to know which conditions (such as shock, heating or contact with water) may set off the dangerous reaction so that appropriate preventive measures can be taken.

DENSITY

The density of a material is its weight for a given volume. Density is usually given in units of grams per millilitre (g/mL) or grams per cubic centimetre (g/cc). Density is closely related to specific gravity (relative density). The volume of a material in a container can be calculated from its density and weight.

DOT

DOT stands for the U.S. Department of Transportation.

EMBRYO

An embryo is an organism in the early stages of its development prior to birth. In humans, the embryo is the developing child from conception to the end of the second month of pregnancy. (See also Fetus/Foetus.)

EMBRYOTOXIC, EMBRYOTOXICITY

Embryotoxic means harmful to the embryo. Embryotoxicity is the ability of a substance to cause harm to the embryo.

The Canadian Controlled Products Regulations describe technical criteria for identifying materials which have teratogenicity and embryotoxicity. (See also Fetotoxicity and Reproductive Effects.) Under the U.S. OSHA HAZCOM standard, embryotoxic effects are included as Target Organ Effects.

ENGINEERING CONTROLS

Engineering controls help reduce exposure to potential hazards either by isolating the hazard or by removing it from the work environment. Engineering controls include mechanical ventilation and process enclosure. They are important because they are built into the work process.

Engineering controls are usually preferred to other control measures such as the use of personal protective equipment.

EPA

EPA stands for the U.S. Environmental Protection Agency.

EU

EU stands for the European Union, formerly known as the EEC (European Economic Community) and the EC (European Community).

EVAPORATION RATE

The evaporation rate is a measure of how quickly the material becomes a vapour at normal room temperature. Usually, the evaporation rate is given in comparison to certain chemicals, such as butyl acetate, which evaporate fairly quickly. For example, the rate might be given as "0.5 (butyl acetate=1)." This means that, under specific conditions, 0.5 grams of the material evaporates during the same time that 1 gram of butyl acetate evaporates. Often, the evaporation rate is given only as greater or less than 1, which means the material evaporates faster or slower than the comparison chemical. In general, a hazardous material with a higher evaporation rate presents a greater hazard than a similar compound with a lower evaporation rate.

EXPLOSION DATA

Explosion data is information on the explosive properties of a material. Quantitative explosion data is seldom available and is usually given in descriptive terms such as low, moderate or high.

The following types of information can be used to describe the explosive hazard of a material:

- Sensitivity to mechanical impact. This information indicates whether or not the material will burn or explode on shock (for example, dropping a package) or friction (for example, scooping up spilled material).

- Sensitivity to static discharge. This information indicates how readily the material can be ignited by an electric spark.

Detailed information is available on the properties of commercial explosives. In Canada, the storage, transportation and handling of commercial explosives are strictly regulated under the Explosives Act and TDG. Commercial explosives are not regulated by the Controlled Products Regulations.

EXPLOSIVE LIMITS

Explosive limits specify the concentration range of a material in air which will burn or explode in the presence of an ignition source (spark or flame). Explosive limits may also be called flammable limits or explosion limits.

The lower explosive limit (LEL), or lower flammable limit (LFL), is the lowest concentration of gas or vapour which will burn or explode if ignited. The upper explosive limit (UEL), or upper flammable limit (UFL), is the highest concentration of gas or vapour which will burn or explode if ignited. From the LEL to the UEL, the mixture is explosive. Below the LEL, the mixture is too lean to burn. Above the UEL, the mixture is too rich to burn. However, concentrations above the UEL are still very dangerous because, if the concentration is lowered (for example, by introducing fresh air), it will enter the explosive range.

In reality, explosive limits for a material vary since they depend on many factors such as air temperature. Therefore, the values given on an MSDS are approximate.

The explosive limits are usually given as the percent by volume of the material in the air. One percent by volume is 10,000 ppm. For example, gasoline has a LEL of 1.4% and a UEL of 7.6%. This means that gasoline vapours at concentrations of 1.4% to 7.6% (14,000 to 76,000 ppm) are flammable or explosive.

EXPOSURE LIMITS (OR OCCUPATIONAL EXPOSURE LIMITS (OELs))

An exposure limit is the concentration of a chemical in the workplace air to which most people can be exposed without experiencing harmful effects. Exposure limits should not be taken as sharp dividing lines between safe and unsafe exposures. It is possible for a chemical to cause health effects, in some people, at concentrations lower than the exposure limit.

Exposure limits have different names and different meanings depending on who developed them and whether or not they are legal limits. For example, Threshold Limit Values (TLVs) are exposure guidelines developed by the American Conference of Governmental Industrial Hygienists (ACGIH). They have been adopted by many Canadian governments as their legal limits. Permissible Exposure Limits (PELs) are legal exposure limits in the United States. Sometimes, a manufacturer will recommend an exposure limit for a material.

Exposure limits have not been set for many chemicals, for many different reasons. For example, there may not be enough information available to set an exposure limit. Therefore, the absence of an exposure limit does not necessarily mean the material is not harmful. There are three different types of exposure limits in common use:

1) Time-weighted average (TWA) exposure limit is the time-weighted average concentration of a chemical in air for a normal 8-hour work day and 40-hour work week to which nearly all workers may be exposed day after day without harmful effects. Time-weighted average means that the average concentration has been calculated using the duration of exposure to different concentrations of the chemical during a specific time period. In this way, higher and lower exposures are averaged over the day or week.

2) Short-term exposure limit (STEL) is the average concentration to which workers can be exposed for a short period (usually 15 minutes) without experiencing irritation, long-term or irreversible tissue damage, or reduced alertness. The number of times the concentration reaches the STEL and the amount of time between these occurrences can also be restricted.

3) Ceiling (C) exposure limit is the concentration which should not be exceeded at any time.

SKIN notation (SKIN) means that contact with the skin, eyes and moist tissues (for example, the mouth) can contribute to the overall exposure. The purpose of this notation is to suggest that measures be used to prevent absorption by these routes; for example, the use of protective gloves. If absorption occurs through the skin, then the airborne exposure limits are not relevant.

EXTINGUISHING MEDIA

Extinguishing media are agents which can put out fires involving the material. Common extinguishing agents are water, carbon dioxide, dry chemical, "alcohol" foam, and halogenated gases (Halon). It is important to know which extinguishers can be used so they can be made available at the worksite. It is also important to know which agents cannot be used since an incorrect extinguisher may not work or may create a more hazardous situation. If several materials are involved in a fire, an extinguisher effective for all of the materials should be used.

FDA

FDA stands for the Food and Drug Administration (U.S.).

FETOTOXIC, FETOTOXICITY

Fetotoxic means the substance is harmful to the fetus/foetus. Fetotoxicity describes the ability of a substance to harm the fetus. (See also Embryotoxicity, Teratogenicity and Reproductive Effects.)

FETUS/FOETUS

A fetus is an organism in the later stages of development prior to birth. In humans, it is the unborn child from the end of the second month of pregnancy to birth. (See also Embryo.)

FIRST AID

First aid is emergency care given immediately to an injured person. The purpose of first aid is to minimize injury and future disability. In serious cases, first aid may be necessary to keep the victim alive.

FLAMMABLE, FLAMMABILITY

Flammable means able to ignite and burn readily. Flammability is the ability of a material to ignite and burn readily. (See also Combustible.) Under the Canadian Controlled Products Regulations and the U.S. HAZCOM Standard, there are specific technical criteria for identifying flammable materials. (See Flammable Aerosol, Flammable Gas, Flammable Liquid, Flammable Solid and Reactive Flammable Material.)

There are closely related criteria for the classification of certain flammable materials under the Canadian Transportation of Dangerous Goods (TDG) Regulations and the U.S. Department of Transportation regulations. (See TDG Flammability Classification.) In Canada, at least, local, provincial and national fire codes also classify and regulate the use of flammable materials in workplaces. (See also Combustible.)

FLAMMABLE AEROSOL

Under the Canadian Controlled Products Regulations, a material is identified as a flammable aerosol if it is packaged in an aerosol container which can release a flammable material. A flammable aerosol is hazardous because it may form a torch (explosive ignition of the spray) or because a fire fueled by the flammable aerosol may flash back.

The U.S. OSHA HAZCOM Standard has a specific definition. Refer to the regulations for detailed information.

FLAMMABLE AND COMBUSTIBLE MATERIAL

Under the Canadian Controlled Products Regulations, a material may be classified as a flammable and combustible material if it meets specific criteria for a flammable gas, flammable liquid, combustible liquid, flammable solid, flammable aerosol or reactive flammable material.

FLAMMABLE GAS

A flammable gas is a gas which can ignite readily and burn rapidly or explosively. Under the Canadian Controlled Products Regulations and under the US Hazard Communication Standard, there are certain technical criteria for the identification of materials as flammable gases for the purposes of each regulation. Flammable gases can be extremely hazardous in the workplace.

FLAMMABLE LIMITS

See Explosive Limits.

FLAMMABLE LIQUID

A flammable liquid gives off a vapour which can be readily ignited at normal working temperatures. Under the Canadian Controlled Products Regulations, a flammable liquid is a liquid with a flash point (using a closed cup test) below 37.8 degrees C (100 degrees F). The US Hazard Communication Standard uses a similar, but not identical, definition.

Flammable liquids can be extremely hazardous in the workplace; for example:

- If there is inadequate ventilation, vapours can travel considerable distances to a source of ignition and flash back to the flammable liquid.
- It may be difficult to extinguish a burning flammable liquid with water because water may not be able to cool the liquid below its flash point.

FLAMMABLE SOLID

A flammable solid is a material which can ignite readily and burn vigorously and persistently. There are certain technical criteria in the Canadian Controlled Products Regulations and in the US OSHA Hazard Communication Standard for the identification of flammable solids for the purposes of each regulation. These criteria are based on ease of ignition and rate of burning. Flammable solids may be hazardous because heat from friction (for example, surfaces rubbing together) or heat from processing may cause a fire. Flammable solids in the form of a dust or powder may be particularly hazardous because they may explode if ignited.

FLASH BACK

Flash back occurs when a trail of flammable gas, vapour or aerosol is ignited by a distant spark, flame or other source of ignition. The flame then travels back along the trail of gas, vapour or aerosol to its source. A serious fire or explosion could result.

FLASH POINT

The flash point is the lowest temperature at which a liquid or solid gives off enough vapour to form a flammable air-vapour mixture near its surface. The lower the flash point, the greater the fire hazard. The flash point is an approximate value and should not be taken as a sharp dividing line between safe and hazardous conditions. The flash point is determined by a variety of test methods which give different results. Two types of methods are abbreviated as OC (open cup) and CC (closed cup).

FREEZING POINT

See Melting Point.

FUMES

Fumes are very small, airborne, solid particles formed by the cooling of a hot vapour. For example, a hot zinc vapour may form when zinc-coated steel is welded. The vapour then condenses to form fine zinc fume as soon as it contacts the cool surrounding air. Fumes are smaller than dusts and are more easily breathed into the lungs.

GAS

A gas is a material without a specific shape or volume. Gases tend to occupy an entire space uniformly at normal room pressure and temperature. The terms vapour and fume are sometimes confused with gas.

GENERAL VENTILATION

As used in an MSDS, general ventilation, also known as dilution ventilation, is the removal of contaminated air from the general area and the bringing in of clean air. This dilutes the amount of contaminant in the work environment. General ventilation is usually suggested for non-hazardous materials. (See also Mechanical Ventilation, Local Exhaust Ventilation and Ventilation.)

GI

GI stands for gastrointestinal (relating to the stomach and intestines).

HAZARD, HAZARDOUS

Hazard is the potential for harmful effects. Hazardous means potentially harmful. The hazards of a material are evaluated by examining the properties of the material, such as toxicity, flammability and chemical reactivity, as well as how the material is used. How a material is used can vary greatly from workplace to workplace and, therefore, so can the hazard.

In Canada and the U.S., the term hazardous is used by many different regulatory agencies. Definitions may vary. For example, OSHA defines a hazardous chemical as any chemical which is a physical hazard or a health hazard according to the OSHA Hazard Communication (Hazcom) criteria.

HAZARDOUS COMBUSTION PRODUCTS

Hazardous combustion products are chemicals which may be formed when a material burns. These chemicals may be toxic, flammable or have other hazards. The chemicals released and their amounts vary, depending upon conditions such as the temperature and the amount of air (or more specifically, oxygen) available. The combustion chemicals may be quite different from those formed by heating the same material during processing (thermal decomposition products). It is important to know which chemicals are formed by hazardous combustion in order to plan the response to a fire involving the material.

HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous decomposition products are formed when a material decomposes (breaks down) because it is unstable, or reacts with common materials such as water or oxygen (in air). This information should be considered when planning storage and handling procedures.

HAZARDOUS INGREDIENT

Under the Canadian Hazardous Products Act, a chemical must be listed in the Hazardous Ingredients Section of an MSDS if:

- it meets the criteria for a controlled product;
- it is on the Ingredient Disclosure List;
- there is no toxicological information available; or
- the supplier has reason to believe it might be hazardous.

Certain chemicals may be exempt from disclosure on an MSDS if they meet specific criteria set out in the Hazardous Materials Information Review Act.

HAZCOM

HAZCOM stands for the Hazard Communication Standard (U.S.) (29CFR1910.1200).

HEPATOTOXIN

Hepatotoxins are agents that can cause toxic effects on the liver.

HIGHLY TOXIC

Under the U.S. OSHA HAZCOM Standard, there are specific criteria for materials which must be identified as toxic. The corresponding term under Canadian WHMIS is "Very Toxic" (criteria are not the same).

HR

HR stands for hour.

IARC

IARC stands for the International Agency for Research on Cancer. IARC evaluates information on the carcinogenicity of chemicals, groups of chemicals and chemicals associated with certain industrial processes. IARC has published lists of chemicals which are generally recognized as human carcinogens, probable human carcinogens or carcinogens in animal tests.

IATA

IATA stands for International Air Transport Association.

IDLH

IDLH stands for Immediately Dangerous to Life or Health. For the purposes of respirator selection, NIOSH defines the IDLH concentration as the airborne concentration that poses a threat of exposure to airborne contaminants when that exposure is likely to cause death or immediate or delayed permanent adverse health effects or prevent escape from such an environment. The purpose of establishing an IDLH exposure concentration is to ensure that the worker can escape from a given contaminated environment in the event of failure of the respiratory protection equipment. In the event of failure of respiratory protective equipment, every effort should be made to exit immediately.

IMPERVIOUS

On an MSDS, impervious is a term used to describe protective gloves and other protective clothing. If a material is impervious to a chemical, then that chemical cannot readily penetrate through the material or damage the material. Different materials are impervious (resistant) to different chemicals. No single material is impervious to all chemicals. If an MSDS recommends wearing impervious gloves, you need to know the type of material from which the gloves should be made. For example, neoprene gloves are impervious to butyl alcohol but not to ethyl alcohol.

INCOMPATIBLE MATERIALS

Incompatible materials can react with the product or with components of the product and may:

- destroy the structure or function of a product;
- cause a fire, explosion or violent reaction; or
- cause the release of hazardous chemicals.

INERT INGREDIENT

An inert ingredient is anything other than the active ingredient of a product. It may be a solvent, colorant, filler or dispersing agent. In some cases, inert ingredients may be hazardous.

INGESTION

Ingestion means taking a material into the body by mouth (swallowing).

INHALATION

Inhalation means taking a material into the body by breathing it in.

IRRITANCY, IRRITATION

Irritancy is the ability of a material to irritate the skin, eyes, nose, throat or any other part of the body that it contacts. Signs and symptoms of irritation include tearing in the eyes and reddening, swelling, itching and pain of the affected part of the body.

Irritancy is often described as mild, moderate or severe, depending on the degree of irritation caused by a specific amount of the material. Irritancy may also be described by a number on a scale of 0 to 4, where 0 indicates no irritation and 4 means severe irritation. Irritancy is usually determined in animal experiments.

The Canadian Controlled Products Regulations and the U.S. OSHA Hazcom Standard describe technical criteria for identifying materials which are skin or eye irritants for the purposes of each regulation.

ISO

ISO stands for the International Standards Organization.

KG

KG stands for kilogram

LC50

LC stands for lethal concentration. LC50 is the concentration of a material in air which causes the death of 50% (one half) of a group of test animals. The material is inhaled over a set period of time, usually 1 or 4 hours. The LC50 helps determine the short-term poisoning potential of a material.

LD50

LD stands for lethal dose. LD50 is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. The LD50 can be determined for any route of entry, but dermal (applied to skin) and oral (given by mouth) LD50's are most common. The LD50 is one measure of the short-term poisoning potential of a material. (See also LC50.)

LCLO

LCLO stands for lowest lethal airborne concentration tested. (See also LC50 and LD50.)

LDLO

LDLO stands for lowest lethal dose tested. (See also LC50 and LD50.)

LEL

See Explosive Limits.

LFL

See Explosive Limits.

LOCAL EXHAUST VENTILATION

Local exhaust ventilation is the removal of contaminated air directly at its source. This type of ventilation can help reduce worker exposure to airborne materials more effectively than general ventilation. This is because it does not allow the material to enter the work environment. It is usually recommended for hazardous airborne materials. (See also Mechanical Ventilation and Ventilation.)

LOWER EXPLOSION LIMIT

See Explosive Limits.

LOWER EXPLOSIVE LIMIT

See Explosive Limits.

LOWER FLAMMABLE LIMIT

See Explosive Limits.

MATERIAL CAUSING IMMEDIATE AND SERIOUS TOXIC EFFECTS

The Canadian Controlled Products Regulations describe technical criteria for identifying materials which cause immediate and serious toxic effects. These criteria use information such as the LD50 or LC50 for a material. Based on the specific information, a material may be identified as toxic or very toxic in the class Poisonous and Infectious Material.

MATERIAL CAUSING OTHER TOXIC EFFECTS

The Canadian Controlled Products Regulations describe technical criteria for identifying materials which cause toxic effects such as skin or respiratory sensitization, mutagenicity and carcinogenicity. Based on the specific information, a material may be identified as toxic or very toxic in the class Poisonous and Infectious Material.

MEANS OF EXTINCTION

See Extinguishing Media.

MECHANICAL VENTILATION

Mechanical ventilation is the movement of air by mechanical means (for example, a wall fan). There are two kinds of mechanical ventilation: general ventilation and local exhaust ventilation. (See also Ventilation.)

MELTING POINT

The melting point is the temperature at which a solid material becomes a liquid. The freezing point is the temperature at which a liquid material becomes a solid. Usually one value or the other is given on the MSDS.

It is important to know the freezing or melting point for storage and handling purposes. For example, a frozen or melted material may burst a container. As well, a change of physical state could alter the hazards of the material.

mg/m³

The abbreviation mg/m³ stands for milligrams (mg) of a material per cubic metre (m³) of air. It is a unit of metric

measurement for concentration (weight/volume). The concentrations of any airborne chemical can be measured in mg/m³, whether it is a solid, liquid, gas or vapour.

MIN

MIN can stand for minute or minimum.

MISCIBLE

Miscible means able to be mixed. Two liquids are said to be miscible if they are partially or completely soluble in each other. Commonly, the term miscible is understood to mean that the two liquids are completely soluble in each other. (See also Solubility.)

MIST

A mist is a collection of liquid droplets suspended in air. A mist can be formed when spraying or splashing a liquid. It can also be formed when a vapour condenses into liquid droplets in the air. (See also Aerosol.)

ML

ML stands for millilitres (mL).

mm Hg

The abbreviation mm Hg stands for millimeters (mm) of mercury (Hg). It is a common unit of measurement for the pressure exerted by gases such as air. Normal atmospheric pressure is 760 mm Hg.

MOLECULAR FORMULA

See Chemical Formula.

MOLECULAR WEIGHT

The molecular weight of a chemical is a number showing how heavy one molecule (or unit) of the chemical is compared to the lightest element, hydrogen, which has a weight of 1. The molecular weight has various technical uses, such as calculating conversions from parts per million (ppm) to milligrams per cubic metre (mg/m³) in air.

MUTAGEN, MUTAGENIC, MUTAGENICITY

A mutagen is a substance which can cause changes in the DNA of cells (mutations). Mutagenic means able to cause mutations. Mutagenicity is the ability of a substance to cause mutations.

DNA determines the characteristics that children inherit from their parents. DNA also determines how cells in the body divide or reproduce.

A number of mutagenicity tests are used to screen chemicals for possible carcinogenicity or reproductive effects. This is because there is some evidence that mutations may increase the risk of cancer and reproductive problems such as infertility or birth defects. However, mutagenicity test results are not very reliable predictors of these effects. One reason for this is that the human body can repair mutations while most mutagenicity tests cannot.

Mutagenicity is included on MSDSs because it is an early indicator of potential hazard, and often there is very little other evidence available on possible carcinogenic or reproductive effects. The Canadian Controlled Products Regulations describes technical criteria for identifying materials which are mutagenic. The U.S. OSHA HAZCOM Standard includes mutagenic effects as reproductive target organ effects.

NA NUMBER

See UN Number.

NATURAL VENTILATION

Natural ventilation is a type of general ventilation which depends on natural instead of mechanical means for air movement. Natural ventilation can depend on the wind or the difference in temperature from one area to another to move air through a building. Therefore, it is unpredictable and unreliable. (See also Local Exhaust Ventilation, Mechanical Ventilation and Ventilation.)

NEPHROTOXINS

Nephrotoxins are agents that can cause toxic effects on the kidney.

NEUROTOXINS

Neurotoxins are agents that can cause toxic effects on the nervous system.

NFPA

NFPA stands for National Fire Protection Association (U.S.).

NIOSH

NIOSH stands for National Institute for Occupational Safety and Health. NIOSH is a branch of the United States government which undertakes research and develops occupational health and safety standards.

NOEL

NOEL stands for No Observable Effect Level.

NOS

NOS stands for not otherwise specified.

NUISANCE DUST, NUISANCE PARTICULATE (see Particulates Not Otherwise

Classified) Nuisance particulate is a term used historically by the ACGIH to describe airborne materials (solids and liquids) which have little harmful effect on the lungs and do not produce significant disease or harmful effects when exposures are kept under reasonable control. Nuisance particulates may also be called nuisance dusts. High levels of nuisance particulates in the air may reduce visibility and can get into the eyes, ears and nose. Removal of this material by washing or rubbing may cause irritation.

OC

OC stands for open cup.

ODOUR THRESHOLD

The odour threshold is the lowest concentration of a chemical in air that is detectable by smell. The odour threshold should only be regarded as an estimate. This is because odour thresholds are commonly determined under controlled laboratory conditions using people trained in odour recognition.

As well, in the workplace, the ability to detect the odour of a chemical varies from person to person and depends on conditions such as the presence of other odorous materials.

Odour cannot be used as a warning of unsafe conditions since workers may become used to the smell (adaptation), or the chemical may numb the sense of smell, a process called olfactory fatigue. However, if the odour threshold for a chemical is well below its exposure limit, odour can be used to warn of a problem with your respirator.

OECD

OECD stands for Organization for Economic Cooperation and Development. The OECD is an international agency which supports programs designed to facilitate trade and development.

The OECD has published "Guidelines for Testing of Chemicals." These guidelines contain recommended procedures for testing chemicals for toxic and environmental effects and for determining physical and chemical properties.

OEL

OEL stands for Occupational Exposure Limit. (See Exposure Limits for a general explanation.)

OSHA

OSHA stands for Occupational Safety and Health Administration. It is the branch of the United States government which sets and enforces occupational health and safety regulations. For example, OSHA sets the legal exposure limits in the United States, which are called Permissible Exposure Limits (PELs). OSHA also specifies what information must be given on labels and Material Safety Data Sheets for materials which have been classified as hazardous using their criteria.

OXIDIZING AGENT, OXIDIZING MATERIAL

An oxidizing agent or material gives up oxygen easily or can readily oxidize other materials. Examples of oxidizing agents are oxygen, chlorine and peroxide compounds. These chemicals will support a fire and are highly reactive. Under the Canadian Controlled Products Regulations and under the U.S. OSHA Hazcom Standard, there are specific criteria for the classification of materials as oxidizing materials.

PARTITION COEFFICIENT

See Coefficient of Oil/Water Distribution.

PEL

PEL stands for Permissible Exposure Limit. PELs are legal limits in the United States set by the Occupational Safety and Health Administration (OSHA). (See Exposure Limits for a general explanation.)

PENSKY-MARTENS CLOSED CUP

Pensky-Martens Closed Cup (PMCC) is a specific method for determining flash points.

PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment is clothing or devices worn to help isolate a person from direct exposure to a hazardous material or situation. Recommended personal protective equipment is often listed on an MSDS. This can include protective clothing, respiratory protection and eye protection.

The use of personal protective equipment is the least preferred method of protection from hazardous exposures. It can be unreliable and, if it fails, the person can be left completely unprotected. This is why engineering controls are preferred. Sometimes, personal protective equipment may be needed along with engineering controls. For example, a ventilation system (an engineering control) reduces the inhalation hazard of a chemical, while gloves and an apron (personal protective equipment) reduce skin contact. In addition, personal protective equipment can be an important means of protection when engineering controls are not practical: for example, during an emergency or other temporary conditions such as maintenance operations.

pH

The pH is a measure of the acidity or basicity (alkalinity) of a material when dissolved in water. It is expressed on a scale from 0 to 14. Roughly, pH can be divided into the following ranges:

pH 0 - 2	Strongly acidic
pH 3 - 5	Weakly acidic
pH 6 - 8	Neutral
pH 9 - 11	Weakly basic
pH 12 - 14	Strongly basic

Under the Canadian Controlled Products Regulations, materials with pH values of 0-2 or 11.5-14 may be classified corrosive. Corrosive materials must be stored and handled with great care.

PIN

See UN Number.

PMCC

See Pensky-Martens Closed Cup

POISONOUS AND INFECTIOUS MATERIAL

Under the Canadian Controlled Products Regulations, a Poisonous and Infectious Material is any material which meets the criteria for a Material Causing Immediate and Serious Toxic Effects, a Material Causing Other Toxic Effects, or a Biohazardous Infectious Material.

POLYMER

A polymer is a natural or man-made material formed by combining units, called monomers, into long chains. The word polymer means many parts. Examples of polymers are starch (which has many sugar units), polyethylene (which has many ethylene units) and polystyrene (which has many styrene units).

Most man-made polymers have low toxicity, low flammability and low chemical reactivity. In these ways, polymers tend to be less hazardous than the chemicals (monomers) from which they are made.

POLYMERIZE, POLYMERIZATION

Polymerization is the process of forming a polymer by combining large numbers of chemical units or monomers into long chains. Polymerization can be used to make some useful materials. However, uncontrolled polymerization can be extremely hazardous. Some polymerization processes can release considerable heat, can generate enough pressure to burst a container or can be explosive. Some chemicals can polymerize on their own without warning. Others can polymerize upon contact with water, air or other common chemicals. Inhibitors are normally added to products to reduce or eliminate the possibility of uncontrolled polymerization. Most MSDSs have a section called "Hazardous Polymerization" which indicates whether hazardous polymerization reactions can occur.

ppb

ppb stands for parts per billion.

ppm

The abbreviation ppm stands for parts per million. It is a common unit of concentration of gases or vapour in air. For example, 1 ppm of a gas means that 1 unit of the gas is present for every 1 million units of air. One ppm is the same as 1 minute in 2 years or 1 cent in \$10,000.

PROCESS ENCLOSURE

As used on an MSDS, process enclosure means that the operation in which the material is used is completely enclosed. A physical barrier separates the worker from the potential health or fire hazard. Process enclosure is usually recommended if the material is very toxic or flammable.

PSI

PSI stands for pounds per square inch and is a unit of pressure.

PYROPHORIC

Pyrophoric chemicals are defined in the U.S. OSHA Hazcom Standard as chemicals which will ignite spontaneously in air at a temperature of 130 degrees F (54.4 degrees C) or below. Regulatory definitions in other jurisdictions may differ.

RCRA

RCRA stands for Resource Conservation and Recovery Act (U.S.) It is a statute regulating waste that is administered by the U.S. EPA.

REACTIVE FLAMMABLE MATERIAL

Under the Canadian Controlled Products Regulations, a reactive flammable material is a material which is a dangerous fire risk because it can react readily with air or water. This category includes any material which:

- is spontaneously combustible, that is, a material which can react with air until enough heat builds up that it begins to burn;
- can react vigorously with air under normal conditions without actually catching fire;
- gives off dangerous quantities of flammable gas on reaction with water; or
- becomes spontaneously combustible when it contacts water or water vapour.

Reactive flammable materials must be kept dry and isolated from oxygen (in air) or other oxidizing agents. Therefore, they are often stored and handled in an atmosphere of unreactive gas, such as nitrogen or argon.

RELATIVE DENSITY

See Specific Gravity.

REPRODUCTIVE EFFECTS

Reproductive effects are problems in the reproductive process which may be caused by a substance. Possible reproductive effects include reduced fertility in the male or female, menstrual changes, miscarriage, embryotoxicity, fetotoxicity, teratogenicity, or harmful effects to the nursing infant from chemicals in breast milk.

Most chemicals can cause reproductive effects if there is an extremely high exposure. In these cases, the exposed person would experience other noticeable signs and symptoms caused by the exposure. These signs and symptoms act as a warning of toxicity. Chemicals which cause reproductive effects in the absence of other significant harmful effects are regarded as true reproductive hazards. Very few workplace chemicals are known to be true reproductive hazards.

REPRODUCTIVE TOXICITY

The Canadian Controlled Products Regulations describe technical criteria for identifying materials which have reproductive toxicity. These criteria refer to adverse effects on fertility. (See also Reproductive Effects.) Other jurisdictions likely have corresponding criteria, which may differ.

Under the U.S. OSHA HAZCOM Standard, Reproductive Toxicity is a Target Organ Effect, and includes mutagens, embryotoxins, teratogens and reproductive toxins.

RESPIRATORY SENSITIZATION

See Sensitization.

RTECS

RTECS stands for Registry of Toxic Effects of Chemical Substances.

SARA

SARA stands for Superfund Amendments and Reauthorization Act of 1986 (U.S.).

SEC

SEC stands for second or section.

SENSITIZATION

Sensitization is the development, over time, of an allergic reaction to a chemical. The chemical may cause a mild response on the first few exposures but, as the allergy develops, the response becomes worse with subsequent exposures. Eventually, even short exposures to low concentrations can cause a very severe reaction.

There are two different types of occupational sensitization: skin and respiratory. Typical symptoms of skin sensitivity are swelling, redness, itching, pain, and blistering. Sensitization of the respiratory system may result in symptoms similar to a severe asthmatic attack. These symptoms include wheezing, difficulty in breathing, chest tightness, coughing and shortness of breath.

The Canadian Controlled Products Regulations and the U.S. OSHA HAZCOM Standard describe technical criteria for identifying materials which are respiratory tract sensitizers or skin sensitizers.

SKIN NOTATION

See Exposure Limits for a general explanation.

SOLUBILITY

Solubility is the ability of a material to dissolve in water or another liquid. Solubility may be expressed as a ratio or may be described using words such as insoluble, very soluble or miscible.

Often, on an MSDS, the "Solubility" section describes solubility in water since water is the single most important industrial solvent. Solubility information is useful for planning spill clean-up and fire fighting procedures.

SOLVENT

A solvent is a material, usually a liquid, which is capable of dissolving another chemical. Chemicals commonly called solvents can dissolve many different chemicals. Examples of common solvents are water, ethanol, acetone, hexane and toluene.

SPECIFIC GRAVITY

Specific gravity is the ratio of the density of a material to the density of water. The density of water is about 1 gram per cubic centimetre (g/cc). Materials which are lighter than water (specific gravity less than 1.0) will float. Most materials have specific gravities exceeding 1.0, which means they are heavier than water and so will sink. Knowing the specific gravity is important for planning spill clean-up and fire fighting procedures. For example, a light flammable liquid such as gasoline may spread and, if ignited, burn on top of a water surface.

STABILITY

Stability is the ability of a material to remain unchanged in the presence of heat, moisture or air. An unstable material may decompose, polymerize, burn or explode under normal environmental conditions. Any indication that the material is unstable gives warning that special handling and storage precautions may be necessary.

STEL

STEL stands for Short-Term Exposure Limit. (See Exposure Limits for a general explanation.)

STP

STP stands for Standard Temperature and Pressure (0 degrees Celsius and one atmosphere pressure).

SYNERGISTIC, SYNERGISM

As used on an MSDS, synergism means that exposure to more than one chemical can result in health effects greater than expected when the effects of exposure to each chemical are added together. Very simply, it is like saying $1 + 1 = 3$.

When chemicals are synergistic, the potential hazards of the chemicals should be re-evaluated, taking their synergistic properties into consideration.

SYNONYMS

Synonyms are alternative names for the same chemical. For example, methanol and methyl hydrate are synonyms for methyl alcohol. Synonyms may help in locating additional information on a chemical.

TARGET ORGAN EFFECTS

Under the U.S. OSHA HAZCOM Standard, chemicals are identified as having target organ effects if there is statistically significant evidence of an acute or chronic health effect determined in a scientifically valid study. The following agents would be included (note, the list is not all-inclusive): hepatotoxins, agents which damage the lungs (including irritants), agents which act on the hematopoietic system, neurotoxins, nephrotoxins, reproductive toxins (mutagens, embryotoxins, teratogens and reproductive toxins), cutaneous hazards (chemicals which affect the dermal layer of the skin) and eye hazards (chemicals which affect the eye or visual capacity). There are no maximum dose criteria for chronic toxicity studies, as specified in the Canadian Controlled Products Regulations.

TCC

TCC stands for Tagliabue closed cup; a standard method of determining flash points. Generally, this appears in abbreviated form as Tag closed cup.

TCLO

TCLO stands for lowest toxic airborne concentration tested (see also LCLO and LC50).

TDG

TDG stands for Transportation of Dangerous Goods. In Canada, the transportation of potentially hazardous materials is regulated under the federal Transportation of Dangerous Goods Act and Regulations which are administered by Transport Canada. The TDG Act and Regulations set out criteria for the classification of materials as dangerous goods and state how these materials must be packaged and shipped.

TDG FLAMMABILITY CLASSIFICATION

Under the Canadian TDG Act and Regulations, chemicals are classified as flammable materials if they have certain properties. Consult the regulation for detailed information.

TDLO

TDLO stands for lowest toxic dose tested (see also LDLO and LD50).

TERATOGEN, TERATOGENIC, TERATOGENICITY

A teratogen is a substance which can cause birth defects. Teratogenic means able to cause birth defects. Teratogenicity is the ability of a chemical to cause birth defects. Teratogenicity results from a harmful effect to the embryo or the fetus/foetus.

The Canadian Controlled Products Regulations describe technical criteria for identifying materials which have teratogenicity and embryotoxicity. (See also Reproductive Effects.) Other jurisdictions may also have defined specific criteria.

Under the U.S. OSHA HAZCOM Standard, materials which have teratogenic effects are included under reproductive Target Organ Effects.

THERMAL DECOMPOSITION PRODUCTS

Thermal decomposition products are chemicals which may be formed when the material is heated but does not burn. These chemicals may be toxic, flammable or have other hazards. The chemicals released and their amounts vary depending upon conditions such as the temperature. The thermal decomposition products may be quite different from the chemicals formed by burning the same material (hazardous combustion products). It is important to know which chemicals are formed by thermal decomposition because this information is used to plan ventilation requirements for processes where a material may be heated.

TLV

TLV stands for Threshold Limit Value. It is the occupational exposure limit established by the American Conference of Governmental Industrial Hygienists (ACGIH). TLV is a registered trademark of ACGIH. TLVs are adopted by some governments as their legal limits. (See Exposure Limits for a general explanation.)

TLV-C

TLV-C stands for the ACGIH Threshold Limit Value-Ceiling. See also TLV.

TOC

TOC stands for Tagliabue open cup; a standard method of determining flash points. Generally, this appears in abbreviated form as Tag open cup.

TOXIC, TOXICITY

Toxic means able to cause harmful health effects. Toxicity is the ability of a substance to cause harmful health effects. Descriptions of toxicity (e.g. low, moderate, severe, etc.) depend on the amount needed to cause an effect or the severity of the effect.

Under the Canadian Controlled Products Regulations and the U.S. OSHA HAZCOM Standard, there are specific technical criteria for identifying a material as toxic for the purpose of each regulation. (See also Very Toxic and Highly Toxic.)

TRADE NAME

A trade name is the name under which a product is commercially known. Some materials are sold under common names, such as Stoddard solvent or degreaser, or internationally recognized trade names, like Varsol. Trade names are sometimes identified by symbols such as (R) or (TM).

TSCA

TSCA stands for Toxic Substances Control Act (U.S.).

TWA

TWA stands for Time-Weighted Average. (See Exposure Limits for a general explanation.)

UEL

See Explosive Limits.

UFL

See Explosive Limits.

uG

uG stands for microgram, a unit of mass.

UN NUMBER

UN number stands for United Nations number. The UN number is a four-digit number assigned to a potentially hazardous material (such as gasoline, UN 1203) or class of materials (such as corrosive liquids, UN 1760). These numbers are used by firefighters and other emergency response personnel for identification of materials during

transportation emergencies. UN (United Nations) numbers are internationally recognized. NA (North American) numbers are used only for shipments within Canada and the United States. PINs (Product Identification Numbers) are used in Canada. UN, NA and PIN numbers have the same uses.

UNSTABLE (REACTIVE)

Under the U.S. OSHA HAZCOM standard, a chemical is identified as unstable (reactive) if in the pure state, or as produced or transported, it will vigorously polymerize, decompose, condense, or will become self-reactive under conditions of shock, pressure or temperature.

UPPER EXPLOSION LIMIT

See Explosive Limits.

UPPER EXPLOSIVE LIMIT

See Explosive Limits.

UPPER FLAMMABLE LIMIT

See Explosive Limits.

USEPA

See EPA.

VAPOUR

A vapour is the gaseous form of a material which is normally solid or liquid at room temperature and pressure. Evaporation is the process by which a liquid is changed into a vapour. Sublimation is the process by which a solid is changed directly into the vapour state.

VAPOUR DENSITY

Vapour density is the weight per unit volume of a pure gas or vapour. On an MSDS, the vapour density is commonly given as the ratio of the density of the gas or vapour to the density of air. The density of air is given a value of 1. Light gases (density less than 1) such as helium rise in air. If there is inadequate ventilation, heavy gases and vapours (density greater than 1) can accumulate in low-lying areas such as pits and along floors.

VAPOUR PRESSURE

Vapour pressure is a measure of the tendency of a material to form a vapour. The higher the vapour pressure, the higher the potential vapour concentration. In general, a material with a high vapour pressure is more likely to be an inhalation or fire hazard than a similar material with a lower vapour pressure.

VENTILATION

Ventilation is the movement of air. One of the main purposes of ventilation is to remove contaminated air from the workplace. There are several different kinds of ventilation. (See General Ventilation, Local Exhaust Ventilation, Mechanical Ventilation and Natural Ventilation.)

More detailed information is available in the CCOHS publication "A Basic Guide to Industrial Ventilation."

VERY TOXIC

Under the Canadian Controlled Products Regulations, there are specific technical criteria for identifying a very toxic material. There are specific criteria for short-term lethality, long-term toxicity, teratogenicity and embryotoxicity, reproductive toxicity, carcinogenicity, respiratory sensitization and mutagenicity. (See also Toxic.)

VOC

VOC stands for volatile organic compound.

VOLATILE, VOLATILITY

Volatile means a material can evaporate. Volatility is the ability of a material to evaporate. The term volatile is commonly understood to mean that a material evaporates easily.

On an MSDS, volatility is commonly expressed as the "% volatile." The percent volatile can vary from 0% (none of the material will evaporate) to 100% (all of the material will evaporate if given enough time).

If a product contains volatile ingredients, there may be a need for ventilation and other precautions to control vapour concentrations.

WATER REACTIVE

Under the U.S. OSHA HAZCOM standard, a chemical is identified as water reactive if it reacts with water to release a gas that is either flammable or presents a health hazard.

WHMIS

WHMIS stands for Workplace Hazardous Materials Information System. It is a Canadian program designed to protect workers by providing them and their employers with vital information about hazardous materials. The following are key features of WHMIS:

- Criteria to identify controlled products and to provide information about them in the workplace;
- A cautionary labelling system for containers of controlled products;
- Requirements for the disclosure of information by the use of material safety data sheets;
- Worker education programs;
- A mechanism to protect trade secrets.

WHMIS is implemented by a series of federal, provincial and territorial acts and regulations.

H. CCOHS INFORMATION GUIDE



INFORMATION PATHFINDER

CCOHS is the path to health and safety with its wealth of worldwide information!

Health & Safety Information on...	CD-ROM Products – Databases & Collections						
	MSDS plus CHEMINFO	CHEMpendium™	FTSS	OSH RESEARCHER	OSHLIN™ with NIOSHTIC®/ NIOSHTIC-2	RTECS®	
Chemicals/ Chemical Safety	CHEMINFO MSDS	CHEMINFO CHEMINDEX CESARS CHRIS DSL/NDL HSD® NJ HSFS NPG TRANSP. 49CFR TRANSP. TDG	FTSS (F)	CISILO ♦ HSELINE ♦ CANADIANA INRS- BIBLIO ♦(F)	OSHLIN™ NIOSHTIC® NIOSHTIC-2♦	RTECS®	
Pesticides	CHEMINFO MSDS	CHEMINFO CESARS HSD®	FTSS (F)		OSHLIN™ NIOSHTIC® NIOSHTIC-2♦	RTECS®	
Emergency Response	CHEMINFO MSDS	CHEMINFO CHRIS HSD® TRANSP. 49CFR TRANSP. TDG	FTSS (F)				
Environment	MSDS	CESARS HSD®	FTSS (F)	CISILO ♦ HSELINE ♦ CANADIANA INRS- BIBLIO ♦(F)	OSHLIN™ NIOSHTIC® NIOSHTIC-2♦	RTECS®	
Toxicology	CHEMINFO	CHEMINFO CESARS HSD® NJ HSFS		HSELINE ♦ CANADIANA	OSHLIN™ NIOSHTIC® NIOSHTIC-2♦	RTECS®	
Occupational Hygiene	CHEMINFO MSDS	CHEMINFO CHRIS HSD® NJ HSFS NPG *	FTSS (F)	CISILO ♦ HSELINE ♦ CANADIANA	OSHLIN™ NIOSHTIC® NIOSHTIC-2♦	RTECS®	
Transportation Of Dangerous Goods	CHEMINFO MSDS	CHEMINFO HSD® TRANSP. 49CFR TRANSP. TDG	FTSS (F)			RTECS®	
Regulations/ Legislation	CHEMINFO	CHEMINFO DSL/NDL HSD® TRANSP. 49CFR TRANSP. TDG		CISILO ♦ HSELINE ♦		RTECS®	
Physical Hazards/ Safety				CISILO ♦ HSELINE ♦ CANADIANA INRS- BIBLIO ♦(F)	OSHLIN™ NIOSHTIC® NIOSHTIC-2♦		
Ergonomics				CISILO ♦ HSELINE ♦ CANADIANA INRS- BIBLIO ♦(F)	OSHLIN™ NIOSHTIC® NIOSHTIC-2♦		

Detailed information about individual products is provided in the CCOHS Catalogue of Products and Services, database fact sheets, and from the CCOHS Web Site – www.ccohs.ca.

LEGEND: Type of information: direct/full text data, bibliographic (♦), or directory (#). French data (F).

(As of: October 2003)



INFORMATION PATHFINDER

CCOHS is the path to health and safety with its wealth of worldwide information!

Health & Safety Information on...	CD-ROM Products – Databases & Collections				
	Canadian enviroOSH Legislation (Also available as regional discs)	Legislation Plus Standards (Also available as regional discs)	IPCS INTOX CD-ROM	IPCS INCHEM CD-ROM	
Chemicals/ Chemical Safety			International data for the prevention of and response to poisoning	Consolidation of international data for the sound management of chemicals	
Pesticides			International data for the prevention of and response to poisoning	Consolidation of international data for the sound management of chemicals	
Emergency Response			International data for the prevention of and response to poisoning	Consolidation of international data for the sound management of chemicals	
Environment	All Canadian (Fed. & Prov.) Health, Safety, & Environmental Legislation	CSA & CGSB Standards All Canadian (Fed. & Prov.) Health, Safety, & Environmental Legislation	International data for the prevention of and response to poisoning	Consolidation of international data for the sound management of chemicals	
Toxicology			International data for the prevention of and response to poisoning	Consolidation of international data for the sound management of chemicals	
Occupational Hygiene			International data for the prevention of and response to poisoning	Consolidation of international data for the sound management of chemicals	
Transportation Of Dangerous Goods	All Canadian (Fed. & Prov.) Health, Safety, & Environmental Legislation	CSA & CGSB Standards All Canadian (Fed. & Prov.) Health, Safety, & Environmental Legislation			
Regulations/ Legislation	All Canadian (Fed. & Prov.) Health, Safety, & Environmental Legislation	CSA & CGSB Standards All Canadian (Fed. & Prov.) Health, Safety, & Environmental Legislation		Consolidation of international data for the sound management of chemicals	
Physical Hazards/ Safety				Consolidation of international data for the sound management of chemicals	

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(As of: October 2003)

I. Glove Selection Guide

Gloves are probably the most commonly used type of PPE in labs. Selecting the right glove is important. You may find the following information useful in selecting the right kind of glove for your application.

Reuse

- Some gloves are designed for single use. If your lab uses disposable gloves, do not wash and re-use them, they are meant for single use.
- Your lab will have specific requirements that will dictate whether you use disposable or reusable gloves. Follow these requirements when making your choice.

Fit

- Fit is important because a glove that is too big or too small will not be worn as it should.
- Gloves that are too small will cut off circulation and be uncomfortable.
- Gloves that are too big will be cumbersome and contribute to spills and accidents.

Lining

Linings add comfort and durability to a glove; they also however, add cost. Some gloves are manufactured with a lining by necessity; here you will have no choice.

Powdering

Powdering is added to some gloves to allow them to be slipped on and off more easily. Powdering can contribute to latex sensitivity (in latex gloves) and latex protein can leach into the powder. If this powder is released into the air and inhaled by a sensitized person, results can be severe.

Thickness

Thickness of a glove influences its resistance to physical and chemical attack; in general, the thicker the glove the better the protection. Increased thickness, however, leads to decreased sensitivity and dexterity.

Material

Material selection is the most important criteria with respect to chemical resistance. See the table below as a guideline to be used in selecting materials. More information may be obtained from glove manufacturers, see them for final confirmation of material selection. Compatibility, breakthrough times, permeation rates and degradation are some factors to be considered.

Material Of Construction	Good Against	Comments	Price
Viton – Fluoroelastomer	PCBs, benzene, chlorinated and aromatic solvents, gas.	Limited physical strength but very flexible.	Very Expensive
Neoprene – Synthetic rubber	Most organic and inorganic acids, caustic, alcohols, solvents, oils.	Resistance is very dependant on method of manufacture (solvent or water dipped).	Moderately Expensive
Latex – Natural rubber	Acids, alkalis, salts, ketones.	Contain sensitizing proteins. Use only if necessary. Form fitting (elastic).	Inexpensive
Multi-Layer - Silvershield, Norfoil	Vinyl chloride, acetone, ethyl ether, superb resistance against a wide variety of compounds.	Virtually no cut resistance, lightweight, flexible.	Very Expensive
Vinyl (PVC) – Thermoplastic	Excellent resistance to most acids, and petroleum hydrocarbons.	Good abrasion resistance.	Inexpensive
Nitrile – Synthetic rubber	Good general resistance; alcohols, acids.	Good physical strength, good flexibility, form fitting.	Inexpensive

BIOLOGICAL and CHEMICAL SAFETY

Fire Department/Ambulance: 9 911

Security: 966-5555

Spills: 966-8497 or 966-8493 (after hours 966-5555)

Safety Requirements

1. Comply with Occupational Health & Safety Act & Regulations and follow University Safety Policies and Procedures
2. Keep unauthorized persons out of laboratory
3. Keep laboratory locked when unattended
4. Individuals handling hazardous substances shall be trained
5. Follow Research Granting Agency's Condition for working with hazardous biological, chemical and physical substances
6. Use good laboratory practices, responsible care, and safety precautions to keep all individuals safe

Safety Precautions

1. Do not eat, drink, store food or smoke in laboratory
2. Do not pipette solutions by mouth
3. Wear gloves or other appropriate protective equipment when working with hazardous substances to limit exposures
4. Wash hands thoroughly before leaving any laboratory
5. Maintain an up-to-date inventory and disposal records
6. Keep laboratory neat and tidy
7. Review and monitor procedures and substitute less hazardous substances wherever possible. Minimize exposure risks by reviewing safety information (MSDS)
8. Use a fume hood when handling volatile, toxic or flammable chemicals and a biosafety cabinet or proper containment for infectious agents. Ensure all units are tested when new, yearly or when moved
9. Clearly identify working areas and equipment used for handling hazardous substances
10. Develop written Exposure Control Plan when working with biohazardous materials (infectious materials)
11. Where possible use disposable absorbent liners on trays or surfaces to contain any spills of hazardous substances
12. Labs using Notifiable Biologicals(GMO's) or Chemical Substances, biohazardous materials in Risk Group Level 2 or higher are to have an operating permit
13. "Equipment and Area Release Form" must be filled out by the lab user and attached to equipment that is serviced, sold, or relocated
14. Disinfect equipment and work areas after use to minimize spread of contamination
15. Develop an Emergency Response Plan (ERP) for spills and medical emergencies. Equipment for spill clean up must be maintained and ready

Storage and Waste Disposal

1. WHMIS label and date all containers and place warning signs or symbols on all hazardous storage facilities.
2. Handling procedures for waste shall be in accordance with University Waste Disposal Procedures Manuals. Individuals who handle, package & dispose of waste must be trained.
3. Ensure that procedures are in place to prevent unnecessary storage (accumulation) of substances in the laboratory, especially if the substances become unstable with time.
4. Store hazardous substances in compatible groups, in secure areas and in proper storage containment.
5. Do not dispose or release any hazardous waste to the environment (e.g. sewer)
6. Needles (sharps) must be put into specific puncture & leak proof waste containers. If this material is contaminated, autoclave or chemically disinfect prior to pickup
7. All non-incinerable sharps waste (e.g. glass, metal,) must be decontaminated prior to packaging in secure puncture proof waste containers. These can go to the normal garbage

Spills/Incidents/Accidents

1. Comply with your written Emergency Response Plan
2. Warn people in the immediate area of the situation
3. Take immediate steps to protect your health and safety
4. Exit area and obtain assistance. Post the area to restrict entry
5. Use proper PPE for spill clean up. If spill gets out of control call Waste Management for assistance (8497 or 8493)
6. Fill out a Spill or Incident Report Form and/or a WCB form (if loss of work time) and return it to the HSE Department

Laboratory Spill Response Materials

1. Compatible absorbent material (e.g. bentonite, pads, etc.)
2. Inactivation or neutralizing material and warning Signs
3. Waste Containers, pH paper, plastic bags, tape, tweezers
4. Personal Protective Equipment (PPE) to protect hands, eyes, body, and respiratory system
5. Call Waste Management for assistance (8497 or 8493)

Right to Know and to Refuse

Under the law, it is your right to take the time to obtain the proper training & knowledge of the substances you work with before you start to work with them. You also have the right to refuse to do hazardous work on reasonable grounds without being discriminated against.

K. Equipment & Area Release Form

Guidelines for Cleaning Equipment or Area Destined for Release or Service

1. Objective

The objective of the Equipment or Area Release Form and guidelines are to protect the health and safety of all staff and the public at large from being exposed unnecessarily to equipment or areas that may contain hazardous biological, chemical or radioactive substances. It is hoped that the person requesting service in an area or on a piece of equipment will take the time to evaluate the associated risks from the point of view of the service technician who will be completing the work.

2. Biological Substances

The minimum for cleaning any area or equipment contaminated with biological agents is for the technical staff in the laboratory to wipe down the area or equipment with a disinfectant that is effective on that biological substance. Often 70% alcohol or javex bleach diluted 1:9 is effective. There are many other ways to disinfect areas and equipment depending on the extent of the contamination and the biological substance. The Biosafety Office (966-8496) is equipped with a paraformaldehyde gas generator for equipment that can be encapsulated to hold the gas for a period of time. The generator also neutralizes the formaldehyde gas with ammonium carbonate.

3. Chemical Substances

Technical staff is to wipe down areas or equipment to remove any surface contamination. It is costly to do quantitative measurements on any surface for chemical contamination therefore, the removal procedure must be repeated several times (3) to ensure minimum contamination. Personal protective equipment may be required in extreme cases to ensure safety.

4. Radioactive Substances

The minimum for cleaning any area or equipment contaminated with radioactive substances is for the technical staff to decontaminated the area or equipment. Wipe tests must be completed to determine if the area or equipment is free from contamination.

5. Personal Protective Equipment and Precautions

Personal protective equipment and precautions shall be taken in handling equipment or upon entering a certain area. Ask technical staff what you should wear when handling or entering the designated area to work. Always protect your eyes and hands. Wash your hands and tools upon completion of the work.

6. Consultation

The Department of Health, Safety and Environment will provide consultation if there is any unresolved safety concerns – phone 966-8493.

7. Examples

Some types of equipment that may need decontamination and/or cleaning as well as the use of personal protective equipment and good hygiene techniques are as follows. *Note:* The type of department where the equipment is used with often indicate what risks may exist.

biosafety cabinets	water baths	chemical storage cupboards	fume hoods
autoclaves	vacuum pumps	sinks and drains	incubators
pipette washers	homogenizers	cage washers	shaker units
vortexers	toilet snakes	sonicators	centrifuges
plugged toilets	drinking troughs	washing machines	

Some types of areas that may need decontamination and/or cleaning as well as the use of personal protective equipment and good hygiene techniques are as follows:

roof venting areas	ventilation ducts	plenums	isolation rooms
animal rooms	radioactive work areas	chemical storage rooms	pits
compressor rooms	refrigeration rooms	incinerator	fume hood discharge areas



EQUIPMENT OR AREA RELEASE FORM

Work Order #: _____

Service Technician: _____

Introductory Notes:

Under the Saskatchewan Occupational Health and Safety Act and Regulations, every employer has a duty to conduct his business so as not expose persons to undue risk to their health and safety. Warnings must be given to any persons if there are likely to be any hazardous biological, chemical, or radioactive agents present in/on equipment or devices offered for service, resale or disposal.

Service staff has the right to refuse acceptance of, or service work on, any equipment or devices, which they have, reason to believe may be hazardous to their health and safety until they receive proper safety instructions. The Department of Health, Safety and Environment will provide consultation if there is any unresolved safety concerns - phone 966-8493.

Supervisors or Person-in-Charge releasing areas for service work or equipment for service, resale or disposal which by its' design may contain hazardous residues within or on its' surface shall complete an Equipment/Area Release Form. This form shall be attached to such equipment or given to the service employee.

Failure to complete this form or comply with this procedure may lead to refusal to handle the equipment for service, resale or disposal.

Equipment / Area Details:

Room # :	Building:
Description of Equipment/Area:	Model # :
	Serial # :
	University ID # :

Destination:

<input type="checkbox"/> Disposal	<input type="checkbox"/> Relocation	<input type="checkbox"/> Resale	<input type="checkbox"/> Service
Comments:			

Safety Details:

Were any hazardous substances used in this equipment or area? <input type="checkbox"/> Biological <input type="checkbox"/> Chemical <input type="checkbox"/> Radioactive <input type="checkbox"/> None Details of any hazardous substances that could be present:
Has this equipment or area been decontaminated or purged of any hazardous substances, which could cause harm to service employees? <input type="checkbox"/> Yes <input type="checkbox"/> No If yes, describe procedure:
What personal protective equipment (PPE) or precautions should be taken in handling this equipment or working in this area? <input type="checkbox"/> gloves <input type="checkbox"/> eye protection <input type="checkbox"/> respiratory protection <input type="checkbox"/> foot protection <input type="checkbox"/> ear protection <input type="checkbox"/> disposable coveralls <input type="checkbox"/> other: _____
What action should be taken in the event of human contact with the hazardous substance?

Declaration:

I have decontaminated or cleaned this equipment or area specified above so that it does not present any hazard associated with hazardous substances.

Print Name:	Date:
<input type="checkbox"/> Supervisor <input type="checkbox"/> Person-In-Charge <input type="checkbox"/> Senior Technician	Telephone # :
Signature:	

L.

Incident Report

This report is to be completed by each person involved in an incident or near miss incident while engaged in activities at, or conducting work for, the University of Saskatchewan.

Complete this report and return a copy to the Department of Health, Safety and Environment
(Room 150, Research Annex or by fax to 966-8394) within 24 hours of the incident.

Definitions of terms and instructions are available on the DHSE website at: www.usask.ca/dhse.

☐ Incident

☐ Near Miss Incident

Identifying Information (complete all that apply)	Type of Occurrence	<input type="checkbox"/> Serious or Major Injury	<input type="checkbox"/> Minor Injury	<input type="checkbox"/> Exposure	<input type="checkbox"/> Spill	<input type="checkbox"/> No Loss Incident	<input type="checkbox"/> Property Damage	<input type="checkbox"/> Construction
	Personal Information	Name:			Ph. #:			
		Department:			Location of Incident:			
		Occupation:		Years of Job Experience:	Supervisor in Charge (name & ph. #):			
		Incident Date:		Time of Incident:	a.m. / p.m.	Equipment Involved:		
	Incident Information	Injury or Illness		Spill		Exposure		Other Loss Type
		Nature of Illness or Injury:		Material:		Material/Noise Exposed to:		Type:
		Object Inflicting Harm:		Quantity:		Quantity:		Est. Cost of Loss:
		Part of Body (indicate left, right, upper, etc.):		Reason for Spill:		Reason for Exposure:		Nature of Loss:
		Medical Attention Received: Y or N	Days Lost: Y or N	Risk: High Moderate Low		Risk: High Moderate Low		Object Causing Loss:
Additional Details Required:					Signature: Date:			

Immediate Causes (check all that apply)	Identification of Substandard Conditions	<input type="checkbox"/> Inadequate Warning System/Alarm <input type="checkbox"/> Inadequate Guards or Barriers <input type="checkbox"/> Defective Tools/Equipment/Materials <input type="checkbox"/> High/Low Temperature (specify) <input type="checkbox"/> Failure of PPE Specify/Explain:		<input type="checkbox"/> Fire or Explosion Hazard <input type="checkbox"/> Poor Housekeeping <input type="checkbox"/> Noise <input type="checkbox"/> Hazardous Environmental Conditions: gases, dust, smoke, vapours (specify) <input type="checkbox"/> Other (explain):		<input type="checkbox"/> Congestion or Restricted Action <input type="checkbox"/> Inadequate/Improper PPE <input type="checkbox"/> Inadequate Ventilation <input type="checkbox"/> Inadequate or Excessive Illumination (specify)	
	Substandard Acts or Practices	<input type="checkbox"/> Operating Equipment without Authority/ Training (specify) <input type="checkbox"/> Failure to Warn Others <input type="checkbox"/> Failure to Secure <input type="checkbox"/> Operating at Improper Speed <input type="checkbox"/> Failure to Follow Procedures <input type="checkbox"/> Failing to Use PPE Specify/Explain:		<input type="checkbox"/> Removing or Disabling Safety Device <input type="checkbox"/> Using Defective Equipment <input type="checkbox"/> Using Equipment Improperly <input type="checkbox"/> Improper Loading <input type="checkbox"/> Under Influence of Alcohol/Drugs (specify) <input type="checkbox"/> Other (explain):		<input type="checkbox"/> Improper Placement <input type="checkbox"/> Horseplay <input type="checkbox"/> Improper Materials Handling <input type="checkbox"/> Improper Position for Task <input type="checkbox"/> Servicing Equipment while Operating	

Identification of Basic Causes (check all that apply)	Job/System Factors	<input type="checkbox"/> Inadequate Work Standards	<input type="checkbox"/> Inadequate Supervision	<input type="checkbox"/> Inadequate Equipment	<input type="checkbox"/> Inadequate Maintenance
	Personal Factors	<input type="checkbox"/> Inadequate Engineering	<input type="checkbox"/> Inadequate Tools	<input type="checkbox"/> Wear and Tear	<input type="checkbox"/> Abuse/Misuse
		<input type="checkbox"/> Inadequate Purchasing	<input type="checkbox"/> Other (specify)		
		Specify/Explain:			
		<input type="checkbox"/> Inadequate Capability	<input type="checkbox"/> Lack of Skill	<input type="checkbox"/> Rushing	<input type="checkbox"/> Personal Challenge
		<input type="checkbox"/> Lack of Knowledge/training	<input type="checkbox"/> Stress	<input type="checkbox"/> Improper Motivation	<input type="checkbox"/> Other (specify)
		Specify/Explain:			

Identification of Contact (check all that apply)	Type of Contact/Exposure	<input type="checkbox"/> Struck By/Against	<input type="checkbox"/> Poke/Puncture	<input type="checkbox"/> Caught In	<input type="checkbox"/> Fall on Same Level
	Contact With/Exposure To	<input type="checkbox"/> Caught Between	<input type="checkbox"/> Slip/Trip	<input type="checkbox"/> Inhalation Of	<input type="checkbox"/> Fall to Below
		<input type="checkbox"/> Cut By	<input type="checkbox"/> Bit By	<input type="checkbox"/> Scratched By	<input type="checkbox"/> Other
		<input type="checkbox"/> Electricity	<input type="checkbox"/> Machinery/Equipment	<input type="checkbox"/> Harmful Substance	<input type="checkbox"/> Radiation
		<input type="checkbox"/> Biohazard	<input type="checkbox"/> Chemical	<input type="checkbox"/> Heat/Cold	<input type="checkbox"/> Structure (railing, wall)
		<input type="checkbox"/> Noise	<input type="checkbox"/> Material (specify)	<input type="checkbox"/> Object (specify)	<input type="checkbox"/> Animal (specify)
		Specify/Explain:			

Description	Describe How the Event Occurred:

Action Plan	Remedial Action (list all actions to control potential future incidents based on causes):		
	Action to Be Taken (completed by Supervisor – ensure changes do not bring about new hazards):		
	Immediate:		
	Long Term:		
	Set Target Date for Action(s):	Supervisor Signature:	Date:

Attach if Applicable/Available: Sketch of Site, Photograph, and/or Additional Explanation as required.

Final Review	Concluding Reviewers Comments (to be completed by DHSE):		
	Dangerous Occurrence? Y or N	Date Reported to Sask.Labour:	SL File #:
	Signature of Reviewer:	Date Reviewed:	Follow Up Date:
	DHSE File #:	WCB File # (if any):	Date Concluded:

M.**Safety Audit Checklist –Generic Sample**

Date: _____ Area: _____

I. Laboratory Work Practices		Comments
✓ No smoking, food & beverages rules are observed.	Yes/No	
✓ Food and beverages are not stored in the laboratory areas, refrigerators or in glassware that is also used for laboratory operations.	Yes/No	
✓ Pipetting is performed by mechanical means.	Yes/No	
✓ Laboratory surfaces are cleaned; disinfected or decontaminated after work is performed.	Yes/No	
✓ Required PPE is being worn.	Yes/No	
✓ Used needles are stored in appropriate sharps containers		
✓ Syringes comply with modern requirements.	Yes/No	
✓ No recapping of needles is performed.	Yes/No	
✓ Hoods are not being used for storage.	Yes/No	

II. Housekeeping		Comments
✓ Laboratory and storage areas uncluttered and orderly (including benchtops).	Yes/No	
✓ Aisles & exits are free from obstruction.	Yes/No	
✓ Work surfaces are protected from contamination.	Yes/No	
✓ Electrical cords are in good condition and are CSA or ULC listed.	Yes/No	
✓ Tools and equipment are in good repair and electrically grounded.	Yes/No	
✓ Tops of cabinets and shelves are free from inappropriate stored items.	Yes/No	
✓ Heavy objects are confined to lower shelves.	Yes/No	
✓ Glassware is free from cracks, chips, sharp edges and other defects.	Yes/No	
✓ Broken glass containers are available and in use.	Yes/No	

III. Personal Protective Equipment		Comments
✓ Protective gloves are available and matched to hazards involved.	Yes/No	
✓ Eye protection is available and in use in all laboratories.	Yes/No	
✓ Lab coats, tyvek garments etc. are available and in use.	Yes/No	
✓ Lab coats are only worn in the laboratory and are removed before entering offices, lunchrooms, rest rooms, conference rooms and other non-laboratory general use areas. (This includes disposable protective clothing).	Yes/No	
✓ Dirty lab coats/uniforms are stored in a covered container until removed for laundering.	Yes/No	
✓ Appropriate protective clothing is available and in use when working with hazardous materials.	Yes/No	
✓ Respirators are provided when necessary, and selected on the basis of hazard present.	Yes/No	
✓ Respirators are used correctly, cleaned after every use and stored in a convenient, clean and sanitary area. Users have been trained.	Yes/No	

IV. Hazard Communication		Comments
✓ Primary & secondary transport containers are labeled with identity, appropriate hazard warnings, and expiration dates.	Yes/No	
✓ Signs on storage areas (e.g. Refrigerators) and laboratories are consistent with hazards within.	Yes/No	
✓ MSDSs are available (paper or electronic) for WHMIS agents used and stored.	Yes/No	
✓ Employees know the location of the MSDS binders for their work area.	Yes/No	
✓ Satellite MSDS collections are complete and readily available at all times to labs.	Yes/No	

V. Chemical Storage		Comments
✓ Incompatible materials are segregated.	Yes/No	
✓ Corrosives and flammables are stored below eye level.	Yes/No	
✓ Hazardous materials used/stored in the laboratory are limited to small quantities.	Yes/No	
✓ Unnecessary, unused, or outdated materials are removed from laboratories and chemical storage areas.	Yes/No	
✓ Safety carriers are available and in use while transporting chemicals.	Yes/No	
✓ Hazardous materials storage areas include trays, doors or lips on shelves.	Yes/No	
✓ All lab carts have side-rails.	Yes/No	
✓ All containers are properly labeled with: Name, Date, Contents, Lab #	Yes/No	

VI. Flammable Liquids Storage & Handling		Comments
✓ Flammable liquids are stored and used away from ignition sources.	Yes/No	
✓ Bulk quantities of flammable liquids are stored in approved storage cabinets.	Yes/No	
✓ Flammable liquid storage cabinets are properly labeled.	Yes/No	
✓ Flammable liquid storage cabinets close properly.	Yes/No	
✓ Flammables stored on open shelves in glass or plastic containers are within permissible quantities (limit =)	Yes/No	
✓ Safety cans used to handle small quantities of flammable liquids are properly labeled.	Yes/No	
✓ Solvent waste cans are labeled properly with Name, Contents, Lab #.	Yes/No	
✓ Nothing flammable is stored on top of flammable cabinets.	Yes/No	

VII. Compressed Gas Cylinders		Comments
✓ Gas cylinders are properly secured from falling.	Yes/No	
✓ Cylinder caps are in place when cylinders are not in use or being moved.	Yes/No	
✓ Gas cylinders are secured on carts for any transport.	Yes/No	
✓ Gas cylinders are stored away from excessive heat.	Yes/No	
✓ Flammable and toxic gas cylinders are stored in approved area and not above allowable maximums.	Yes/No	
✓ All gas cylinders are properly marked as to their contents.	Yes/No	
✓ Full and empty cylinders are stored separately.	Yes/No	
✓ Empty gas cylinders are labeled "EMPTY".	Yes/No	
✓ Gas lines, piping, manifold, etc. are labeled with the identity of their	Yes/No	

contents.		
✓ Hoses, tubing and regulators are in good working condition.	Yes/No	

VIII. Waste Handling: Hazardous, Non-Hazardous & Biological		Comments
✓ No liquid waste is disposed of in the sinks or the sewer.	Yes/No	
✓ Hazardous wastes are not accumulated for longer than is necessary in the laboratory.	Yes/No	
✓ Waste streams are separated as necessary: ex. Solid vs. liquid, hazardous vs. non-hazardous, halogenated vs. non-halogenated, etc.	Yes/No	
✓ Waste containers are appropriately tagged before placing in waste room.	Yes/No	
✓ Containers of hazardous waste are labeled properly with the date and name of person discarding waste.	Yes/No	
✓ Biological waste is appropriately marked with a biohazard symbol.	Yes/No	
✓ Syringes and other sharp waste are disposed of into labeled sharps container.	Yes/No	
✓ Waste material is not allowed to accumulate on the floors, in corners or under shelves/tables in laboratories.	Yes/No	
✓ Radioactive waste is properly marked with radiation symbol.	Yes/No	

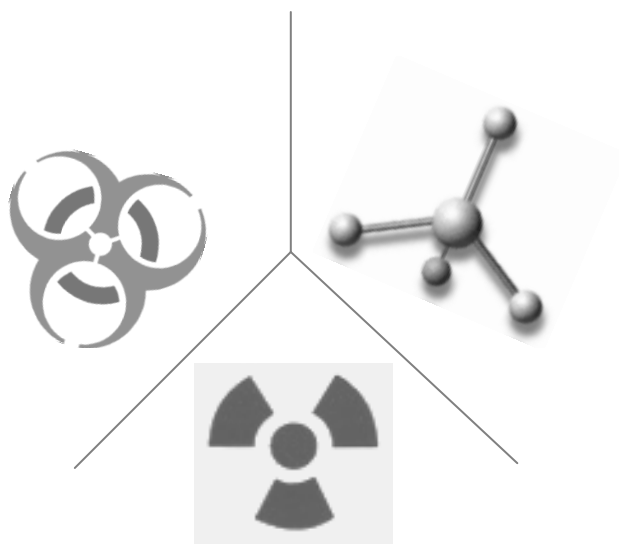
IX. Means of Egress and Emergency		Comments
✓ Exits are clearly marked.	Yes/No	
✓ Exits are free from obstruction.	Yes/No	
✓ All fire doors are self-closing and are kept closed.	Yes/No	
✓ A fire emergency response plan is in place and communicated.	Yes/No	
✓ Fire alarms are provided.	Yes/No	
✓ Telephones are labeled with emergency numbers.	Yes/No	
✓ Emergency evacuation routes are clearly posted.	Yes/No	
✓ Emergency evacuation routes are posted in common hallways.	Yes/No	
✓ Emergency exit lights are working and clear of obstruction.	Yes/No	
✓ Spill Response Materials and Equipment are available.	Yes/No	

X. Safety Equipment		Comments
✓ Safety showers and eye wash stations are located within 100' of all laboratories.	Yes/No	
✓ Safety showers and eye wash stations are clearly labeled, and these areas are clear from obstruction.	Yes/No	
✓ All showers and eye wash stations are clean, covers are replaced and they are in good working condition.	Yes/No	
✓ Showers and eye washes are checked monthly and recorded. Date of last check: _____	Yes/No	
✓ Fire extinguishers are available.	Yes/No	
✓ Fire extinguishers are the appropriate type for the hazard in the work area.	Yes/No	
✓ Fire extinguishers are checked annually. Date of last check: _____	Yes/No	
✓ Fire detection devices, smoke alarms, sprinkler systems, lighted exit signs are in good working condition.	Yes/No	
✓ First-aid supplies are readily available and clearly visible.	Yes/No	
✓ Spill team list is clearly posted in laboratories.	Yes/No	

NOTES

NOTES

Hazardous Waste Disposal Manual



August 2007

Emergency Numbers

SECURITY SERVICES (24 hours)	5555
Ambulance	9 - 911
(if using a pay phone)	911
Fire (Pull an alarm and call)	9 - 911
(if using a pay phone)	911
Safety Related Incidents (Mon – Fri: 8:30 to 4:30)	8493
Spills (Mon – Fri: 8:30 to 4:30)	8497

Department of Health, Safety and Environment

General Inquiries	8493
Fax.....	8394
Hazardous Waste Disposal	8497
Fax.....	6146
Safety Training Inquiries	8492
Biosafety.....	8496
Biosafety Cabinet Testing Inquiries.....	8510
Chemical/Environmental Safety	8512
Safety Inspections Inquiries	8738
Environmental Program Inquiries	2379
Community Safety	1957
Fire Safety.....	8838
Contractor Safety Inquiries.....	6076
Occupational Hygiene / Injury Prevention	8511
Radiation Safety.....	8494
Radiological Inquiries	8491
Director.....	8463
Health, Safety and Environment Management System Coordinator	2370

Other Essential Services

Bloodborne Pathogen Exposures (Sharps Puncture)	
Department of Immunology and Infectious Disease	655-1777
Public Health Consultation	655-4620
Employee Assistance Program	4300
Facilities Management (Mon – Fri: 8:00 to 4:30)	4496

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1. General Information

The objective of this manual is to help you collect, segregate, handle, treat, store, transport, and dispose of all hazardous waste in a safe and efficient manner.

The University of Saskatchewan (U of S) is committed to the proper management of hazardous waste generated by research, educational and business activities. Proper management of waste will minimize the risk to the University community, the general public and the environment, and reduce the financial cost to the University.

The University provides a hazardous waste management service through the Department of Health, Safety and Environment (DHSE). The Waste Management Facility (WMF) personnel collect, process, and package hazardous waste generated at the University. Federal, Provincial, and Municipal Acts and Regulations regulate these activities.

In order to comply with the specific regulations of these acts, it is imperative that the WMF personnel know whether the waste is biological, chemical, radioactive, mixed or unusual waste. They must also have specific details regarding the waste.

All hazardous waste is University of Saskatchewan property; therefore it shall be disposed of according to the procedures outlined in this manual.

Any person (or any waste generator sending hazardous waste to an approved external waste facility, or sending waste to the University from an off campus site must be trained in accordance with the Transportation of Dangerous Goods regulations.

POLICY: Hazardous waste from University laboratories will be removed and disposed of in a safe manner: waste must be identified, segregated into compatible groups, collected in appropriate containers, labelled, and the corresponding forms filled out as per University's waste disposal procedures. DHSE reserves the right to charge a fee or decline services in the event these procedures are not followed.

2 *Categories of Hazardous Waste*

There are primarily four methods available for the disposal of hazardous waste: incineration, landfill, sewer dilution or shipment to an approved external waste facility. The method of disposal is determined by factors such as:

- the type of waste;
- the nature of the hazard to the public and to the environment;
- the ease of destruction of any hazard if present; and
- the volume reduction and cost of disposal.

Therefore, it is essential that each type of waste be properly segregated according to the categories listed below to ensure safe and environmentally responsible disposal of the hazardous waste.

2.1 **Biohazardous Waste**

A Biohazard refers to a biological substance that may pose a threat to the health and safety of humans, animals or the environment. Anything that comes in contact with a Biohazard is normally deemed contaminated. Any contaminated items must be disposed of in accordance with the Saskatchewan Biomedical Waste Guidelines. There are different methods of disposal such as: autoclaving, chemical inactivation or incineration. There are also different categories of Biohazardous waste such as: medical waste, animal carcasses, animal husbandry, laboratory waste, sharps, blood soaked towels or gauze, petri dishes, or any contaminated laboratory ware. This waste must be segregated and disposed of according to the information presented in this section.

See Appendix A for the Biological Waste Flow Chart.

2.1.1 *Animal waste*

Waste that is destined for incineration must not contain highly combustible materials (e.g. culture plates, gloves, test tubes) because they are not allowed to be incinerated under the permit. Therefore, it is important that animal waste be collected separately in properly labelled containers.

Definition:

This type of waste consists of animal tissues, organs, body parts, and carcasses. This waste must be:

- Stored in a freezer/refrigerator until the day of pick up.
- Segregated from other waste (e.g. glass, metals, gloves).
- Treated as radioactive animal waste if contaminated with a nuclear substance.(see section 2.3.1.2)

2.1.1.1 Infectious animal waste

- This type of waste includes animal tissues, organs, body parts, and whole carcasses that contain infectious agents.
- Collect this waste in a lined Biohazard box.
- Keep the weight of the container below 20 kg.
- Label the box according to the specifications in section 3.1.2.
- Store this waste in a secure, refrigerated area until WMF personnel picks it up for incineration.

2.1.1.2 Non infectious animal waste

- This type of waste includes animal tissues, organs, body parts, and whole carcasses that do not contain infectious agents.
- Collect this waste in a heavy-duty cardboard box lined with double plastic bags or a Biohazard box with the biohazard warning symbol defaced.
- Keep the weight of the container below 20 kg.
- Label the box according to the specifications in section 3.1.2.
- Store this waste in a secure, refrigerated area until WMF personnel picks it up for incineration.
- When exceptionally large or heavy animals are involved, contact the Biosafety Manager for more information on disposal arrangements.

2.1.2 *Animal husbandry waste*

Definition:

This type of waste includes stable or cage bedding, waste feed, litter, etc.

- ***Infectious:*** waste must be autoclaved or inactivated then treated as non-infectious and put into a regular garbage bin.
- ***Non-infectious:*** waste that is not contaminated with radioactivity or chemicals can be directly disposed of into a regular garbage bin.

2.1.3 *Microbiological laboratory waste*

Definition:

This type of waste includes laboratory cultures, weigh boats, gloves, paper towels, absorbent pads, bench top covers, plastic products (tubes, flasks, petri dishes), non penetrable waste (metal pans, blunt objects), etc.

2.1.3.1 Not contaminated

- Do not put in a Biohazard box or Biohazard bag.
- Must be placed into plastic bags and closed by the technical staff before custodial employees handle it.
- Waste can be disposed of into the regular garbage bin(s).

2.1.3.2 Contaminated

- This waste should be autoclaved and then disposed of with general waste provided it is contained in a package that is labelled **decontaminated** and there is no evidence of a recognizable Biohazard sign anywhere.
- Do not send autoclaved waste to the approved external waste facility or to WMF.
- If the waste is not autoclaved, then it should be collected in a Biohazard box or appropriate designated biowaste bin(s).
- This waste should be collected and labelled in accordance with sections 3.1.1 and 3.1.2.
- If contaminated with radioactivity, treat as radioactive waste and see section 2.3.1.
- If contaminated with volatile or extremely toxic material, contact Biosafety Manager for consultation.

2.1.4 *Liquid waste*

Definition:

This type of waste includes human and/or animal blood and body fluids.

- Small quantities of infectious liquid waste must be decontaminated or inactivated *prior to* diluting them down the drain. Due care must be taken to ensure this waste has truly been inactivated.
- Ensure that all containers with residual blood or body fluids are rinsed prior to disposal.
- If a large amount of infectious liquid is being produced, contact the Biosafety Manager for advice.
- Note that for **prion** contaminated formalin or formic acid waste consult the Biosafety Manager for appropriate disposal procedure.

2.2 **Chemical Waste**

Chemical waste is divided into several categories, with each type of waste requiring storage, handling, and disposal practices appropriate to the type of chemicals present in the waste. It is the responsibility of the person generating the chemical waste to ensure that all procedures are followed to ensure safe and environmentally responsible disposal of the waste. For each chemical to be disposed of refer to the MSDS to be aware of the safety and environmental considerations.

2.2.1 *Unused chemicals and chemical mixtures waste*

Definition:

Pure chemicals or mixtures of chemicals that will not be used for their intended purpose or are waste products.

- Refer to the MSDS to recognize all hazards associated with each chemical.
- Waste chemicals must be collected in sealed, leak proof containers that are appropriate for each chemical being disposed of. Containers that do not seal (e.g. pails or containers with bungs or corks), containers that have spigots, or leaking containers will not be accepted.
- Containers must have screw type lids that seal securely.
- **Containers designed to store solid chemicals must not be used to hold liquid chemicals.**
- Chemicals or chemical mixtures with incompatibility must not be packaged in the same package.
- **Use containers of appropriate size for the amount of waste generated. Maximum container size is 10 L (see exceptions in section 3.2.1).**
- **Maximum container size for collecting flammable waste is 5L (flammable waste is waste that has $\geq 40\%$ flammable content).**
- Ensure that containers are not overfilled. Leave space in the container to allow for expansion and contraction of the contents of the container, as well as to allow the contents to be poured without spilling.
- Label each container according to the specification in section 3.2.2.
- Ensure container lids are secured tightly prior to shipping.
- Packages must be strong enough to bear the aggregate weight of all containers within.
- All tops and bottoms of boxes must be taped shut.
- Liquid chemicals must not be contaminated with debris. Ensure that all pipette tips, tubes, etc are segregated from liquid waste.

10L maximum container capacity allows for hand processing by Waste Management. Only tight sealing original chemical containers or liquid waste jugs supplied by Waste Management are acceptable due to safe transportation and handling requirements.

2.2.2 *Lubricating oils waste*

Definition:

Lubricating oils include: various automotive oils, pump oils, hydraulic fluid, etc.

- Packaging requirements are the same as for other liquid chemicals.
- In the case of transformer oil, indicate whether or not PCBs are suspected or known (or if the oil is known to be PCB-free from analysis or purchasing information). Consult with WMF personnel for proper handling and labeling of PCB contaminated waste.

2.2.3 *Gel waste*

Definition:

Gels containing trace amounts of chemicals must be disposed of as hazardous waste.

- Gels must be packaged in wide-mouth, leak-proof, sealable plastic containers that are appropriate for the chemicals present in the gel (consult MSDS).
- Twenty litre pails are acceptable as a container for gels provided that the lid seals water tight and the weight of the container and contents is less than 20 kg.
- Paper, gloves, plastic pipette tips, etc. must not be disposed of with the gels.

2.2.4 *Plastics with chemical residue waste*

Definition:

Plastics that are contaminated with a hazardous chemical residue.

- These plastics must be disposed of according to the hazard of the chemicals present on the plastics
- Collect dry plastic waste in a plastic lined cardboard box.
- Collect wet plastic waste in a wide-mouthed leak-proof container appropriate for the chemicals present (consult MSDS).
- Plastics contaminated with microorganisms must be disposed of as biological waste according to section 2.1.3.

2.2.5 *Equipment containing mercury waste*

Definition:

Any equipment containing mercury such as thermometers, barometers, blood pressure gauges, flasks of mercury, etc.

- Equipment containing mercury must be collected separately from other types of waste.
- Collect this equipment in a puncture-proof, sealable container of appropriate size to contain the mercury and equipment. Remember that the container will also be disposed of as hazardous waste, so do not use an oversize container.
- Label the container according to section 3.2.2.

2.2.6 *Contaminated debris (including empty chemical containers) waste*

Definition:

Contaminated debris refers to any material not otherwise specified in this document that is contaminated with hazardous chemical residues, including plastic bags, paper, gloves, etc, and also includes empty chemical containers.

- Appropriate disposal will depend on the hazards associated with the chemicals and each waste generator should consult the MSDS to determine what the hazards are.

- If the waste generator determines that debris is contaminated with highly toxic or hazardous substance, or if the amount of contamination is great, this waste shall be categorized as hazardous waste and shipped to WMF.
- Contaminated debris that is shipped to WMF may be packaged with contaminated plastics without segregating.
- If it is determined that minute amounts of contamination of low toxicity, corrosivity, flammability or environmental hazardous chemicals are present, this debris can be disposed of in the regular garbage bin after being sealed in a garbage bag.
- Empty chemical containers should be treated the same way as contaminated debris. If the containers contain only trace amounts of low-toxicity, corrosivity, flammability or environmental hazardous chemicals, the containers can be disposed of in the regular garbage bin after the labels are removed or defaced and containers are slashed or broken to prevent reuse. If concentrated contamination is present, triple rinse the containers, collect the rinsate and dispose of the rinsate as hazardous waste. Rinsed clean containers can be disposed of in the regular garbage bin.
- Prior to disposing of any container into the regular garbage bin, deface labels and slash or break the container to prevent reuse.
- Package garbage appropriately to ensure custodial staff are not exposed to any hazards in the laboratory.

2.2.7 *Empty pesticide containers*

Definition:

This type of waste includes any container contaminated with pesticide residue.

- Triple rinse all empty pesticide containers prior to disposal.
- Rinsate must also be collected and disposed of through WMF.
- Empty pesticide containers must not be disposed of with regular garbage.
- **Send rinsed containers to WMF for disposal.**

2.2.8 *Unknown chemicals*

Definition:

The chemical composition characteristics of the chemicals are unknown.

- To determine the general characteristics of the chemical a sample of the chemical must be sent to an analytical lab for analysis.
- It is the responsibility of the waste generator to have the chemical properly analyzed so that the chemical can be disposed of in a safe and environmentally responsible manner.
- Contact the Chemical Safety Manager for more information on disposal of this type of waste.

2.2.9 *Unstable chemicals*

Definition:

Certain chemicals may decompose or dry into potentially unstable compounds that may react or become explosive under certain conditions. Peroxide forming compounds and picric acid are (along with several other compounds) examples of potentially unstable chemicals that require special handling and management.

- Refer to MSDS for precautionary measures with potentially unstable chemicals
- Contact the Chemical Safety Manager for handling and disposal advice.
- Do not handle or disturb potentially unstable compounds until given clearance to do so by the Chemical Safety Manager.

2.2.10 *Gas cylinders*

Definition:

Any cylinder used to hold a gas or **any** container used to hold a liquefied gas at or above atmospheric pressure.

- Gas cylinders are returned by the waste generator to the manufacturer after being used or if no longer needed.
- Many manufacturers will accept returned cylinders, if the cylinders meet the manufacturer's return criteria.
- Cylinders that can not be returned to the manufacturer (cylinders older than ten years or not eligible for re-filling) must be disposed of through a hazardous waste contractor. Disposing of cylinders through a contractor is expensive and should therefore be avoided by promptly returning empty cylinders.
- Any costs associated with the disposal of gas cylinders are the responsibility of the waste generator.
- WMF personnel will act in a **consulting** role with individuals on proper disposal procedures for gas cylinders on a case by case basis.

2.3 **Radioactive Waste**

Radioactive waste is divided into 8 categories with each type of waste requiring storage, handling and disposal practices appropriate for the amount of radioactivity present in the waste. It is the responsibility of the person generating the radioactive waste to ensure that all procedures are followed to ensure safe and environmentally responsible disposal of the waste.

2.3.1 *Dry waste*

2.3.1.1 Dry (DRY)

□ **Definition:**

This type of waste includes laboratory wares (i.e. pipette tips, Petri plates, test tubes, etc.) gloves, absorbent materials, etc. Liquid content must be removed from all containers deemed as dry waste.

- Collect dry waste in a cardboard box lined with a heavy-duty plastic bag. Ensure that the plastic bag is closed prior to sealing the box.
- All tops and bottoms of boxes must be taped shut.
- Remove all radiation warning symbols from material prior to disposing it as waste.
- Specifically describe the physical and chemical nature of the waste on the form.

2.3.1.2 Animal (COM)

Definition:

This type of waste consists of animal tissue, carcasses, body parts, and/or organs.

- The method of disposal of dead animals (whole or parts) is incineration.
- The Radiation Safety Manager will determine the number of animals that can be packaged together to ensure that during incineration the environmental release limit is not exceeded.
- Collect animal waste in a sturdy plastic bag(s).
- Label each bag with the date, name of the radioisotope, and the activity remaining.
- No other objects shall be packed with the animal (i.e. no gloves, scalpels, etc.).
- Animal waste waiting for disposal must be kept in a freezer or designated cold room until removal by WM technicians. Freezers and cold rooms shall be labelled with appropriate radiation signs.
- Animal waste with a substantial amount of activity may have to decay to an acceptable level before it can be incinerated. Therefore, researchers shall ensure that there is adequate space to store the animal waste to allow for decay.
- Animal waste that is infectious and radioactive shall have the biohazard warning label affixed to the bag.

2.3.2 *Liquid scintillation vials (LSV) waste*

Definition:

Plastic or glass vials containing the radioactive sample and liquid scintillant.

- Collect this waste in either plastic pails (with lids) or cardboard boxes lined with a heavy duty plastic bag.
- All tops and bottoms of boxes must be taped shut.

- Plastic vials and glass vials shall be collected separately.
- Do not separate the scintillant from the vial.
- When describing the *chemical nature* of the scintillant, identify the chemical composition.

2.3.3 *Liquid waste*

Liquid waste is waste that is purely in liquid form. It does not contain tissue, paper matter, tubes or any other solid material in the liquid. To avoid contaminating the outside of the waste container use a funnel during the transfer of the waste to the waste container.

2.3.3.1 Aqueous (AQU)

Definition:

This category of waste is comprised of all liquids that are water-soluble, non-toxic and contain a nuclear substance.

- Do not mix this waste with any other non-aqueous liquid waste.
- All liquid waste must be collected in plastic containers of suitable size with lids that tighten securely.
- Recycled plastic containers are acceptable provided the container was previously used to contain a liquid and has a securable lid. (Exception is 4 L milk containers).
- Liquid waste generated from the third and fourth washing of cells or equipment may be released directly to the sewer.

2.3.3.2 Non Aqueous (NAQ)

Definition:

This category of waste includes substances such as flammable, toxic, and/or corrosive liquids ($5.5 > \text{pH} > 9.5$) that contain a nuclear substance. This type of waste is not water soluble.

- Collect this waste in a container (teflon lined plastic) that is compatible with the waste. Ensure the lid tightens securely.
- All liquid waste must be collected in plastic jugs with lids that tighten securely.
- Indicate the chemical composition on the Radioactive Waste Disposal form.

2.3.4 *Unused or Partially Used Radioactive Materials (URM)*

Definition:

Any radioisotope partly used or unused that is futile (i.e. chemically broken down, bacteriological contamination, old or no longer required for research).

- Radionuclides with an activity greater than 100 uCi (370 kBq) must remain in its original shipping vials. Record the volume and activity (correct for decay) on the form.
- Radionuclides with an activity less than 101 uCi (370 kBq) must be pipetted from the shipping vials and added to the aqueous waste. The shipping vials shall be triple rinsed (add rinse to aqueous waste). Deface the radiation warning symbol and dispose of container as “dry waste”.

2.3.5 *Protein Iodination Waste (PRO)*

Definition:

This waste is generated during separation of the desired product from the waste using size exclusion chromatography.

- Discard all waste products such as fraction columns, pipette tips, syringes, gloves, liquids, etc. into a sealable plastic container.
- If the column needs to be broken to fit in the plastic container, break it inside a plastic bag.
- The plastic container(s) used to collect the waste must be small enough to fit into a 4 litre can. The dimensions of the can are 15 cm in height and 16.5 cm in diameter.
- Shield and store these containers in the fume hood until pick up.
- WM technicians will place the waste containers into a metal can at the time of collection.

2.3.6 *Transuranic Waste (TRU)*

Definition:

This waste consists of radioactive elements with an atomic number greater than 89.

This category of waste includes materials such as:

- uranium / thorium ore
 - uranium / thorium / radium chemical compounds
 - uranium tailings
- For collection and packaging consult the Radiation Safety Manager.

2.3.7 *Shipping Vials and Holders*

Definition:

A shipping vial is the primary container that the radioisotope is shipped in. The shipping vial holder is the secondary container which holds the primary container. Some shipping vials holders maybe lead lined.

- Empty shipping vials shall be triple rinsed, radiation warning symbols defaced and then disposed of as dry waste.

- Plastic shipping vial holders must also be defaced of the radiation warning symbol and disposed of to the dry waste container.
- Shipping vial holders with lead lining shall be disposed of through the WMF. These holders should be wipe tested to ensure they are free of contamination prior to pick up.
- If any shipping vial holders remain in the laboratory, ensure that the radiation warning symbol is removed if it is not used to hold nuclear substances.

2.3.8 Unusual Waste (UNW)

For any unusual waste not described in this manual, please consult the Radiation Safety Manager

2.4 Penetrable Waste (sharps, broken glass, etc.)

Definition:

This waste consists of any object that can penetrate the skin. This includes: needles, syringes, scalpel blades, lancets, capillary tubes, broken pipettes, broken glassware (contaminated), broken blood tubes, and broken culture dishes, slides, cover slips, tubing with needles attached, wooden applicator sticks or any other objects that can puncture skin or a plastic bag.

- Items that have come in contact with infectious substances should be autoclaved or inactivated prior to disposal. If this waste can not be autoclaved, the container must have a Biohazard label as well as a sharps label affixed to it. **Do not** attempt to autoclave chemically contaminated sharps.
- All contaminated sharps whether chemical, radiological, or biological can be collected in the same sharps container.
- Collect all sharps waste in specially designed puncture proof plastic containers that are labelled appropriately. (Affix sharps labels and biohazard labels if applicable. See Appendix H)
- The sharps can be picked up by WMF personnel on their weekly biological waste pick up or the sharps containers can be placed inside the Biowaste bin that are picked up by an external waste contractor. If they are being sent to an external waste facility, they must remain in the sharps container separate from the inner sealed bag.
- **Never discard sharps waste loosely into another waste vessel. Sharps must always remain in a sharps container.**
- Collect broken glass in a lined heavy-duty cardboard box and seal all seams with tape. Label the box as “*Waste: Broken Glass*” and leave for the custodial staff to dispose of.

- If broken glass is classified as ‘infectious’ it should be autoclaved and disposed of as above. If it is not autoclaved, this waste should be disposed of in a sharps container and labelled with the sharps and biohazardous labels.
- **Never** discard sharp waste into the regular garbage bin or mix it with other categories of waste.

2.5 Mixed Waste

There are three principal classes of hazardous waste: biological, chemical, and radiological. Each principal class of hazardous waste should be collected separately. In some instances it may not be possible to separate the waste due to the nature of the experiment. Any hazardous waste which contains more than one principal class of hazardous waste is called mixed waste. The table below gives guidelines for mixed waste. Consult a Safety Manager prior to undertaking an experiment which will generate mixed waste.

Infectious and radioactive waste	Identify the waste as biohazardous, affix a Biohazard label, and dispose of the waste according to the procedures for radioactive waste.
Prions and chemical waste	Contact the Biosafety Manager for the appropriate disposal procedures.
Biological and chemical waste	Separate the two classes as much as possible. If the waste is chemical in nature, where infectious agents could survive, consult with the Biosafety Manager.
Chemical and radioactive waste	Treat as radioactive waste and identify the chemicals present.
Biological, chemical and radioactive	If the waste is infectious consult with the Biosafety Manager. If the waste is not infectious follow the procedures given for the disposal of radioactive waste.

3. *Processing Instructions*

3.1 Biohazardous Waste

3.1.1 *Collecting the waste*

Waste that is collected and packaged for disposal must be stored in a cold area (fridge, freezer) until the day of pick up. The waste is then transferred to a designated and locked storage location (facility) by the waste generator. Waste that is designated for the external facility is transported off campus and therefore must be packaged in accordance with the Transportation of

Dangerous Goods regulations and the person offering the container for transport must be TDG certified.

For waste that is designated for WMF, waste generators must ensure that the packages are ready for transport once the hazardous waste disposal form is sent to the WMF.

Adhere to the following guidelines for either category of waste:

- Containers must not weigh more than 20 kg.
- Use the appropriate size and container for collecting the waste.
- Ensure that all containers are structurally capable of withstanding the aggregate weight of all containers within.
- Do not overfill containers. Three quarters full is usually the acceptable quantity for sharps and external biohazard bins.
- Ensure that all inner containers (e.g. plastic bags) are sealed or tied shut.
- Ensure that the lids on containers are secured tightly and boxes are taped shut. For external bins, ensure that the outer lids are tie wrapped on.
- Ensure that all the containers that are offered for pick up are included on the hazardous waste disposal form.

3.1.2 *Labelling the waste*

All waste containers must be properly labelled according to the following requirements:

For shipments off campus:

- The waste generator must determine the type of waste that their bin will fall under (see Appendix C).
- Affix the label corresponding to that type of waste and the external waste contractor's Bar Code label to the bin. These labels incorporate the transportation of dangerous goods UN numbers.
- In order to comply with regulations the generator should record the name, department, building, room number and telephone number of the person that generated the waste. This will be the contact person if more information is required regarding the contents of the container.

For shipments to WMF:

- Labels can be purchased from WMF.
- Each container must be labelled with the appropriate hazardous waste label (see appendix D for Hazardous waste label).
- All fields on the label must be completed in full as follows:
 - Waste Class(es): Circle all the appropriate hazard class(es) for the contents of the container.
 - Chemical or infectious agent or mixture name: Record all chemical or infectious agents that are shipped in the container (use proper shipping names).

- Waste shipment number: Corresponds to the number on the Hazardous Waste Disposal form (see Appendix F).
- Package number: Number of the container within the shipment (e.g. 1 of 20).
- Generator: Record the name, department, building, room number and telephone number of the person that generated the waste. This will be the contact person if WMF personnel require more information about the contents of the container.
- Shipper: Record the name, department, and phone number of the person shipping the waste if the shipper is different from the waste generator, otherwise indicate it is the same individual. This is the person who packaged the waste and arranged for disposal.
- Ensure that the biological waste label is securely attached to the container so that it will not come off during transport.
- All other labels must be removed from the container or otherwise made illegible (painted, scratched out).
- Waste that must be transported from locations off campus must be transported according to federal Transportation of Dangerous Goods (TDG) regulations. There are special requirements for packaging and labelling. Please consult the Biosafety Manager for packaging and labelling and training requirements on a case by case basis.

3.1.3 Storing waste containers

- Store biologicals in a location that provides the maximum amount of safety for personnel in the lab. Remember that custodial and maintenance staff may come into contact with packages of biological waste so ensure that all precautions are taken to avoid unsafe working conditions for all personnel.
- Store biological waste in a cool or cold location (e.g. fridge or freezer if possible) until the day of pick up.
- If there is a designated, non-refrigerated, locked storage facility in your area, transport the biological waste to it on the day of pick up. If there is a designated refrigerated locked facility available, the waste may be transported to it at any time.
- If there isn't a designated storage area assigned to your department or there isn't an internal procedure in place, ensure that WMF and the external waste personnel are aware of the waste storage location so that the waste containers get picked up.

3.1.4 Waste disposal form

- Hazardous waste disposal forms can be purchased from WMF and must be used for all biological waste shipments. (See Appendix F for an example of the form). Each form is uniquely numbered for tracking purposes, so use only one form per shipment. Photocopy the original sheet if there is not sufficient room on one sheet to enter all containers for a shipment.

This will ensure that all containers within a given shipment are labelled with the same waste shipment number.

- Complete all fields on the top of the form as follows:
 - Date: Record the date the form is sent to WMF
 - Record the name, department, building, room number, and telephone number of the person generating the waste (not the shipper if shipper and generator are different).
 - Pick up location: Record the room that the waste is stored in. This is the room that WMF personnel will go to on pick up day.
 - Record the number of containers in the shipment.
- Record an accurate description of the biologicals in each container. This must be the proper biological name of all constituents in the container
- Record the number of containers that correspond to the waste description within each package.
- Total quantity refers to the total quantity of biologicals of a given waste description in the container.
- Record the number of the containers according to the waste label attached to the package.

3.1.5 Requesting waste removal

WMF personnel will pick up biological waste at least one day each week (typically Thursday). In order to transport the biological waste safely and legally, WMF personnel must produce a shipping document for each shipment of biological waste using the information provided on the hazardous waste disposal form. Hazardous waste disposal forms must be received by WMF personnel by 1300 hours on Tuesday to arrange for waste pick up for that week. Forms that are not received by this deadline will not be picked up until the following week. Forms can be either mailed directly to the WMF through interdepartmental mail (Waste Management Facility, 113 North Road), or faxed to 966-6146.

3.2 Chemical Waste

Waste generators must ensure that the packages are ready for transport to WMF once the hazardous waste disposal form is sent to the WMF.

3.2.1 Collecting the waste

Definition:

A container is the primary containment for liquid or solid waste. In the case of 4 litre or 10 litre **plastic** containers, 20 litre pails, and 210 litre drums, the container is also the package and shall have the chemical waste label affixed to it. If multiple containers are included in the same box, this box is considered a package.

- Do not pack incompatible chemical waste in the same package or box (consult MSDS for each chemical).
- Packages must not weigh more than 20 kg (except in the case of drum shipments).
- Use the appropriate size and the correct container for collecting the waste.
 - Maximum 5L for flammable waste.
- If reusing an empty -container for waste collection ensure that the original label is defaced.
- Ensure that all packages are structurally capable of withstanding the aggregate weight of all containers within.
- Ensure that all containers are properly packaged. All containers other than 4 or 10 litre plastic containers, 20 litre pails, or 210 litre drums must be enclosed in a package with sufficient packing material to ensure that the container(s) will not be damaged during transport.
- Ensure that lids on containers are secured tightly and boxes are taped shut.
- Avoid contaminating the outside of the container during filling. Containers and packages with visible signs of external contamination will not be accepted.
- Ensure that all the containers and packages that are offered for pick up are included on the hazardous waste disposal form.
- 20 packages is the maximum that will be accepted per shipment.

3.2.2 *Labelling the waste*

Labels can be purchased from WMF. All waste containers and packages must be properly labelled according to the following requirements:

- Each package must be labelled with the appropriate hazardous waste label (see Appendix D for Hazardous Waste label).
- Each container must always be labelled with the container number and contents prior to being packed. Numbers and content labels must correspond to the information on the Hazardous Waste Disposal form which corresponds to the package in which the containers are held. This label may be hand written as long as it is legible.
- When more than one container is in a package, each container within must be labelled according to this section.
- Use proper chemical names on all labels. Acronyms, trade names, or chemical formulas are not acceptable.
- All fields on the label must be completed in full as follows:
 - Waste Class(es): Circle all the appropriate hazard class(es) for the contents of the container.
 - Chemical or infectious agent or mixture name: Record all chemical or infectious agents that are shipped in the container (use proper shipping names).
 - Waste shipment number: Corresponds to the number on the Hazardous Waste Disposal form (see Appendix F).
 - Package number: Number of the container within the shipment (e.g. 1 of 20).

- Generator: Record the name, department, building, room number and telephone number of the person that generated the waste. This will be the contact person if WMF personnel require more information about the contents of the container.
- Shipper: Record the name, department, and phone number of the person shipping the waste if the shipper is different from the waste generator, otherwise indicate it is the same individual. This is the person who packaged the waste and arranged for disposal.
- Ensure that the chemical waste label is securely attached to the container so that it will not come off during transport.
- All other labels must be removed from the container or otherwise made illegible (painted, scratched out).
- Waste that must be transported from locations off campus must be transported according to federal Transportation of Dangerous Goods (TDG) regulations. There are special requirements for packaging and labelling. Please consult with the WMF person for packaging and labelling and training requirements on a case by case basis.

3.2.3 *Storing waste containers*

- Store waste chemicals in a location that provides the maximum amount of safety for personnel in the lab. Remember that custodial and maintenance staff may come into contact with packages of chemical waste, so ensure that all precautions are taken to avoid unsafe working conditions for all personnel.
- Store chemical waste according to compatibility with other chemical waste in the storage area. Never store incompatible chemical waste in a manner that will allow reactions to occur in the event of a spill or release (refer to MSDS).
- Store waste chemicals for the shortest possible length of time. Do not store waste chemicals for extended periods.
- Ensure that WMF personnel are aware of the waste storage location so that the waste containers get picked up. Choose your location carefully.
- Consider spill containment trays for your waste storage area.

3.2.4 *Waste disposal form*

- Hazardous waste disposal forms can be purchased from WMF and must be used for all chemical waste shipments. (See Appendix F for example of form). Each form is uniquely numbered for tracking purposes, so use only one form per shipment. Photocopy the original sheet if there is not sufficient room on one sheet to enter all packages for a shipment. This will ensure that all packages within a given shipment are labelled with the same waste shipment number.
- Complete all fields on the top of the form as follows:
 - Date: Record the date the form is sent to WMF

- Record the name, department, building, room number, and telephone number of the person generating the waste (not the shipper if shipper and generator are different).
- Pick up location: Record the room that the waste is stored in. This is the room that WMF personnel will go to on pick up day.
- Record the number of packages in the shipment.
- Record an accurate description of the chemicals in each container. This must be the proper chemical name of all constituents in the container. If the chemical is a mixture, record all chemicals that are included in the mixture. Acronyms, trade names, or chemical formulas are not suitable information and must not be used on the hazardous waste disposal form or on the labels on containers or packages.
- Record the proportion of all chemicals in a mixture in the appropriate column. This information must be a percentage (%), do not use concentrations. This information must be accurate to ensure safe disposal of the waste.
- Record the number of containers that correspond to the waste description within each package.
- Total quantity refers to the total quantity of chemicals of a given waste description in the same package.
- Record the number of the package according to the waste label attached to the package.

3.2.5 *Requesting waste removal*

WMF personnel will pick up chemical waste at least one day each week. In order to transport the chemical waste safely and legally, WMF personnel must produce a shipping document for each shipment of chemical waste using the information provided on the hazardous waste disposal form. Hazardous waste disposal forms must be received by WMF personnel by **1300 hours Friday** to arrange for waste pick up the following week. Forms that are not received by this deadline will not be picked up until the second following week. Forms can be either mailed directly to the WMF through interdepartmental mail (Waste Management Facility, 113 North Road), or faxed to 966-6146.

3.3 **Radioactive Waste**

Radioactive waste must **never** be placed in regular garbage bins. It is the responsibility of the Permit Holder and generator to ensure that waste is properly segregated, identified and labelled for disposal.

3.3.1 *Collecting the waste*

- Do not mix beta emitter with gamma emitter waste.
- Do not mix long lived ($T_{1/2} > 1$ year) radioisotope waste with short lived ($T_{1/2} < 1$ year) radioisotope waste.

- Collect all radioactive waste separately according to the categories identified in section 2.3.
- Use the appropriate size and the correct container for collecting waste.
- Ensure that the container is structurally capable of withstanding the aggregate weight of its contents.
- Ensure that lids on containers are secured tightly and boxes are taped shut.
- Containers shall not weigh more than 20 kg.

3.3.2 *Labelling the waste*

Labels can be purchased from DHSE. All waste containers must be properly labelled according to the following requirements:

- Affix a Radioactive Waste Label (see Appendix E) to the container prior to its use. Complete the “type of waste” and “isotope” section. This will reduce the chance of mixing different radioisotopes and categories of waste. Also laboratory workers and custodial staff can readily identify the containers.
- When the waste container is full, complete the rest of the Radioactive Waste label.
- All other labels must be removed from the container or otherwise made illegible (defaced).

3.3.3 *Storing waste containers*

- Store radioactive waste in a location that provides the maximum amount of safety for personnel in the lab. Remember that custodial and maintenance staff may come into contact with packages of hazardous waste, so ensure that all precautions are taken to avoid unsafe working conditions for all personnel.
- Each laboratory shall establish a location for the consolidation of radioactive waste. This location should be in the same laboratory where the waste is generated.
- All liquid waste shall be placed in a tray or on top of enough absorbent material to absorb the liquid in the event of a spill.
- Radioactive waste must be shielded and stored in such a manner that the dose to personnel is as low as reasonably possible. Under no circumstances shall the dose exceed 2.5 $\mu\text{Sv/hr}$ in a radioactive work area.
- Any form of shielding material used around radioactive waste must be designed, constructed, and handled in such a way that it can be easily moved by WM technicians at the time of waste pick up.
- Avoid storing radioactive waste in the laboratory for decay purposes.
- If research has stopped for a time period greater than 1 month, dispose of all radioactive waste immediately, regardless if the container is full or not.

3.3.4 *Waste disposal form*

- Radioactive Waste Disposal forms can be purchased from DHSE. (see Appendix G for an example of the form).
- When completing the form provide specific information for the physical and chemical nature descriptions.
- The total activity for each container shall be determined by the procedures outlined in section 3.3.4.1.
- If the number of containers to be picked up is greater than the space provided on one form, continue onto a new form.
- If the waste is also infectious or contains chemicals, indicate this on the form.
- To request radioactive waste removal from the laboratory, complete a Radioactive Waste Disposal form.
- Mail the first two copies of the form to DHSE. The third copy should be retained for your records. No pick up is authorized unless the Radiation Safety Office receives this form.

3.3.4.1 Calculating the activity in waste

The following is a list of methods one may use to determine the amount of activity in each waste container at the time of disposal.

3.3.4.1.1 *Dry*

Dry waste usually consists of material that may have come in contact with a nuclear substance. Therefore, the activity will be estimated and the estimate should be very low. However, if you dispose of gels or cleaned a spill then the activity can be calculated and recorded accordingly.

3.3.4.1.2 *Liquid*

The activity of liquid waste can be determined by taking a sample (1 ml aliquot) of the waste and counting the sample in a liquid scintillation counter or gamma counter. The total activity can be calculated as follows:

$$\text{total activity in liquid waste} = \frac{\text{Net CPM} \times V}{E \times v \times 2.2} \times 10^{-6} \text{ uCi} \quad (1)$$

where Net CPM = sample CPM - background CPM
 v = volume of the sample (ml)
 V = total volume of the waste (ml)
 E = counting efficiency of equipment

3.3.4.1.3 *Liquid scintillation vials*

There are two methods for determining the amount of activity for liquid scintillation vial waste.

Either keep a running total of the activity disposed to the waste container or determine the average activity and multiply by the number of vials disposed to the waste container. Regardless of which method is used, one must correct for decay.

3.3.4.1.4 Protein iodination

Since all protein iodination waste goes into one waste container, the original amount of activity minus the activity used in the experiment will be the activity disposed.

3.3.4.1.5 Unused nuclear substance

The amount of activity remaining in the shipping vial can be determined from the inventory sheet. Correct for decay.

$$A = A_0 - U$$

Where A_0 = purchased activity
U = total activity used in experiment
A = activity remaining in the shipping vial

3.3.4.1.6 Transuranic waste

Consult the Radiation Safety Manager.

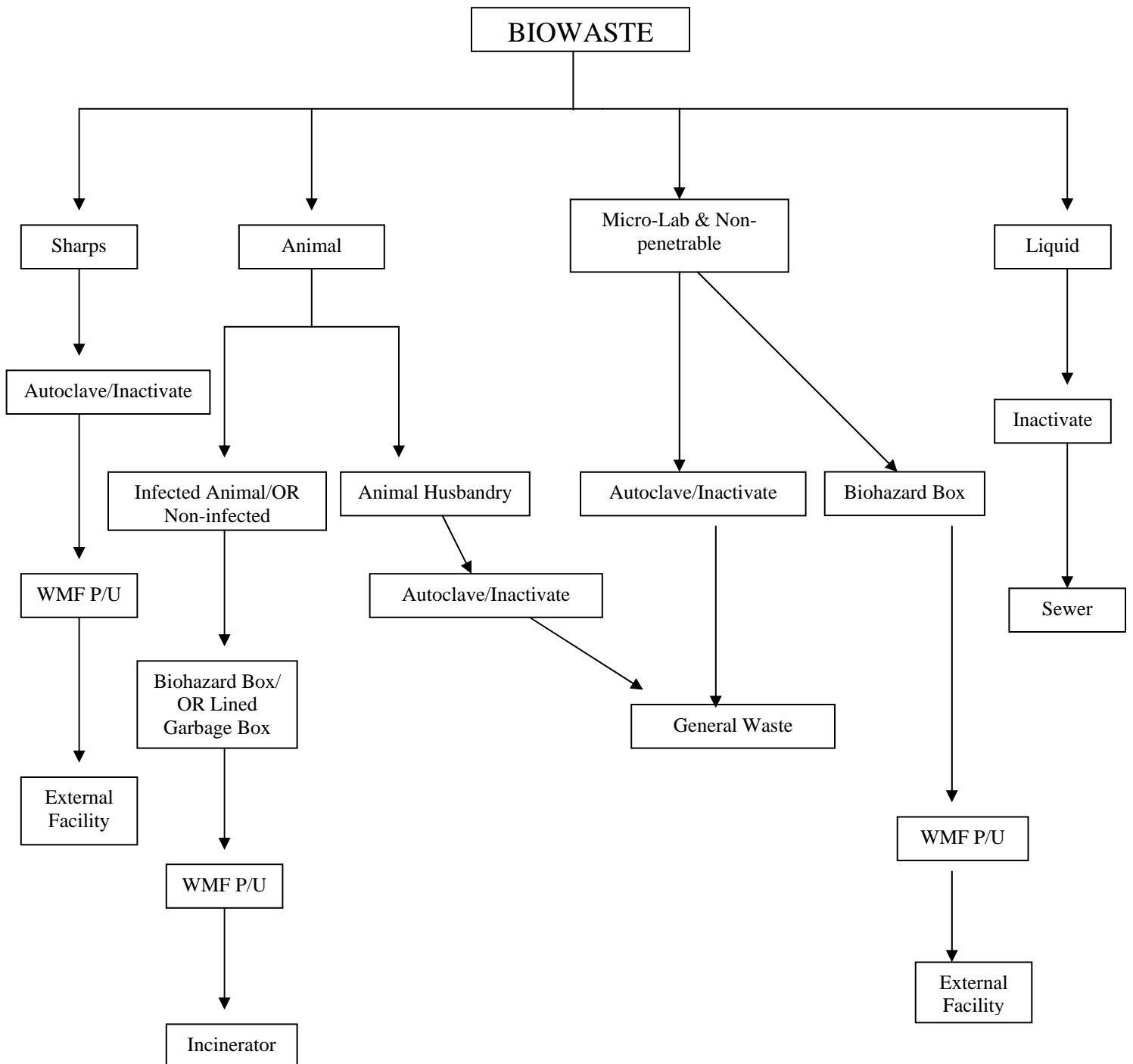
3.3.5 Requesting waste removal

- Removal of radioactive waste from laboratories takes place every Monday. If Monday is a holiday, pick up is the following Wednesday.
- To request removal of radioactive waste from the laboratory forward the Radioactive Waste Disposal form to the Radiation Safety Office, DHSE.
- The Radioactive Waste Disposal form must be received by the Radiation Safety Office by 9:00 a.m. Monday in order to have the waste removed that day.
- WM technicians have the right to refuse pick up of any improperly packaged or labelled waste to ensure their safety.

Appendices

Appendix A	Biological Waste Flowchart
Appendix B	Radioactive Waste Summary Chart
Appendix C	Biological Waste Segregation Labels
Appendix D	Hazardous Waste Label
Appendix E	Radioactive Waste Label
Appendix F	Hazardous Waste Disposal Form
Appendix G	Radioactive Waste Disposal Form
Appendix H	Sharps and Biohazard Labels

Appendix A: Biological Waste Flowchart



Appendix B: Radioactive Waste Summary Table

Category of Waste	Examples	Container Type	Max. Weight	Comments
Dry waste (DRY)	Glass, gloves, bench cover, plastic or metal articles, etc.	Plastic lined cardboard box	20 kg	Do not add liquid waste to this type of waste. Only residual liquid is acceptable.
Animal carcasses (COM)	Carcasses part or whole	Individually packaged in plastic and stored in a fridge/freezer	Consult RSM	Do not package scalpels, gloves, towels, etc. with the animal
Aqueous (AQU)	Soluble liquid which is not poisonous or toxic	Plastic container with a secure lid	10 L	Do not add any test tubes, gels or other solids to this waste.
Non Aqueous (NAQ)	Liquid which is poisonous and/or toxic	Plastic container compatible with waste. Secure lid.	10 L	Do not add any test tubes, gels or other solids to this waste.
Protein Iodination (PRO)	All the waste generated from the procedure.	Plastic container(s) small enough to fit inside a one-gallon can.	n/a	Collect all waste together. Store waste in the fume hood and shield.
Liquid scintillation vials (LSV)	10 ml or 20 ml glass or plastic vials with cocktail solution.	Plastic container with a secure lid or a plastic lined cardboard box.	10 L or 20 kg	Do not mix plastic and glass vials in same waste container.
Unused radioactive material (URM)	Complete or partial shipment.	Original shipping vial and holder.	n/a	Refer to section 2.3.3.3 for instructions.
Transuranic waste (TRU)	Uranium, thorium chemical compounds, ore, waste rock	Metal drums or 20 L plastic pails.	n/a	Contact the RSM
Shipping vial holders	Plastic or lead lined	n/a	n/a	Dispose of plastic holders to the dry waste. Collect the lead holders and send to WMF for recycling.
Sharps	Scalpels, needles, syringes	Sharps container.	4 L	Ensure syringes are totally empty prior to disposal. Not classified as radioactive waste.

Appendix C: Biological Waste Segregation Labels

**INFECTIOUS SUBSTANCE,
AFFECTING HUMANS**

UN 2814

RG 2

HYDROCLAVE

**INFECTIOUS SUBSTANCE,
AFFECTING HUMANS**

UN 2814

**RG 3
or TSE Waste**

INCINERATE


**Human Anatomical
Waste**

INCINERATE




**Cytotoxic or
Pharmaceutical
Waste**

INCINERATE

Appendix D: Hazardous Waste Label

CAUTION - HAZARDOUS WASTE				
Circle Waste Class(es)	Flammable	Pesticide	Poison	Animal Carcasses
	Oxidizer	Corrosive	Infectious	
				DHSE DEPARTMENT OF HEALTH SAFETY & ENVIRONMENT
Chemical or Infectious Agent or Mixture name: _____				
Waste Shipment Form Number _____		Package # _____ of _____		
<u>Generator</u>		<u>Shipper</u>		
Name _____ Dept _____		Name _____		
Ph # _____ - _____ Room _____ Bldg _____		Dept _____ Ph # _____ - _____		
Don't handle if leaking. Wear gloves & avoid contact with package contents.				
Report all incidents to				
Waste Management Facility @ 966-8497 or after hours @ 966-5555				

Appendix E: Radioactive Waste Label

 CAUTION  RAYONNEMENT - DANGER - RADIATION	
TYPE OF WASTE _____	
Waste Disposal Form # _____	Container # _____
Radioisotope _____	Activity _____
Permit Holder _____	
 UNIVERSITY OF SASKATCHEWAN Department of Health, Safety & Environment	In case of an emergency contact: Radiation Safety, DHSE 966-8493 Dept. of Campus Safety 966-5555 (24 hour)
<div style="border: 1px solid black; padding: 5px;">DHSE Use Only Received Date: _____ Disposal Date: _____</div>	

Appendix F: Hazardous Waste Disposal Form

WASTE MANAGEMENT SECTION

HAZARDOUS WASTE DISPOSAL FORM

PLEASE PRINT

Date: _____

Department: _____

Building & Room: _____

Pick up Location _____
(if other than above)

WASTE

SHIPMENT NO.: 13375

Number of packages
in this shipment:

Contact

Name: _____

Telephone: _____

[illegible]

* State type of container such as glass, metal, plastic, etc.

MIXTURES (more than 2 components): Print "**MIXTURE**" and state **total** quantity in container.

Then list all hazardous components and their concentrations within this mixture.

WARNING: DO NOT package incompatibles in the same container! Separate: **FLAMMABLES** **OXIDIZERS** **CORROSIVES**
PESTICIDES **POISONS**

Appendix G: Radioactive Waste Disposal Form

**RADIOACTIVE WASTE DISPOSAL
AUTHORIZATION FORM**

PERMIT HOLDER _____ DEPT. _____

LAS PERMIT NO. _____ BUILDING _____ PHONE NO. _____ DATE _____

SEND TOP TWO COPIES TO
RADIATION SAFETY OFFICE

DESIGNATED WORKER RESPONSIBLE
FOR PACKING RADIOACTIVE WASTE _____

Nº 000847

1	2	3	4	5	6	7	8	9	10
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									

CONTAINER:
List type of container: eg, 55 gal. drum, 4L or 10L plastic jug.
(Indicate jug and drum, available from Waste Management Agency)

TYPE OF WASTE:
List type of waste: eg, radioactive, non-radioactive, LSA, extremely, highly, fissile, fissionable, mixed, etc.
(Indicate type of waste: eg, radioactive, non-radioactive, LSA, extremely, highly, fissile, fissionable, mixed, etc.)

PHYSICAL NATURE:
List type of material: eg, plastic, rubber, glass, shape.
NOTE: If the container is made of glass and shape must be indicated separately.

CHEMICAL NATURE:
List name of chemical: eg, HCl, H₂SO₄, etc.
NOTE: If the container is made of glass and shape must be indicated separately.

VOLUME WEIGHT:
List approximate weight or volume of container in application.

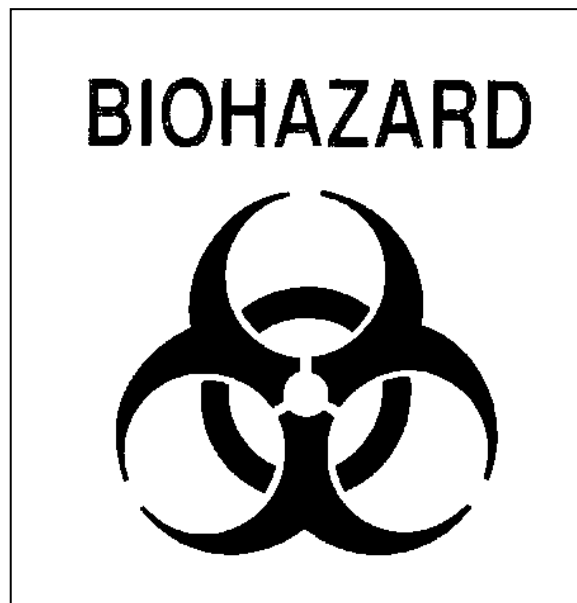
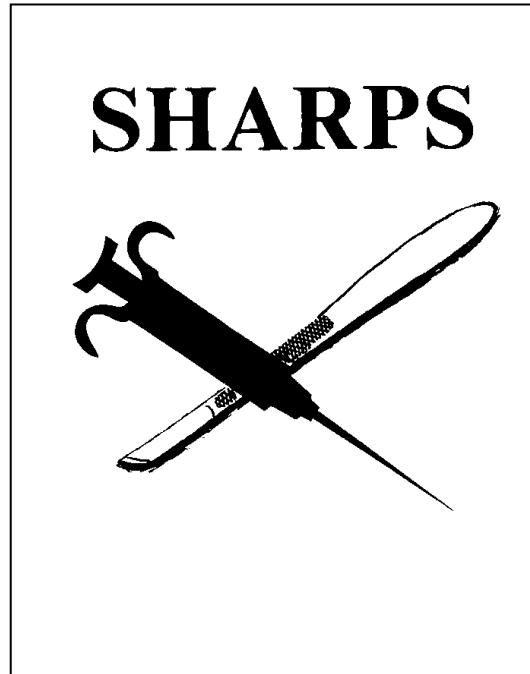
ISOTOPE:
List name of isotope: eg, H-3, C-14, etc.

ACTIVITY:
List activity: Bq or Ci per gram, in each container using necessary Sheet and Flow Chart. (See U of S Radiation Safety Manual for details)

REMARKS:
List additional information or remarks: eg, date, time, etc.

Where waste is not in container - indicate required amount of waste - eg, 100g, 100ml, etc.

Appendix H: Sharps and Biohazard Labels



APPENDIX E

QUALITY ASSURANCE MANUAL FOR COLUMBIA ANALYTICAL SERVICES

QUALITY ASSURANCE MANUAL

Columbia Analytical Services, Inc.

1317 South 13th Avenue

Kelso, Washington 98626

(360) 577-7222

Effective Date: March 1, 2009


Approved by:

Laboratory Director/Technical Director:



Jeff Christian

Quality Assurance Manager:



Julie Gish

Technical Director - Metals:




Jeff Coronado

Technical Director - Inorganics:



Harvey Jacky

Technical Director - Organics:



Jeff Grindstaff

Technical Director - Microbiology:



Lynda Huckestein

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DOCUMENT CONTROL

NUMBER: _____

Initials: _____ Date: _____

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3.0 INTRODUCTION AND COMPANY QUALITY ASSURANCE POLICY

Columbia Analytical Services, Inc. (CAS) is an employee-owned professional analytical services laboratory which performs chemical and microbiological analyses on a wide variety of sample matrices, including drinking water, groundwater, surface water, wastewater, soil, sludge, sediment, tissue, industrial and hazardous waste, and other material.

It is a policy at CAS that there will be sufficient Quality Assurance (QA) activities conducted in the laboratory to ensure that all analytical data generated and processed will be scientifically sound, legally defensible, of known and documented quality, and will accurately reflect the material being tested. This goal is achieved by ensuring that adequate Quality Control (QC) procedures are used throughout the monitoring process, and by establishing a means to assess performance of these Quality Control and other QA activities. Policies and procedures are established in order to meet the quality objectives of clients, accrediting authorities, and certifying organizations. The Quality System is established to meet the requirements of The NELAC Institute (TNI) National Environmental Laboratory Accreditation Program (NELAP).

CAS maintains control of analytical results by adhering to written standard operating procedures (SOPs) and by observing sample custody requirements. All analytical results are calculated and reported in units consistent with project specifications to allow comparability of data.

We recognize that quality assurance requires a commitment to quality by everyone in the organization - individually, within each operating unit, and throughout the entire laboratory.

CAS is a network of laboratories. In addition to the Kelso, WA facility, to which this manual is applicable, CAS also operates laboratories in California, Florida, New York, Arizona, and Texas.

The information in this document has been organized according to the format described in *EPA Requirements for Quality Management Plans, EPA QA/R-2*, USEPA, 2001; and *EPA Requirements for Quality Assurance Project Plans, EPA QA/R-5*, USEPA, 2001.

4.0 PROGRAM DESCRIPTION

The purpose of the QA program at CAS is to ensure that our clients are provided with analytical data that is scientifically sound, legally defensible, and of known and documented quality. The concept of Quality Assurance can be extended, and is expressed in the mission statement of CAS:

"The mission of Columbia Analytical Services, Inc., is to provide high quality, cost-effective, and timely professional testing services to our customers. We recognize that our success as a company is based on our ability to maintain customer satisfaction. To do this requires constant attention to customer needs, maintenance of state-of-the-art testing capabilities and successful management of our most important asset - our people - in a way that encourages professional growth, personal development and company commitment."

In support of this mission, our QA program addresses all aspects of laboratory operations, including laboratory organization and personnel, standard operating procedures, sample management, sample and quality control data, calibration practices, standards traceability data, equipment maintenance records, method proficiency data (such as method detection limit studies and control charts), document control/storage and staff training records.

4.1 Facilities and Equipment

CAS features over 45,000 square feet of laboratory and administrative workspace. The laboratory has been designed and constructed to provide safeguards against cross-contamination of samples and is arranged according to work function, which enhances the efficiency of analytical operations. The ventilation system has been specially designed to meet the needs of the analyses performed in each work space. Also, CAS minimizes laboratory contamination sources by employing janitorial and maintenance staff to ensure that good housekeeping and facilities maintenance are performed. In addition, the segregated laboratory areas are designed for safe and efficient handling of a variety of sample types. These specialized areas (and access restrictions) include:

- Shipping and Receiving/Purchasing
- Sample Management Office, including controlled-access sample storage areas
- Inorganic/Metals Sample Preparation Laboratories (2)
- Inorganic/Metals "clean room" sample preparation laboratory
- ICP-AES Laboratory
- ICP-MS Laboratory
- AA Laboratory
- Water Chemistry & General Chemistry Laboratories (3)
- Semi-volatile Organics Sample Preparation Laboratory
- Gas Chromatography/High Performance Liquid Chromatography Laboratories (2)

- Gas Chromatography/Mass Spectrometry Laboratory
- Petroleum Hydrocarbon Laboratory
- Semi-volatile Organics Drinking Water Laboratories (2)
- Volatile Organics Laboratory
 - Separate sample preparation laboratory
 - Access by semi-volatile sample preparation staff only after removing lab coat and solvent-contaminated gloves, etc.
- Microbiology Laboratory
- Laboratory Deionized Water Systems (2)
- Laboratory Management, Client Service, Report Generation and Administration
- Data Archival, Data Review and support functions areas
- Information Technology (IT) and LIMS

In addition, the designated areas for sample receiving, refrigerated sample storage, dedicated sample container preparation and shipping provide for the efficient and safe handling of a variety of sample types. Figure 4-1 shows the facility floor plan. The laboratory is equipped with state-of-the-art analytical and administrative support equipment. The equipment and instrumentation are appropriate for the procedures in use. Appendix C lists the major equipment, illustrating the laboratory's overall capabilities and depth.

4.2 Technical Elements of the Quality Assurance Program

The Quality Assurance Program provides a platform on which technical operations are based. The program provides laboratory organization, procedures, and policies by which the laboratory operates. The necessary certifications and approvals administered by external agencies are maintained. This includes method approvals and audit administration. In addition, internal audits are performed to assess compliance with policies and procedures. Standard Operating Procedures (SOPs) are maintained for technical and administrative functions. A document control system is used for SOPs, as well as laboratory notebooks, and this QA Manual. A list of QA Program documents is provided in Appendix A.

Acceptable calibration procedures are defined in the SOP for each test procedure. Calibration procedures for other laboratory equipment (balances, thermometers, etc.) are also defined. Quality Control (QC) procedures are used to monitor the testing performed. Each analytical procedure has associated QC requirements to be achieved in order to demonstrate data quality. The use of method detection limit studies, control charting, technical training and preventative maintenance procedures further ensure the quality of data produced. Proficiency Testing (PT) samples are used as an external means of monitoring the quality and proficiency of the laboratory. PT samples are obtained from qualified vendors and are performed on a regular basis. In addition to method proficiency, documentation of analyst training is performed to ensure proficiency and competency of laboratory analysts and technicians. Sample handling and custody procedures are defined in SOPs. Procedures are also in place to monitor the sample storage areas. The technical elements of the QA program are discussed in further detail in later sections of this QA manual.

4.3 Operational Assessments

There are a number of methods used to assess the laboratory and its daily operations. In addition to the routine quality control (QC) measurements to measure quality, the senior laboratory management examines a number of other indicators to assess the overall ability of the laboratory to successfully perform analyses for its clients. On-time performance, report quality, training, and Quality Assurance are a few of the items that are used to assess performance from an external perspective. A frequent, routine assessment must also be made of the laboratory's facilities and resources in anticipation of accepting an additional or increased workload.

CAS utilizes a number of different methods to ensure that adequate resources are available in anticipation of the demand for service. Regularly scheduled senior staff meetings, tracking of outstanding proposals and an accurate, current synopsis of incoming work all assist the senior staff in properly allocating resources to achieve the required results. All Requests for Proposal (RFP) documents are reviewed by the Project Chemist and appropriate managerial staff to identify any project specific requirements that differ from the standard practices of the laboratory. Any requirements that cannot be met are noted and communicated to the client, as well as requesting the client to provide any project specific Quality Assurance Plans (QAPPs) if available. A weekly status meeting is also conducted with the laboratory staff by the Client Services Manager to inform the staff of the status of incoming work, future projects, or project requirements.

4.4 Document Control

Procedures for control and maintenance of documents are described in the *SOP for Document Control (ADM-DOC_CTRL)*. The procedures described in the SOP include distribution, tracking, filing, and copyrighting of CAS controlled documents. The requirements of the SOP apply to all standards preparation logbooks, instrument maintenance logbooks, run logbooks, certificates of analysis, standard operating procedures (SOPs), quality assurance manuals (QAMs), quality assurance project plans (QAPPs), Environmental Health & Safety (EHS) manuals, and other controlled CAS documents.

Each controlled copy of a controlled document will be released only after a document control number is assigned and the recipient is recorded on a document distribution list. Filing and distribution is performed by the Quality Assurance Manager, or designee, and ensure that only the most current version of the document is distributed and in use. A document control number is assigned to logbooks. Completed logbooks that are no longer in use are archived in a master logbook file.

CAS maintains a records system that ensures all laboratory records (including raw data, reports, and supporting records) are retained and available. The archiving system is described in the *SOP for Data Archiving (ADM-ARCH)*.

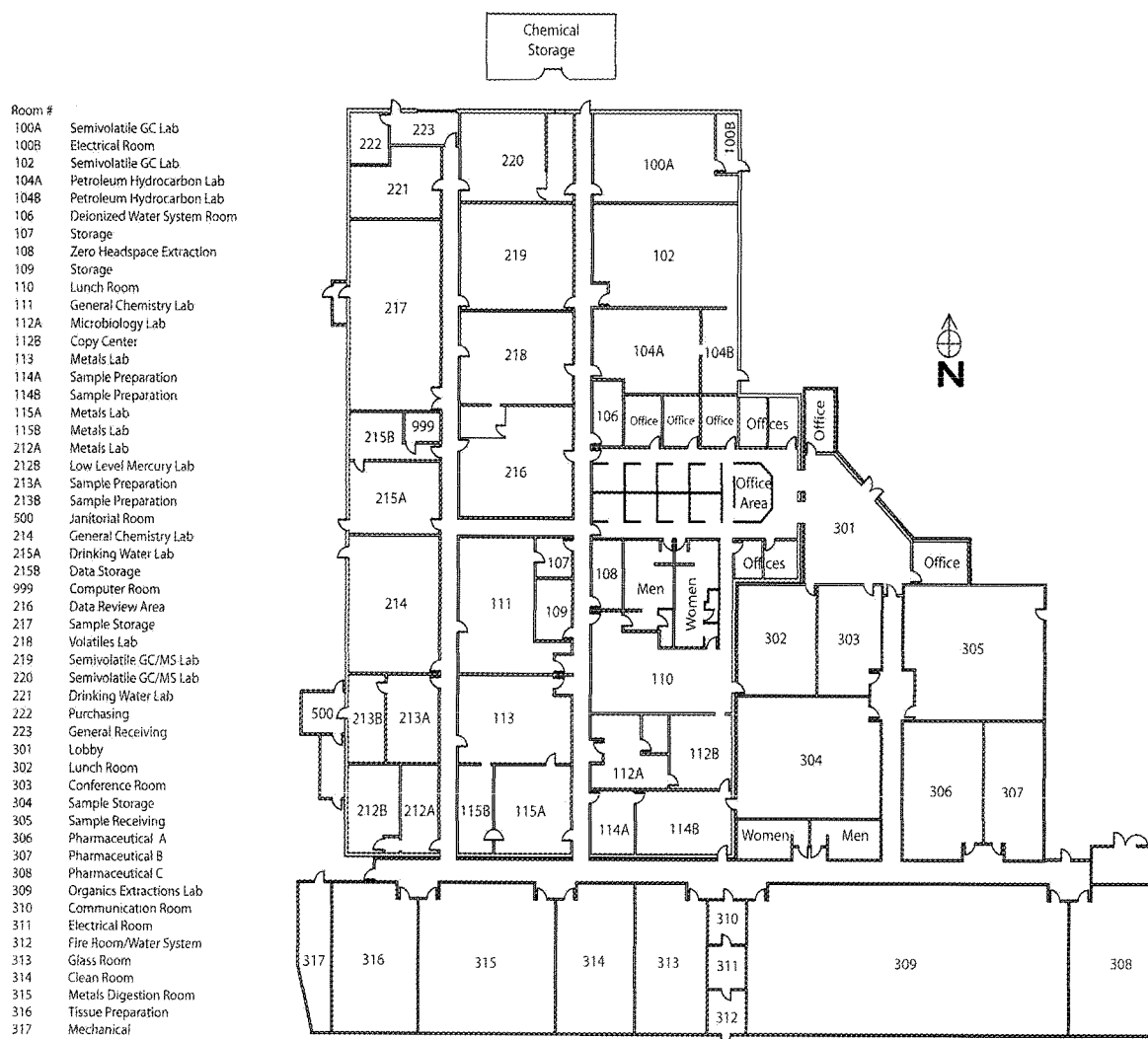
4.5 Subcontracting

Analytical services are subcontracted when CAS/Kelso needs to balance workload or when the requested analyses are not performed by CAS/Kelso. Subcontracting is only done with the knowledge and approval of the client. Subcontracting to another CAS laboratory is preferred over external-laboratory subcontracting. Further, sub-contracting is done using capable and qualified laboratories. Established procedures are used to qualify external subcontract laboratories. These procedures are described in the *SOP for Qualification of Subcontract Laboratories Outside of CAS Network (ADM-SUBLAB)*. The Corporate Quality Assurance staff is responsible for qualifying and oversight of subcontract laboratories.

4.6 Procurement

The quality level of reagents and materials (grade, traceability, etc.) required is specified in analytical SOPs. Department supervisors ensure that the proper materials are purchased. Inspection and verification of material ordered is performed at the time of receipt by receiving personnel. The receiving staff labels the material with the date received. Expiration dates are assigned (by the laboratory user) as appropriate for the material. Storage conditions and expiration dates are specified in the analytical SOP. The procedures for purchasing and procurement are described in the *SOP for Purchasing through CAS Purchasing Department in Kelso (SOP ADM-PUR)*. Also, refer to section 10.4 for a discussion of reference materials.

Figure 4-1
CAS/Kelso Laboratory Floor Plan



5.0 PROFESSIONAL CONDUCT AND ETHICAL PRACTICES

One of the most important aspects of the success of CAS is the emphasis placed on the integrity of the data provided and services performed. To promote product quality, employees are required to comply with certain standards of conduct and ethical practices. The following examples of CAS policy are representative of these standards, and are not intended to be limiting or all-inclusive:

- Under no circumstances is the willful act of fraudulent manipulation of analytical data condoned. Such acts are to be reported immediately to senior management for appropriate corrective action. Unless specifically required in writing by a client, alteration, deviation or omission of written contractual requirements is not permitted. Such changes must be in writing and approved by senior management.
- Falsification of data in any form will not be tolerated. While much analytical data is subject to professional judgment and interpretation, outright falsification, whenever observed or discovered, will be documented, and appropriate remedies and punitive measures will be taken toward those individuals responsible. Employee discipline is progressive in its severity and each situation is handled individually in that the discipline is designed to fit the circumstances. Potential disciplinary actions may include a verbal warning, written warning, a second written notice (more severe and more strongly worded than a warning), suspension without pay, demotion, or termination.
- It is the responsibility of all CAS employees to safeguard sensitive company and client information. The nature of our business and the well being of our company and of our clients is dependent upon protecting and maintaining proprietary company/client information. All information, data, and reports (except that in the public domain) collected or assembled on behalf of a client is treated as confidential. Information may not be given to third parties without the consent of the client. Unauthorized release of confidential information about the company or its clients is taken seriously and is subject to formal disciplinary action.

All employees are required to sign and adhere to the requirements set forth in the CAS *Confidentiality and Conflicts of Interest Employee Agreement* and the CAS *Commitment to Excellence in Data Quality Policy*. All employees receive in-house ethics training and are periodically reminded of their data quality and ethical conduct responsibilities.

CAS makes every attempt to ensure that employees are free from any commercial, financial, or other undue pressures that might affect their quality of work. Related policies are described in the CAS Employee Handbook. This includes the CAS Ombudsman Program, the CAS Open Door Policy, and the use of flexible work hours. Operational assessments are regularly made to ensure that project planning is performed and that adequate resources are available during anticipated periods of increased workloads (Section 4.3). Procedures for subcontracting work are established, and within the CAS laboratory network additional capacity is typically available for subcontracting, if necessary.

6.0 ORGANIZATION AND RESPONSIBILITIES

The CAS/Kelso staff, consisting of approximately 130 employees, includes chemists, technicians and support personnel. They represent diverse educational backgrounds and experience, and provide the comprehensive skills that the laboratory requires. During seasonal workload increases, additional temporary employees may be hired to perform specific tasks.

CAS is committed to providing an environment that encourages excellence. Everyone within CAS shares responsibility for maintaining and improving the quality of our analytical services. The responsibilities of key personnel within the laboratory are described below. Table 6-1 lists the CAS/Kelso personnel assigned to these key positions. Managerial staff members are provided the authority and resources needed to perform their duties. An organizational chart of the laboratory, as well as the resumes of these key personnel, can be found in Appendix B.

- The role of the **Laboratory Director** is to provide technical, operational, and administrative leadership through planning, allocation and management of personnel and equipment resources. The Laboratory Director provides leadership and support for the QA program and is responsible for overall laboratory efficiency and the financial performance of the Kelso facility. The Laboratory Director has the authority to stop work in response to quality problems. The Laboratory Director also provides resources for implementation of the QA program, reviews and approves this QA Manual, reviews and approves standard operating procedures (SOPs), and provides support for business development by identifying and developing new markets through continuing support of the management of existing client activities.
- The responsibility of the **Quality Assurance Manager (QAM)** is to oversee implementation of the quality program and to coordinate QA activities within the laboratory. The QAM works with laboratory production units to establish effective quality control and assessment plans. The QAM has the authority to stop work in response to quality problems. The QAM is responsible for maintaining the QA Manual and performing an annual review of it; reviewing and approving SOPs and coordinating the annual review of each SOP; maintaining QA records such as metrological records, archived logbooks, PT sample results, etc.; document control; conducting PT sample studies; approving nonconformity and corrective action reports; maintaining the laboratory's certifications and approvals; performing internal QA audits; preparing QA activity reports; etc. The QAM reports directly to the Laboratory Director. The QAM also interacts with the CAS Quality Assurance Director. It is important to note that when evaluating data, the QAM does so in an objective manner and free of outside, or managerial, influence.

The Chief Quality Officer (CQO) is responsible for the overall QA program at all the CAS laboratories. The CQO is responsible for ensuring that annual internal audits are performed at each CAS laboratory; maintaining a data base of information about state certifications and accreditation programs; writing laboratory-wide SOPs; maintaining a data base of CAS-approved subcontract laboratories; providing assistance to the laboratory QA staff and laboratory managers; preparing a quarterly QA activity report; etc.

- In the case of absence of the Laboratory Director or QA Manager, deputies are assigned to act in that role. Default deputies for these positions are the Client Services Manager or Organics Department Manager (for the Laboratory Director) and the CQO or Laboratory Director (for the QA Manager).
- The **Environmental Health and Safety Officer** (EH&S) is responsible for the administration of the laboratory health and safety policies. This includes the formulation and implementation of safety policies, the supervision of new-employee safety training, the review of accidents, incidents and prevention plans, the monitoring of hazardous waste disposal and the conducting of departmental safety inspections. The EH&S officer is also designated as the Chemical Hygiene Officer. The EH&S Officer has a dotted-line reporting responsibility to CAS' EH&S Director.
- The **Client Services and Sample Management Office Manager** is responsible for the Client Services Department (customer services/project chemists, and Electronic Data Deliverables group) and the sample management office/bottle preparation sections. The Client Services Department provides a complete interface with clients from initial project specification to final deliverables. The sample management office handles all the activities associated with receiving, storage, and disposal of samples. The Client Services Manager has the authority to stop subcontractor work in response to quality problems.
- The **Project Chemist** is a senior-level scientist assigned to each client to act as a technical liaison between the client and the laboratory. The project chemist is responsible for ensuring that the analyses performed by the laboratory meet all project, contract, and regulatory-specific requirements. This entails coordinating with the CAS laboratory and administrative staff to ensure that client-specific needs are understood, and that the services CAS provides are properly executed and satisfy the requirements of the client.
- The Analytical Laboratory is divided into operational units based upon specific disciplines. Each department is responsible for establishing, maintaining and documenting a quality control program based upon the unique requirements within the department. Each **Department Manager and Supervisor** has the responsibility to ensure that quality control functions are carried out as planned, and to guarantee the production of high quality data. Department managers and bench-level supervisors have the responsibility to monitor the day-to-day operations to ensure that productivity and data quality objectives are met. Each department manager has the authority to stop work in response to quality problems in their area. Analysts have the responsibility to carry out testing according to prescribed methods, SOPs, and quality control guidelines particular to the laboratory in which he/she is working.
- The **Sample Management Office** plays a key role in the laboratory QA program by maintaining documentation for all samples received by the laboratory, and by assisting in the archival of all laboratory results. The sample management office staff is also responsible for the proper disposal of samples after analysis.
- **Information Technology** (IT) staff are responsible for the administration of the Laboratory Information Management System (LIMS) and other necessary support services. Other functions of the IT staff include laboratory network maintenance, IT systems development and implementation, education of analytical staff in the use of scientific software, Electronic Data Deliverable (EDD) generation, and data back-up, archival and integrity operations.

Table 6-1
Summary of Technical Experience and Qualifications

Personnel	Years of Experience	Project Role
Jeff Christian, B.S.	30	Laboratory Director
Julie Gish, M.S.	18	Quality Assurance Manager
Lynda Huckestein, B.S.	20	Client Services Manager Sample Management Office Manager
Jeff Coronado, B.S.	19	Metals Department Manager
Harvey Jacky, B.S.	20	General Chemistry Department Manager
Gregory Salate, Ph.D.	9	Extractions Department Manager
Jeff Grindstaff, B.S.	20	Organics Chromatography & Mass Spectrometry Department Manager
Loren Portwood, B.S.	18	Organics Drinking Water Department Manager
Eileen Arnold, B.A.	27	Environmental Health and Safety Officer
Mike Sullivan, B.S.	8	CAS Information Technology Director
Lee Wolf, B.S.	23	CAS Chief Quality Officer
Steve Vincent, B.S.	33	CAS President

7.0 INFORMATION MANAGEMENT

The generation, compilation, reporting, and archiving of electronic data is a critical component of laboratory operations. In order to generate data of known and acceptable quality, the quality assurance systems and quality control practices for electronic data systems must be complete and comprehensive and in keeping with the overall quality assurance objectives of the organization. CAS management provides the tools and resources to implement electronic data systems and establishes information technology standards and policies. Appendix C lists major automated data processing equipment.

7.1 Software Quality Assurance Plan

CAS has defined practices for assuring the quality of the computer software used throughout all laboratory operations to generate, compile, report, and store electronic data. These practices are described in the CAS Software Quality Assurance Plan (SQAP). The purpose of the SQAP is to describe the policies and practices for the procurement, configuration management, development, validation and verification, data security, maintenance, and use of computer software. The policies and practices described in the plan apply to purchased computer software as well as to internally developed computer software. Key components of configuration management plan are policies for controlling the software version that is in use in the laboratory.

7.2 IT Support

The local CAS Information Technology (IT) department is established to provide technical support for all computing systems. The IT department staff continually monitors the performance and output of operating systems. The IT department oversees routine system maintenance and data backups to ensure the integrity of all electronic data. A software inventory is maintained. Additional IT responsibilities are described in the SQAP.

In addition to the local IT department, CAS corporate IT provides support for network-wide systems. CAS also has personnel assigned to information management duties such as development and implementation of reporting systems; data acquisition, and Electronic Data Deliverable (EDD) generation.

7.3 Information Management Systems

CAS has various systems in place to address specific data management needs. The CAS Laboratory Information Management System (LIMS) is used to manage sample information and invoicing. Access is controlled by password. This system is used to establish and define sample identification, analysis specifications, and provide a means of sample tracking. This system is used during sample login to generate the internal Service Request. The Service

Request provides a summary of client information, sample information, required analyses, work instructions, deliverable requirements and other necessary information provided on the chain of custody. The LIMS also is the basis for valuable sample tracking mechanisms used throughout the laboratory. Laboratory analysts generate responsibility reports from the LIMS and perform internal chain of custody via the LIMS.

Where possible, instrument data acquired locally is immediately moved to a server (Microsoft Windows2003® domain). This provides a reliable, easily maintained, high-volume acquisition and storage system for electronic data files. With password entry, users may access the system from many available computer stations, improving efficiency and flexibility. The server is also used for data reporting, EDD generation, and administrative functions. Access to these systems is controlled by password. A standardized EDI (electronic data interchange) format is used as a reporting platform, providing functionality and flexibility for end users. With a common standardized communication platform, the EDI provides data reporting in a variety of hardcopy and electronic deliverable formats, including Staged Electronic Data Deliverable (SEDD) format.

7.4 Backup and Security

CAS laboratory data is either acquired directly to the centralized acquisition server or acquired locally and then transferred to the server. All data is eventually moved to the centralized data acquisition server for reporting and archiving. Differential backups are performed on all file server information once per day, Sunday through Thursday. Full backups are performed each Friday night. Tapes are physically stored in a locked media cabinet within a locked, temperature controlled computer room, with every other full backup also securely stored offsite.

Access to sample information and data is on a need-to-know basis. Access is restricted to the person's areas of responsibility. Passwords are required on all systems. No direct external, non-CAS access is allowed to any of our network systems.

The external e-mail system and Internet access is established via a single gateway to discourage unauthorized entry. CAS uses a closed system for company e-mail. Files, such as electronic deliverables, are sent through the external e-mail system only via a trusted agent. The external messaging system operates through a single secure gateway. Email attachments sent in and out of the gateway are subject to a virus scan. Because the Internet is not regulated, we use a limited access approach to provide a firewall for added security. Virus screening is performed continuously on all network systems.

8.0 SAMPLE MANAGEMENT

8.1 Sampling and Sample Preservation

The quality of analytical results is highly dependent upon the quality of the procedures used to collect, preserve and store samples. CAS recommends that clients follow sampling guidelines described in 40 CFR 136, 40 CFR 141, USEPA SW-846, and state-specific sampling guidelines, if applicable. Sampling factors that must be taken into account to insure accurate, defensible analytical results include:

- Amount of sample taken
- Type of container used
- Type of sample preservation
- Sample storage time
- Proper custodial documentation

CAS uses the sample preservation, container, and holding-time recommendations published in a number of documents. The primary documents of reference are: USEPA SW-846, Third Edition and Updates I, II, IIA, IIB, III, IV for hazardous waste samples; USEPA 600/4-79-020, 600/4-91-010, 600/4-82-057, 600/R-93/100, 600/4-88-039, 600/R-94-111, and Supplements; EPA 40CFR parts 136 and 141; and *Standard Methods for the Examination of Water and Wastewater* for water and wastewater samples (see Section 18 for complete citations). The container, preservation and holding time information for these references is summarized in Table 8-1 for soil, water, and drinking water. The current EPA CLP Statement of Work should be referred to for CLP procedures. Where allowed by project sampling and analysis protocols (such as Puget Sound Protocols) the holding time for sediment, soil, and tissue samples may be extended for a defined period when stored frozen at -20°C.

CAS routinely provides sample containers with appropriate preservatives for our clients. Containers are purchased as precleaned to a level 1 status, and conform to the requirements for samples established by the USEPA. Certificates of analysis for the sample containers are available to clients if requested. Reagent water used for sampling blanks (trip blanks, etc.) and chemical preservation reagents are tested by the laboratory to ensure that they are free of interferences and documented. Our sample kits typically consist of foam-lined, precleaned shipping coolers, (cleaned inside and out with appropriate cleaner, rinsed thoroughly and air-dried), specially prepared and labeled sample containers individually wrapped in protective material, (VOC vials are placed in a specially made, foam holder), chain-of-custody (COC) forms, and custody seals. Container labels and custody seals are provided for each container.

Figure 8-1 shows the chain-of-custody form routinely used at CAS and included with sample kits. For large sample container shipments, the containers may be shipped in their original boxes. Such shipments will consist of several boxes of labeled sample containers and sufficient materials (bubble wrap, COC forms, custody seals, shipping coolers, etc.) to allow the sampling personnel to process the sample containers and return them to CAS. The proper preservative is added to the sample containers prior to shipment, unless otherwise instructed by the client.

If any returning shipping cooler exhibits an odor or other abnormality after receipt and subsequent decontamination by laboratory personnel, a second, more vigorous decontamination process is employed. Containers exhibiting an odor or abnormality after the second decontamination process are promptly and properly discarded. CAS keeps client-specific shipping requirements on file and utilizes major transportation carriers to guarantee that sample shipping requirements (same-day, overnight, etc.) are met. CAS also provides courier service that makes regularly scheduled trips to the Greater Portland, Oregon Metropolitan area.

When CAS ships environmental samples to other laboratories for analysis each sample bottle is wrapped in protective material and placed in a plastic bag (preferably Ziploc®) to avoid any possible cross-contamination of samples during shipping. The sample management office (SMO) follows formalized procedures for maintaining the chain of custody of the sample(s) (*SOP for Chain of Custody for Sample Transfer between Laboratories* [SOP ADM-COC]), proper packaging and shipment, specification of proper methodology, etc. Blue or gel ice is the only temperature preservative used by CAS, unless otherwise specified by the client or receiving laboratory.

8.2 Sample Receipt and Handling

Standard Operating Procedures are established for the receiving of samples into the laboratory. These procedures ensure that samples are received and properly logged into the laboratory, and that all associated documentation, including chain of custody forms, is complete and consistent with the samples received. Complete documentation of all sample storage is maintained in order to preserve the integrity of the samples.

Once samples are delivered to the CAS sample management office (SMO), a Cooler Receipt and Preservation Check Form (CRF - See Figure 8-2 for an example) is used to assess the shipping cooler and its contents as received by the laboratory personnel. Verification of sample integrity includes the following activities:

- Assessment of custody seal presence/absence, location and signature;
- Temperature of sample containers upon receipt;
- Chain of custody documents properly used (entries in ink, signature present, etc.);
- Sample containers checked for integrity (broken, leaking, etc.);

- Sample is clearly marked and dated (bottle labels complete with required information);
- Appropriate containers (size, type) are received for the requested analyses;
- The minimum amount of sample material is provided for the analysis.
- Sample container labels and/or tags agree with chain of custody entries (identification, required analyses, etc.);
- Assessment of proper sample preservation (if inadequate, corrective action is employed); and
- VOC containers are inspected for the presence/absence of bubbles. (Assessment of proper preservation of VOC containers is performed by lab personnel).

Samples are logged into a Laboratory Information Management System (LIMS). Any anomalies or discrepancies observed during the initial assessment are recorded on the CRF and COC documents. Potential problems with a sample shipment are addressed by contacting the client and discussing the pertinent issues. When the Project Chemist and client have reached a satisfactory resolution, the login process may continue and analysis may begin. During the login process, each sample is given a unique laboratory code and a service request form is generated. The LIMS generates a Service Request that contains client information, sample descriptions, sample matrix information, required analyses, sample collection dates, analysis due dates and other pertinent information. The service request is reviewed by the appropriate Project Chemist for accuracy, completeness, and consistency of requested analyses and for client project objectives.

Samples are stored as per method requirements until they undergo analysis, unless otherwise specified, using various refrigerators or freezers, or designated secure areas. CAS has five walk-in cold storage units which house the majority of sample containers received at the laboratory. In addition, there are four additional refrigerators, including dedicated refrigerated storage of VOC samples. The dedicated storage areas for VOC samples are monitored using storage blanks, as described in the *SOP for VOA Storage Blanks (VOC-BLAN)*. CAS also has seven sub-zero freezers capable of storing samples at -20° C primarily used for tissue and sediment samples requiring specialized storage conditions. The temperature of each sample storage unit is monitored daily and the data recorded in a bound logbook. Continuous-graph temperature recorders have also been placed in the walk-in refrigerators to provide a permanent record of the storage conditions to which samples are exposed.

CAS adheres to the method-prescribed or project-specified holding times for all analyses. The sampling date and time are entered into the LIMS system at the time of sample receipt and login. Analysts then monitor holding times by obtaining analysis-specific reports from the LIMS. These reports provide holding time information on all samples for the analysis, calculated from the sampling date and the holding time requirement. To document holding time compliance, the date and time analyzed is printed or written on the analytical raw data. For analyses with a holding time prescribed in hours it is essential that the sample collection time is provided, so holding time compliance can be demonstrated. If not, the sample collection time is assumed as the earliest in the day (i.e. the most conservative).

Unless other arrangements have been made in advance, upon completion of all analyses and submittal of the final report, aqueous samples and sample extracts are retained at ambient temperature for 30 days, soil samples are retained at ambient temperature for 60 days, and tissue samples are retained frozen for 3 months. Upon expiration of these time limits, the samples are either returned to the client or disposed of according to approved disposal practices. All samples are characterized according to hazardous/non-hazardous waste criteria and are segregated accordingly. All hazardous waste samples are disposed of according to formal procedures outlined in the *CAS Environmental Health and Safety Manual*. All waste produced at the laboratory, including the laboratory's own various hazardous waste streams, is treated in accordance with applicable local and Federal laws. Documentation is maintained for each sample from initial receipt through final disposal to ensure that an accurate history of the sample from "cradle to grave" is available.

8.3 Sample Custody

Sample custody transfer at the time of sample receipt is documented using chain-of-custody (COC) forms accompanying the samples. During sample receipt, it is also noted if custody seals were present. This is described in the *SOP for Sample Receiving (SMO-GEN)*. Figure 8-1 is a copy of the chain-of-custody form routinely used at CAS.

Facility security and access is important in maintaining the integrity of samples received at CAS/Kelso. Access to the laboratory facility is limited by use of locked exterior doors with a coded entry, except for the reception area and sample receiving doors, which are manned during business hours and locked at all other times. In addition, the sample storage area within the laboratory is a controlled access area with locked doors with a coded entry. The CAS facility is equipped with an alarm system and CAS employs a private security firm to provide nighttime and weekend security.

A barcoding system is used to document internal sample custody. Each person removing or returning samples from/to sample storage while performing analysis is required to document this custody transfer. The system uniquely identifies the sample container and provides an electronic record of the custody of each sample. For sample extracts and digestates the analyst documents custody of the sample extract or digestate by signing on the benchsheet, or custody record, that they have accepted custody. The procedures are described in the *SOP for Sample Tracking and Internal Chain of Custody (SMO-SCOC)*.

8.4 Project Setup

The analytical method(s) to be used for sample analysis are chosen based on the client's requirements. Unless specified otherwise, the most recent versions of reference methods are used. For SW-846 methods, some projects may require the most recent *promulgated* version, and some projects may require the most recent *published* version. The Project Chemist will ensure that the correct method version is used. LIMS codes are chosen to identify the analysis method used for analysis. The Project Chemist ensures that the correct methods are selected for analysis, deliverable requirements are identified, and due dates are specified on the LIMS generated Service Request. To communicate and specify project-specific requirements, a Tier V form (Figure 8-3) is used and accompanies the service request form.

Table 8-1
Sample Preservation and Holding Times

DETERMINATION ^a	MATRIX ^b	CONTAINER ^c	PRESERVATION	MAXIMUM HOLDING TIME
Bacterial Tests				
Coliform, Colilert (Standard Methods)	W, DW	P, Bottle or Bag	Cool, 4°C, 0.008% Na ₂ S ₂ O ₃ ^d	6-24 hours ^e
Coliform, Fecal and Total (Standard Methods)	W, DW	P,G	Cool, 4°C, 0.008% Na ₂ S ₂ O ₃ ^d	6-24 hours ^e
Fecal Streptococci (SM 9230B)	W	P,G	Cool, 4°C, 0.008% Na ₂ S ₂ O ₃ ^d	6-24 hours ^e
Inorganic Tests				
Acidity (SM 2310B)	W	P,G	Cool, 4°C	14 days ^{EPA}
Alkalinity (SM 2320B)	W, DW	P,G	Cool, 4°C	14 days ^{EPA}
Ammonia (SM 4500NH ₃)	W, DW	P,G	Cool, 4°C, H ₂ SO ₄ to pH<2	28 days
Biochemical Oxygen Demand (SM 5210B)	W	P,G	Cool, 4°C	48 hours
Bromate (EPA 300.1)	W, DW	P,G	50mg/L EDA, cool to 4°C	28 days
Bromide (EPA 300.1)	W, DW	P,G	None Required	28 days
Chemical Oxygen Demand (SM 5220C)	W	P,G	Cool, 4°C, H ₂ SO ₄ to pH<2	28 days
Chloride (EPA 300.0)	W, DW	P,G	None Required	28 days
Chloride (EPA 9056)	W	P,G	Cool, 4°C	Analyze immediately
Chlorine, Total Residual (SM 4500Cl F)	W, DW	P,G	None Required	24 hours
Chlorite (EPA 300.1)	W, DW	P,G	50mg/L EDA, cool to 4°C	14 days
Chlorophyll-A (SM 11200H)	W	G Amber	Cool, 4°C	Analyze immediately
Chromium VI (EPA 7196A)	W	P,G	Cool, 4°C	24 hours
Color (SM 2120B)	W, DW	P,G	Cool, 4°C	48 hours
Cyanide, Total and Amenable to Chlorination (EPA 335.4, 9010, 9012) (SM 4500CN E,G)	W, DW	P,G	Cool, 4°C, NaOH to pH>12, plus 0.6 g Ascorbic Acid	14 days
Cyanide, Weak Acid Dissociable (SM 4500CN I)	W	P,G	Cool, 4°C, NaOH to pH >12	14 days
Ferrous Iron (CAS SOP)	W, DW	G Amber	Cool, 4°C	24 hours
Fluoride (EPA 300.0)	W, DW	P,G	None Required	28 days
Fluoride (EPA 9056)	W	P,G	Cool, 4°C	Analyze immediately
Hardness (SM 2340C)	W, DW	P,G	HNO ₃ to pH<2	6 months
Hydrogen Ion (pH) (SM 4500H B)	W, DW	P,G	None Required	Analyze immediately
Kjeldahl and Organic Nitrogen (ASTM D3590-89)	W	P,G	Cool, 4°C, H ₂ SO ₄ to pH<2	28 days

Table 8-1 (continued)
Sample Preservation and Holding Times^a

DETERMINATION^a	MATRIX^b	CONTAINER^c	PRESERVATION	MAXIMUM HOLDING TIME
Nitrate (EPA 300.0)	W, DW	P,G	Cool, 4°C	48 hours
Nitrate (EPA 353.2)	W, DW	P,G	Cool, 4°C, H ₂ SO ₄ to pH<2	48 hours
Nitrate (EPA 9056)	W	P,G	Cool, 4°C	Analyze immediately
Nitrate-Nitrite (EPA 353.2)	W, DW	P,G	Cool, 4°C, H ₂ SO ₄ to pH<2	28 days
Nitrite (EPA 300.0)	W, DW	P,G	Cool, 4°C	48 hours
Nitrite (EPA 353.2)	W, DW	P,G	Cool, 4°C, H ₂ SO ₄ to pH<2	48 hours
Nitrite (EPA 9056)	W	P,G	Cool, 4°C	Analyze immediately
Orthophosphate (EPA 365.3)	W, DW	P,G	Cool, 4°C	Analyze immediately
Oxygen, Dissolved (Probe) (SM 4500O G)	W, DW	G, Bottle and Top	None Required	Analyze immediately
Oxygen, Dissolved (Winkler)	W, DW	G, Bottle and Top	Fix on Site and Store in Dark	8 hours
Perchlorate (EPA 314.0)	W, DW	P,G	Protect from temp. extremes	28 days
Phenolics, Total (EPA 420.1)	W	G Only	Cool, 4°C, H ₂ SO ₄ to pH<2	28 days
Phosphorus, Total (EPA 365.3)	W	P,G	Cool, 4°C, H ₂ SO ₄ to pH<2	28 days
Residue, Total (EPA 160.3 & SM 2540B)	W	P,G	Cool, 4°C	7 days
Residue, Filterable (TDS) (SM 2540C)	W	P,G	Cool, 4°C	7 days
Residue, Nonfilterable (TSS) (SM 2540D)	W	P,G	Cool, 4°C	7 days
Residue, Settleable (SM 2540F)	W	P,G	Cool, 4°C	48 hours
Residue, Volatile (EPA 160.4)	W	P,G	Cool, 4°C	7 days
Silica (SM 4500SiO ₂ C)	W	P Only	Cool, 4°C	28 days
Specific Conductance (EPA 120.1 & SM 2510B)	W, DW	P,G	Cool, 4°C	28 days
Sulfate (EPA 300.0)	W, DW	P,G	Cool, 4°C	28 days
Sulfate (EPA 9056)	W	P,G	Cool, 4°C	Analyze immediately
Sulfide (SM 4500S ₂ F)	W	P,G	Cool, 4°C, Add Zinc Acetate plus Sodium Hydroxide to pH>9	7 days
Sulfite (SM 4500SO ₃ B)	W	P,G	None Required	24 hours
Surfactants (MBAS) (SM 5540C)	W	P,G	Cool, 4°C	48 hours
Tannin and Lignin (SM 5550B)	W	P,G	Cool, 4°C	28 days
Turbidity (EPA 180.1)	W, DW	P,G	Cool, 4°C	48 hours

Table 8-1 (continued)
Sample Preservation and Holding Times^a

DETERMINATION^a	MATRIX^b	CONTAINER^c	PRESERVATION	MAXIMUM HOLDING TIME
Metals				
Metals, except CrVI and Mercury (EPA 200.7, 200.8, 200.9, 6010, 6020)	W, DW	P,G	HNO ₃ to pH<2	6 months
	S	G, Teflon-Lined Cap	Cool, 4°C	6 months
Chromium VI (EPA 7195/7191)	W	P,G	Cool, 4°C	24 hours
Mercury (EPA 245.1, 7470, 7471, 1631E)	W	P,G	HNO ₃ to pH<2	28 days
	S	P,G	Cool, 4°C	28 days
Organic Tests				
Oil and Grease, Hexane Extractable Material (EPA 1664)	W	G, Teflon-Lined Cap	Cool, 4°C, H ₂ SO ₄ to pH<2	28 days
Organic Carbon, Total (EPA 415.1, 9060 & SM 5310C)	W	P,G	Cool, 4°C, H ₂ SO ₄ to pH<2	28 days
Organic Halogens, Total (EPA 9020)	W	G, Teflon-Lined Cap	Cool, 4°C, H ₂ SO ₄ to pH<2, No headspace	28 days
Organic Halogens, Adsorbable (EPA 1650B)	W	G, Teflon-Lined Cap	Cool, 4°C, HNO ₃ to pH<2	6 months
Petroleum Hydrocarbons, Total (EPA 8015)	W	G, Teflon-Lined Cap	Cool, 4°C, HCl or H ₂ SO ₄ to pH<2	7 days until extraction; 40 days after extraction
	S	G, Teflon-Lined Cap	Cool, 4°C	14 days until extraction; 40 days after extraction

Table 8-1 (continued)
Sample Preservation and Holding Times^a

DETERMINATION ^a	MATRIX ^b	CONTAINER ^c	PRESERVATION	MAXIMUM HOLDING TIME
Volatile Organics				
Petroleum Hydrocarbons, Volatile (Gasoline-Range Organics) (EPA 8015)	W	G, Teflon-Lined Septum Cap	Cool, 4°C, HCl to pH<2 No Headspace	14 days
	S	G, Teflon-Lined Cap	Cool, 4°C Minimize Headspace	14 days
Purgeable Halocarbons (EPA 624, 8021, 8260)	W	G, Teflon-Lined Septum Cap, No Headspace	No Residual Chlorine Present: HCl to pH<2, Cool, 4°C, No Headspace Residual Chlorine Present: 10% Na ₂ S ₂ O ₃ , HCl to pH<2, Cool, 4°C	14 days
	S	G, Teflon-Lined Cap	Cool, 4°C, Minimize Headspace	14 days
	S	Method 5035	Encore, Freeze at -20°C Methanol, Cool, 4°C Sodium Bisulfate Cool, 4°C	7 days 48 hrs to prepare from Encore, 14 days after preparation. 48 hrs to prepare from Encore, 14 days after preparation.
Purgeable Aromatic Hydrocarbons (including BTEX and MTBE) (EPA 624, 8021, 8260)	W	G, Teflon-Lined Septum Cap, No Headspace	No Residual Chlorine Present: HCl to pH<2, Cool, 4°C, No Headspace Residual Chlorine Present: 10% Na ₂ S ₂ O ₃ , HCl to pH<2, Cool 4°C	14 days
	S	G, Teflon-Lined Cap	Cool, 4°C, Minimize Headspace	14 days
	S	Method 5035	Encore, Freeze at -20°C Methanol, Cool, 4°C Sodium Bisulfate Cool, 4°C	7 days 48 hrs to prepare from Encore, 14 days after preparation. 48 hrs to prepare from Encore, 14 days after preparation.
Acrolein, Acrylonitrile, Acetonitrile (EPA 624, 8260)	W	G, Teflon-Lined Septum Cap	Adjust pH to 4-5, Cool, 4°C, No Headspace	14 days
EDB and DBCP (EPA 8260)	W,S	G, Teflon-Lined Cap	Cool, 4°C, 3 mg Na ₂ S ₂ O ₃ , No Headspace	28 days

Table 8-1 (continued)
Sample Preservation and Holding Times^a

DETERMINATION^a	MATRIX^b	CONTAINER^c	PRESERVATION	MAXIMUM HOLDING TIME
Semivolatile Organics				
Petroleum Hydrocarbons, Extractable (Diesel-Range Organics) (EPA 8015)	W,S	G, Teflon-Lined Cap	Cool, 4°C	7 days until extraction; ^f 40 days after extraction
Alcohols and Glycols (EPA 8015)	W,S	G, Teflon-Lined Cap	Cool, 4°C ^g	7 days until extraction; ^f 40 days after extraction
Acid Extractable Semivolatile Organics (EPA 625, 8270)	W,S	G, Teflon-Lined Cap	Cool, 4°C ^g	7 days until extraction; ^f 40 days after extraction
Base/Neutral Extractable Semivolatile Organics (EPA 625, 8270)	W,S	G, Teflon-Lined Cap	Cool, 4°C ^g	7 days until extraction; ^f 40 days after extraction
Polynuclear Aromatic Hydrocarbons (EPA 625, 8270, 8310)	W,S	G, Teflon-Lined Cap	Cool, 4°C, Store in Dark ^g	7 days until extraction; ^f 40 days after extraction
Organochlorine Pesticides and PCBs (EPA 608, 8081)	W,S	G, Teflon-Lined Cap	Cool, 4°C	7 days until extraction; ^f 40 days after extraction
Organophosphorus Pesticides (EPA 8141)	W,S	G, Teflon-Lined Cap	Cool, 4°C ^g	7 days until extraction; ^f 40 days after extraction
Nitrogen- and Phosphorus-Containing Pesticides (EPA 8141)	W,S	G, Teflon-Lined Cap	Cool, 4°C ^g	7 days until extraction; ^f 40 days after extraction
Chlorinated Herbicides (EPA 8151)	W,S	G, Teflon-Lined Cap	Cool, 4°C ^g	7 days until extraction; ^f 40 days after extraction
Organotins (CAS SOP)	W,S	G, Teflon-Lined Cap	Cool, 4°C	7 days until extraction; ^f 40 days after extraction
Chlorinated Phenolics (EPA 1653A)	W	G, Teflon-Lined Cap	H ₂ SO ₄ to pH<2, Cool, 4°C ^g	30 days until extraction; 30 days after extraction
Resin and Fatty Acids (NCASI 85.02)	W	G, Teflon-Lined Cap	NaOH to pH ≥10, Cool, 4°C ^g	30 days until extraction; 30 days after extraction

Table 8-1 (continued)
Sample Preservation and Holding Times^a

DETERMINATION^a	MATRIX^b	CONTAINER^c	PRESERVATION	MAXIMUM HOLDING TIME
Drinking Water Organics				
Purgeable Organics (EPA 524.2)	DW	G, Teflon-Lined Septum Cap	Ascorbic Acid, HCl to pH \leq 2, Cool, 4°C, No Headspace	14 days
EDB, DBCP, and TCP (EPA 504.1)	DW	G, Teflon-Lined Septum Cap	Cool, 4°C, 3 mg Na ₂ S ₂ O ₃ , No Headspace	14 days
Carbamates, Carbamoyloximes (EPA 531.1)	DW	G, Amber, Teflon-Lined Cap	1.8 mL monochloroacetic acid to pH $<$ 3; 80 mg/L Na ₂ S ₂ O ₃ if Res.Cl.; Cool, 4°C	28 days
Chlorinated Herbicides (EPA 515.4)	DW	G, Amber, Teflon-Lined Cap	If Res.Cl, 2mg/40mL NaS; Cool, $<$ 6°C	14 days until extraction; 21 days after extraction
Chlorinated Pesticides (EPA 508.1, 525.2)	DW	G, Amber, Teflon-Lined Cap	50 mg/L NaS, HCl to pH \leq 2; Cool, 4°C	14 days until extraction; 30 days after extraction
Diquat and Paraquat (EPA 549.2)	DW	G, Amber, Teflon-Lined Cap	100 mg/L Na ₂ S ₂ O ₃ if Res.Cl., Cool, 4°C,	7days until extraction; 21 days after extraction
Endothall (EPA 548.1)	DW	G, Amber, Teflon-Lined Cap	Cool, 4°C	7 days until extraction; 14 days after extraction
Glyphosate (EPA 547)	DW	G, Amber, Teflon-Lined Cap	100 mg/L Na ₂ S ₂ O ₃ , Cool, 4°C	14 days
Haloacetic Acids (EPA 552.2)	DW	G, Amber, Teflon-Lined Cap	100 mg/L NH ₄ Cl, Cool, 4°C	14 days until extraction; 7 days after extraction
Semivolatile Organics (EPA 525.2)	DW	G, Amber, Teflon-Lined Cap	50 mg/L NaS, HCl to pH \leq 2; Cool, 4°C	14 days until extraction; 30 days after extraction
Toxicity Characteristic Leaching Procedure (TCLP)				
Mercury (EPA 1311/7470)	HW	P,G	Sample: Cool, 4°C TCLP extract: HNO ₃ to pH $<$ 2	28 days until extraction; 28 days after extraction
Metals, except Mercury (EPA 1311/6010)	HW	P,G	Sample: Cool, 4°C TCLP extract: HNO ₃ to pH $<$ 2	180 days until extraction; 180 days after extraction
Volatile Organics (EPA 1311/8260)	HW	G, Teflon-Lined Cap	Sample: Cool, 4°C Minimize Headspace TCLP extract: Cool, 4°C, HCl to pH $<$ 2, No Headspace	14 days until extraction; 14 days after extraction

Table 8-1 (continued)
Sample Preservation and Holding Times^a

DETERMINATION ^a	MATRIX ^b	CONTAINER ^c	PRESERVATION	MAXIMUM HOLDING TIME
Toxicity Characteristic Leaching Procedure (TCLP)				
Semivolatile Organics (EPA 1311/8270)	HW	G, Teflon-Lined Cap	Sample: Cool, 4°C, Store in Dark ^g TCLP extract: Cool, 4°C, Store in Dark ^g	14 days until TCLP ext'n; 7 days until extraction; 40 days after extraction
Organochlorine Pesticides (EPA 1311/8081)	HW	G, Teflon-Lined Cap	Sample: Cool, 4°C TCLP extract: Cool, 4°C	14 days until TCLP ext'n; 7 days until extraction; 40 days after extraction
Chlorinated Herbicides (EPA 1311/8151)	HW	G, Teflon-Lined Cap	Sample: Cool, 4°C TCLP extract: Cool, 4°C	14 days until TCLP ext'n; 7 days until extraction; 40 days after extraction

- a For EPA SW-846 methods the method number is listed generically, without specific revision suffixes.
b DW = Drinking Water, W = Water; S = Soil or Sediment; HW = Hazardous Waste
c P = Polyethylene; G = Glass
d For chlorinated water samples
e The maximum holding time is dependent upon the geographical proximity of sample source to the laboratory.
f Fourteen days until extraction for soil, sediment, and sludge samples.
g If the water sample contains residual chlorine, 10% sodium thiosulfate is used to dechlorinate.

QAM_2009.DOC

Figure 8-2

Columbia Analytical Services, Inc.
Cooler Receipt and Preservation Form

PC _____

Client / Project: _____ Service Request **K09**

Received: _____ Opened: _____ By: _____

1. Samples were received via? *US Mail Fed Ex UPS DHL GH GS PDX Courier Hand Delivered*

2. Samples were received in: (circle) *Cooler Box Envelope Other* _____ *NA*

3. Were custody seals on coolers? *NA Y N* If yes, how many and where? _____
 If present, were custody seals intact? *Y N* If present, were they signed and dated? *Y N*

4. Is shipper's air-bill filed? If not, record air-bill number: _____ *NA Y N*

5. **Temperature of cooler(s) upon receipt (°C):** _____
Temperature Blank (°C): _____
Thermometer ID: _____

6. If applicable, list Chain of Custody Numbers: _____

7. Packing material used. *Inserts Baggies Bubble Wrap Gel Packs Wet Ice Sleeves Other* _____

8. Were custody papers properly filled out (ink, signed, etc.)? *NA Y N*

9. **Did all bottles arrive in good condition (unbroken)?** *Indicate in the table below.* *NA Y N*

10. Were all sample labels complete (i.e analysis, preservation, etc.)? *NA Y N*

11. Did all sample labels and tags agree with custody papers? *Indicate in the table below* *NA Y N*

12. **Were appropriate bottles/containers and volumes received for the tests indicated?** *NA Y N*

13. Were the pH-preserved bottles tested* received at the appropriate pH? *Indicate in the table below* *NA Y N*

14. Were VOA vials and 1631 Mercury bottles received without headspace? *Indicate in the table below.* *NA Y N*

15. **Are CWA Microbiology samples received with >1/2 the 24hr. hold time remaining from collection?** *NA Y N*

16. Was C12/Res negative? *NA Y N*

Sample ID on Bottle	Sample ID on COC	Sample ID on Bottle	Sample ID on COC

Sample ID	Bottle Count	Bottle Type	Out of Temp	Head-space	Broken	pH	Reagent	Volume added	Reagent Lot Number	Initials

*Does not include all pH preserved sample aliquots received. See sample receiving SOP (SMO-GEN).

Additional Notes, Discrepancies, & Resolutions: _____

Page 1 of: 1 2

Figure 8-3 Tier V Form

Client : Project Chemist :
Project Name : Service Request :
Project Number : SMO LimsTemplate ID :
Project Description :

QAPP/SOW Information :

Reporting

Tier Level : PDF: Report to :
In result field use : EDO :
Flagging Requirements :
Other Requirements :

Sample Considerations

Sample Limitations :
Sample Prep/Analysis :
Non-Standard Holdtimes :
Historical Data :
Comments :

9.0 ANALYTICAL PROCEDURES

CAS employs methods and analytical procedures from a variety of sources. The primary method references are: USEPA SW-846, Third Edition and Updates I, II, IIA, IIB, III, IVA, IVB, and online updates for hazardous waste samples, and USEPA 600/4-79-020, 600/4-91-010, 600/4-82-057, 600/R-93/100, 600/4-88-039, 600/R-94-111, and Supplements; and *Standard Methods for the Examination of Water and Wastewater* for water and wastewater samples. Complete citations for these references can be found in Section 18.0. Other published procedures, such as state-specific methods, program-specific methods (such as Puget Sound Protocols), or in-house methods may be used. Several factors are involved with the selection of analytical methods to be used in the laboratory. These include the method detection limit, the concentration of the analyte being measured, method selectivity, accuracy and precision of the method, the type of sample being analyzed, and the regulatory compliance objectives. The implementation of methods by CAS is described in SOPs specific to each method. A list of NELAP-accredited methods are given in Appendix E. Further details are described below.

9.1 Standard Operating Procedures (SOPs) and Laboratory Notebooks.

CAS maintains SOPs for use in both technical and administrative functions. SOPs are written following standardized format and content requirements. Each SOP is reviewed and approved by a minimum of two managers (the Laboratory Director and/or Department Manager and the Quality Assurance Manager). All SOPs undergo a documented annual review to make sure current practices are described. The QA Manager maintains a comprehensive list of current SOPs. The document control process ensures that only the most currently prepared version of an SOP is being used. The QA Manual, QAPPs, SOPs, standards preparation logbooks, maintenance logbooks, et al., are controlled documents. The procedures for document control are described in the *SOP for Document Control* (ADM-DOC_CTRL). In addition to SOPs, each laboratory department maintains a current file, accessible to all laboratory staff, of the current methodology used to perform analyses. Laboratory notebook entries are standardized following the guidelines in the *SOP for Making Entries into Logbooks and onto Benchsheets* (ADM-DATANTRY). Entries made into laboratory notebooks are reviewed and approved by the appropriate supervisor at a regular interval.

9.2 Deviation from Standard Operating Procedures

When a customer requests a modification to an SOP (such as a change in reporting limit, addition or deletion of target analyte(s), etc.), the project chemist handling that project must discuss the proposed deviation with the department manager in charge of the analysis and obtain their approval to accept the project. The project chemist is responsible for documenting the approved or allowed deviation from the SOP by placing a detailed description of the deviation attached to the quotation or in the project file and also providing an appropriate comment on the service request when the samples are received.

For circumstances when a deviation or departure from company policies or procedures involving any non-technical function is found necessary, approval must be obtained from the appropriate supervisor, manager, the laboratory director, or other level of authority. Frequent departure from policy is not encouraged. However, if frequent departure from any policy is noted, the laboratory director will address the possible need for a change in policy.

9.3 Modified Procedures

CAS strives to perform published methods as described in the referenced documents. If there is a material deviation from the published method, the method is cited as a "Modified" method in the analytical report. Modifications to the published methods are listed in the standard operating procedure. Standard operating procedures are available to analysts and are also available to our clients for review, especially those for "Modified" methods. Client approval is obtained for the use of "Modified" methods prior to the performance of the analysis.

9.4 Analytical Batch

The basic unit for analytical quality control is the analytical batch. The definition that CAS has adopted for the analytical batch is listed below. The overriding principle for describing an analytical batch is that all the samples in a batch, both field samples and quality control samples, are to be handled exactly the same way, and all of the data from each analysis is to be manipulated in exactly the same manner. The minimum requirements of an analytical batch are:

- 1) The number of (field) samples in a batch is not to exceed 20.
- 2) All (field) samples in a batch are of the same matrix.
- 3) The QC samples to be processed with the (field) samples include:
 - a) Method Blank (a.k.a. Laboratory Reagent Blank)
Function: Determination of laboratory contamination.
 - b) Laboratory Control Sample (a.k.a. Laboratory Fortified Blank)
Function: Assessment of method performance
 - c) Matrix Spiked (field) Sample (a.k.a. Laboratory Fortified Sample Matrix)*
Function: Assessment of matrix bias
 - d) Duplicate Matrix Spiked (field) Sample or Duplicate (field) Sample (a.k.a. Laboratory Duplicate)*
Function: Assessment of batch precision

* A sample identified as a field blank, an equipment blank, or a trip blank is not to be matrix spiked or duplicated.

- 4) A single lot of reagents is used to process the batch of samples.
- 5) Each operation within the analysis is performed by a single analyst, technician, chemist, or by a team of analysts/technicians/chemists.
- 6) Samples are analyzed in a continuous manner over a timeframe not to exceed 24-hours.
- 7) (Field) samples are assigned to batches commencing at the time that sample processing begins. For example: for analysis of metals, sample processing begins when the samples are digested. For analysis of organic constituents, it begins when the samples are extracted.
- 8) The QC samples are to be analyzed in conjunction with the associated field samples prepared with them. However, for tests which have a separate sample preparation step that defines a batch (digestion, extraction, etc.), the QC samples in the batch do not require analysis each time a field sample within the preparation batch is analyzed (multiple instrument sequences to analyze all field samples in the batch need not include re-analyses of the QC samples).
- 9) The batch is to be assigned a unique identification number that can be used to correlate the QC samples with the field samples.
- 10) Batch QC refers to the QC samples that are analyzed in a batch of (field) samples.
- 11) Project-specific requirements may be exceptions. If project, program, or method requirements are more stringent than these laboratory minimum requirements, then the project, program, or method requirements will take precedence. However, if the project, program, or method requirements are less stringent than these laboratory minimum requirements, these laboratory minimum requirements will take precedence.

9.5 Specialized Procedures

CAS not only strives to provide results that are scientifically sound, legally defensible, and of known and documented quality; but also strives to provide the best solution to analytical challenges. Procedures using specialized instrumentation and methodology have been developed to improve sensitivity (provide lower detection limits), selectivity (minimize interferences while maintaining sensitivity), and overall data quality for low concentration applications. Examples are trace-level Mercury and methylmercury analyses, reductive precipitation metals analysis, specialized GC/MS analyses, LC/MS analyses, and ultra-low level organics analyses (including PAHs, pesticides and PCBs).

9.6 Sample Cleanup

CAS commonly employs several cleanup procedures to minimize known common interferences prior to analysis. EPA methods(3620, 3630, 3640, 3660, 3665) for cleanup of sample extracts for organics analysis are routinely used to minimize or eliminate interferences that may adversely affect sample results and data usability.

10.0 CALIBRATION PROCEDURES AND FREQUENCY

All equipment and instruments used at CAS are operated, maintained and calibrated according to the manufacturer's guidelines and recommendations, as well as to criteria set forth in the applicable analytical methodology. Operation and calibration are performed by personnel who have been properly trained in these procedures. Documentation of calibration information is maintained in appropriate reference files. Brief descriptions of the calibration procedures for our major laboratory equipment and instruments are described below. Calibration verification is performed according to the applicable analytical methodology. Calibration verification procedures and criteria are listed in laboratory Standard Operating Procedures. Documentation of calibration verification is maintained in appropriate reference files. Records are maintained to provide traceability of reference materials.

Equipment which has been subjected to overloading or mishandling, or has been shown by verification or otherwise to be defective; is taken out of service until it has been repaired. The equipment is placed back in service only after verifying by calibration that the equipment performs satisfactorily. An evaluation of the effect of this defect on previous calibrations or tests is made and documented appropriately.

10.1 Temperature Control Devices

Temperatures are monitored and recorded for all of the temperature-regulating support equipment such as sample refrigerators, freezers, and standards refrigerators. Bound record books are kept which contain daily-recorded temperatures, identification and location of equipment, acceptance criteria and the initials of the technician who performed the checks. The procedure for performing these measurements is provided in the *SOP for Support Equipment Monitoring and Calibration (SOP ADM-SEMC)*. The SOP also includes the use of acceptance criteria and correction factors.

Where the operating temperature is specified as a test condition (such as ovens, incubators, evaporators) the temperature is recorded on the raw data. All thermometers are identified according to serial number, and the calibration of these thermometers is checked annually against a National Institute of Standards and Technology (NIST) certified thermometer. The NIST thermometer is recertified by a professional metrology organization on an annual basis.

10.2 Analytical Balances

The calibration of each analytical balance is checked by the user each day of use with three Class S or S-1 weights, which assess the accuracy of the balance at low, mid-level and high levels bracketing the working range. Records are kept which contain the recorded measurements, identification of the balance, acceptance criteria, and the initials of user who performed the check. The procedure for performing these measurements and use of acceptance criteria is described in the SOP ADM-SEMC. The weights are recertified using NIST traceable standards by a professional metrology organization on an annual basis.

As needed, the balances are recalibrated using the manufacturers recommended operating procedures. Analytical balances are serviced on a semi-annual basis by a professional metrology organization. New certificates of calibration for each balance are issued to the laboratory on a semi-annual basis.

10.3 Water Purification Systems

CAS uses two independent water purification systems is designed to produce deionized water meeting method specifications. One system consists of a series of pumps, filters, and resin beds designed to yield deionized water meeting the specifications of ASTM Type II water, and *Standard Methods for the Examination of Water and Wastewater* (SM1080, 20th Ed.) *High Quality* water. Activated carbon filters are also in series with the demineralizers to produce "organic-free" water. A second system consists of pumps, filters, and treatment components designed to yield deionized water meeting the specifications of ASTM Type I water, and *Standard Methods for the Examination of Water and Wastewater* (SM1080, 20th Ed.) *High Quality* water. Following a written SOP, the status of each system is monitored continuously for conductivity and resistivity with an on-line meter and indicator light, and readings recorded daily in a bound record book. The meter accuracy is verified annually. Deionizers are rotated and replaced on a regular schedule. Microbiology water is checked at a point downstream of the purification system at a tap in the laboratory, and monitoring documented.

10.4 Source and Preparation of Standard Reference Materials

All analytical measurements generated at CAS are performed using materials and/or processes that are traceable to a reference material. Metrology equipment (analytical balances, thermometers, etc.) is calibrated using reference materials traceable to the National Institute of Standards and Technology (NIST). These primary reference materials are themselves recertified on an annual basis. All sampling containers provided to the client by the laboratory are purchased as precleaned (Level 1) containers, with certificates of analysis available for each bottle type. This information is provided to the client when requested.

Consumable reference materials routinely purchased by the laboratories (e.g., analytical standards) are purchased from nationally recognized, reputable vendors. All vendors have fulfilled the requirements for ISO 9001 certification and/or are accredited by A₂LA. CAS relies on a primary vendor for the majority of its analytical supplies. Consumable primary stock standards are obtained from certified commercial sources or from sources referenced in a specific method. Supelco, Ultra Scientific, AccuStandard, Chem Services, Inc., Aldrich Chemical Co., Baker, Spex, etc. are examples of the vendors used. Reference material information is recorded in the appropriate logbook(s) and materials are stored under conditions that provide maximum protection against deterioration and contamination. The logbook entry includes such information as an assigned logbook identification code, the source of the material (i.e. vendor identification), solvent (if applicable) and concentration of analyte(s), reference to the certificate of analysis and an assigned expiration date. The date that the standard is received in the laboratory is marked on the container. When the reference material is used for the first time, the date of usage and the initials of the analyst are also recorded on the container.

Stock solutions and calibration standard solutions are prepared fresh as often as necessary according to their stability. All standard solutions are properly labeled as to analyte concentration, solvent, date, preparer, and expiration date; these entries are also recorded in the appropriate notebook(s) following the *SOP for Making Entries into Logbooks and onto Benchsheets* (SOP No. ADM-DATANTRY). Prior to sample analysis, all calibration reference materials are verified with a second, independent source of the material (see section 11.3.5).

10.5 Inductively Coupled Plasma-Atomic Emission Spectrograph (ICP-AES)

Each emission line on the ICP is calibrated daily against a blank and against standards. Analyses of calibration standards, initial and continuing calibration verification standards, and inter-element interference check samples are carried out as specified in the applicable method SOP and analytical method (i.e. EPA 200.7, 6010B, 6010C, CLP SOW, etc.).

10.6 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS)

Each element of interest is calibrated for using a blank and a single standard. Prior to calibration, a short-term stability check is performed on the system. Following calibration, an independent check standard is analyzed, and a continuing calibration verification standard (CCV) is analyzed with every ten samples.

10.7 Atomic Absorption Spectrophotometers (AAS)

These instruments are calibrated daily using a minimum of four standards and a blank. Calibration is validated using reference standards, and is verified at a minimum frequency of once every ten samples. Initial calibration points cannot be "dropped" from the resulting calibration curve.

10.8 GC/MS Systems

All GC/MS instruments are calibrated at a minimum of five different concentration levels for the analytes of interest (unless specified otherwise) using procedures outlined in Standard Operating Procedures and/or appropriate USEPA method citations. All reference materials used for this function are vendor-certified standards. Calibration verification is performed at method-specified intervals following the procedures in the SOP and reference method. Compounds selected as system performance check compounds (SPCCs) must show a method-specified response factor in order for the calibration to be considered valid. Calibration check compounds (CCCs) must also meet method specifications for percent difference from the multipoint calibration. For isotope dilution procedures, the internal standard response(s) and labeled compound recovery must meet method criteria. Method-specific instrument tuning is regularly checked using bromofluorobenzene (BFB) for volatile organic chemical (VOC) analysis, or decafluorotriphenylphosphine (DFTPP) for semi-volatile analysis. Mass spectral peaks for the tuning compounds must conform both in mass numbers and in relative intensity criteria before analyses can proceed. Calibration policies for organics chromatographic analyses are described in the *SOP for Calibration of Instruments for Organics Chromatographic Analyses (SOP SOC-CAL)*.

10.9 Gas Chromatographs and High Performance Liquid Chromatographs

Calibration and standardization follow SOP guidelines and/or appropriate USEPA method citations. All GC and HPLC instruments are calibrated at a minimum of five different concentration levels for the analytes of interest (unless specified otherwise). The lowest standard is equivalent to the method reporting limit; additional standards define the working range of the GC or LC detector. Results are used to establish response factors (or calibration curves) and retention-time windows for each analyte. Calibration is verified at a minimum frequency of once every ten samples, unless otherwise specified by the reference method. *SOP for Calibration of Instruments for Organics Chromatographic Analyses (SOP SOC-CAL)*.

10.10 LC/MS Systems

Calibration and tuning procedures are included in analytical SOPs written specifically for these tests. In general, multiple concentration levels for the analytes of interest are used to generate calibration curves. All reference materials used for this function are vendor-certified standards. Calibration and tuning verification is performed at SOP-defined intervals. Any other system performance checks are described in the applicable SOP. Calibration policies for organics chromatographic analyses are described in the *SOP for Calibration of Instruments for Organics Chromatographic Analyses (SOP SOC-CAL)*.

10.11 UV-Visible Spectrophotometer (manual colorimetric analyses)

Routine calibrations for colorimetric and turbidimetric analyses involve generating a 5-point calibration curve including a blank. Initial calibration points cannot be "dropped" from the resulting calibration curve. Correlation coefficients must meet method or SOP specifications before analysis can proceed. Independent calibration verification standards (ICVs) are analyzed with each batch of samples. Continuing calibration is verified at a minimum frequency of once every ten samples. Typical UV-Visible spectrophotometric methods at CAS include total phenolics, phosphates, surfactants and tannin-lignin.

10.12 Flow Injection Analyzer (automated colorimetric analysis)

A minimum of six standards and a blank are used to calibrate the instrument for cyanide analysis. A blank and (minimum of) five standards are used to calibrate the instrument for all other automated chemistries. Initial calibration points cannot be "dropped" from the resulting calibration curve. Standard CAS acceptance limits are used to evaluate the calibration curve prior to sample analysis.

10.13 Ion Chromatographs

Calibration of the ion chromatograph (IC) involves generating a calibration curve with the method-specified number of points (or more). Initial calibration points cannot be "dropped" from the resulting calibration curve. A correlation coefficient of ≥ 0.995 for the curve is required before analysis can proceed. Quality Control (QC) samples that are routinely analyzed include blanks and laboratory control samples. The target analytes typically determined by the IC include nitrate, nitrite, chloride, fluoride, sulfate and drinking water inorganic disinfection byproducts. Calibration verification is performed at method-specified intervals following the procedures in the SOP and reference method.

10.14 Turbidimeter

Calibration of the turbidimeter requires analysis of three Nephelometric Turbidity Unit (NTU) formazin standards. Quality Control samples that are routinely analyzed include blanks, Analytical Products Group® QC samples (or equivalent) and duplicates.

10.15 Ion-selective electrode

The method-prescribed numbers of standards are used to calibrate the electrodes before analysis. The slope of the curve must be within acceptance limits before analysis can proceed. Quality Control samples that are routinely analyzed include blanks, LCSs and duplicates.

10.16 Pipets

The calibration of pipets and autopipettors used to make critical-volume measurements is verified following the *SOP for Checking Pipet Calibration*. Both accuracy and precision verifications are performed, at intervals applicable to the pipet and use. The results of all calibration verifications are recorded in bound logbooks.

10.17 Other Instruments

Calibration for the total organic carbon (TOC), total organic halogen (TOX), and other instruments is performed following manufacturer's recommendations and applicable SOPs.

11.0 QUALITY CONTROL

A primary focus of Columbia Analytical Services Quality Assurance (QA) Program is to ensure the accuracy, precision and comparability of all analytical results. Prior to using a procedure for the analysis of field samples, acceptable method performance is established by performing demonstration of capability analyses and performance characteristics are established by performing method detection limit studies and assessing accuracy and precision according to the reference method. CAS has established Quality Control (QC) objectives for precision and accuracy that are used to determine the acceptability of the data that is generated. These QC limits are either specified in the methodology or are statistically derived based on the laboratory's actual historical data obtained from the various QC measurements for each analytical method. The Quality Control objectives are defined below.

11.1 Quality Control Objectives

11.1.2 Demonstration of Capability - Where required by mandatory test method, regulation, or accreditation protocols, a demonstration of capability (DOC) is made prior to using any test method. This demonstration is made following regulatory, accreditation, or method specified procedures. In general, this demonstration does not test the performance of the method in real world samples, but in the applicable clean matrix free of target analytes and interferences.

A quality control reference material or quality control sample is obtained. The analyte(s) is (are) diluted in a volume of clean matrix (for analytes which do not lend themselves to spiking, e.g., TSS, the demonstration of capability may be performed using quality control samples). Where specified, the method-required concentration levels are used. Four aliquots are prepared and analyzed according to the test procedure. The mean recovery and standard deviations are calculated and compared to the corresponding acceptance criteria for precision and accuracy in the test method or laboratory-generated acceptance criteria (if there are not established mandatory criteria). All parameters must meet the acceptance criteria. Where spike levels are not specified, actual Laboratory Control Sample results or MDL study results may be used to meet this requirement, provided acceptance criteria is met.

11.1.3 Accuracy - Accuracy is a measure of the closeness of an individual measurement (or an average of multiple measurements) to the true or expected value. Accuracy is determined by calculating the mean value of results from ongoing analyses of laboratory-fortified blanks, standard reference materials, and standard solutions. In addition, laboratory-fortified (i.e. matrix-spiked) samples are also measured; this indicates the accuracy or bias in the actual sample matrix. Accuracy is expressed as percent recovery (% REC.) of the measured value, relative to the true or expected value. If a measurement process produces results whose mean is not the true or expected value, the process is said to be biased. Bias is the systematic error either inherent in a method of analysis (e.g., extraction efficiencies) or

caused by an artifact of the measurement system (e.g., contamination). CAS utilizes several quality control measures to eliminate analytical bias, including systematic analysis of method blanks, laboratory control samples and independent calibration verification standards. Because bias can be positive or negative, and because several types of bias can occur simultaneously, only the net, or total, bias can be evaluated in a measurement

11.1.4 Precision - Precision is the ability of an analytical method or instrument to reproduce its own measurement. It is a measure of the variability, or random error, in sampling, sample handling and in laboratory analysis. The American Society of Testing and Materials (ASTM) recognizes two levels of precision: repeatability - the random error associated with measurements made by a single test operator on identical aliquots of test material in a given laboratory, with the same apparatus, under constant operating conditions, and reproducibility - the random error associated with measurements made by different test operators, in different laboratories, using the same method but different equipment to analyze identical samples of test material.

"Within-batch" precision is measured using replicate sample or QC analyses and is expressed as the relative percent difference (RPD) between the measurements. The "batch-to-batch" precision is determined from the variance observed in the analysis of standard solutions or laboratory control samples from multiple analytical batches.

11.1.5 Control Limits - The control limits for accuracy and precision originate from two different sources: For analyses having enough QC data, control limits are calculated at the 99% confidence limits. For analyses not having enough QC data, or where the method is prescriptive, control limits are taken from the method on which the procedure is based. If the method does not have stated control limits, then control limits are assigned method-default or reasonable values. Control limits are updated periodically when new statistical limits are generated for the appropriate surrogate, laboratory control sample, and matrix spike compounds (typically once a year) or when method prescribed limits change. The updated limits are reviewed by the Quality Assurance Manager. The new control limits replace the previous limits and data is assessed using the new values. The current acceptance limits for accuracy and precision are available from the laboratory and on the accompanying CD-ROM. For inorganics, the precision limit values listed are for laboratory duplicates. For organics, the precision limit values listed are for duplicate laboratory control samples or duplicate matrix spike analyses.

11.1.6 Representativeness - Representativeness is the degree to which the field sample, being properly preserved, free of contamination, and analyzed within holding time, represents the overall sample site or material. This can be extended to the sample itself, in that representativeness is the degree to which the subsample that is analyzed represents the entire field sample submitted for analysis. CAS has sample handling procedures to ensure that the sample used for analysis is representative of the entire sample. These include the *SOP for Subsampling and Compositing of Samples* and the *SOP for Tissue Sample Preparation*. Further, analytical SOPs specify appropriate sample handling and sample sizes to further ensure the sample aliquot that is analyzed is representative in entire sample.

11.1.7 Comparability – Comparability expresses the confidence with which one data set can be compared to another and is directly affected by data quality (accuracy and precision) and sample handling (sampling, preservation, etc). Only data of known quality can be compared. The objective is to generate data of known quality with the highest level of comparability, completeness, and usability. This is achieved by employing the quality controls listed below and standard operating procedures for the handling and analysis of all samples. Data is reported in units specified by the client and using CAS or project-specified data qualifiers.

11.2 Method Detection Limits and Method Reporting Limits

Method Detection Limits (MDL) for methods performed at CAS/Kelso are determined annually, and may change slightly from year to year. If an MDL study is not performed annually, an MDL verification check is performed quarterly on every instrument used in the analysis. The MDLs are determined by following the *SOP for the Determination of Method Detection Limits and Limits of Detection*, which is based on the procedure in 40 CFR Part 136, Appendix B. As required by NELAP and DoD protocols, the validity of MDLs is verified using MDL verification samples. The Method Reporting Limit (MRL) is the lowest amount of an analyte in a sample that can be quantitatively determined with stated, acceptable precision and accuracy under stated analytical conditions (i.e. the lower limit of quantitation). Therefore, analyses are calibrated to the MRL, or lower. To take into account day-to-day fluctuations in instrument sensitivity, analyst performance, and other factors, the MRL is established at three times the MDL (or greater). The current MDLs and MRLs are available from the laboratory.

11.3 Quality Control Procedures

The specific types, frequencies, and processes for quality control sample analysis are described in detail in method-specific standard operating procedures and listed below. These sample types and frequencies have been adopted for each method and a definition of each type of QC sample is provided below. In addition, a number of other quality control processes that may impact analytical results are also described below.

11.3.1 Method Blank (a.k.a. Laboratory Reagent Blank)

The method blank is an analyte-free matrix (water, soil, etc.) subjected to the entire analytical process. When analyte-free soil is not available, anhydrous sodium sulfate, organic-free sand, or an acceptable substitute is used. The method blank is analyzed to demonstrate that the analytical system itself does not introduce contamination. The method blank results should be below the Method Reporting Limit (MRL) or, if required for DoD projects, $< \frac{1}{2}$ MRL for the analyte(s) being tested. Otherwise, corrective action must be taken. A method blank is included with the analysis of every sample preparation batch, every 20 samples, or as stated in the method, whichever is more frequent.

11.3.2 Calibration Blanks

For some methods, calibration blanks are prepared along with calibration standards in order to create a calibration curve. Calibration blanks are free of the analyte of interest and, where applicable, provide the zero point of the calibration curve. Additional project-specific requirements may also apply to calibration blanks.

11.3.3 Continuing Calibration Blanks

Continuing calibration blanks (CCBs) are solutions of either analyte-free water, reagent, or solvent that are analyzed in order to verify the system is contamination-free when CCV standards are analyzed. The frequency of CCB analysis is either once every ten samples or as indicated in the method, whichever is greater. Additional project-specific requirements may also apply to continuing calibration blanks.

11.3.4 Calibration Standards

Calibration standards are solutions of known concentration prepared from primary standard or stock standard materials. Calibration standards are used to calibrate the instrument response with respect to analyte concentration. Standards are analyzed in accordance with the requirements stated in the particular method being used.

11.3.5 Initial (or Independent) Calibration Verification Standards

Initial (or independent) calibration verification standards (ICVs) are standards that are analyzed *after* calibration with newly prepared standard(s) but *prior to* sample analysis, in order to verify the validity and accuracy of the standards used in the calibration. Once it is determined that there is no reference material defect or systematic error in preparation of the calibration standard(s), standards are considered valid and may be used for subsequent calibrations and quantitative determinations (as expiration dates and methods allow). The ICV standards are prepared from materials obtained from a source independent of that used for preparing the calibration standards ("second-source"). ICVs are also analyzed in accordance with method-specific requirements.

11.3.6 Continuing Calibration Verification Standards

Continuing calibration verification standards (CCVs) are midrange standards that are analyzed in order to verify that the calibration of the analytical system is still acceptable. The frequency of CCV analysis is either once every ten samples, or as indicated in the method.

11.3.7 Internal Standards

Internal standards are known amounts of specific compounds that are added to each sample prior to instrument analysis. Internal standards are generally used for GC/MS and ICP-MS procedures to correct sample results that have been affected by changes in instrument conditions or changes caused by matrix effects. The requirements for evaluation of internal standards are specified in each method and SOP.

11.3.8 Surrogates

Surrogates are organic compounds which are similar in chemical composition and chromatographic behavior to the analytes of interest, but which are not normally found in environmental samples. Depending on the analytical method, one or more of these compounds is added to method blanks, calibration and check standards, and samples (including duplicates, matrix spike samples, duplicate matrix spike samples and laboratory control samples) prior to extraction and analysis in order to monitor the method performance on each sample. The percent recovery is calculated for each surrogate, and the recovery is a measurement of the overall method performance.

$$\text{Recovery (\%)} = (M/T) \times 100$$

Where: M = The measured concentration of analyte,
T = The theoretical concentration of analyte added.

11.3.9 Laboratory Control Samples (a.k.a. Laboratory Fortified Blanks)

The laboratory control sample (LCS) is an aliquot of analyte-free water or analyte-free solid (or anhydrous sodium sulfate or equivalent) to which known amounts of the method analyte(s) is (are) added. A reference material of known matrix type, containing certified amounts of target analytes, may also be used as an LCS. An LCS is prepared and analyzed at a minimum frequency of one LCS per 20 samples, with every analytical batch or as stated in the method, whichever is more frequent. The LCS sample is prepared and analyzed in exactly the same manner as the field samples.

The percent recovery of the target analytes in the LCS is compared to established control limits and assists in determining whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements at the required reporting limit. Comparison of batch-to-batch LCS analyses enables the laboratory to evaluate batch-to-batch precision and accuracy.

$$\text{Recovery (\%)} = (M/T) \times 100$$

Where: M = The measured concentration of analyte,
T = The theoretical concentration of analyte added.

11.3.10 Matrix Spikes (a.k.a. Laboratory Fortified Sample Matrix)

Matrix spiked samples are aliquots of samples to which a known amount of the target analyte (or analytes) is(are) added. The samples are then prepared and analyzed in the same analytical batch, and in exactly the same manner as are routine samples. For the appropriate methods, matrix spiked samples are prepared and analyzed and at a minimum frequency of one spiked sample (and one duplicate spiked sample, if appropriate) per twenty samples. The spike recovery measures the effects of interferences caused by the sample matrix and reflects the accuracy of the method for the particular matrix in question. Spike recoveries are calculated as follows:

$$\text{Recovery (\%)} = (S - A) \times 100 \div T$$

Where: S = The observed concentration of analyte in the spiked sample,
A = The analyte concentration in the original sample, and
T = The theoretical concentration of analyte added to the spiked sample.

11.3.11 Laboratory Duplicates and Duplicate Matrix Spikes

Duplicates are additional replicates of samples that are subjected to the same preparation and analytical scheme as the original sample. Depending on the method of analysis, either a duplicate analysis (and/or a matrix spiked sample) or a matrix spiked sample and duplicate matrix spiked sample (MS/DMS) are analyzed. The relative percent difference between duplicate analyses or between an MS and DMS is a measure of the precision for a given method and analytical batch. The relative percent difference (RPD) for these analyses is calculated as follows:

$$\text{Relative Percent Difference (RPD)} = (S1 - S2) \times 100 \div S_{ave}$$

Where S_1 and S_2 = The observed concentrations of analyte in the sample and its duplicate, or in the matrix spike and its duplicate matrix spike, and

S_{ave} = The average of observed analyte concentrations in the sample and its duplicate, or in the matrix spike and its duplicate matrix spike.

Depending on the method of analysis, either duplicates (and/or matrix spikes) or MS/DMS analyses are performed at a minimum frequency of one set per 20 samples. If an insufficient quantity of sample is available to perform a laboratory duplicate or duplicate matrix spikes, duplicate LCSs will be prepared and analyzed.

11.3.12 Interference Check Samples

An interference check sample (ICS) is a solution containing both interfering and analyte elements of known concentration that can be analyzed to verify background and interelement correction factors in metals analyses. The ICS is prepared to contain known concentrations (method or program specific) of elements that will provide an adequate test of the correction factors. The ICS is analyzed at the beginning and end of an analytical run or at a method-specified frequency. Results must meet method criteria and any project-specific criteria.

11.3.13 Post Digestion Spikes

Post digestion spikes are samples prepared for metals analyses that have an analyte spike added to determine if matrix effects may be a factor in the results. The spike addition should produce a method-specified minimum concentration above the method reporting limit. A post digestion spike is analyzed with each batch of samples and recovery criteria are specified for each method.

11.3.14 Control Charting

The generation of control charts is routinely performed at CAS. Surrogate, Matrix Spike and LCS recoveries are all monitored and charted. In addition, the laboratory also monitors the Relative Percent Difference (RPD) measurement of precision. Control charts are available to each individual laboratory unit to monitor the data generated in its facility using control charts that have been programmed to identify various trends in the analytical results. If trends in the data are perceived, various means of corrective action may then be employed in order to prevent future problems with the analytical system(s). Finally, data quality reports using control charts are generated for specific clients and projects pursuant to contract requirements. The control charting procedure is described in the SOP for *Control Charting Quality Control Data* (ADM-CHRT).

11.3.15 Glassware Washing

Glassware washing and maintenance play a crucial role in the daily operation of a laboratory. The glassware used at CAS undergoes a rigorous cleansing procedure prior to every usage. A number of SOPs have been generated that outline the various procedures used at CAS; each is specific to the end-use of the equipment as well as to the overall analytical requirements of the project. In addition, other equipment that may be routinely used at the laboratory is also cleaned following instructions in the appropriate SOP.

12.0 DATA REDUCTION, VALIDATION, AND REPORTING

CAS reports the analytical data produced in its laboratories to the client via the certified analytical report (CAR). This report includes a transmittal letter, a case narrative, client project information, specific test results, quality control data, chain of custody information, and any other project-specific support documentation. The following procedures describe our data reduction, validation and reporting procedures.

12.1 Data Reduction and Review

Results are generated by the analyst who performs the analysis and works up the data. All data is initially reviewed and processed by analysts using appropriate methods (e.g., chromatographic software, instrument printouts, hand calculation, etc.). Equations used for calculation of results are found in the applicable analytical SOPs. The resulting data set is either manually entered (e.g., titrimetric or microbiological data) into an electronic report form or is electronically transferred into the report from the software used to process the original data set (e.g., chromatographic software). Once the complete data set has been transferred into the proper electronic report form(s), it is then printed. The resulting hardcopy version of the electronic report is then reviewed by the analyst for accuracy. Once the primary analyst has checked the data for accuracy and acceptability, the hardcopy is forwarded to the supervisor or second qualified analyst, who reviews the data for errors. Where calculations are not performed using a validated software system, the reviewer rechecks a minimum of 10% of the calculations. When the entire data set has been found to be acceptable, a final copy of the report is printed and signed by the laboratory supervisor, departmental manager or designated laboratory staff. The entire data package is then placed into the appropriate service request file, and an electronic copy of the final data package is forwarded to the appropriate personnel for archival. Data review procedures are described in the *SOP for Laboratory Data Review Process*.

Policies and procedures for manual editing of data are established. The analyst making the change must initial and date the edited data entry, without obliteration of the original entry. The policies and procedures are described in the *SOP for Making Entries into Logbooks and onto Benchsheets* (SOP ADM-DATANTRY).

Policies and procedures for electronic manual integration of chromatographic data are established. The analyst performing the integration must document the integration change by printing both the "before" and "after" integrations and including them in the raw data records. The policies and procedures are described in the *SOP for Manual Integration of Chromatographic Peaks* (SOP ADM-INT).

12.2 Confirmation Analysis

12.2.1 Gas Chromatographic and Liquid Chromatographic Analyses

For gas chromatographic (GC) and liquid chromatographic (LC) analyses, all positive results are confirmed by a second column, a second detector, a second wavelength (HPLC/UV), or by GC/MS analysis, unless exempted by one of the following situations:

- The analyte of interest produces a chromatogram containing multiple peaks exhibiting a characteristic pattern, which matches appropriate standards. This is limited to petroleum hydrocarbon analyses (e.g., gasoline and diesel) and does not include polychlorinated biphenyls.
- The sample meets all of the following requirements:
 1. All samples (liquid or solid) come from the same source (e.g., groundwater samples from the same well) for continuous monitoring. Samples of the same matrix from the same site, but from different sources (e.g., different sampling locations) are not exempt.
 2. All analytes have been previously analyzed in sample(s) from the same source (within the last year), identified and confirmed by a second column or by GC/MS. The chromatogram is largely unchanged from the one for which confirmation was carried out. The documents indicating previous confirmation must be available for review.

12.2.2 Confirmation Data

Confirmation data will be provided as specified in the method. Identification criteria for GC, LC or GC/MS methods are summarized below:

- GC and LC Methods
 1. The analyte must fall within plus or minus three times the standard deviation (established for the analyte/column) of the retention time of the daily midpoint standard in order to be qualitatively identified. The retention-time windows will be established and documented, as specified in the appropriate Standard Operating Procedure (SOP).
 2. When sample results are confirmed by two dissimilar columns or detectors, the agreement between quantitative results must be evaluated. The relative percent difference between the two results is calculated and evaluated against SOP and/or method criteria.

- GC/MS Methods - Two criteria are used to verify identification:
 1. Elution of the analyte in the sample will occur at the same relative retention time (RRT) as that of the analyte in the standard.
 2. The mass spectrum of the analyte in the sample must, in the opinion of a qualified analyst or the department manager, correspond to the spectrum of the analyte in the standard or the current GC/MS reference library.

12.3 Data Review and Validation

The integrity of the data generated is assessed through the evaluation of the sample results, calibrations, and QC samples (method blanks, laboratory control samples, sample duplicates, matrix spikes, trip blanks, etc.). A brief description of the evaluation of these analyses is described below, with details listed in applicable SOPs. The criteria for evaluation of QC samples are listed within each method-specific SOP. Other data evaluation measures may include (as necessary) a check of the accuracy check of the QC standards and a check of the system sensitivity. Data transcriptions and calculations are also reviewed.

Note: Within the scope of this document, all possible data assessment requirements for various project protocols cannot be included in the listing below. This listing gives a general description of data evaluation practices used in the laboratory in compliance with NELAP Quality Systems requirements. Additional requirements exist for certain programs, such as projects under the DoD QSM protocols, AFCEE QAPP protocols, and project-specific QAPPs.

- Method Calibration – Following the analysis of calibration blanks and standards according to the applicable SOP the calibration correlation coefficient, average response factor, etc. is calculated and compared to specified criteria. If the calibration meets criteria analysis may continue. If the calibration fails, any problems are isolated and corrected and the calibration standards reanalyzed. Following calibration and analysis of the independent calibration verification standard(s) the percent difference for the ICV is calculated. If the percent difference is within the specified limits the calibration is complete. If not, the problem associated with the calibration and/or ICV are isolated and corrected and verification and/or calibration is repeated.
- Continuing Calibration Verification (CCV) – Following the analysis of the CCV standard the percent difference is calculated and compared to specified criteria. If the CCV meets the criteria analysis may continue. If the CCV fails, routine corrective action is performed and documented and a 2nd CCV is analyzed. If this CCV meets criteria, analysis may continue, including any reanalysis of samples that were associated with a failing CCV. If the routine corrective action failed to produce an immediate CCV within criteria, then either acceptable performance is demonstrated (after additional corrective action) with two consecutive calibration verifications, or a new initial calibration is performed. For DoD projects, the concentration of these two consecutive must be varied as required by the DoD QSM, Version 3.

- Method Blank – Results for the method blank are calculated as performed for samples. If results are less than the MRL ($< \frac{1}{2}$ MRL for DoD projects), the blank may be reported. If not, associated sample results are evaluated to determine the impact of the blank result. If possible, the source of the contamination is determined. If the contamination has affected sample results the blank and samples are reanalyzed. If positive blank results are reported, the blank (and sample) results are flagged with an appropriate flag, qualifier, or footnote.
- Sample Results (Inorganic) – Following sample analysis and calculations (including any dilutions made due to the sample matrix) the result is verified to fall within the calibration range. If not, the sample is diluted and analyzed to bring the result into calibration range. When sample and sample duplicates are analyzed for precision, the calculated RPD is compared to the specified limits. The sample and duplicate are reanalyzed if the criteria are exceeded. The samples may require re-preparation and reanalysis. For metals, additional measures as described in the applicable SOP, may be taken to further evaluate results (dilution tests and/or post-digestion spikes). Results are reported when within the calibration range, or as estimates when outside the calibration range. When dilutions are performed the MRL is elevated accordingly and qualified. Efforts are made to meet the project MRL's including alternative analysis.
- Sample Results (Organic) – For GC/MS analyses, it is verified that the analysis was within the prescribed tune window. If not, the sample is reanalyzed. Following sample analysis and calculations (including any dilutions made due to the sample matrix) peak integrations, retention times, and spectra are evaluated to confirm qualitative identification. Internal standard responses and surrogate recoveries are evaluated against specified criteria. If internal standard response does not meet criteria, the sample is diluted and reanalyzed. Results outside of the calibration range are diluted to within the calibration range. For GC and HPLC tests, results from confirmation analysis are evaluated to confirm positive results and to determine the reported value. The procedure to determine which result to report is described in the SOP *Confirmation Procedure for GC and HPLC Analysis(SOC-CONF)*. If obvious matrix interferences are present, additional cleanup of the sample using appropriate procedures may be necessary and the sample is reanalyzed. When dilutions are performed the MRL is elevated accordingly and qualified. Efforts are made to meet the project MRL's including additional cleanup.
- Surrogate Results (Organic) – Following sample analysis and data reduction, the percent recovery of each surrogate is compared to specified control limits. If recoveries are acceptable, the results are reported. If recoveries do not fall within control limits, the sample matrix is evaluated. When matrix interferences are present or documented, the results are reported with a qualifier that matrix interferences are present. If no matrix interferences are present and there is no cause for the outlier, the sample is reprepared and reanalyzed. However, if the recovery is above the upper control limit with non-detected target analytes, the sample may be reported. All surrogate recovery outliers are appropriately qualified on the report.

- Duplicate Sample and/or Duplicate Matrix Spike Results – The RPD is calculated and compared to the specified control limits. If the RPD is within the control limits the result is reported. If not, an evaluation of the sample is made to verify that a homogenous sample was used. Despite the use of homogenizing procedures prior to sample preparation or analysis, the sample may not be homogenous or duplicate sample containers may not have been sample consistently. If non-homogenous, the result is reported with a qualifier about the homogeneity of the sample. Also, the results are compared to the MRL. If the results are less than five times the MRL, the results are reported with a qualifier that the high RPD is due to the results being near the MRL. If the sample is homogenous and results above five times the MRL, the samples and duplicates are reanalyzed. If re-analysis also produces out-of-control results, the results are reported with an appropriate qualifier.
- Laboratory Control Sample Results – Following analysis of the LCS the percent recovery is calculated and compared to specified control limits. If the recovery is within control limits, the analysis is in control and results may be reported. If not, this indicates that the analysis is not in control. Samples associated with the 'out of control' LCS, shall be considered suspect and the samples re-extracted or re-analyzed or the data reported with the appropriate qualifiers. For analysis where a large number of analytes are in the LCS, it becomes more likely that some analytes (marginal exceedences) will be outside the control limits. The procedure described in the 2003 NELAC standards, Appendix D.1.1.2.1 are used to determine if the LCS is effective in validating the analytical system and the associated samples.
- Matrix Spike Results – Following analysis of the MS the percent recovery is calculated and compared to specified control limits. If the recovery is within control limits the results may be reported. If not, and the LCS is within control limits, this indicates that the matrix potentially biases analyte recovery. It is verified that the spike level is at least five times the background level. If not, the results are reported with a qualifier that the background level is too high for accurate recovery determination. If matrix interferences are present or results indicate a potential problem with sample preparation, steps may be taken to improve results; such as performing any additional cleanups, dilution and reanalysis, or re-preparation and reanalysis. Results that do not meet acceptance limits are reported with an appropriate qualifier.

12.4 Data Reporting

When an analyst determines that a data package has met the data quality objectives (and/or any client-specific data quality objectives) of the method and has qualified any anomalies in a clear, acceptable fashion, the data package is reviewed by a trained chemist. Prior to release of the report to the client, the project chemist reviews and approves the entire report for completeness and to ensure that any and all client-specified objectives were successfully achieved. The original raw data, along with a copy of the final report, is filed in project files by service request number for archiving. CAS maintains control of analytical results by adhering to standard operating procedures and by observing sample custody requirements. All data are calculated and reported in units consistent with project specifications, to enable easy comparison of data from report to report.

To the extent possible, samples shall be reported only if all QC measures are acceptable. If a QC measure is found to be out of control, and the data is to be reported, all samples associated with the failed quality control measure shall be reported with the appropriate data qualifier(s). The *SOP for Data Reporting and Report Generation* addresses the flagging and qualification of data. The CAS-defined data qualifiers, state-specific data qualifiers, or project-defined data qualifiers are used depending on project requirements. A case narrative may be written by the project chemist to explain problems with a specific analysis or sample, etc.

For subcontracted analyses, the Project Chemist verifies that the report received from the subcontractor is complete. This includes checking that the correct analyses were performed, the analyses were performed for each sample as requested, a report is provided for each analysis, and the report is signed. The Project Chemist accepts the report if all verification items are complete. Acceptance is demonstrated by forwarding the report to the CAS client.

12.5 Documentation

CAS maintains a records system which ensures that all laboratory records of analysis data retained and available. Analysis data is retained for 5 years from the report date unless contractual terms or regulations specify a longer retention time. The archiving system is described in the *SOP for Data Archiving*.

12.5.1 Documentation and Archiving of Sample Analysis Data

The archiving system includes the following items for each set of analyses performed:

- Benchsheets describing sample preparation (if appropriate) and analysis;
- Instrument parameters (or reference to the data acquisition method);
- Sample analysis sequence;
- Instrument printouts, including chromatograms and peak integration reports for all samples, standards, blanks, spikes and reruns;
- Logbook ID number for the appropriate standards;
- Copies of report sheets submitted to the work request file; and
- Copies of Nonconformity and Corrective Action Reports, if necessary.

Individual sets of analyses are identified by analysis date and service request number. Since many analyses are performed with computer-based data systems, the final sample concentrations can be automatically calculated. If additional calculations are needed, they are written on the integration report or securely stapled to the chromatogram, if done on a separate sheet.

For organics analysis, data applicable to all analyses within the batch, such as GCMS tunes, CCVs, batch QC, and analysis sequences; are kept using a separate documentation system. This system is used to archive data on a batch-specific basis and is segregated according to the date of analysis. This system also includes results for the most recent calibration curves, as well as method validation results.

12.6 Deliverables

In order to meet individual project needs, CAS provides several levels of analytical reports. Standard specifications for each level of deliverable are described in Table 12-1. Variations may be provided based on client or project specifications. This includes (but is not limited to) to following specialized deliverables:

- ADEC – Alaska Department of Conservation specified data package
- ACOE/HTRW – Army Corps of Engineers specified data package and reporting requirements (HTRW, CERP, FUDS, etc.)
- AFCEE – Air Force Center for Environmental Excellence project-specific reporting

When requested, CAS provides Electronic Data Deliverables (EDDs) in the format specified by client need or project specification. CAS is capable of generating EDDs with many different formats and specifications. The EDD is prepared by report production staff using the electronic version of the laboratory report to minimize transcription errors. User guides and EDD specification outlines are used in preparing the EDD. The EDD is reviewed and compared to the hard-copy report for accuracy.

Table 12-1
Descriptions of CAS Standard Data Deliverables

Tier I. Routine Certified Analytical Report (CAR) includes the following:

1. Transmittal letter
2. Sample analytical results
3. Method blank results
4. Surrogate recovery results and acceptance criteria for applicable organic methods
5. Chain of custody documents
6. Dates of sample preparation and analysis for all tests

Tier II and IIA. In addition to the Tier I Deliverables, this CAR includes the following:

1. Matrix spike result(s) with calculated recovery and including associated acceptance criteria
2. Duplicate or duplicate matrix spike result(s) (as appropriate to method), with calculated relative percent difference
3. Tier IIA also includes Laboratory Control Sample (LCS) result(s) with calculated recovery and including associated acceptance criteria

Tier III. Data Validation Package. In addition to the Tier II Deliverables, this CAR includes the following:

1. Case narrative
2. Calibration records and results of initial and continuing calibration verification standards, with calculated recoveries
3. Results of laboratory control sample (LCS) or Quality Control check sample, with calculated recovery and/or associated acceptance limit criteria
4. Results of calibration blanks or solvent blanks (as appropriate to method)
5. Summary forms for associated QC and calibration parameters
6. Copies of all raw data, including extraction/preparation bench sheets, chromatograms, and instrument printouts. For GC/MS, this includes tuning criteria and mass spectra of all positive hits. Results and spectra of TIC compounds will be included upon request.

Tier IV. CLP-Level Data Validation Package.

A complete Data Validation Package containing all sample results, quality control and calibration results, and raw data necessary to fulfill all deliverable requirements of an EPA Contract Laboratory Program (CLP) data package.

13.0 PERFORMANCE AND SYSTEM AUDITS

Quality audits are an essential part of CAS/Kelso's quality assurance program. There are two types of audits used at the facility: System Audits are conducted to qualitatively evaluate the operational details of the QA program, while Performance Audits are conducted by analyzing proficiency testing samples in order to quantitatively evaluate the outputs of the various measurement systems.

13.1 System Audits

The system audit examines the presence and appropriateness of laboratory systems. External system audits of CAS/Kelso are conducted regularly by various regulatory agencies and clients. Table 13-1 summarizes some of the major programs in which CAS/Kelso participates. Programs and certifications are added as required. Additionally, internal system audits of CAS/Kelso are conducted regularly under the direction of the Quality Assurance Manager. The internal audit procedures are described in the *SOP for Internal Audits*. The internal audits are performed as follows:

- Comprehensive lab-wide system audit – performed annually. This audit is conducted such that systems, technical operations, hardcopy data, and electronic data are assessed.
- Hardcopy report audits – minimum of 3 per quarter.
- Electronic audit trail reviews – each applicable instrument per quarter.

All audit findings, and corrective actions are documented. The results of each audit are reported to the Laboratory Director and Department Managers for review. Any deficiencies identified are summarized in the audit report. Managers must respond with corrective actions correcting the deficiency within a defined timeframe. Should problems impacting data quality be found during an internal audit, any client whose data is adversely impacted will be given written notification within the corrective action period (if not already provided).

Electronic data audits may be performed in conjunction with hardcopy data audits. The electronic audits focus on organic chromatographic data and include an examination of audit trails, peak integrations, calibration practices and files, GCMS tuning data, peak response data, use of appropriate files, and other components of the analysis. The audit also verifies that the electronic data supports the hardcopy reported data.

Additional internal audits or data evaluations may be performed as needed to address any potential data integrity issues that may arise.

13.2 Performance Audits

CAS/Kelso also participates in the analysis of interlaboratory proficiency testing (PT) samples. Participation in PT studies is performed on a regular basis and is designed to evaluate all analytical areas of the laboratory. CAS routinely participates in the following studies:

- Water Pollution (WP) and additional water parameters, 2 per year.
- Water Supply (WS) PT studies, 2 per year.
- Hazardous Waste/Soil PT studies, 2 per year.
- Underground Storage Tank PT studies, 2 per year.
- Microbiology (WS and WP) PT studies, 2 per year.
- Other studies as required for specific certifications, accreditations, or validations.

PT samples are processed by entering them into the LIMS system as samples (assigned Service Request, due date, testing requirements, etc.) and are processed the same as field samples. The laboratory sections handle samples the same as field samples, performing the analyses following method requirements and performing data review. The laboratory sections submit results to the QA Manager for subsequent reporting to the appropriate agencies or study provider. Results of the performance evaluation samples and audits are reviewed by the Quality Assurance Manager, Laboratory Director, the laboratory staff, and the CAS Quality Assurance Director. For any results outside acceptance criteria, the analysis data is reviewed to identify a possible cause for the deficiency, and corrective action is taken and documented.

Table 13-1
Current CAS Performance and System Audit Programs

Federal and National Programs

- The TNI (The NELAC Institute) National Environmental Laboratory Accreditation Program (NELAP) Accredited Drinking Water, Non-Potable Water, Solid & Hazardous Waste, and Biological Tissue Laboratory
- Naval Facilities Engineering Service Center Validated Laboratory for NFESC Parameters
- U.S. Air Force, Air Force Center for Environmental Excellence (AFCEE) Approved Laboratory for AFCEE Projects
- U.S. Army Corps of Engineers Approved Laboratory for USACE Projects
- U.S. EPA Region 8 Approved Drinking Water Laboratory

State and Local Programs

- State of Alaska, Department of Environmental Conservation
UST Laboratory, Lab I.D. UST040
- State of Arizona, Department of Health Services
License No. AZ0339
- State of Arkansas, Department of Environmental Quality
Certified Environmental Laboratory, Lab I.D. 88-0637
- State of California, Department of Health Services, Environmental Laboratory Accreditation Program
Certification No. 2286
- State of Colorado, Department of Public Health and Environment
Certified Drinking Water Laboratory
- State of Florida, Department of Health
Primary NELAP Accreditation No. E87412
- State of Georgia, Department of Natural Resources
Certified Drinking Water Laboratory
- State of Hawaii, Department of Health
Certified Drinking Water Laboratory
- State of Idaho, Department of Health and Welfare
Certified Drinking Water Laboratory
- State of Indiana, Department of Health
Certified Drinking Water Laboratory, Lab I.D. C-WA-01
- State of Louisiana, Department of Environmental Quality
Accredited Environmental Laboratory, Lab I.D. 3016
- State of Louisiana, Department of Health and Hospitals
Accredited Drinking Water Laboratory, Lab I.D. LA080001
- State of Maine, Department of Human Services
Certified Environmental Laboratory, Lab I.D. WA0035
- State of Michigan, Department of Environmental Quality
Certified Drinking Water Laboratory, Lab I.D. 9949

Table 13-1 (continued)
State and Local Programs (continued)

- State of Minnesota, Department of Health
Certified Environmental Laboratory, Lab I.D. 053-999-368
- State of Montana, Department of Health and Environmental Sciences
Certified Drinking Water Laboratory, Lab I.D. 0047
- State of Nevada, Division of Environmental Protection
Certified Drinking Water Laboratory, Lab I.D. WA35
- State of New Jersey, Department of Environmental Protection
Accredited Environmental Laboratory, Lab I.D. WA005
- State of New Mexico, Environment Department
Certified Drinking Water Laboratory
- State of North Carolina, Department of Environment and Natural Resources
Certified Environmental Laboratory, Lab I.D. 605
- State of Oklahoma, Department of Environmental Quality
General Water Quality/Sludge Testing, Lab I.D. 9801
- State of Oregon, ORELAP Laboratory Accreditation Program
Accredited Environmental Laboratory, Lab I.D. WA200001
- State of South Carolina, Department of Health and Environmental Control
Certified Environmental Laboratory, Lab I.D. 61002
- State of Utah, Department of Health, Division of Laboratory Services
Accredited Environmental Laboratory
- State of Washington, Department of Ecology, Environmental Laboratory Accreditation Program
Accreditation No. C1203
- State of Wisconsin, Department of Natural Resources
Accredited Environmental Laboratory, Lab I.D. 998386840

14.0 PREVENTIVE MAINTENANCE

Preventive maintenance is a crucial element of the Quality Assurance program. Instruments at CAS (e.g., ICP/MS and ICP systems, GC/MS systems, atomic absorption spectrometers, analytical balances, gas and liquid chromatographs, etc.) are maintained under commercial service contracts or by qualified, in-house personnel. All instruments are operated and maintained according to the instrument operating manuals. All routine and special maintenance activities pertaining to the instruments are recorded in instrument maintenance logbooks. The maintenance logbooks used at CAS contain extensive information about the instruments used at the laboratory.

An initial demonstration of analytical control is required on every instrument used at CAS before it may be used for sample analysis. If an instrument is modified or repaired, a return to analytical control is required before subsequent sample analyses can occur. When an instrument is acquired at the laboratory, the following information is noted in a bound maintenance notebook specifically associated with the new equipment:

- The equipment's serial number;
- Date the equipment was received;
- Date the equipment was placed into service;
- Condition of equipment when received (new, used, reconditioned, etc.); and
- Prior history of damage, malfunction, modification or repair (if known).

Equipment records also include a copy of the manufacturer's manual(s) and dates and results of calibrations.

Preventive maintenance procedures, frequencies, etc. are available for each instrument used at CAS. They may be found in the various SOPs for routine methods performed on an instrument and may also be found in the operating or maintenance manuals provided with the equipment at the time of purchase.

Responsibility for ensuring that routine maintenance is performed lies with the section supervisor. The supervisor may perform the maintenance or assign the maintenance task to a qualified bench level analyst who routinely operates the equipment. In the case of non-routine repair of capital equipment, the section supervisor is responsible for providing the repair, either by performing the repair themselves with manufacturer guidance or by acquiring on-site manufacturer repair. Each laboratory section maintains a critical parts inventory. The parts inventories include the items needed to perform the preventive maintenance procedures listed in Appendix D.

This inventory or "parts list" also includes the items needed to perform any other routine maintenance and certain in-house non-routine repairs such as gas chromatography/mass spectrometry jet separators and electron multipliers and ICP/MS nebulizer. When performing maintenance on an instrument (whether preventive or corrective), additional information about the problem, attempted repairs, etc. is also recorded in the notebook. Typical logbook entries include the following information:

- Details and symptoms of the problem;
- Repairs and/or maintenance performed;
- Description and/or part number of replaced parts;
- Source(s) of the replaced parts;
- Analyst's signature and date; and
- Demonstration of return to analytical control.

See the table in Appendix D for a list of preventive maintenance activities and frequency for each instrument.

15.0 CORRECTIVE ACTION

To the extent possible, samples shall be reported only if all quality control measures are acceptable. If a quality control measure is found to be out of control, and the data is to be reported, all samples associated with the failed quality control measure shall be reported with the appropriate data qualifier(s). Failure to meet established analytical controls, such as the quality control objectives outlined in Section 11, prompts corrective action. In general, corrective action may take several forms and may involve a review of the calculations, a check of the instrument maintenance and operation, a review of analytical technique and methodology, and reanalysis of quality control and field samples. If a potential problem develops that cannot be solved directly by the responsible analyst, the supervisor, team leader, the department manager, and/or the Quality Assurance Manager may examine and pursue alternative solutions. In addition, the appropriate project chemist may be notified in order to ascertain if contact with the client is necessary.

In the event that analyses produce nonconformances with data or results, the problem and the corresponding corrective actions taken are documented on Nonconformity and Corrective Action Reports (See Figure 15-1) following the requirements in the SOP for Corrective Action (SOP No. ADM-CA). This form is utilized to document corrective actions in response to out-of-control situations. The Quality Assurance Manager reviews each problem, ensuring that appropriate corrective action has been taken by the appropriate personnel. The Nonconformity and Corrective Action Report (NCAR) is filed in the associated service request file and a copy is kept by the Quality Assurance Manager. The Quality Assurance Manager periodically reviews all NCARs looking for chronic, systematic problems that need more in-depth investigation and alternative corrective action consideration. In addition, the appropriate project chemist is promptly notified of any problems in order to inform the client and proceed with any action the client may want to initiate.

In addition to internal communication of data issues, the laboratory also maintains a system for dealing with customer complaints. The person who initially receives the feedback (typically the project chemist) is responsible for documenting the complaint. If the project chemist is unable to satisfy the customer, the complaint is brought to the attention of the Client Services Manager, Laboratory Director, or QA Manager for final resolution. The complaint and resolution are documented. The procedure is described in the *SOP for Handling Customer Feedback* (ADM-FDBK).

Corrective action due to a performance audit or a proficiency(PT) sample finding is initiated by the Quality Assurance Manager; the affected laboratory supervisors and managers are promptly informed of performance audit results requiring corrective action.

Figure 15-1

Columbia Analytical Services, Inc.

Nonconformity and Corrective Action Report

NONCONFORMITY

NCAR No. _____

PROCEDURE (SOP or METHOD): _____	EVENT DATE: _____
EVENT: <input type="checkbox"/> Missed Holding Time <input type="checkbox"/> QC Failure <input type="checkbox"/> Lab Error (spilled sample, spiking error, etc.) <input type="checkbox"/> Method Blank Contamination <input type="checkbox"/> Login Error <input type="checkbox"/> Project Management Error <input type="checkbox"/> Equipment Failure <input type="checkbox"/> Unacceptable PT Sample Result <input type="checkbox"/> SOP Deviation <input type="checkbox"/> Other (describe): _____	
SAMPLES / PROJECTS / CUSTOMERS / SYSTEMS AFFECTED _____	
DETAILED DESCRIPTION _____	
ORIGINATOR: _____	DATE: _____
PROJECT MANAGER(S): _____ NOTIFIED BY: _____	DATE: _____

CORRECTIVE ACTION AND OUTCOME

Re-establishment of conformity must be demonstrated and documented. Describe the steps that were taken, or are planned to be taken, to correct the particular Nonconformity and prevent its reoccurrence. Include Project Manager instructions here.

Is the data to be flagged in the Analytical Report with an appropriate qualifier? ☐ No ☐ Yes

APPROVAL AND NOTIFICATION

Supervisor Verification and Approval of Corrective Action _____	Date: _____
Comments: _____	
QA PM Verification and Approval of Corrective Action _____	Date: _____
Comments: _____	
Customer Notified by <input type="checkbox"/> Telephone <input type="checkbox"/> Fax <input type="checkbox"/> E-mail <input type="checkbox"/> Narrative <input type="checkbox"/> Not notified	
Project Manager Verification and Approval of Corrective Action _____	Date: _____
Comments: _____	
(Attach record or cite reference where record is located.)	

16.0 QUALITY ASSURANCE REPORTS

Quality assurance requires an active, ongoing commitment by CAS personnel at all levels of the organization. Communication and feedback mechanisms are designed so that analysts, supervisors and managers are aware of QA issues in the laboratory. Analysts performing routine testing are responsible for generating a data quality narrative or data review document with every analytical batch processed. This report also allows the analyst to provide appropriate notes and/or a case narrative if problems were encountered with the analyses. A Non-Conformity and Corrective Action Report (NCAR) (see Section 15.0) may also be attached to the data prior to review. Supervisors or qualified analysts review all of the completed analytical batches to ensure that all QC criteria have been examined and any deficiencies noted and corrected if possible.

It is the responsibility of each laboratory unit to provide the project chemist with a final report of the data, accompanied by signature approval. Footnotes and/or narrative notes must accompany any data package if problems were encountered that require further explanation to the client. Each data package is submitted to the appropriate project chemist, who in turn reviews the entire collection of analytical data for completeness. The project chemist must also review the entire body of data to ensure that any and all client-specified objectives were successfully achieved. A case narrative may be written by the project chemist to explain any unusual problems with a specific analysis or sample, etc.

The Quality Assurance Manager (QAM) provides overview support to the project chemists as required (e.g., contractually specified, etc.). The QAM is also responsible for the oversight of all internal and external audits, for all proficiency testing sample and analysis programs, and for all laboratory certification/accreditation responsibilities. The QAM provides the Laboratory Director with quarterly reports that summarize the various QA/QC activities that occurred during the previous quarter. The report addresses such topics as the following:

- Status, schedule, and results of internal and external audits;
- Status, schedule, and results of internal and external proficiency testing studies;
- Status of certifications, accreditations, and approvals;
- Status of QA Manual and SOP review and revision;
- Status of MDLs studies;
- Discussion of QC problems in the laboratory;
- Discussion of corrective action program issues;
- Status of staff training and qualification; and
- Other topics as appropriate.

Any operational or quality assurance problems noted by the Laboratory Director are then addressed during the senior staff operations meetings with all appropriate department managers. The Laboratory Director also performs a documented management review annually of the quality and management systems to identify any necessary changes or improvements to the quality system or quality assurance policies.

17.0 PERSONNEL TRAINING

Technical position descriptions are available for all employees, regardless of position or level of seniority. These documents are maintained by the Human Resources personnel and are available for review. In order to assess the technical capabilities and qualifications of a potential employee, all candidates for employment at CAS are evaluated, in part, against the appropriate technical description.

Training begins the first day of employment at CAS when the company policies are presented and discussed. Safety and QA/QC requirements are integral parts of all technical SOPs and, consequently, are integral parts of all training processes at CAS. Safety training begins with the reading of the *Environmental Health and Safety Manual*. Employees are also required to attend periodic safety meetings where additional safety training may be performed by the Environmental, Health and Safety Officer. Employees are responsible for complying with the requirements of the QA Manual and QA/QC requirements associated with their function(s).

Each employee participates in Ethics training, which is part of the CAS Improper Practices Prevention Program. CAS also encourages its personnel to continue to learn and develop new skills that will enhance their performance and value to the Company. Ongoing training occurs for all employees through a variety of mechanisms. The "CAS University" education system, external and internal technical seminars and training courses, and laboratory-specific training exercises are all used to provide employees with professional growth opportunities.

A training plan is developed whenever an employee starts a new procedure to new position. The training plan includes a description of the step-by-step process for training an employee and for initial demonstration of proficiency. Where the analyst performs the entire procedure, a generic training plan may be used. In cases where work cells are used, a training plan specific to the work cell is established.

17.1 Initial Demonstration of Capability (IDOC)

Training in analytical procedures typically begins with the reading of the Standard Operating Procedure (SOP) for the method. Hands-on training begins with the observation of an experienced analyst performing the method, followed by the trainee performing the method under close supervision, and culminating with independent performance of the method on quality control samples. Successful completion of the applicable Demonstration of Capability analysis qualifies the analyst to perform the method independently. Demonstration of Capability is performed by one of the following:

- Successful completion of an Initial Precision and Recovery (IPR) study (required where mandated by the method).
- Analysis of 4 consecutive Laboratory Control Samples, with acceptable accuracy and precision.
- Where spiking is not possible but QC standards are used ("non-spiked" Laboratory Control Samples), analysis of 4 consecutive Laboratory Control Samples with acceptable accuracy and precision.
- Where one of the three above is not possible, special requirements are as follows:
 - Total Settleable Solids: Successful single-blind PT sample analysis and duplicate results with RPD<10%.
 - Color: Four consecutive prepared LCSs with acceptable accuracy and precision of <10% RSD.
 - Physical Tests (Grain size, Corrosivity to Steel, etc.): Supervisor acknowledgement of training and approval.

A flowchart identifying the Demonstration of Proficiency requirements is given in Figure 17-1. The flowchart identifies allowed approaches to assessing Demonstration of Capability when a 4-replicate study is not mandated by the method, when spiking is not an option, or when QC samples are not readily available.

17.2 Continuing Demonstration of Proficiency

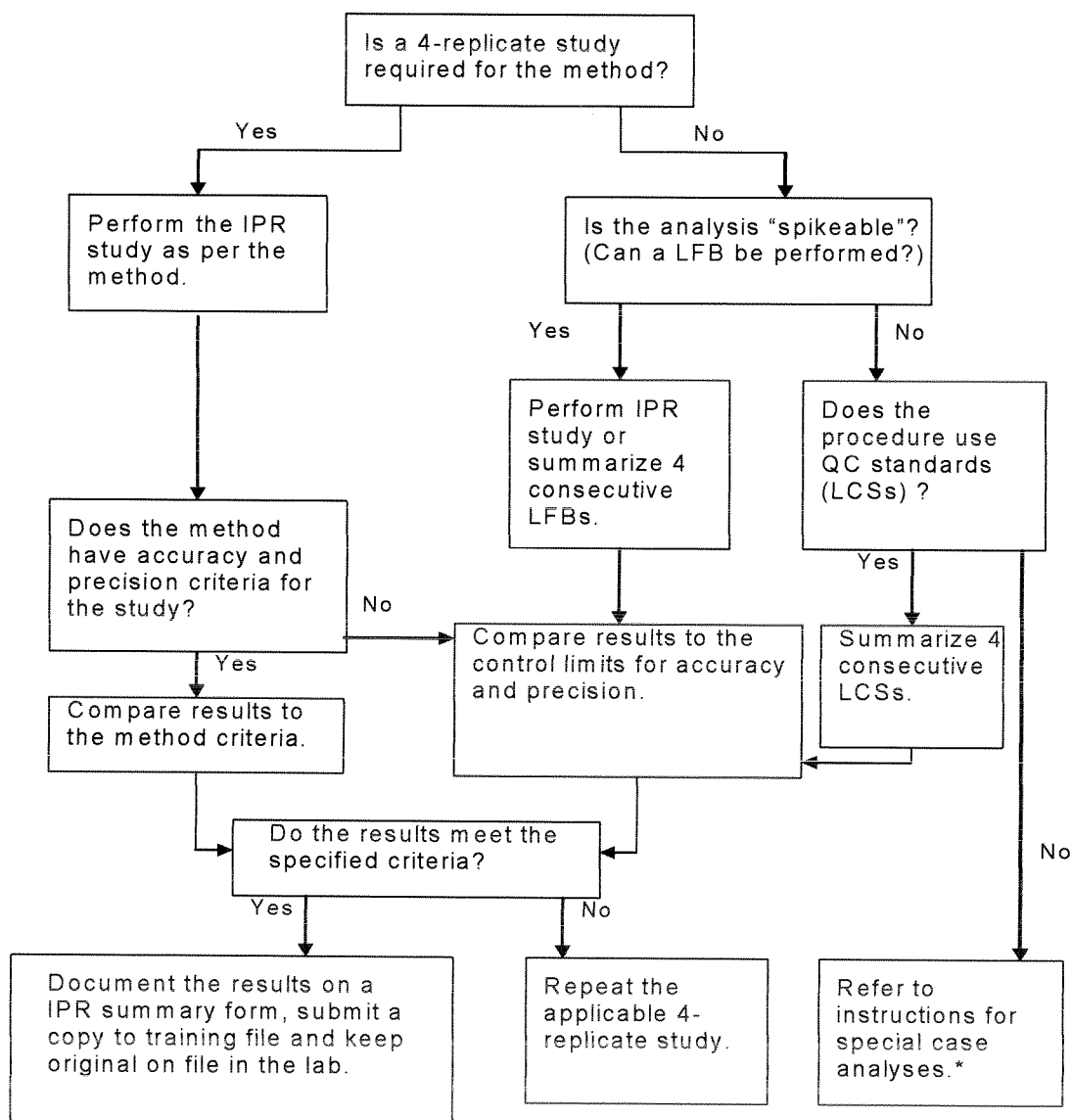
A periodic demonstration of proficiency is required to maintain continuing qualification. Continuing Demonstration of Proficiency is required each year, and may be performed one of the following ways:

- Successful performance on external (independent) single-blind PT sample analyses using the test method, or a similar test method using the same technology.
- Performing Initial Demonstration of Capability as described above, with acceptable levels of precision and accuracy.
- Analysis of at least 4 consecutive LCSs with acceptable levels of accuracy and precision from in-control analytical batches.
- For methods for which PT samples are not available and a spiked analysis (LFB, MDL, etc.) is not possible, analysis of field samples that have been analyzed by another analyst with statistically indistinguishable results.

17.3 Documentation of Training

Records are maintained to indicate the employee has the necessary training, education, and experience to perform their functions. Information of previously acquired skills and abilities for a new employee is maintained in Human Resources personnel files and CAS resumes. A database is used to record the various technical skills and training acquired while employed by CAS. Information includes the employee's name, a description of the skill including the appropriate method and SOP reference, the mechanism used to document proficiency, and the date the training was completed. General procedures for documenting technical training are described in the *SOP for Documentation of Training (SOP No. ADM-TRANDOC)*.

Figure 17-1
Initial Demonstration of Capability Requirements^a



^a For IDOC IPR or LFB studies, "second-source" reference materials are used, as per NELAP requirements

*Total Settleable Solids: Successful PT sample analysis and duplicate results with RPD<10%.

*Color: Four consecutive prepared LCSs with acceptable accuracy and precision of <10% RSD.

* Physical Tests (Grain size, Corrosivity to Steel, etc.): Supervisor acknowledgement of training and approval.

18.0 REFERENCES FOR ANALYTICAL PROCEDURES

The analytical methods used at CAS generally depend upon the end-use of the data. Since most of our work involves the analysis of environmental samples for regulatory purposes, specified federal and/or state testing methodologies are used and followed closely. Typical methods used at CAS are taken from the following references:

- *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*, SW-846, Third Edition, (September 1986) and Updates I (July 1992), II (September 1994), IIA (August 1993), IIB (January 1995), III (December 1996), Final Update IV (February 2007), and updates posted online at <http://www.epa.gov/epaoswer/hazwaste/test/sw846.htm>. See Chapters 1, 2, 3, and 4.
- *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, (Revised March 1983).
- *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA/600/R-93/100 (August 1993).
- *Methods for the Determination of Metals in Environmental Samples*, EPA/600/4-91/010 (June 1991) and Supplements.
- *Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater*, EPA 600/4-82-057 (July 1982) and 40 CFR Part 136, Appendix A.
- *Methods for the Determination of Organic Compounds in Drinking Water*, EPA/600/4-88/039 (December 1988) and Supplements.
- *Standard Methods for the Examination of Water and Wastewater*, 18th Edition (1992); 19th Edition (1995), 20th Edition (1998). See Introduction in Part 1000.
- 40 CFR Part 136, Guidelines for Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act.
- 40 CFR Part 141, National Primary Drinking Water Regulations.
- *Analytical Methods for Petroleum Hydrocarbons*, ECY 97-602, Washington State Department of Ecology, June 1997.
- State-specific total petroleum hydrocarbon methods for the analysis of samples for gasoline, diesel, and other petroleum hydrocarbon products (Alaska, Arizona, California, Oregon, Washington, Wisconsin, etc.).

- Annual Book of ASTM Standards, Part 31, Water.
- EPA Contract Laboratory Program, Statement of Work for Organic Analysis, SOW Nos. OLM03.1, OLM03.2, OLM04.2, and OLM04.3.
- EPA Contract Laboratory Program, Statement of Work for Inorganic Analysis, SOW No. ILM04.0, ILM04.1, and ILM05.2.
- *U. S. EPA Contract Laboratory Program National Functional Guidelines for Organic Data Review*, EPA-540/R-94/012 (February 1993).
- *U. S. EPA Contract Laboratory Program National Functional Guidelines for Inorganic Data Review*, EPA-540/R-94/013 (February 1994).
- National Institute for Occupational Safety and Health (NIOSH) *Manual of Analytical Methods*, Third Edition (August 1987); Fourth Edition (August 1994).
- *Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound*, for USEPA and USACE (March 1986), with revisions through April 1997.
- WDOE 83-13, *Chemical Testing Methods for Complying with the State of Washington Dangerous Waste Regulations* (March 1982) and as Revised (July 1983 and April 1991).
- *Identification and Listing of Hazardous Waste*, California Code of Regulations, Title 22, Division 4.5, Chapter 11.
- *Analytical Methods for the Determination of Pollutants in Pulp and Paper Industry Wastewater*, EPA 821-R-93-017 (October 1993).
- *Analytical Methods for the Determination of Pollutants in Pharmaceutical Manufacturing Industry Wastewaters*, EPA 821-B-98-016 (July 1998).
- National Council of the Pulp and Paper Industry for Air and Stream Improvement (NCASI).
- *Good Automated Laboratory Practices, Principles and Guidance to Regulations For Ensuring Data Integrity In Automated Laboratory Operations*, EPA 2185 (August 1995).
- *Manual for the Certification of Laboratories Analyzing Drinking Water*, 4th Edition, EPA 815-B-97-001 (March 1997).
- National Environmental Laboratory Accreditation Program (NELAP), 2003 Quality Standards.
- *Department of Defense Quality Systems Manual for Environmental Laboratories*, Final Version 3 (January 2006).

APPENDIX A

LIST of QA PROGRAM DOCUMENTS

and

STANDARD OPERATING PROCEDURES

Quality Assurance Manual	2/6/2009
Software Quality Assurance Plan	7/11/05
CAS-Kelso Certifications/Accreditations	Cert_kel.xls
Columbia Analytical Services MDL Tracking Spreadsheet	Mdl_list.xls
Technical Training Summary Database	TrainDat.mdb
Approved Signatories List	AppSignatories.pdf
Personnel resumes/qualifications	HR Department
Personnel Job Descriptions	HR Department
Quality Control Acceptance Criteria	Qclimits.xls
Master Logbook of Laboratory Logbooks	Masterlog-001
Standard Operating Procedure Database	TrainDat.mdb
ADMINISTRATIVE STANDARD OPERATING PROCEDURES	
<u>ADMINISTRATIVE - CORPORATE</u>	<u>FILE NAME</u>
CHECKING NEW LOTS OF CHEMICALS FOR CONTAMINATION	ADM-CTMN
CONTROL LIMITS	ADM-CTRL_LIM
CORRECTIVE ACTION	ADM-CA
DATA RECALL	ADM-DATARECALL
HANDLING CUSTOMER FEEDBACK	ADM-FDBK
DETERMINATION OF METHOD DETECTION LIMITS AND LODS	ADM-MDL
DOCUMENT CONTROL	ADM-DOCCTRL
DOCUMENTATION OF TRAINING	ADM-TRANDOC
ELECTRONIC DATA AUDITING	ADM-E_DATAAUDIT
ESTIMATION OF UNCERTAINTY OF MEASUREMENTS	ADM-UNCERT
MAKING ENTRIES INTO LOGBOOKS AND ONTO BENCHSHEETS	ADM-DATANTRY
MANAGERIAL REVIEW OF THE LABORATORY'S QUALITY SYSTEM	ADM-MGMTRVW
MANUAL INTEGRATION OF CHROMATOGRAPHIC PEAKS	ADM-INT
PREPARATION OF ELECTRONIC DATA FOR ORGANIC ANALYSES ELECTRONIC DATA AUDITS	ADM-EDATA
PREPARATION OF STANDARD OPERATING PROCEDURES	ADM-SOP
PROFICIENCY TESTING SAMPLE ANALYSIS	ADM-PTS
PURCHASING THROUGH CAS PURCHASING DEPARTMENT IN KELSO	ADM-PUR
QUALIFICATION OF SUBCONTRACT LABORATORIES OUTSIDE OF CAS NETWORK	ADM-SUBLAB

SIGNIFICANT FIGURES	ADM-SIGFIG
CONFIRMATION of ORGANIC ANALYTE IDENTIFICATION and QUANTITATION	ADM-CONFIRM
PREVENTATIVE ACTION	ADM-PA

<u>ADMINISTRATIVE – LOCAL LABORATORY</u>	<u>FILE NAME</u>
CHECKING PIPETTE CALIBRATION	ADM-CPIP
CONTINGENCY PLAN FOR LABORATORY EQUIPMENT FAILURE	ADM-ECP
CONTROL CHARTING QUALITY CONTROL DATA	ADM-CHRT
DATA ARCHIVING	ADM-ARCH
DATA REPORTING AND REPORT GENERATION	ADM-RG
DEPARTMENT OF DEFENSE PROJECTS LABORATORY PRACTICES AND PROJECT MANAGEMENT	ADM-DOD
ELECTRONIC DATA BACKUP AND ARCHIVING	ADM-EBACKUP
INTERNAL QUALITY ASSURANCE AUDITS	ADM-IAUD
LABORATORY BALANCE MONITORING AND CALIBRATION	ADM-BAL
LABORATORY DATA REVIEW PROCESS	ADM-DREV
METHOD DETECTION LIMIT DOCUMENTATION AND CONTROL	ADM-MDLC
PROJECT MANAGEMENT	ADM-PCM
REAGENT LOGIN AND TRACKING	ADM-RLT
SUPPORT EQUIPMENT MONITORING AND CALIBRATION	ADM-SEMC
SAMPLE BATCHES	ADM-BATCH
<u>SAMPLE MANAGEMENT SOPS</u>	<u>FILE NAME</u>
BOTTLE ORDER PREPARATION AND SHIPPING	SMO-BORD
FOREIGN SOILS HANDLING TREATMENT	SMO-FSHT
SAMPLE DISPOSAL	SMO-SDIS
SAMPLE RECEIVING	SMO-GEN
SAMPLE TRACKING AND LABORATORY CHAIN OF CUSTODY	SMO-SCOC
<u>TECHNICAL STANDARD OPERATING PROCEDURES</u>	
SOP TABLE OF CONTENTS	SOPLIST.XLS

COLUMBIA ANALYTICAL SERVICES, INC. , KELSO, WA.
STANDARD OPERATING PROCEDURES TABLE OF CONTENTS

January 19, 2009

<u>SOP NAME</u>	<u>FILE NAME</u>
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COLIFORM, FECAL	BIO-9221FC
COLIFORM, TOTAL	BIO-9221TC
COLIFORM, FECAL (MEMBRANE FILTER PROCEDURE)	BIO-9222D
COLILERT® and COLITAG	BIO-9223
FECAL STREPTOCOCCUS/ENTEROCOCCUS	BIO-9230B
COLILERT® COMPLETED TEST VERIFICATION OF E. COLI IN MUG CULTURES	BIO-CCT
ENTEROLERT	BIO-ENT
HEPTEROTROPHIC PLATE COUNT	BIO-HPC
MICROBIOLOGY QUALITY ASSURANCE AND QUALITY CONTROL	BIO-QAQC
SHEEN SCREEN/OIL DEGRADING MICROORGANISMS	BIO-SHEEN
EPA CLP ORGANICS ANALYSES	CLP_ORGA
SEPARATORY FUNNEL LIQUID-LIQUID EXTRACTION	EXT-3510
CONTINUOUS LIQUID - LIQUID EXTRACTION	EXT-3520
SOLID PHASE EXTRACTION	EXT-3535
SOLID PHASE EXTRACTION - Method 3535A	EXT-3535A
SOXHLET EXTRACTION	EXT-3540
AUTOMATED SOXHLET EXTRACTION	EXT-3541
ULTRASONIC EXTRACTION	EXT-3550
WASTE DILUTION EXTRACTION	EXT-3580
SILICA GEL CLEANUP	EXT-3630
REMOVAL OF SULFUR USING COPPER	EXT-3660
REMOVAL OF SULFUR USING MERCURY	EXT-3660M
SULFURIC ACID CLEANUP	EXT-3665
DIAZOMETHANE PREPARATION	EXT-DIAZ
FLORISIL CLEANUP	EXT-FLOR
ORGANIC EXTRACTIONS GLASSWARE CLEANING	EXT-GC
PREPARATION OF REAGENTS AND BLANK MATRICES USED IN SEMIVOLATILE ORGANICS ANALYSIS	EXT-REAG
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MEASURING SAMPLE WEIGHTS AND VOLUMES FOR ORGANIC ANALYSIS	EXT-WVOL
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OPERATION AND MAINTENANCE OF LABORATORY REAGENT WATER SYSTEMS	FAC-WATER
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SULFIDES, ACIDS VOLATILE	GEN-AVS
HEAT OF COMBUSTION	GEN-BTU
CYANIDE, WEAK ACID DISSOCIABLE	GEN-CNWAD
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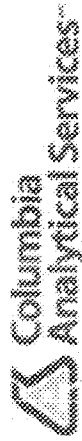
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TRACE METALS IN WATER BY PRECONCENTRATION USING REDUCTIVE PRECIPITATION FOLLOWED BY ICP-MS	MET-MEP
WASTE EXTRACTION TEST (WET) PROCEDURE (STLC) for NONVOLATILE and SEMIVOLATILE PARAMETERS	MET-RPMS
METALS AND SEMIVOLATILES TCLP EXTRACTION (EPA METHOD 1311)	MET-STLC
SAMPLE PREPARATION OF BIOLOGICAL TISSUES FOR METALS ANALYSIS BY GFAA, ICP-OES, AND ICP-MS	MET-TCLP
TISSUE SAMPLE PREPARATION	MET-TDIG
GRAVIMETRIC DETERMINATION OF HEAXANE EXTRACTABLE MATERIAL (1664)	MET-TISP
GASOLINE RANGE ORGANICS BY GAS CHROMATOGRAPHY	PET-1664
ANALYSIS OF WATER, SOLIDS AND SOLUBLE WASTE SAMPLES FOR SEMI-VOLATILE FUEL HYDROCARBONS	PET-GRO
	PET-SVF

ANALYSIS OF SOLID AND AQUEOUS SAMPLES FOR STATE OF WISCONSIN DIESEL RANGE ORGANICS	PHC-WIDRO
CHLORINATED PHENOLICS IN WASTE WATER BY IN-SITU	SOC-1653A
PHARMACEUTICALS, PERSONAL CARE PRODUCTS AND ENDOCRINE DISRUPTING COMPOUNDS IN WATER BY HPLC/TANDEM MASS SPECTROMETRY (HPLC/MS/MS)	SOC-1694
1,8-DIHYDROXYANTHRAQUINONE BY GC/MS SIM	SOC-
GEL PERMEATION CHROMATOGRAPHY	18DHYDRAQ
ACETAMIDE HERBICIDE DEGRADATES IN DRINKING WATER BY SPE AND HPLC/MS/MS	SOC-3640A
ORGANOCHLORINE PESTICIDES AND PCBs (METHOD 608)	SOC-535
SEMIVOLATILE ORGANIC COMPOUNDS BY GC/MS	SOC-608
GLYCOLS	SOC-625
ORGANOCHLORINE PESTICIDES BY GAS CHROMATOGRAPHY: CAPILLARY COLUMN TECHNIQUE	SOC-8015M
PCBS AS AROCLORS	SOC-8081
PCBS AS AROCLORS - METHOD 8082A	SOC-8082A
CONGENER-SPECIFIC DETERMINATION OF PCBS BY GC/ECD - METHOD 8082A	SOC-8082AAr
CONGENER-SPECIFIC DETERMINATION OF PCBS BY GC/ECD	SOC-8082ACo
DETERMINATION OF NITROGEN OR PHOSPHORUS CONTAINING PESTICIDES	SOC-8082C
CHLORINATED HERBICIDES	SOC-8141
CHLORINATED PHENOLS METHOD 8151 MODIFIED	SOC-8151
SEMIVOLATILE ORGANIC COMPOUNDS BY GC/MS	SOC-8151M
SEMIVOLATILE ORGANIC COMPOUNDS BY GC/MS - METHOD 8270D	SOC-8270C
SEMIVOLATILE ORGANIC COMPOUNDS BY GC/MS - LOW LEVEL PROCEDURE	SOC-8270D
POLYNUCLEAR AROMATIC HYDROCARBONS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY SIM	SOC-8270L
SEMIVOLATILE ORGANIC COMPOUNDS BY GC/MS SELECTED ION MONITORING	SOC-8270P
POLYNUCLEAR AROMATIC HYDROCARBONS BY HPLC	SOC-8270S
ALDEHYDES BY HPLC	SOC-8310
NITROAROMATICS AND NITRAMINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY	SOC-8315A
NITROGLYCERIN AND PETN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY	SOC-8330
RESIN AND FATTY ACIDS BY GC/MS - NCASI METHOD 85.02 MODIFIED	SOC-8332
METHANOL IN PROCESS LIQUIDS AND STATIONARY SOURCE EMISSIONS	SOC-85.02
HAZARDOUS AIR POLLUTANTS (HAPS) IN PULP AND PAPER INDUSTRY CONDENSATES	SOC-9403
HAPS AND OTHER COMPOUNDS IN IMPINGER/CANISTER SAMPLES FROM WOOD PRODUCTS FACILITIES	SOC-9901
BUTYLINS	SOC-9902
CALIBRATION OF INSTRUMENTS FOR ORGANICS CHROMATOGRAPHIC ANALYSES	SOC-BUTYL
CALIBRATION OF INSTRUMENTS FOR ORGANICS CHROMATOGRAPHIC ANALYSES USING EPA 8000C	SOC-CAL
CARBON CLEANUP	SOC-CAL8000C
CONFIRMATION PROCEDURE FOR GC AND HPLC ANALYSES	SOC-CARCU
DIMP	SOC-CONF
DMD SYNTHESIS	SOC-DIMP
TOTAL OLEANOLIC ACID SAPONINS IN WATER BY ACID HYDROLYSIS AND HPLC/MS/MS	SOC-DMD
PERCENT LIPIDS IN TISSUE	SOC-LCMS3
MONOCHLOROACETIC ACID BY GC-ECD	SOC-LIPID
NITROGUANIDINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY	SOC-MCA
NONYLPHENOLS ISOMERS AND NONYLPHENOL ETHOXYLATES	SOC-NITG
ORGANIC ACIDS IN AQUEOUS MATRICES BY HPLC	SOC-NONYL
EXTRACTION METHOD FOR ORGANOTINS IN SEDIMENTS, WATER, AND TISSUE	SOC-OALC
CHLORINATED PESTICIDES BY GC/MS/MS, EPA METHOD 1699 MODIFIED	SOC-OSWT
PERFLUORINATED COMPOUNDS BY HPLC/MS/MS	SOC-PESTMS2
	SOC-PFC

PICRIC ACID AND PICRAMIC ACID BY HPLC	SOC-PICRIC
POLYBROMINATED DIPHENYL ETHERS (PBDEs) AND POLYBROMINATED BIPHENYLS (PBBs) BY GC/MS	
SEMI-VOLATILE ORGANICS SCREENING	SOC-ROHS
1,2-DIBROMOETHANE, 1,2-DIBROMO-3-CHLOROPROPANE, AND 1,2,3-TCP BY GC	SOC-SCR
ORGANOCHLORINE PESTICIDES AND PCBS IN DRINKING WATER	SVD-504
CHLORINATED HERBICIDES IN DRINKING WATER	SVD-508_1
N-NITROSAMINES BY GC/MS/MS	SVD-515_4
SEMIVOLATILE ORGANIC COMPOUNDS BY GC/MS (METHOD 525.2)	SVD-521
SELECTED PESTICIDES AND FLAME RETARDANTS IN DRINKING WATER BY GC/MS (EPA METHOD 527)	SVD-525
DETERMINATION OF EXPLOSIVES AND RELATED COMPOUNDS IN DRINKING WATER BY GC/MS	SVD-527
CARBAMATES AND CARBAMOYLOXIMES IN WATER BY POST-COLUMN DERIVATIZATION HPLC	SVD-529
GLYPHOSATE IN DRINKING WATER BY HPLC	SVD-531 -1
ENDOTHALL IN DRINKING WATER BY GC/MS	SVD-547
DIQUAT AND PARAQUAT BY HPLC	SVD-548
HALOACETIC ACIDS IN DRINKING WATER	SVD-549
PURGE AND TRAP FOR AQUEOUS SAMPLES	SVD-552
PURGE AND TRAP/EXTRACTION FOR VOC IN SOIL AND WASTE SAMPLES , CLOSED SYSTEM	VOC-5030
VOLATILE ORGANIC COMPOUNDS BY GC/MS	VOC-5035
AROMATIC VOLATILE ORGANICS (BTEX) BY GC - METHOD 602	VOC-524.2
VOLATILE ORGANIC COMPOUNDS BY GC/MS	VOC-602BTEX
AROMATIC VOLATILE ORGANICS (BTEX) BY GC - METHOD 8021	VOC-624
VOLATILE ORGANIC COMPOUNDS BY GC/MS	VOC-8021BTEX
VOLATILE ORGANIC COMPOUNDS BY GC/MS SELECTIVE ION MONITORING	VOC-8260
VOA STORAGE BLANKS	VOC-8260S
SAMPLE SCREENING FOR VOLATILE ORGANIC COMPOUNDS IN SOIL, WATER AND MISC. MATRICES	VOC-BLAN
WA-DOH DRINKING WATER PROTOCOL	VOC-BVOC
ZERO HEADSPACE EXTRACTION (EPA METHOD 1311)	VOC-WA.DOH
	VOC-ZHE

APPENDIX B

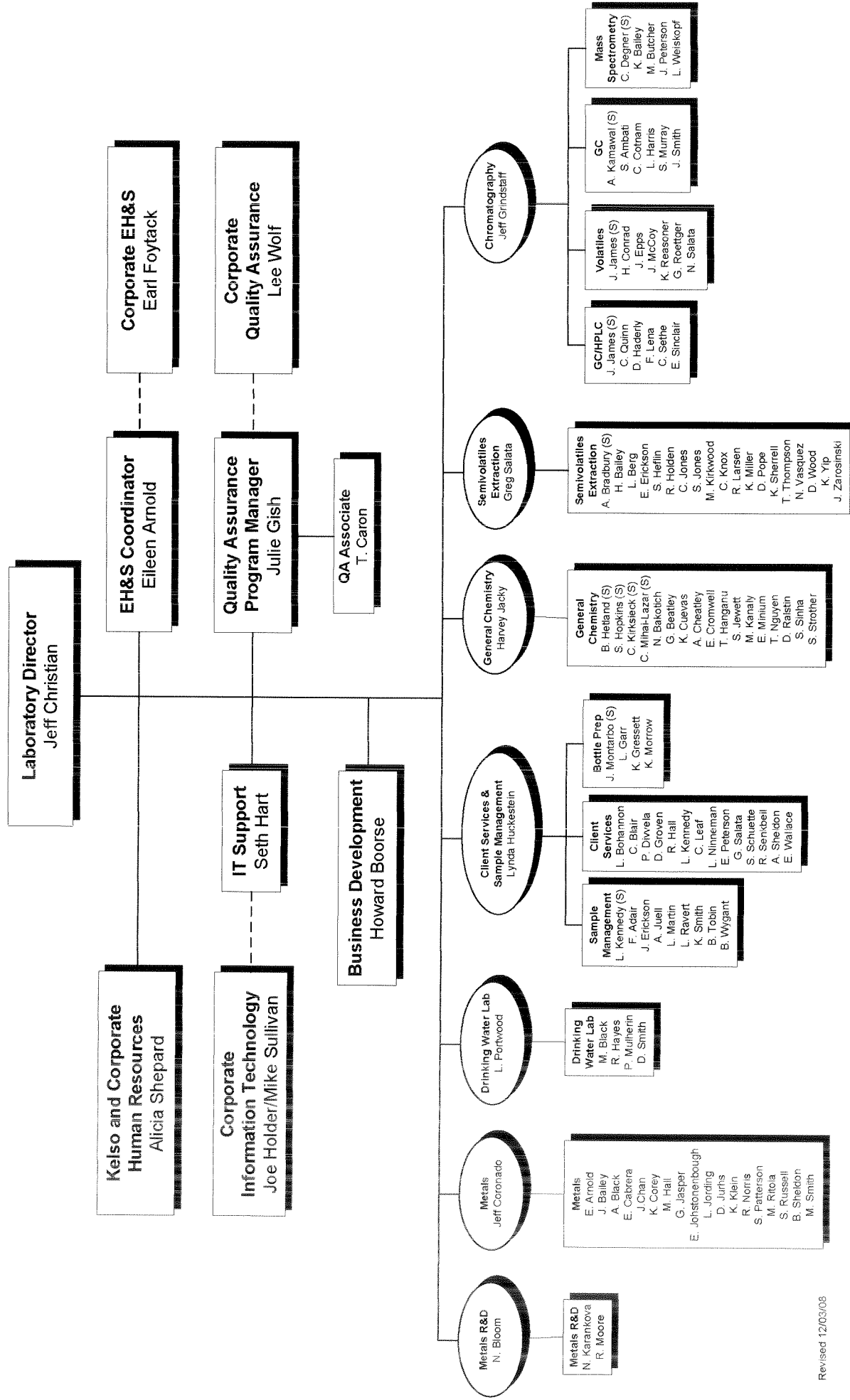
**ORGANIZATIONAL CHARTS and RESUMES OF KEY
PERSONNEL**

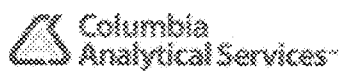


Environmental and General Testing Division

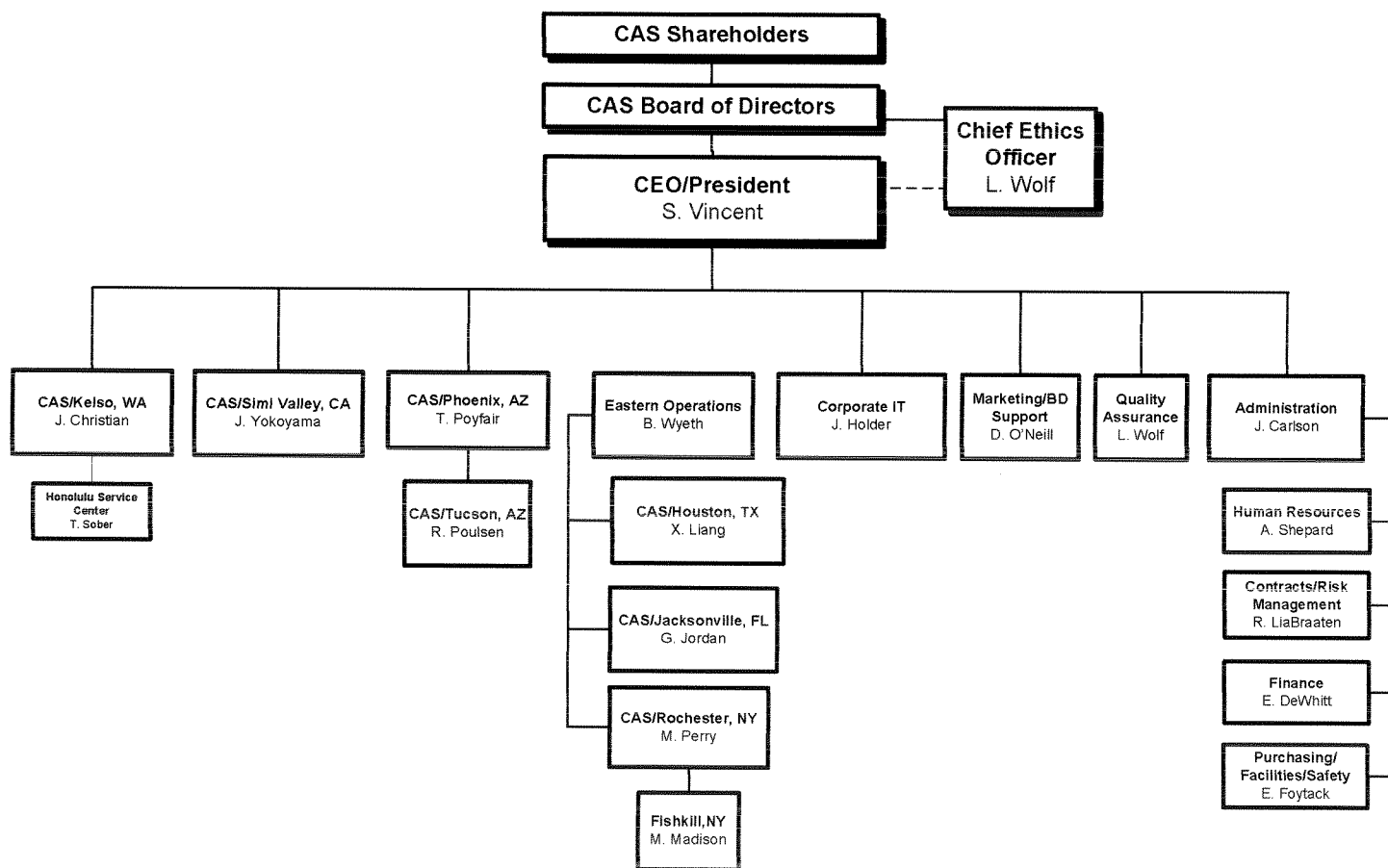
Kelso, Washington

Laboratory Organization





Laboratory Division Organization



JEFFREY D. CHRISTIAN

1989 TO PRESENT

Columbia Analytical Services, Inc., 1317 S. 13th Avenue, Kelso, WA 98626 (360) 577-7222

Current Position

VICE PRESIDENT/NW REGIONAL DIRECTOR – 1996 to Present

Responsibilities

Responsible for all phases of laboratory operations at the Kelso (WA) and Redding (CA) facilities, including project planning, budgeting, and quality assurance. Primary duties include the direct management of the Kelso laboratory (i.e. serves as the Kelso Laboratory Director, 1993-present). Also responsible for additional duties acquired as a member of the Columbia Analytical Services Holdings, Inc., Board of Directors.

Experience

Laboratory Director, Kelso Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1993-1995. Responsible for all phases of laboratory operations, including project planning, budgeting, and quality assurance.

Operations Manager, Kelso Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1992-1993. Responsibilities included directing the daily operation of the Kelso laboratory. Other responsibilities and duties included functioning as a technical consultant to clients, providing assistance in developing and planning analytical schemes to match client objectives, and writing and developing analytical procedures/methods. Also, served as Project Manager for State of Alaska Department of Environmental Conservation contract and Coordinator for EPA Special Analytical Services (SAS) contracts.

Project Chemist and Manager, Metals Analysis Laboratory, Columbia Analytical Services, Kelso, Washington, 1989-1992. Responsible for directing the daily operation of the Metals Laboratory, including the sample preparation, AAS, ICP-OES, and ICP-MS Laboratories.

Scientist, Weyerhaeuser Technology Center, Federal Way, Washington, 1986-1989. Responsibilities included supervising atomic spectroscopy laboratory which included flame and furnace AAS, ICP-OES, and sample preparation capabilities to handle a wide variety of sample types. Interfaced with internal and external clients to provide technical support. Wrote and developed analytical procedures/methods.

Lead Technician, Metals Lab, Weyerhaeuser Technology Center, Federal Way, Washington, 1981-1986. Responsibilities included primary ICP and AAS analyst for EPA-CLP contract work. Extensive experience in wide variety of environmental and product-related testing.

Research Assistant, ITT Rayonier, Olympic Research Division, Shelton, Washington, 1978-1981. Responsibilities included performing water quality tests, product-related analytical tests, corrosion tests (i.e., potentiometric polarization techniques), and operated pilot equipment specific to the pulp and paper industry.

Education

B.S., Chemistry, Evergreen State College, Olympia, Washington, 1993.

ICP/MS Training Course, VG-Elemental, 1992.

Coursework, Pacific Lutheran University, Tacoma, Washington. 1988-1989.

Coursework, Tacoma Community College, Tacoma, Washington. 1970-1971, 1988-1989.

Perkin-Elmer Advanced Furnace, Norwalk, Connecticut, 1986.

CERTIFICATION, Chemistry, L.H. Bates Technical, Tacoma, Washington, 1978.

Coursework, Central Washington University, Ellensburg, Washington. 1969-1970.

Publications/ Presentations

On request.

JULIE GISH
1996 TO PRESENT



Columbia Analytical Services, Inc., 1317 South 13th Ave., Kelso, WA 98626 360.577.7222

Current Position	TECHNICAL MANAGER I, KELSO LAB QUALITY ASSURANCE MANAGER – 2008 to Present
Responsibilities	Responsible for the overall implementation of the laboratory QA program. Responsible for the Quality Assurance Manual, certifications, documenting SOPs, and maintaining proficiency testing (PT) records. Oversee balance calibration and sample storage temperature control. Maintain certifications/accreditations for regulatory agencies and client certifications or approval programs. Act as primary point of contact during laboratory audits and provides audit responses and initiates any corrective actions. Coordinate the analysis and reporting of PT samples. Conduct internal audits and make recommendations for corrective action.
Experience	<p>Scientist IV, Semi-Volatile Mass Spectrometry Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 2002-2008. Primary responsibilities were analysis, interpretation and report generation for semivolatile organics by GC/MS. Analyses included EPA 625, 8270, SIM, and other miscellaneous methodology.</p> <p>Technical Manager I, Semi-Volatile GC Organics Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1999-2002. Primary responsibilities include supervision and oversight of semi-volatile GC department. This includes initiating new methods, staff training, workload management, and instrument maintenance/troubleshooting. Duties include departmental compliance with CAS QA and Safety policies. Responsible for analysis, interpretation and report generation for pesticides and PCB's by EPA Methods 608, 8080, 8081, 8082, EPA 8141A, Organotins, and CLP Pesticides.</p> <p>Scientist III, Semi-Volatile Organics Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1996-1999. Primary responsibilities were analysis, interpretation and report generation for pesticides and PCB's by EPA Methods 608, 8080, 8081, 8082, and CLP-Pesticides. Secondary responsibilities include organics semi-volatile sample preparation.</p> <p>Scientist, Volatile Organics Sample Preparation, Employer's Overload, Longview, Washington – assigned to the Columbia Analytical Services, Inc., Kelso, Washington facility, 1996. Primary duties included the preparation of water, soil, sediment and tissue samples using EPA Methods 3510, 3520, 3540, 3550, and 3545. Other duties were the further clean up of extracts using EPA Methods 3620 (Florsil), 3610 (Alumina), 3630 (Silica gel), 3650 (Acid/Base Partitioning), and 3660 (Sulfur).</p> <p>Organics Chemist and GC/MS Chemist, Coffey Laboratories, Portland, Oregon, 1990-1996. Primary responsibilities included sample preparation and analysis for EPA FID, ECD, and HPLC using various EPA SW-846 and 500-series methods, as well as other methodology. Later, moved to GC/MS position which included sample preparation, analysis, and associated instrument maintenance for EPA Methods 625, 8027, and 525 BNA's. Also responsible for data review and approval of data packages.</p> <p>QC Manager/QC Supervisor and Product Manager, Corn Products, Frito-Lay, Inc., Vancouver, Washington, 1982-1990. Manager of the QC department overseeing three supervisors and approximately 30 technicians. Responsible for department cost, accuracy, timeliness of data and safety performance. Later, responsible for production oversight of brand name snacks. Responsible for cost, quality and safety performance over three shifts. Managed four supervisors directly and approximately 60 employees indirectly.</p> <p>Food Technologist, QA Department, Kraft, Inc., Buena Park, California, 1978-1981. Responsible for audits, formulations, finished product evaluation, batch reviews and technical support.</p>
Education	<p>MS, Food Science, Minor in Industrial Engineering, Oregon State Univ. Corvallis, Oregon, 1978.</p> <p>BS, Food Science, Minor in Business Administration, Utah State University, Logan, Utah, 1975</p>
Publications/ Presentations	<p><i>Quality Improvement Team Leader, Coffey Laboratories, Portland, Oregon. 1991</i></p> <p><i>Methods Improvement Program, Coffey Laboratories, Portland, Oregon. Seminars on Development and Implementation 1990.</i></p> <p><i>Statistical Process Control and Total Quality Management, Frito-Lay, Vancouver, Washington. Routine Training Classes 1986-1988</i></p>

LYNDA A. HUCKESTEIN

1989 TO PRESENT

Columbia Analytical Services, Inc., 1317 S. 13th Avenue, Kelso, WA 98626 (360) 577-7222

Current Position

CLIENT SERVICES MANAGER IV – 1998 to Present

Responsibilities

Management of the Client Services Departments: Project Management, Electronic Data Deliverables and Report Generation, and Sample Management. Personally responsible for approximately 1.5 million dollars of client work annually performing technical project management and client service. Provides technical and regulatory interpretation assistance as-well-as project organization to work received by the laboratory.

Experience

Documentation of Demonstration of Capabilities is available for review.

Project Chemist, Columbia Analytical Service, Inc., Kelso, Washington, 1992-1998. Primary responsibilities included technical project management and client service in areas of pulp & paper, marine services, mining, and DOD. Also responsible for providing technical and regulatory interpretation assistance as-well-as project organization to work received by the laboratory

Project Chemist and Department Manager, General Chemistry Laboratory, Columbia Analytical Services, Inc., 1989-1992. Responsible for management of the General Chemistry laboratory for routine wastewater, bioassay, and microbiological analyses. Also responsible for supervision of staff, data review, and reporting.

Analyst III, Columbia Analytical Services, Inc., Kelso, Washington, 1989. Primary responsibilities included coliform testing, total recoverable petroleum hydrocarbon extractions and analysis, BODs, ammonias, and TKN, in addition to miscellaneous wet chemistry analyses.

Microbiologist/Chemist, Coffey Laboratories, Portland, Oregon, 1983. Coliform analysis; water chemistry.

Laboratory Assistant, Oregon State University, Corvallis, Oregon, 1983. Wheat spike dissection and tissue culture.

Education

BS, Microbiology, Oregon State University, Corvallis, Oregon, 1983.

JEFFREY A. CORONADO
1989 TO PRESENT

Columbia Analytical Services, Inc., 1317 S. 13th Avenue, Kelso, WA 98626 (360) 577-7222

Current Position

TECHNICAL MANAGER IV, METALS DEPARTMENT MANAGER – 2001 to Present

Responsibilities

Primary responsibilities include management of the Metals laboratory department. Responsible for training oversight, data review, report accuracy and timeliness QA/QC implementation, tracking department workload, and scheduling and performance of the Metals department. Also responsible for departmental budgets, method development efforts, and resource allocation.

Experience

Documentation of Demonstration of Capabilities is available for review.

Metals Department Manager, Columbia Analytical Services, Inc., Kelso, Washington, 1992-2001. Responsibilities included management of all aspects of the metal laboratory operation, including personnel training and evaluation, review of all metals data, and report generation. Also responsible for client service on a number of ongoing CAS accounts. Technical duties include primary analytical responsibility for trace level metals analysis by ICP/MS. Analyses range from routine water and soil analysis, to marine tissues, as well as industrial applications such as ultra-trace QA/QC work for various semiconductor clients. Also responsible for a number of specialized sample preparation techniques including trace metals in seawater by reductive precipitation, and arsenic and selenium speciation by ion-exchange chromatography. Developed methodology for performing mercury analysis at low part per trillion levels by cold vapor atomic fluorescence..

Supervisor, GFAA Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1989-1992. Responsibilities included supervision of metals analysis by graphite furnace atomic absorption following SW-846 and EPA CLP methodologies. Duties include workload scheduling, data review, instrument maintenance, personnel training and evaluation.

Education

Field Immunoassay Training Course, EnSys Inc., 1995.

Winter Conference on Plasma Spectrochemistry, San Diego, California, 1994.

ICP-MS Training Course, VG-Elemental, 1992.

BS, Chemistry, Western Washington University, Bellingham, Washington, 1988.

BA, Business Administration, Western Washington University, Bellingham, Washington, 1985.

JEFFREY A. GRINDSTAFF
1991 TO PRESENT

Columbia Analytical Services, Inc., 1317 S. 13th Avenue, Kelso, WA 98626 (360) 577-7222

Current Position

TECHNICAL MANAGER III, CHROMATOGRAPHY AND MASS SPECTROMETRY LABORATORIES – 1997 to Present

Responsibilities

Primary responsibilities include management of the GC/MS SemiVoa and VOA laboratory departments. Responsible for training oversight, data review, report accuracy and timeliness QA/QC implementation, tracking department workload, and scheduling and performance of the GC/MS departments. Also responsible for departmental budgets, method development efforts, and resource allocation. Also performs GC/MS maintenance and troubleshooting.

Experience

Documentation of Demonstration of Capabilities is available for review.

Manager, GC/MS VOA Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1994-1997. Responsible for supervision of GC/MS VOA staff, method development, training, data review, tracking department workload, scheduling analyses, and general maintenance and troubleshooting of GC/MS systems.

Scientist III, GC/MS VOA Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1991-1994. Responsibilities included scheduling workload, data review, instrument maintenance and troubleshooting, and personnel training and evaluation. Also responsible for supervision of extraction personnel and instrument analysts. Additional supervisory duties included report generation and data review for GC analyses. Responsibilities also included project management and customer service.

Chemist, Enseco-CRL, Ventura, California, 1990-1991. Established GC/MS department including inventory maintenance, preparation of state certification data packages, method development, SOPs, and extended data programs. Performed daily maintenance and troubleshooting of GC and GC/MS instrumentation. Scheduled and performed routine and non-routine VOA analyses.

GC/MS Chemist, VOA Laboratory Coast-to-Coast Analytical Service, San Luis Obispo, California, 1990-1991. Responsible for standard preparation for VOA analyses and instrument calibration, tuning, and maintenance. Also implemented and further developed EPA methods for quantitative analysis of pesticides and priority pollutants..

Education

Mass Selective Detector Maintenance, Hewlett-Packard Education Center, 1993.

Interpretation of Mass Spectra I, Hewlett-Packard Analytical Education Center, 1992.

B.S., Chemistry, California Polytechnic State University, San Luis Obispo, California, 1989.

A.A., Liberal Arts, Allan Hancock College, Santa Maria, California. 1986

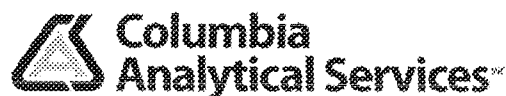
**Publications/
Presentations**

Alternate Method to Lower Detection Limits to Satisfy Regulatory Action Levels for Volatiles in Groundwater, with David Edelman, Kairas Parvez, and Paul Laymon. TAPPI National Meeting, Orlando, Florida. 1996

Affiliations

American Chemical Society. 1989

HARVEY L. JACKY
1999 TO PRESENT



Columbia Analytical Services, Inc., 1317 South 13th Ave., Kelso, WA 98626 360.577.7222

Current Position TECHNICAL MANAGER II – 2008 to Present

Responsibilities Oversee the operation of the General Chemistry and Microbiology groups. Responsible for the quality and timeliness of the inorganic laboratories analytical reports, departmental budgets, workload coordination, method development efforts, cost-effectiveness, and resource allocation.

Documentation of Demonstration of Capabilities is available for review.

Experience **Project Manager III, Columbia Analytical Services, Inc., Kelso, WA, 1999-2008.** Responsible for technical project management, ensuring overall data quality and compliance with customer requirements, and providing technical support to clients regarding laboratory application to projects. Additionally, acts as a consultant to clients regarding industrial/environmental compliance issues; serving as liaison between clients and regulatory agencies.

Director of Project Management, Coffey Laboratories, Portland, Oregon, 1997-1999. Responsible for technical project management. Communicated with clients to determine needs and expectations. Monitored laboratory production and ensured the timely completion of analytical projects. Technical consultant for clients regarding environmental compliance. Supervised and managed other members of the project management team. Served as a member of the senior management team for oversight of general operations, strategic planning, finances, and policy.

Project Manager/Chemist, Coffey Laboratories, Portland, Oregon, 1997-1999. Served as primary liaison between Coffey Laboratories and major clients. Ensured that work was completed in a timely manner and done to client specifications. Served as technical consultant regarding environmental chemistry, soil remediation, and waste water industrial compliance. Clients included the Oregon Department of Transportation, Hazmat Unit, Portland, Oregon; Raytheon Demilitarization Co., Umatilla, Oregon; Hydroblast - Wastewater Evaporator Systems, Vancouver, Washington; and Union Pacific Railroad, Northwest Region, Klamath Falls, Oregon.

Technical Sales Representative, Coffey Laboratories, Portland, Oregon, 1995-1997. Responsible for marketing and sales, including actively prospecting for new potential clients. Additional responsibilities included procurement and preparation of all major project bids; ensuring that client expectations were met; and maintaining customer satisfaction. Served as consultant regarding industrial compliance issues, environmental remediation projects, and hazardous waste management.

Senior Chemist/Laboratory Chemical Hygiene Officer, Coffey Laboratories, Portland, Oregon, 1988-1995. Performed analytical tests including Anions by Ion Chromatography (EPA 300.0), PAHs by HPLC (EPA 8310), Cyanides (EPA 335), and other inorganic, wet chemistry, and organic analytical tests on a wide variety of sample matrices. Responsible for the initial quality assurance review of work performed, supervised and managed personnel. Developed and implemented Laboratory Chemical Hygiene Plan. Directed personnel in regards to safety issues and hazardous waste management. Served as consultant and teacher regarding analytical methodology, environmental compliance, and industrial hygiene.

Education **40-Hour Hazmat Certification, PBS Environmental, 1996.**

Industrial Emergency Response, SFSP Seminar, 1991

BS, Zoology, Oregon State University, Corvallis, Oregon, 1988.

BS, General Science, Oregon State University, Corvallis, Oregon, 1988.

COURSEWORK, General Studies, Linfield College, McMinnville, Oregon, 1981-1982.

Publications/ Presentations *Biochemical and Physical Factors Involved in the Application and Measurement of a Soil Bioremediation System.* Biogeochemistry, Portland State University, 1996

LOREN E. PORTWOOD

1992 TO PRESENT

Columbia Analytical Services, Inc., 1317 S. 13th Avenue, Kelso, WA 98626 (360) 577-7222

Current Position	SCIENTIST IV, DRINKING WATER LABORATORY MANAGER – 2008 to Present
Responsibilities	<p>Responsible for the overall operation and supervision of the Organic Drinking Water department, including oversight of UCMR2 analyses. Perform analyses and conduct data review. Perform method development. Work with project management of drinking water accounts. Development of Standard Operating Procedures for drinking water methods. Operation of Varian GC/MS, Agilent GC/ECD and Agilent HPLC.</p> <p>Documentation of Demonstration of Capabilities is available for review.</p>
Experience	<p>Scientist IV, Drinking Water Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 2002-2008. Plan, conduct, and, as lead analyst, supervise analyses using advanced instrumentation such as HPLC with post column derivatization, GC/MS, and GC/ECD. Responsible for data interpretation, QC, and data reporting. Also responsible for preparation of SOPs; handling routine and advanced maintenance and troubleshooting of instrumentation; and assisting in the training of staff department analysts. Assists the department manager and/or other senior scientists in setting up more complex procedures.</p> <p>Technical Manager I, Petroleum Hydrocarbon Laboratory Supervisor, Primary responsibilities included oversight of the PHC laboratory, including initiating new processes and staff development and training. Responsible for CAS QA compliance, routine system checks. Technical mentor to PHC staff. Also duties listed below under Scientist II and Scientist III.</p> <p>Scientist III, Petroleum Hydrocarbon Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1997-1998. Duties primarily as listed below.</p> <p>Scientist II, Petroleum Hydrocarbon Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1996-1997. Duties primarily as listed below, and including HPLC methods 8310, 8315, and 8330.</p> <p>Scientist I, Petroleum Hydrocarbon Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1993-1996. Primary responsibilities included the analysis, reporting, and archiving of water, soil, and product samples for semi-volatile petroleum hydrocarbons. Methods of analysis include EPA method 8015 and various state modifications thereof (OR, WA, CA, AK). Additional responsibilities include sample preparation, instrument maintenance, and assistance with other departmental analyses.</p> <p>Bench Chemist I, Organic Extractions Laboratory, Columbia Analytical Services, Inc., Kelso, Washington, 1992-1993. Primary responsibilities included performing a wide range of organics extractions and cleanups for water, soil, and oil to be analyzed in the GC, GC/MS, and PHC laboratories.</p> <p>Chemist, Treclen Laboratories, Spokane, Washington, 1990-1992. Primary responsibilities included inorganic water and soil testing by EPA methods. Developed testing which was accredited by the EPA, including metal digestions, phosphates, and TSS/ TDS.</p>
Education	<p>BS, Chemistry, Emphasis in Biochemistry, Whitworth College, Spokane, Washington, 1990.</p> <p>Several vendor chromatography, GC, HPLC, and Quality training courses, 1993-2002.</p>

EILEEN M. ARNOLD
1987 TO PRESENT

Columbia Analytical Services, Inc., 1317 S. 13th Avenue, Kelso, WA 98626 (360) 577-7222

Current Position

SCIENTIST IV, METALS LABORATORY, KELSO HEALTH AND SAFETY OFFICER – 1994 to Present

Responsibilities

Duties include the operation and maintenance of the Inductively Coupled Argon Plasma (ICAP) Emission Spectrometer. This involves digestion, instrumental analysis, and report generation for environmental samples using approved EPA techniques. Health and Safety Officer responsibilities included development and implementation of the Kelso Health and Safety program, including accident investigation and incident review, maintenance of all safety related equipment and documents, and performance of monthly safety audits.

Documentation of Demonstration of Capabilities is available for review.

Experience

Project Chemist, Client Services Group, Kelso Health and Safety Officer, Columbia Analytical Services, Inc., Kelso, Washington, 1992-1994. Duties included technical project management and customer service. Responsible for meeting the clients' needs of timely and appropriate analyses, and to act as liaison for all client-related activities within Columbia Analytical Services, Inc. Health and Safety Officer responsibilities included development and implementation of the Kelso Health and Safety program, including accident investigation and incident review, maintenance of all safety related equipment and documents, and performance of monthly safety audits.

Scientist IV, Metals Laboratory, Health and Safety Officer, Columbia Analytical Services, Inc., Kelso, Washington, 1987-1992. Duties include the operation and maintenance of the Inductively Coupled Argon Plasma (ICAP) Emission Spectrometer. This involves digestion, instrumental analysis, and report generation for environmental samples using approved EPA techniques. Health and Safety Officer responsibilities included development and implementation of the Kelso Health and Safety program, including accident investigation and incident review, maintenance of all safety related equipment and documents, and performance of monthly safety audits.

Chemist, Dow Corning Corporation, Springfield, Oregon, 1986-1987. Responsibilities included ICP and atomic absorption work in silicon manufacturing. Methods development for ICP analysis of minor impurities found in silicon.

Chemist, Ametek, Inc., Harleysville, Pennsylvania, 1982-1985. Responsibilities included product research and development chemist involved in production of thin-film semiconductors for use as solar cells. Work involved AA and SEM techniques.

Chemist, Janbridge, Inc., Philadelphia, Pennsylvania, 1978-1982. Responsibilities included maintaining electroplating process lines through wet chemical analysis techniques, and performed Quality Assurance testing on printed circuit boards.

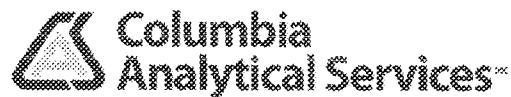
Education

BA, Chemistry, Immaculata College, Immaculata, Pennsylvania, 1977.

Affiliations

American Chemical Society, Member since 1987.

GREGORY G. SALATA
2003 TO PRESENT



Columbia Analytical Services, Inc., 1317 South 13th Ave., Kelso, WA 98626 360.577.7222

Current Position	PROJECT/EXTRACTIONS MANAGER V – 2003 to Present
Responsibilities	Responsibilities include Project Management, including quotation preparation and data reporting, as well as providing technical support to the laboratory as needed. Responsibilities also include oversight of the organic extractions lab, managing resources and providing technical support for all organic preparation work flows. 2003-Present.
Experience	<p>Project Manager, B&B Laboratories, College Station, Texas, 1999-2003. Supervisor/responsible for analysis of TPH (waters, tissues, sediments), organotins (waters, tissues, sediments), Atterberg Limits (sediments), and total organic/inorganic carbon (sediments, waters). Also responsible for report generation on specific projects. Instrumentation operated included GCs with FID and FPD detectors, Combustion TOC, Water TOC, and Dionex Accelerated Solvent Extractor.</p> <p>Graduate Student, Texas A&M University, College Station, Texas, 1991-1999. While working toward MS in Oceanography, performed organic extractions for pesticides, PCBs, PAHs, and butyltins. While working toward Ph.D. in Oceanography determined stable carbon isotope ratios in sediments, waters, and bacterial phospholipid fatty acids. Other responsibilities included field sample collection, and operation/maintenance of FinniganMAT 252 isotope ratio MS.</p> <p>Analytical Chemist, Science Applications International (SAIC), San Diego, California, 1989-1990. Performed organic extraction and GC/FID analysis on sediment/rock samples for the Exxon Valdez oil spill.</p> <p>GC Chemist, Analytical Technologies, San Diego, California, 1987-1989. Responsible for analysis of volatile organics using purge and trap and GC/PID/ELCD.</p>
Education	<p>Ph.D., Oceanography, Texas A&M University, College Station, Texas. 1999</p> <p>MS, Oceanography, Texas A&M University, College Station, Texas. 1993</p> <p>BA, Chemistry, University of California San Diego, Revelle College, La Jolla, California. 1987</p>
Publications/ Presentations	<i>Dr. Salata has a number of publications and published abstracts. For a list of these publications and published abstracts, please contact CAS.</i>
Affiliations	American Chemical Society

LEE E. WOLF
1988 TO PRESENT



Columbia Analytical Services, Inc., 1317 South 13th Ave., Kelso, WA 98626 360.577.7222

Current Position	QUALITY ASSURANCE DIRECTOR AND CHIEF QUALITY OFFICER – 2008 to Present
Responsibilities	Directing the overall corporate-wide quality systems and ethics programs for all CAS facilities. Responsible for ensuring that CAS quality systems and data integrity standards are implemented at all facilities. Act as liaison with government entities involving quality, technical and operational issues. Provide QA input and policy as needed for operations, development initiatives, special projects, planning, and information technology implementation. Provide assistance to QA Program Managers.
Experience	<p>Technical Manager IV, Quality Assurance Program Manager, Columbia Analytical Services, Inc., Kelso, Washington – 2002 to 2008. As part of the management team, responsibilities included the overall management and implementation of the laboratory QA program. This included maintaining accreditations and certifications, and maintaining all necessary documents (QA Manual, SOPs, and QA records). Acted as primary point of contact during laboratory audits and provided audit responses and corrective actions. Coordinated performance audits (PE/PT testing) and conducted internal audits.</p> <p>Scientist IV, Quality Assurance Program Manager, Columbia Analytical Services, Inc., Kelso, Washington, 1996-2002. Duties primarily as listed above.</p> <p>Project Chemist/Principal Organic Scientist, Columbia Analytical Services, Inc., Kelso, Washington, 1994-1996. Responsibilities included GC and GC/MS method development and special projects coordination. Acts as technical advisor to the GC and GC/MS laboratories and GC/MS interpretation specialist and CLP organics specialist. Also responsible for Project Chemist functions, including management of projects for clients, identifying client needs, and preparation of data reports.</p> <p>Semivolatile Organics Department Manager, Columbia Analytical Services, 1988-1994. Responsibilities included overall management of the department. Supervised GC/MS analyses, data review, reporting and related QA/QC functions. Responsible for supervision of staff, training, and scheduling. Beginning in 1992, responsibilities included being a Project Chemist for organics EPA-SAS and other clients. This involved scheduling projects for clients, identifying client requirements, and preparing data reports.</p> <p>GC/MS Chemist, U.S. Testing Co., Richland, Washington, 1985-1988. Responsibilities included GC and GC/MS analysis of water and soil samples for volatiles and semivolatiles by EPA protocol, including Methods 8240, 8270 and CLP. Coordinated extraction and GC-GC/MS areas to manage sample/data flow through the lab. Also performed HPLC analysis and pesticide analysis by GC using EPA Methods.</p> <p>Laboratory Assistant, Eastern Washington University, Cheney, Washington, 1985. Responsibilities included supervision and instruction of organic chemistry labs. Experience with GC and IR operation. Responsible for lab safety.</p>
Education	<p>Pharmaceutical Laboratory Control Systems, Univ. of Wisconsin Short Course, Las Vegas, 2004</p> <p>Test Method Validation in Pharmaceutical Development and Production, Univ. of Wisconsin Short Course, Las Vegas, 2004</p> <p>Documenting Your Quality System, A2LA Short Course, Las Vegas, Nevada, 1998.</p> <p>Internal Laboratory Audits, A2LA Short Course, Las Vegas, Nevada, 1998.</p> <p>Mass Spectra Interpretation, ACS Short Course, Denver, Colorado, 1992.</p> <p>BS, Chemistry, Eastern Washington University, Cheney, Washington, 1985.</p>
Publications/ Presentations	<p><i>Selected Ion Monitoring: Issues for Method Development</i>, Panel Discussion, Association of Official Analytical Chemists, (AOAC) Pacific Northwest Regional Meeting, 1995.</p> <p><i>Method Enhancement Techniques for Achieving Low level Detection of Butyl Tin in Marine Sediments and Tissues</i>, Association of Official Analytical Chemists (AOAC) Pacific Northwest Regional Meeting, 1994.</p> <p><i>The Determination of Low-Level Concentrations of Polynuclear Aromatic Hydrocarbons (PAHs) in Soil and Water Using Gas Chromatography/Mass Spectroscopy Selected Ion Monitoring (GC/MS SIM)</i>, HazMat West, Long Beach, California, 1992.</p>

STEPHEN W. VINCENT

1986 TO PRESENT

Columbia Analytical Services, Inc., 1317 S. 13th Avenue, Kelso, WA 98626 (360) 577-7222

Current Position

PRESIDENT, CAS HOLDINGS INC. – 1986 to Present

Responsibilities

Responsible for the overall growth and profitability of the CAS laboratory network. This includes establishing and implementing long-range objectives, plans, and policies, and representing the company with its major customers, technical community, and the public.

Experience

Laboratory Manager, Weyerhaeuser Company, Federal Way, Washington, 1979-1986. Responsibilities involved all phases of technical and administrative management. This included management of organic, inorganic, and microbiological analyses and management of capital; an annual operating budget of approximately \$2 million; management of thirty staff members; contract procurement, and project management. Projects included an EPA Inorganic CLP contract; an EPA acid rain deposition contract; a contract with the Fish and Wildlife Service to measure trace organic contaminants in animal tissues; and others.

Analytical Chemist, Weyerhaeuser Company, Longview, Washington, 1975-1979.

Responsibilities: Method development, routine analysis and supervision for the Weyerhaeuser Multi-Region Support Lab. Responsible for setting up a company-wide laboratory audit, round robin, and quality assurance program.

Education

Market Strategy for Technology Based Companies, Executives Program, Stanford University, 1994.

Advanced Technical Management Program, University of California at Los Angeles, Department of Business, Engineering and Management, 1991.

Completion of Coursework for MS, Pulp and Paper Technology, University of Washington, Seattle, Washington, 1984.

Post Graduate Coursework, Engineering and Management, University of California at Los Angeles, Graduate School of Engineering and Applied Science, Los Angeles, California, 1981.

BS, Oceanography, University of Washington, Seattle, Washington, 1974.

Publications/ Presentations

Mr. Vincent has a number of publications and presentations. For a list of these publications and presentations, please contact CAS.

Affiliations

American Chemical Society.

Technical Association of the Pulp and Paper Industry.

APPENDIX C

MAJOR ANALYTICAL EQUIPMENT

GENERAL CHEMISTRY/WATER CHEMISTRY LABORATORY			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
Analytical Balances (10): Precisa and Mettler models	1988-2008	MM	15
Autoclave - Market Forge Sterilmatic	1988	LM	5
Autotitrator – Thermo Orion 500	2007	LM	3
Calorimeters (2): Parr 1241 EA Adiabatic	1987	LM	4
Parr 6300 Isoparabolic	2005	LM	4
Centrifuge - Damon/IEC Model K	1992	LM	15
Colony Counter - Quebec Darkfield	1988	LM	4
Conductivity Meters (2): YSI Model 3200	2004	LM	4
VWR	2001	LM	4
Digestion Systems (5): COD (4)	1987, 1989	LM	5
Kjeldahl, Lachat 46-place (1)	1999	LM	3
Dissolved Oxygen Meter - YSI Model 58 (3)	1987, 1988, 1991	LM	5
Distillation apparatus (Midi) - Easy Still (2)	1996, 2000	LM	7
Drying Ovens (11): Shel-Lab and VWR models	1988 - 2003	LM	15
Flash Point Testers (2): ERDCO Setaflash Tester	1991	LM	4
Petroleum Systems Services	2005	LM	4
Flow-Injection Analyzers (2): Bran-Leubbe	2002	LM	4
Lachat 8500	2007	LM	4
Ion Chromatographs (4) Dionex 2000i with Peaknet Data Systems	1988	LM	3
Dionex DX-120 with Peaknet Data System	1998	LM	3
Dionex ICS-2500 with Chromchem Data System	2002	LM	3
Dionex ICS-2000 with Chromchem Data System	2006	LM	3
Ion Selective Electrode Meters (5) Fisher Scientific Accumet Model 50	1997	LM	6
Fisher Scientific Accumet Model 25	1993	LM	6
Fisher Scientific Accumet Model 20	2000	LM	6
Orion Model 920A	1990	LM	6
Corning pH/ion Meter Model 135	1992	LM	6
Microscope - Olympus	1988	LM	1
Muffle Furnace- Sybron Thermolyne Model F-A1730	1991	LM	15
pH Meters (2): Fisher Scientific Accumet Model 20	1993	LM	6
Fisher Scientific Accumet Model AR25	2005	LM	6

GENERAL CHEMISTRY/WATER CHEMISTRY LABORATORY (continued)			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
Shatter Box - GP 1000	1989	LM	5
Sieve Shakers (2):			
CE Tyler - Portable RX 24	1990	LM	5
WS Tyler - RX 86	1991	LM	5
Thomas-Wiley Laboratory Mill, Model 4	1989	LM	7
Total Organic Carbon (TOC) Analyzers (2)			
Coulemetrics Model 5012	1997	LM	3
O-I Corporation Model 1010	2002	LM	3
Total Organic Halogen (TOX) Analyzers (3):			
Mitsubishi TOX-Sigma	1995	LM	4
Mitsubishi TOX-100 (2)	2001	LM	4
Turbidimeter - Hach Model 2100N	1996	LM	8
UV-Visible Spectrophotometers (3):			
Hitachi 100-40 Single Beam	1986	LM	5
Beckman-Coulter DU520	2005	LM	5
Perkin Elmer Lambda 25	2008	LM	5
Vacuum Pumps (2):			
Welch Duo-Seal Model 1376	1990	LM	13
Busch R-5 Series Single Stage	1991	LM	13
Water Baths/Incubators (6):			
Hach Model 15320 Incubator	1986	LM	15
Precision Model L-6 (2)	1989, 1990	LM	15
VWR 1540	1991	LM	15
Fisher 11-680-626M Incubator	1992	LM	15
Fisher Isotemp Incubator	2001	LM	15

METALS LABORATORY			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
Analytical Balance (6)			
Mettler AE 200 analytical balance	1990	MM	12
Various Mettler, Sartorius, and Ohaus models (5)	1988	MM	12
Atomic Absorption Spectrophotometers (5):			
Varian SpectrAA Zeeman/220 AA w/Data Systems (2)	2000	LM	3
CETAC Mercury Analyzer	2000	LM	2
Perkin Elmer AAnalyst 200 Flame AA	2005	MM	2
Atomic Fluorescence Spectrophotometer			
Brooks-Rand Model III (2)	1996, 2005	LM	3
Leeman Mercury Analyzer (1)	2006	LM	2
Centrifuge - IEC Model Clinical Centrifuge	1990	LM	12
Drying Oven - VWR Model 1370F	1990	LM	12
Freeze Dryers (2) - Labconco	1992, 2006	LM	5
Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (3)			
Thermo Jarrell Ash Model 61E	1988	LM	4
Thermo Jarrell Ash, Model IRIS	2000	MM	4
Thermo Scientific Model iCAP 6500	2007	MM	3
Inductively Coupled Plasma Mass Spectrometers (ICP-MS):			
VG Excell	2001	MM	3
Thermo X-Series	2006	MM	2
Muffle Furnace - Thermolyne Furnatrol Model 53600 (2)	1991, 2005	LM	5
Shaker - Burrell Wrist Action Model 75	1990	LM	12
TCLP Extractors (3)	1989, 2002	LM	5

SEMIVOLATILE ORGANICS SAMPLE PREPARATION LABORATORY			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
Analytical Balance (4) Mettler PM480, AE166, BB300 Ohaus EP613	1999 - 2005 2006	MM MM	18 18
Centrifuge - Sorvall Model GLC-1	1988	LM	18
Drying Ovens (2) Fisher Model 655G VWR Model 1305U	1991 1999	LM LM	18 18
Evaporators (14): Organomation N-Evap (7) Organomation S-Evap (7)	1989-98, 2001, 2006 1989-1991, 2006	LM LM	18 18
Extractor Heaters: Lab-Line Multi-Unit Models for Continuous Liquid-Liquid and Soxhlet Extractions (102)	1987-1992, 2007	LM	12
Extractors (52): Branson Model 450 Sonifier (2) Tekmar Sonicator Fisher Scientific Sonicator Soxhtherm (48)	1991 1994 1994 2000, 2008	LM LM LM LM	6 6 6 8
Extractors, TCLP (10): Millipore TCLP Zero Headspace Extractors (10) TCLP Extractor - Tumbler (12 position)	1987-1992 1989	LM LM	2 2
Gel Permeation Chromatography (GPC) (5) ABC single column (3) ABC Autoprep 1000 J2 Scientific	1998, 1999, 2007 1995 2005	LM LM LM	4 4 4
Muffle Furnace - 4	1994-2006	LM	4
Solid Phase Extractors (8) – Horizon SPE-Dex 4790	2003, 2006	LM	4
Ultrasonic Water Bath – VWR 550D	2007	LM	18
Vacuum Pump – Edwards	1992	LM	8

GC SEMIVOLATILE ORGANICS INSTRUMENT LABORATORY			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
Analytical Balance - Mettler AT 250	1989	MM	7
Chromatography Data Systems (12)			7
HP Enviroquant (8)	1994-2002	LM	
Thruput Target (4)	1998-2000	LM	
Gas Chromatographs (11):			
Hewlett-Packard 5890 GC with HP 7673 Autosampler and Dual ECD Detectors (4)	1990 – 1995	LM	7
Hewlett-Packard 5890 GC with HP 7673 Autosampler and Dual FPD Detectors	1991	LM	7
Agilent 6890 GC with Agilent 7683 Autosampler and Dual ECD Detectors (5)	2001, 2005, 2007	LM	7
Agilent 6890 GC with Agilent 7683 Autosampler and Dual FPD Detectors	2003	LM	7
Agilent 7890A Dual ECD Detectors	2008	LM	7
Agilent 7683B autosampler			

GC/MS SEMIVOLATILE ORGANICS INSTRUMENT LABORATORY			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
Accelerated Solvent Extractor - Dionex ASE 200	1996	LM	5
HP Enviroquant Chromatography Data Systems (9)	1994-2002	LM	5
Gas Chromatograph: Hewlett-Packard 5890 with HP 7673 autosampler and FID Detector	1994	LM	5
Semivolatiles GC/MS Systems (9):			
Agilent 6890/5973 with ATAS Optic2 LVI and HP 7673 Autosampler (2)	1997, 2001	LM	5
Agilent 5890/5970 and HP 7673 Autosampler	1990	LM	5
Agilent 5890/5970 with ATAS Optic2 LVI and HP 7673 Autosampler	1994	LM	5
Agilent 5890/5972 with ATAS Optic2 LVI and HP 7673 Autosampler (3)	1993, 1994, 1998	LM	5
Agilent 6890/5973 with ATAS Optic3 LVI and 7683 Autosampler	2004	LM	
Agilent 6890/5973 with Agilent PTV Injector and 7683 Autosampler	2007	LM	4
Semivolatiles GC/MS/MS –			
Waters Quattro Micro GC Micromass with Agilent 6890, Agilent PTV Injector, 7683B Autosampler	2008	MM	1

PETROLEUM HYDROCARBONS GC/HPLC LABORATORY			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
Analytical Balance - Mettler BB240	1994	MM	6
Aspirator pump – GAST	2004	LM	6
Drying Oven - Fisher Model 630F	1991	LM	6
Evaporator - Organomation N-Evap	1990	LM	6
HP Enviroquant Chromatography Data Systems (8)	1994-2002	LM	6
Gas Chromatographs (6):			
Hewlett-Packard 5890 Series II with PID/PID/FID(2)	1991	LM	4
EST-ENCON Purge and Trap Concentrator	1991	LM	4
Dynatech Archon 5100 Autosampler	1992	LM	4
Hewlett-Packard 5890 GC with HP 7673 Autosampler and FID Detector	1995	LM	4
Agilent 6890 with Dual FID Detectors and Agilent 7873 Autosampler (3)	2001, 2005	LM	4
High-Performance Liquid Chromatographs (2):			
HP 1090M Series II with Diode Array UV Detector	1999	LM	4
HP 1050/1100 Series with Fluorescence & Diode Array UV Detectors	2004	LM	4
High-Performance Liquid Chromatograph/Mass(2) Spectrometer - Thermo Electron TSQ Quantum LC/MS/MS and Autosampler	2005	MM	2
API 5000 LC/MS/MS and SIL-20AC Autosampler	2008	MM	2

VOLATILE ORGANICS LABORATORY			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
Analytical Balance - Mettler PE 160	1989	MM	5
Fisher Vortex Mixer	1989	LM	5
HP Enviroquant Chromatography Data Systems (10)	1994-2002	LM	5
Drying Ovens (2):			
Narco 420	1989	LM	5
VWR 1305 U	1991	LM	5
Sonic Water Bath - Branson Model 2200	1989	LM	5
Volatile GC/MS Systems (7):			
Agilent 5890/5970	1989	LM	5
Tekmar 3000 Purge and Trap Concentrator	1995	LM	5
Dynatech ARCHON 5100 Autosampler	1996	LM	5
Agilent 5890/5971	1991	LM	5
Tekmar 3000 Purge and Trap Concentrator	2001	LM	5
Dynatech ARCHON 5100 Autosampler	1995	LM	5
Agilent 5890/5972A	1993	LM	5
Tekmar 3000 Purge and Trap Concentrator	1995	LM	5
Dynatech ARCHON 5100 Autosampler	1996	LM	5
Agilent 6890/5973	2001	LM	5
Tekmar 3100 Purge and Trap Concentrator	2001	LM	5
Varian Archon Autosampler	2001	LM	5
Agilent 6890/5973	2005	LM	5
Tekmar Velocity Purge and Trap Concentrator	2005	LM	5
Tekmar Aquatech Autosampler	2005	LM	5
Agilent 6890/5973 (2)	2007	LM	5
Tekmar 3000 Purge and Trap Concentrator	2007	LM	5
Varian Archon 5100 Autosampler	2007	LM	5

DRINKING WATER ORGANICS LABORATORY			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
Analytical Balance - Mettler BB300	1991	MM	2
Extractors (10) – Horizon SPE-DEX Solid Phase Extractor	2003/2008	LM	2
Aglinet Enviroquant Chromatography Data Systems (2)	2003	LM	2
Varian Saturn Chromatography Data System	2003	LM	2
Evaporator - Organomation N-Evap	2003	LM	2
Agilent 1100 HPLC w/post-column derivitization:	2003	LM	2
UV/Fluorescence detectors	2003	LM	2
Pickering PCX-5200 Post-column derivitization unit	2003	LM	2
Agilent 6890N GC/Dual ECD system w/ autosamplers	2003	LM	2
Agilent 7890 GC/Dual ECD w/autosamplers	2008	LM	2
Varian Ion trap GC/MS:	2003	LM	2
Varian 3800 GC w/CP8400 autosampler	2006	LM	2
Varian Saturn 2100T mass spectrometer	2003	LM	2
Thremo Ion Trap GC/MS w/TriPlus autosampler	2008	LM	2

Metals Method Development Laboratory			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
Perkin-Elmer ICP/MS Elan 9000 w/ Perkin-Elmer AS-93+ Autosampler	2008	LM	2
Perkin-Elmer Series 200 IC	2008	LM	2
Brooks Rand III Atomic Fluorescence Spectrophotometer - 2	2008	LM	2
Oriel Atomic Fluorescence Spectrophotometer – Lab Designed	2008	LM	2
Balances - 4	2008	LM	2
Ovens - 2	2008	LM	2
Buck AA Spectrophotometer Model 205	2008	LM	2
Forma Scientific Bio Freezer	2008	LM	2
Digital Shaker SK-71	2008	LM	2

AUTOMATED DATA PROCESSING EQUIPMENT			
Equipment Description	Year Acquired	Manufacturer or Laboratory Maintained (MM/LM)	# of Trained Operators
1-WAN: LIMS Sample Manager using Oracle 10g DBMS running on Redhat Advanced Server 3.0 (Linux) platform connected/linked on a frame relay WAN environment	1994-2004	LM	NA
1 - Network Server Pentium 4 class, 1 for Reporting and Data Acquisition running Windows 2003 Advanced Server, 1 for Applications running Windows 2003 Advanced Server. Data acquisition capacity at 65GB with redundant tape and disk arrays.	2004	LM	NA
Approximately 50+ HP and Dell Laserjet printers (various types including models III, 4, 5, 8150, 4000, 4050, 4250, 8150, 1720dn, W5300)	1991 - 2007	LM	NA
Approximately 180 Gateway/Dell PC/Workstations running Windows 2000/XP on LAN connected via 10BT/100BT and TCP/IP for LIMs Terminal Emulation	1993 - 2004	LM	NA
Microsoft Office 2003 Professional as the base application for all PC/Workstations. Some systems using Office 2000/97.	1996 - 2004	LM	NA
E-Mail with link to SMTP for internal/external messaging. Web mail via Outlook Web Access interface. Microsoft Outlook 2003.	1994 - 2006	LM	NA
Standard Excel (R) reporting platform application linked to LAN/WAN for data connectivity and EDD generation.	1996 - 2004	LM	NA
Standard Excel (R) reporting platform application linked to LAN/WAN for data connectivity and EDD generation.	1996 - 2004	LM	NA
Facsimile Machines - Brother 4750e (2); Brother SuperG3 (1); Canon CFX-L4000 (1)	1991 - 2007	LM	NA
Copiers/Scanners: Konica BizHub 420 (1), BizHub 600 (1), BizHub 920 (2), BizHub Pro 1050 (3). The 920s and 1050s are accessible via LAN for network scanning.	2000 - 2007	LM	NA
Dot Matrix Epson FX-880, LQ-1050, LX-300	1991 - 2004	LM	NA
Thruput, MARRS, Stealth, Harold, Blackbird, EDDGE, StarLIMS reporting software systems.	1998 - 2004	LM	NA

NA: Not applicable. This equipment administered by IT staff but may be used by all staff.

APPENDIX D

PREVENTIVE MAINTENANCE PROCEDURES

Instrument	Activity	Frequency
Refrigerators and Coolers	Record temperatures Clean coils Check coolant	Daily Annually Annually or if temperature outside limits
Vacuum Pumps	Clean and change pump oil	Every month or as needed
Fume Hoods	Face velocity measured Sash operation Change filters Inspect fan belts	Quarterly As needed Annually Annually
Ovens	Clean Record temperatures	As needed or if temperature outside lim. Daily, when in use
Incubators	Record temperatures	Daily, morning and evening
Water Baths	Record temperatures Wash with disinfectant solution	Daily, morning and evening When water is murky, dirty, or growth appears
Autoclave	Check sterility Check temperature Clean	Every month Every month When mold or growth appears
Analytical Balances	Check alignment Check calibration Clean pans and compartment	Before every use Daily After every use
Dissolved Oxygen Meter	Change membrane	When fluctuations occur
pH probes	Condition probe	When fluctuations occur
Fluoride ISE	Store in storage solution	Between uses
Ammonia ISE	Store in storage solution	Between uses
UV-visible Spectrophotometer	Wavelength check	Annually
Total Organic Carbon Analyzers	Check IR zero Check digestion/condensation vessels Clean digestion chamber Clean permeation tube Clean six-port valves Clean sample pump Clean carbon scrubber Clean IR cell	Weekly Each use Every 2000 hours, or as needed Every 2000 hours, or as needed Every 200 - 2000 hours, or as needed Every 200 - 2000 hours, or as needed Every 200 - 2000 hours, or as needed Every 2000 - 4000 hours, or as needed

Instrument	Activity	Frequency
Total Organic Halogen Analyzers	Change cell electrolyte	Daily
	Change electrode fluids	Daily
	Change pyrolysis tube	As needed
	Change inlet and outlet tubes	As needed
	Change electrodes	As needed
Flow Injection Analyzer	Check valve flares	Each use
	Check valve ports	Each use
	Check pump tubing	Each use
	Check light counts	Each use
	Check flow cell flares	Quarterly
	Change bulb	As needed
	Check manifold tubing	Each use
	Check T's and connectors	Each use
Ion Chromatographs	Change column	Every six months or as needed
	Change valve port face & hex nut	Every six months or as needed
	Clean valve slider	Every six months or as needed
	Change tubing	Annually or as needed
	Eluent pump	Annually
Atomic Absorption Spectro- photometers - FAA and CVAA	Check gases	Daily
	Clean burner head	Daily
	Check aspiration tubing	Daily
	Clean optics	Every three months
	Empty waste container	Weekly
Atomic Absorption Spectro- photometers - GFAA	Check gases	Daily
	Check argon dewar	Daily
	Change graphite tube	Daily, as needed
	Clean furnace windows	Monthly
ICP - AES	Check argon dewar	Daily
	Replace peristaltic pump tubing	Daily
	Empty waste container	Weekly
	Clean nebulizer, spray chamber, and torch	Every two weeks
	Replace water filter	Quarterly
	Replace vacuum air filters	Monthly

Instrument	Activity	Frequency
ICP - MS	Check argon dewar	Daily
	Check water level in chiller	Daily
	Complete instrument log	Daily
	Replace peristaltic pump tubing	Daily
	Clean sample and skimmer cones	As needed
	Clean RF contact strip	As needed
	Inspect nebulizer, spray chamber, and torch	Clean as needed
	Clean lens stack/extraction lens	As needed
	Check rotary pump oil	Monthly
	Change rotary pump oil	Every six months
Gel-Permeation Chromatographs	Clean and repack column	As needed
	Backflush valves	As needed
High Pressure Liquid Chromatographs	Backflush guard column	As needed
	Backflush column	As needed
	Change guard column	As needed when back pressure too high
	Change column	Annually or as needed
	Change in-line filters	As needed
	Leak check	After column maintenance
	Change pump seals	As needed
	Change pump diaphragm	Annually
	Clean flow cell	As needed
	Fluorescence detector check	Daily
	Diode array absorbance check	Daily
Gas Chromatographs, Semivolatiles	Check gas supplies	Daily, replace if pressure reaches 50psi
	Change in-line filters	Quarterly or after 30 tanks of gas
	Change septum	Daily
	Change injection port liner	Weekly or as needed
	Clip first 6-12" of capillary column	As needed
	Change guard column	As needed
	Replace analytical column	As needed when peak resolution fails
	Check system for gas leaks	After changing columns and after any power failure
	Clean FID	Weekly or as needed
	Clean ECD	Quarterly or as needed
	Leak test ECD	Annually

Instrument	Activity	Frequency
Gas Chromatograph/Mass Spectrometers, Semivolatiles	Check gas supplies	Daily, replace if pressure reaches 50psi
	Change in-line filters	Annually or as needed
	Change septum	Daily, when in use
	Change injection port liner	Weekly or as needed
	Clip first 6-12" of capillary column	As needed
	Change guard column	As needed
	Replace analytical column	As needed when peak resolution fails
	Clean source	As needed when tuning problems
	Change pump oil	As specified by service specifications
Purge and Trap Concentrators	Change trap	Every four months or as needed
	Change transfer lines	Every six months or as needed
	Clean purge vessel	Daily
Gas Chromatographs, Volatiles	Check gas supplies	Daily, replace when pressure reaches 50 psi
	Change in-line filters	Quarterly or after 30 tanks of gas
	Change septum	Daily
	Clip first 6-12" of capillary column	As needed
	Change guard column	As needed
	Replace analytical column	As needed when peak resolution fails
	Check system for gas leaks	After changing columns and after any power failure
	Clean PID lamp	As needed
	Clean FID	As needed
	Change ion exchange resin	Every 60 days
	Replace nickel tubing	Quarterly or as needed
Gas Chromatograph/Mass Spectrometers, Volatiles	Check gas supplies	Daily, replace when pressure reaches 50 psi
	Change in-line filters	Annually or as needed
	Change septum	Daily
	Clip first foot of capillary column	As needed
	Change guard column	As needed
	Replace analytical column	As needed when peak resolution fails
	Clean jet separator	As needed
	Clean source	As needed when tuning problems
	Change pump oil	As specified by service specifications

APPENDIX E

LIST OF NELAC ACCREDITED METHODS

APPENDIX F

ADDITIONAL AGENCY-SPECIFIC DOCUMENTS