COMPREHENSIVE QUALITY ASSURANCE PLAN

for

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LABORATORY'S APPROVED SIGNATORIES

Michelle L. Briscoe – President/CEO (effective upon this sign-off)	2/4/16 Date
Hakan Gürleyük – Senior Scientist (Technical Director)	2/4/16 Date
Annie Carter – VP of Operations (Deputy Technical Director)	2/4/16 Date
Frank McFarland – VP of Quality	2/4/16 Date

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2.0 Statement of Policy

The Brooks Applied Labs (BAL) is committed to sound and useful quality assurance/quality control (QA/QC) management practices resulting in the production of accurate analytical data. The principal focus of the analytical laboratory is to provide specialized analytical services for trace metals analysis with an emphasis on ultra-low detection limits, metals speciation, and unusual or non-routine matrices.

Obtaining accurate data is dependent upon an effective and consistent quality assurance program. To meet this need, ISO/IEC 17025:2005 (ISO 17025), The National Environmental Laboratory Accreditation Conference (NELAC) Institute (TNI), and the Department of Defense/Department of Energy Consolidated Quality Systems Manual for Environmental Laboratories (DoD/DOE QSM) standards have been incorporated into BAL's quality assurance program. Internal audits are conducted by BAL, and external audits of BAL's facilities are conducted by ANSI-ASQ National Accreditation Board/ANAB every two years to ensure that BAL meets the requirements of the TNI standards (for TNI accreditation through the Florida Department of Health), the DoD QSM, and ISO 17025 accreditation requirements. BAL is also audited annually under the Department of Energy Consolidated Audit Program (DOECAP). Reciprocal TNI accreditation is granted for several other states. Refer to the BAL website for a complete list of current accreditations. Additionally, periodic external audits initiated by clients serve to ensure that Brooks Applied Labs continually meets the specific requirements of our clientele.

Brooks Applied Labs' management is committed to compliance with ISO 17025, TNI, and DoD/DOE QSM standards. Additionally, BAL is committed to meeting the requirements of the Current Good Manufacturing Practices (CGMP) regulations and the Clinical Laboratory Improvement Amendments (CLIA) for clinical laboratory testing performed on humans in order to meet the needs of our pharmaceutical and clinical laboratory clients. As such, BAL management is committed to continually improving the quality assurance program. The BAL quality assurance program is implemented through a team effort across the entire laboratory. All personnel concerned with environmental testing activities within BAL must familiarize themselves with the quality system documentation [this Comprehensive Quality Assurance Plan (CQAP) and all relevant standard operating procedures (SOP)] and implement the documented policies and procedures in their work. A listing of the general considerations and objectives of the overall program are as follows:

- *Maintenance of sample integrity.* Integrity is maintained by following documented and accepted sample handling procedures for the preservation, custody, storage, labeling, and record keeping associated with samples received by the laboratory.

- *Use of approved analytical methods.* Analytical methods and related procedures approved by the EPA are readily available. These are read and followed by all analysts. In addition, BAL is on the cutting edge of method development for the analysis of trace metals and speciation. All BAL developed methods undergo rigorous testing and validation before they are approved by BAL scientists for use in the analysis of customer samples.

- *Regular evaluation of analytical results.* The results from quality control tests and from sample analyses are continually evaluated to identify method weaknesses and/or to detect a need for further analyst training.

- *Instrumentation performance and maintenance.* Determination of instrument performance level by frequent calibration and the analyses of performance evaluation samples, and through scheduled preventive maintenance, is documented on a real-time basis. Instrument calibration is performed as part of each analytical procedure.

- *Data reduction and report formatting.* Various levels of data review from acquisition to the final report are incorporated to minimize any potential errors in the final data. The report format is variable from a standard format to a customized data package, with or without electronic data deliverables (EDD).

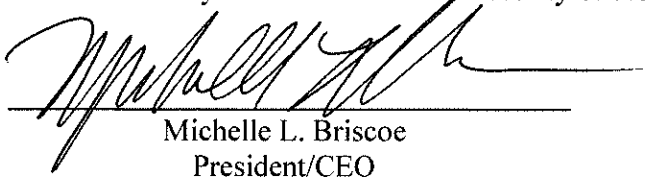
- *Method performance (precision and bias) documentation.* Data from analyses are monitored using control charts to assess performance and to detect trends.

- *Regular evaluation of the quality system.* Brooks Applied Labs management is committed to continually improving the quality system. Reviews of all standard operating procedures and the CQAP each calendar year, as well as routine audits of the laboratory and management reviews are some of the procedures used to find and correct deficiencies in the quality system.

Brooks Applied Labs' management is committed to professional laboratory practices and to the quality of our analytical testing in providing services to our clients. The above considerations are documented to ensure the quality of the data generated by BAL. Subsequent sections of this manual detail the various elements of the QA program developed and practiced by the laboratory.

The QA program is structured such that the CQAP is the primary reference for quality policies and procedures. As long as the CQAP contains all of the necessary requirements of the quality system, then no additional SOP is necessary. If there is a discrepancy between the CQAP and any SOP, the policy or procedure contained in the CQAP takes precedence and the discrepancy must be resolved as quickly as feasible. Any written directions that disagree with the CQAP and SOPs is considered a departure from the approved QA plan and may not be followed unless it has been approved by the VP of Quality and the President/CEO of BAL. Refer to Section 8.5 for details on how any exceptional departure from BAL procedure is handled.

The Statement of Policy is issued under the authority of the President/CEO of Brooks Applied Labs.



Michelle L. Briscoe
President/CEO

2/4/16
Date

3.0 Organization and Responsibility

3.1 Duties and Responsibilities of Personnel

The laboratory staff is organized in such a way that all analytical personnel are trained in a variety of laboratory duties. Individuals are specialized in their area of primary responsibility, but training overlaps so that there are always secondary personnel trained to perform the primary functions of staff that may be absent. Specific responsibilities have different minimum qualification requirements. The descriptions of responsibilities and their minimum qualifications are listed in Table 3.1.

TABLE 3.1 RESPONSIBILITIES AND MINIMUM QUALIFICATIONS

Title	Responsibilities	Deputies	Minimum Qualifications
President/CEO	Oversees operations of the laboratory, including: management of personnel and analytical services, contracting, client services, sales & marketing, QA/QC, R&D, budgeting, and financial controls	VP of Operations and VP of Quality	Bachelor's degree in physical sciences (advanced degree or equivalent experience preferred) and 15 years experience in the environmental analytical lab business, with 10 years in management positions
VP of Operations Lab Manager	Oversees operations of the laboratory, including: management of all lab personnel and all analytical services, R&D, method development, facilities improvements, and scheduling	President/CEO of Brooks Applied Labs	Bachelor's degree in physical sciences and 6 years experience in the environmental lab business, with 5 years in management positions
Technical Director	Oversees operations of the laboratory, including: maintaining instrumentation and equipment, technical oversight, method development, validation, and approval, R&D, identification of method limitations which may result in degradation of representativeness of results	President/CEO of Brooks Applied Labs and the VP of Operations both serve as backup Technical Directors. If backup exceeds 65 consecutive calendar days, the primary accrediting authority must be notified in writing.	Bachelor's degree in physical sciences (advanced degree or equivalent experience preferred) with 24 hours of college chemistry credits, and 3 years experience in the environmental trace metals analytical lab business, with 1 year in a supervisory position
Client Services Manager	Oversees the project management group and manages client projects, including: internal communication of client requirements and reporting to client. Performs report level review of the data prior to issuing reports to clients.	President/CEO of Brook Applied Labs	Bachelor's degree in physical sciences or equivalent and 3 years experience in the environmental lab business; including at least 1 year in Project Management positions

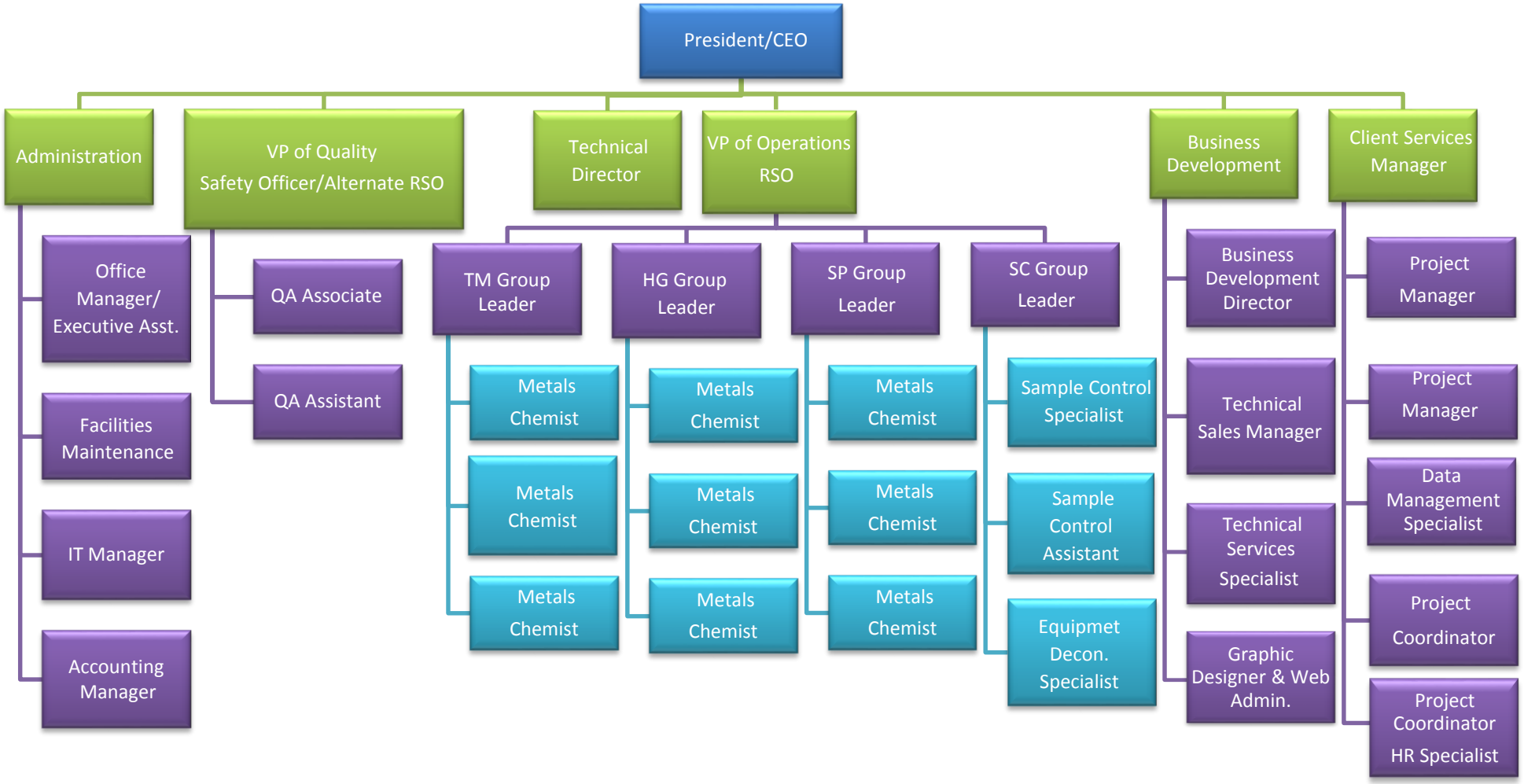
Title	Responsibilities	Deputies	Minimum Qualifications
Project Manager	Manages client projects including: internal communication of client requirements, reporting to client. Performs report level review of the data prior to issuing reports to clients.	Another Project Manager or Project Coordinator	Bachelor's degree in physical sciences or equivalent and 1 year experience in the environmental lab business
VP of Quality QA Manager	Oversees QA group; has the authority and is responsible for implementing, maintaining, and improving the QA program, ensuring that all personnel understand their contribution to the QA program, ensure that communication takes place at all levels regarding the effectiveness of the QA program, evaluate the effectiveness of training, using all available tools to monitor trends and continually improve the QA program, ensure laboratory compliance with all accrediting authority standards. Responsible for ensuring all data undergoes final data review prior to release to clients.	President/CEO of Brook Applied Labs and QA Associate both serve as backup QA Managers	Bachelor's degree in physical sciences (advanced degree preferred) and 3 years lab experience with 1 year of applied QA principles
QA Associate	Be able to mirror VP of Quality in QA related duties. Cover for VP of Quality in his absence. Authorized to perform final review of laboratory data.	VP of Quality	Bachelor's degree in physical sciences and 6 months experience in the environmental lab business or with applied QA principles
QA Assistant (as necessary)	Assist VP of Quality in duties. Performs final review of laboratory data.	QA Associate	Bachelor's degree in physical sciences
HG Group Leader	Oversees the mercury analytical group including: training records, sample preparation, analysis, detection limit studies, troubleshooting, and scheduling within the group. Responsible for ensuring all data produced by the HG Group undergoes primary review prior to turning data in to QA.	VP of Operations	Bachelor's Degree in physical sciences and 1 year of analytical lab experience as a metals chemist
TM Group Leader	Oversees the trace metals analytical group including: training records, sample preparation, analysis, detection limit studies, troubleshooting, and scheduling within the group. Responsible for ensuring all data produced by the TM Group undergoes primary review prior to turning data in to QA.	VP of Operations	Bachelor's Degree in physical sciences and 1 year of analytical lab experience as a metals chemist

Title	Responsibilities	Deputies	Minimum Qualifications
SP Group Leader	Oversees the speciation analytical group including: training records, sample preparation, analysis, detection limit studies, troubleshooting, and scheduling within the group. Responsible for ensuring all data produced by the TM Group undergoes primary review prior to turning data in to QA.	VP of Operations	Bachelor's Degree in physical sciences and 1 year of analytical lab experience as a metals chemist
Metals Chemist and Senior Metals Chemist	Perform and document sample preparations and analyses following SOPs, instrument calibration and reagent/standard preparation. May perform primary review of data for methods trained to perform.	Group Leader	Bachelor's Degree in physical sciences.
Sample Control Group Leader	Oversees sample control group including: training, custody of samples, and scheduling within the group. Oversees classical chemistry method for total suspended solids performed by the sample control group and sample homogenization. Responsible for ensuring all data produced by the SC Group undergoes primary review prior to turning data in to QA.	VP of Operations	Bachelor's Degree in physical sciences and 1 year of analytical lab experience
Sample Control Specialist	Oversees custody of sample, sample receipt, sample log-in, and sample homogenization.	Group Leader	Bachelor's Degree in physical sciences
Equipment Decontamination Specialist	Cleaning and decontamination of laboratory equipment; sample disposal	Group Leader	Bachelor's Degree in physical sciences
Radiation Safety Officer (RSO)	Identify potential radiation safety issues, review all radiation level surveys, ensures safety of laboratory personnel from radiation risks, ensure compliance with all licenses and regulations.	Alternate Radiation Safety Officer	Bachelor's Degree in physical sciences. Current certificate of RSO training.
Alternate RSO	Perform all duties of the RSO when the RSO is not present.	Either the RSO or an Alternate RSO must be available when rad samples are in use at BAL	Bachelor's Degree in physical sciences. Current certificate of RSO training.
Safety Officer	Maintain the Chemical Hygiene Plan (CHP), design and implement safety program including training, safety meetings, preventative measures, and responses to accidents/injuries, ensure monthly safety inspections are performed and any deficiencies are corrected.	BAL President/CEO and VP of Operations	Bachelor's Degree in physical sciences.

3.2 BAL Organization

There is a defined chain of responsibility along which the laboratory staff is organized. The organization of the laboratory personnel is shown in Figure 3.2 (next page). This organizational chart is current as of the finalization of the current version of the CQAP and is subject to change prior to the next revision of the CQAP.

Brooks Applied Labs Organizational Chart



3.3 Managerial Responsibilities for the Detection of Improper, Unethical, and Illegal Actions

Brooks Applied Labs holds management responsible for ensuring that all client data is properly and accurately reported. To this end, management works diligently to ensure that all employees of BAL are free from undue commercial, financial, and any other pressures that may adversely affect the quality of their work. In addition, the management of BAL works proactively to detect any improper, unethical, or illegal actions that might arise due to such pressures before such actions can adversely affect client data.

All information relevant to client results, from sample receipt to the analysis and reporting of data, goes through several levels of review. Management keeps track of all work that appears suspect or contains mistakes and all changes made to the LIMS are electronically tracked and stamped with the date/time of the change and who instigated it. All employees routinely meet with senior management at which time work-related problems are discussed so that any undue pressures can be brought out into the open. Immediate supervisors speak daily with all employees and strive to keep aware of the activities and general attitudes of those employees for which they are responsible. Employee work is additionally reviewed during monthly audits.

All employees receive training to ensure that each is fully aware as to what constitutes improper, unethical, and illegal behavior and what the consequences are for such behavior (refer to BAL SOP BAL-0001) and must sign the “Brooks Applied Labs Ethical and Legal Responsibilities Agreement” form stating that they agree to adhere to all aspects of the ethics program at BAL prior to working with client samples. Any employee who becomes aware of unethical behavior is encouraged to report the behavior to BAL management. Any employee should feel free to report such behavior to any manager, on up to the President/CEO of BAL. BAL management assures that any reporting of improper, unethical, or illegal behavior will remain strictly confidential. If such behavior is detected, the responsible employee is immediately brought before senior management. If the behavior is unintentional and appears to be caused by undue pressures, every effort is made by management to eradicate the pressures. If the behavior is deemed willful, then senior management is responsible for determining the best course of action for BAL and its clients. Under no circumstances is any behavior that might adversely affect the quality of the data produced by BAL tolerated.

All investigations that result in finding inappropriate activity shall be documented and shall include any disciplinary action that was taken, corrective actions taken, and all appropriate notifications of affected clients. All documentation of the investigation’s actions shall be maintained for a minimum of 7 years. Documentation may be stored either electronically or by hardcopy, but must be readily available.

4.0 Training

4.1 Technical Training

Brooks Applied Labs personnel are trained prior to the analysis of client samples. An experienced chemist who has previously demonstrated their capability to perform the procedures for which a new employee is being trained directly supervises all training.

Brooks Applied Labs is also on the cutting edge in the development of new techniques for the analysis of trace metals. In such situations where a scientist is developing a new technique, they must train themselves in the new procedures. The lead scientist is expected to develop the training protocol by which future technicians will be trained in the method.

Regardless of how training takes place, an initial demonstration of capability (IDOC) for each method must be successfully completed as per ISO 17025, TNI, and DoD/DOE QSM standards prior to any analysis of client samples. The IDOC serves as an indicator of the successful completion of training. From then on, the consistent meeting of quality control criteria serves as the ongoing DOC. The VP of Quality reviews the ongoing DOC annually to ensure the continued proficiency of each technician. The conclusions of these reviews are recorded in each employee's training records.

4.2 Safety Training

All BAL employees receive training in laboratory safety that they are required to review on an annual basis. Safety training includes "Right-to-Know" training as to the potential chemical and physical hazards of working in an environmental laboratory and how best to reduce these hazards. Training also includes what procedures to take in the case of a laboratory accident and the locations of all safety and first aid equipment.

4.3 Training in Legal and Ethical Rights and Responsibilities

All Brooks Applied Labs employees receive annual training in their legal and ethical rights and responsibilities. This training includes the following topics: Freedom from undue-pressures; data manipulation; and workplace ethics. This training also specifies the potential punishments and penalties for improper, unethical, or illegal actions performed by BAL employees. The BAL data integrity plan (SOP BAL-0001) discusses in detail the specific legal and ethical rights and responsibilities of employees at BAL. Training on this SOP is required for all personnel.

In conjunction with this training, each employee must attest that they are free from any commercial, financial, and other undue pressures, which might adversely affect the quality of their work prior to working with client samples.

4.4 Documentation of Training

Each employee is responsible for documenting all training in their personal training records and each Group Lead must review the training records for all employees within their group to ensure completeness. At a minimum, the VP of Quality or their designee audits the training records quarterly and reports any deficiencies to the Group Leads and the VP of Operations so that they can be

corrected. Training records are updated at least annually to ensure that all personnel continue to be proficient in their assigned tasks. Records for each type of training (i.e. technical, safety, and legal and ethical) are maintained and stored for a minimum of 7 years from when employment at BAL ends.

4.5 Additional Training

The VP of Quality or their designee reviews each employee's training records quarterly. Any deficiencies found are recorded in the monthly QA audits. In addition, the technical abilities of each employee are constantly monitored through the analysis of quality control samples. If quality control criteria are not consistently met, additional training is required under the supervision of the VP of Operations. Any additional training is fully documented and a new DOC must be successfully completed before the technician may restart analyzing client samples.

5.0 Capabilities and Quality Assurance Objectives

5.1 Acceptance of New Work

The President/CEO (along with any necessary assistance from the Client Services Manager, project managers, VP of Operations, and the VP of Quality, as designated) carefully reviews the specific requirements of every contract before any new work is accepted by BAL. BAL will accept a project only after the President/CEO or her designee has ensured that BAL possesses the appropriate facilities and resources to carry out the work as specified by the client.

5.2 Capabilities of Organization

Brooks Applied Labs is an analytical laboratory primarily focused on providing analytical services for the determination of low-level trace metals and metals speciation in environmental, food samples, biologicals, and pharmaceuticals. BAL's specialties are fourfold: 1) providing the lowest detection limits commercially available, 2) speciation of oxidation state and organometallic forms, 3) analysis of non-routine matrices, and 4) providing our clients with expert consulting to ensure their data objectives are met.

Current scopes of accreditation and licenses can be found on the BAL website at http://www.brooksapplied.com.

Each sample preparation method followed at BAL is dependent upon the analyte of interest and the type of matrix being analyzed. Refer to the specific analytical method or standard operating procedure (SOP) for a description of each particular preparation method utilized at BAL. Other sample preparation methods may be used upon request for specific enforcement or compliance-based contracts.

5.3 Quality Assurance Objectives

Brooks Applied Labs is dedicated to providing the finest services to its clients. To meet this objective, every position at BAL is staffed with trained personnel and competent managers who possess the authority and resources to produce meaningful metals data that meet the needs of our clients.

The primary purpose of the Quality Assurance Program at BAL is to ensure that all data reported to our clients are accurate and reproducible. To this end, BAL implements procedures to ensure that all staff are qualified and fully trained to perform their specific laboratory duties, that laboratory instrumentation is properly maintained and calibrated, and that materials are adequately stocked and tested prior to use in the laboratory. All data reported by the laboratory undergoes several levels of review before being approved for release by the Quality Assurance Department.

5.4 Subcontracted Work

Occasionally, a client may wish to work directly through Brooks Applied Labs even for analyses that BAL does not currently perform. Under these conditions, BAL will subcontract work to other laboratories with the client's approval in writing (typically an email or signed laboratory service

agreement referencing the quotation). The subcontracted laboratories must meet all project specific requirements, including required accreditations, before samples may be delivered.

Subcontract laboratories are selected by BAL based on a culmination of many factors. These include but are not limited to laboratory capabilities, past work experience, data quality (including data presentation), accreditation / certification, price, turnaround time, customer service, and electronic data deliverable (EDD) capabilities. Example reports can be requested from a potential subcontract laboratory.

Once subcontracted work has been contracted between BAL and the client, a purchase order agreement is issued by BAL to the subcontract laboratory.

All samples are logged into the BAL Laboratory Information Management System (LIMS) and a subcontract order is created. When samples are submitted to the subcontract laboratory, they are accompanied with a copy of the subcontract order.

The subcontract laboratory will email the appropriate BAL Project Manager with the final report and EDD (if requested). The Project Manager will then forward the report to the VP of Quality. Data is reviewed by the QA Department and notes are written regarding any issues with the subcontracted data set. If there are outstanding issues, the Project Manager will contact the subcontract laboratory and work to resolve the issue/gain clarification. Depending on the reporting level, BAL may supply a cover letter to the client regarding the data set provided by the subcontract laboratory. Final reports (and EDDs) will be emailed by the Project Manager to the client.

6.0 Sampling Procedures and Requirements

6.1 Sampling Capabilities

Brooks Applied Labs conducts field sampling on a very infrequent basis. Therefore, the following sampling procedure topics are only briefly addressed:

Sampling Equipment - All sampling equipment is decontaminated and/or tested prior to and following every sampling event and stored in a secure designated area. Any equipment requiring calibration or maintenance, as specified by the manufacturer's instructions, is placed on a routine calibration/maintenance schedule.

Field Sample Documentation - During site visits, minimal notes regarding specific field parameter measurements, general observations, hydrologic conditions, and overall suitability are documented, if applicable. These notes are entered as Work Order Comments in BAL's LIMS for the related project(s).

Sample Dispatch - Field samples are relinquished by the sample collection team to BAL's Sample Control Group following a strict chain-of-custody process. Time, date, samplers' and receipt signatures, and any relevant environmental conditions are documented.

Field Reagent and Waste Disposal - All field reagents and wastes generated or used during field sampling activities should be collected and disposed of in accordance with all state and federal regulations.

6.2 List of Equipment Provided by BAL for Sampling

TABLE 6.2 - CONTENTS OF BAL SAMPLING KITS

Equipment	Construction	Use	Parameter Groups
Sample Container(s)	Teflon® – FEP, PFA Fluorinated – FLPE Glass – I-Chem 200 series HDPE (for solids only) Zip-type bags (some biota)	Sampling/Storage	Mercury and monomethyl mercury in water/soil/biota
	HDPE bottles LDPE, HDPE, or PP jars Zip-type bags	Sampling/Storage	Trace Metals and metals species, except Hg and MeHg, in water/soil/biota
	Iodated carbon (IC) Traps or Gold Coated Media in Zip-type bags	Sampling/Storage	Mercury in air

Sampling Equipment	Teflon® tubing, inline filter units	Sampling	Trace metals and mercury in water
Shipping Containers	Plastic cooler or cardboard box	Sample Transport	All parameter groups
Gloves, Clean Room	Vinyl, non-powdered	Sampling	All parameter groups
Trip Blanks	FEP, PFA, FLPE, HDPE bottle (dependent on analysis) filled with reagent water and sealed with custody seal.	Test for potential contamination during transit	Mercury, monomethyl mercury, trace metals and metals species.
Reagent Water	FEP, PFA, FLPE, HDPE bottle (dependent on analysis) filled with reagent water	Rinsing of equipment in the field, preparation of field blanks	Mercury, monomethyl mercury, trace metals and metals species.

6.3 Decontamination Procedures

Client Equipment

Brooks Applied Labs supplies sampling equipment and sample containers for all analyses. Clients may provide their own sample containers at their discretion, but BAL cannot guarantee the cleanliness of containers that have not been cleaned and/or tested by BAL.

Cooler/shipping containers

All coolers are cleaned prior to use for shipping samples or sample containers. Appropriate sample containers are placed in the coolers to make a sampling kit. Sampling kits are sent via freight carrier (UPS or FedEx) to the requested location.

Sample Containers

Due to the possible occurrence of false positive results due to trace metals contamination, it is extremely important that all water samples are collected in rigorously acid-cleaned or pretested containers that are double-bagged in poly bags and suitable to the analyses to be performed.

6.4 Sampling Protocol

Brooks Applied Labs recommends that all samples to be analyzed for trace metals are collected following the guidelines laid out in EPA Method 1669 (7/96): Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels.

6.5 Sample Preservation, Holding Times, Container Types, and Required Volumes

All preservation reagents used by Brooks Applied Labs are reagent grade or better. For total metals analyses, samples not being filtered in the lab may be sent to BAL at ambient temperature via ground shipment. For most speciation parameters, containers are sent to the field pre-preserved or the field sampling crew can add the appropriate preservative (See Table 6.5). Samples can also be sent to BAL on ice via overnight shipping to be preserved at BAL. All samples for “dissolved” analyses must be filtered before preservation.

TABLE 6.5 - SUMMARY OF SAMPLE CONTAINERS & PRESERVATIVES

Parameter	Method	Minimum Volume	Container	Means of Preservation	Holding Time
A. Water[1]					
As Species	EPA 1632A	125 mL	HDPE[2]	HCl to pH<2 at time of collection; Store in dark at 0-4 °C[3]	28 days
As Species, dissolved (Natural Waters)	IC-ICP-MS (BAL-4100)	5 mL	125-mL HDPE	Field-filtration recommended, especially for high solids samples; collect in bottles prepreserved with EDTA/acetic acid solution; minimal headspace, 0-4 ºC and dark immediately	28 days
As Species, dissolved (landfill leachates or anoxic waters)	IC-ICP-MS (BAL-4100)	2 x 9 mL	2 x 9-mL Vacutainer	Field-filtration recommended, especially for high solids samples; collect in bottles prepreserved with EDTA; minimal headspace, 0-4 ºC and dark immediately	14 days
Se Species, dissolved (Se4, Se6, SeCN, MeSe4, and SeMet)	IC-ICP-MS (BAL-4200)	5 mL	125-mL HDPE	Filed filtration recommended; filter with 0.45 µm syringe filter, cryofreeze at -80 ºC	1 year
MeHg (Freshwater)	EPA 1630	250 mL	Fluoropolymer, FLPE, or Glass with Fluoropolymer lined lids	0-4 ºC and dark immediately; Pre-preserved: 0.8% (v/v) 6 M HCl; In-Lab Preservation: 0.4% (v/v) 12 M HCl within 48 hours of collection	6 months
MeHg (Salt Water ≥ 10 ppth salinity)	EPA 1630	250 mL		0-4 ºC and dark immediately; Pre-preserved: 0.4% (v/v) 9 M H_2SO_4; In-Lab Preservation: 0.2% (v/v) 18 M H_2SO_4 within 48 hours of collection	6 months
Total Hg	EPA 1631E	125 mL		Preserve to pH < 2 with BrCl in original sample container within 28 days	90 days
Hg(II), MeHg, EtHg	IC-CV-ICP-MS (BAL-4701)	10 mL	40-mL glass vial	0-4 ºC and dark immediately. 0.8% (v/v) 6 M HCl within 48 hours of collection	6 months
EtHg	GC-CVAFS (in-house)	250 mL	Fluoropolymer, FLPE, or Glass with Fluoropolymer lined lids	0-4 ºC and dark immediately. 0.4% (v/v) 12 M HCl within 48 hours of collection	6 months
Reactive Hg	CVAFS (BAL-3904)	n/a	Gold Trap	Field collect reactive Hg onto gold traps and mail overnight; dark	Analyze gold traps within 7 days of collection
Acid-labile Hg	CVAFS (BAL-3901)	250 mL (500 mL for QC)	Glass with Fluoropolymer lined lids	0-4 ºC and dark immediately. 0.8% (v/v) 12 M HCl within 24 hours of collection or prepreserved	90 days (do not analyze before 21 days from preservation
Total Volatile Hg	CVAFS (BAL-3902)	1-L	Glass septa top Fluoropolymer lined lids	0-4 ºC and dark immediately. No acid preservative.	24 hours

[1] Samples to be analyzed for dissolved analytes must be filtered prior to preservation. This should preferably be done on site in the field. If this is not possible, the sample must be chilled (0-4 ºC) and filtered within 48 hours of collection (mercury samples filtered after 24 hours but within 48 hours will be narrated in the report).
[2] High Density Polyethylene
[3] All temperature ranges of 0-4 ºC assume an acceptable measured temperature of ± 2 ºC from either extreme as long as the samples do not freeze.

Cr(VI), dissolved (drinking water)	IC-ICP-MS (BAL-4300)	125 mL	125-mL HDPE	Container prepreserved with $NH_4OH/(NH_4)_2SO_4$ buffer to pH>8; no headspace; keep in dark at < 25 ºC during shipment, store in refrigerator	14 days
Parameter	**Method**	**Minimum Volume**	**Container**	**Means of Preservation**	**Holding Time**
Cr(VI), dissolved (except drinking water)	IC-ICP-MS (BAL-4300)	125 mL	125-mL HDPE	Field filtration recommended, Container prepreserved with $NH_4OH/(NH_4)_2SO_4$ buffer to pH>9; no headspace; ≤ 6ºC (without freezing) during shipment, store in refrigerator	28 days
Fe(II) and Fe(reducible)	BAL-4500	40 mL	Glass with Teflon-lined lid w/preservation	0-4 ºC, dark, 2% (v/v) degassed HCl at collection (pre-preserved vial)	48 hours for Fe(II)
Total CN	BAL-4400	125 mL	HDPE	Prepreserved containers with 130µL of 50% NaOH to pH>10; ship and store at 0 – 4 ºC	28 days
ICP-MS Metals (Freshwater)	EPA 1638, Mod. EPA 200.8, Mod. EPA 6020A	125 mL	HDPE	HNO_3 to pH < 2 in lab, within 14 days of collection[4]	6 months
Noble Metals (Au, Pd, Pt)	EPA 1638, Mod. EPA 200.8, Mod. EPA 6020A	125 mL	HDPE	HCl + HNO_3 (3% + 1%) in lab upon receipt (closed-vessel digestion in original sample container)	28 days
Os	EPA 1638, Mod. EPA 200.8, Mod. EPA 6020A	125 mL	HDPE	1% HCl	6 months
Total W or Si	EPA 1638, Mod. EPA 200.8, Mod. EPA 6020A	125 mL	HDPE	HF + HNO_3 (0.2% + 1.8%) in lab within 14 days of collection[4]	6 months
ICP-MS Metals (Brackish or Seawater)	EPA 1640, Mod.	1 L	HDPE	0.2% (v/v) HNO_3 (RP prep) or 1% (v/v) HNO_3 (column chelation procedure) in lab within 14 days of collection[4]	6 months
TSS	BAL-0303	1 L	HDPE	Store at 0-4 °C	7 days
Lab pH, hydrogen ion	BAL-0502	125 mL	Glass or HDPE	0-4 °C	Immediately (analyze on the day of receipt)

[4] While Brooks Applied Labs suggests that samples be acidified within 14 days of collection, there is no good scientific evidence to indicate that not doing so will impact data quality as long as samples are acidified in the original collection container for a minimum of 16 hours prior to analysis.

TABLE 6.5 - SUMMARY OF SAMPLE CONTAINERS & PRESERVATIVES (CONTINUED)

Parameter	Method	Minimum Volume	Container	Means of Preservation	Holding Time
B. Wet Sediments and Soils					
Total Metals	Various	20 g	4-oz. FLPE, Glass or HDPE	0-4 ºC during shipping, ≤ 4 °C in lab	1 year[5]
Metal Species	Various	20 g	4-oz. FLPE, Glass or HDPE	If possible, place on dry ice or freeze immediately following collection. Otherwise, maintain at 0-4 °C following collection and during shipping, and ship ASAP (within 48 hours) to the lab; store at -15 °C.	7 days to freeze; 1 year to analyze
Reactive Hg	In-House	20 g	4-oz. Glass or HDPE	Flash frozen or frozen immediately (overnight) in the field, ship frozen on dry ice, ≤ -18 °C in lab	14 days to prep; 28 days to analysis
Volatile Hg	In-House	Fill 4 oz. jar	4-oz. Glass with Teflon-lined lid	Store at < 4 ºC	48 hours
Cr (VI)	SW 3060A IC-ICP-MS	20 g	4-oz. Glass with Teflon-lined lid	0-4 ºC during shipment; ≤ 4 ºC in lab	28 days
% Solids	EPA 160.3	20 g	4-oz. FLPE, Glass or HDPE	0-4 °C	7 Days[6]
C. Dry Sediments and Soils					
Total Metals	Various	10 g	4-oz. FLPE, Glass or HDPE	N/A (Room Temperature is OK)	1 year[5]
Metal Species	Various	10 g	4-oz. FLPE, Glass or HDPE	N/A (Room Temperature is OK)	1 year[5]
D. Wet Tissues					
Total Metals or Species (other than As)	Various	Fill 4 oz. jar	4-oz Glass or plastic jar	0-4 ºC during shipping, -15 °C in lab	1 year[5]
As Species	EPA 1632A	Fill 4 oz. jar	4-oz Glass or plastic jar	0-4 ºC during shipping, < -18 °C in lab	2 years
As Species, dissolved (Juice and Juice Concentrate)	Various	Various	Various	Juice: 0-4 ºC for up to 14 days after opening then : -15 °C Concentrates: -15 °C in lab	28 days
As Species, dissolved (Wine and Spirits)	Various	Various	various	Room temperature, then refrigerate 0-4 ºC after opening	1 year
Big 4 in Foods (Food/Supplement)	AOAC 2015.01 (BAL-5040)	10 g	Various	Store according to label recommendation	N/A
E. Tissues (freeze dried)					
Total Metals	Various	10 g	4-oz Glass or plastic jar	N/A (Room Temperature is OK)	1 year[5]
Metal Species (other than As Species)	Various	10 g	4-oz Glass or plastic jar	N/A (Room Temperature is OK)	1 year[5]

[5] There are no established holding time limitations for solid samples.

[6] Although the standard temperature / holding time requirements for % solids are 0-4 ºC for 7 days, many solid samples are frozen and held for much longer periods prior to analysis for other parameters. Ideally, sample aliquots for each parameter should be removed prior to freezing. If this has not been done and % solids analysis is required for the samples, then the aliquots for % solids should be removed at the same time as the aliquots for the other parameters to ensure similar sample characteristics between aliquots.

As Species	EPA 1632	10 g	4-oz Glass or plastic jar	N/A (Room Temperature is OK)	2 years
Parameter	**Method**	**Minimum Volume**	**Container**	**Means of Preservation**	**Holding Time**
F. Air					
Hg (Natural Gas / Land Fill Gas)	ASTM D6350	1.5 L	Gold traps, capped tightly	Ambient temp OK, keep dark; ends plugged, store in zip-type bag	28 days
Hg (Ambient Air)	IO-5	20 L	Gold traps, capped tightly	Ambient temp OK, keep dark; ends plugged, store in zip-type bag	28 days
Hg (Air/Stack Gas)	EPA 324 / 1631	2 L	Iodated Carbon Trap, capped tightly	Ambient temp OK, keep dark; ends plugged, store in zip-type bag	28 days
G. Biomonitoring					
Hg (Urine)	EPA 1631, modified	90 mL	Polyethylene urine specimen container (transfer immediately to fluoropolymer bottle)	0-4 ºC during shipment; 0-4 ºC or ≤ -15 ºC in lab	28 days (1 year if frozen)
Total Metals (Urine)	Various	90 mL	Polyethylene urine specimen container or 20 – 40 mL vials	0-4 ºC during shipment; 0-4 ºC or ≤ -15 ºC in lab	28 days (1 year if frozen)
As, Cr, Se Speciation (Urine)	IC-ICP-MS	90 mL	Polyethylene urine specimen container or 20 – 40 mL vials	0-4 ºC during shipment; ≤ -15 ºC in lab	1 year
Total Metals and Metals Speciation (Whole Blood)	Various	6 mL	6-mL royal blue top K2 EDTA Vacutainer	0-4 ºC during shipment; ≤ -15 ºC in lab	1 year
Total Metals and Metals Speciation (Plasma/Serum)	Various	6 mL	6-mL royal blue top Vacutainer with clot activator	Separation from whole blood must occur prior to adding sample to the vial; 0-4 °C during shipment; ≤ -15 °C in lab	1 year
Total Metals and Metals Speciation (Hair, Nails, Feathers)	Various	100 mg	Zip-type plastic bag	Dark, room temperature or on ice	1 year
H. Other					
Metals (DGT Units)	Various	N/A	Plastic Bag	Add a few drops of 0.01 NaCl solution; check weekly to ensure probes stay moist	6 months

6.6 BAL's Policy on Accepting Samples

Brooks Applied Labs will only accept samples for analysis from parties with whom a written contract or agreement has been jointly signed or otherwise agreed to in writing, or from long-time clients in good standing with BAL's accounts receivable department. If a signed contract is not in place, samples are still received and placed on "hold." Any time-sensitive work, such as preservation or filtering, is still performed and the samples are stored appropriately. Once the Project Manager establishes the contracting paperwork, the samples are taken off hold status and the agreed to TAT begins.. The terms under which BAL would enter into a contract are explained in section 5.1 of this document.

Once a legal contract is in place, BAL will accept client samples even if the samples have not been preserved or handled properly, but all evidence of improper preservation and/or handling will be fully documented by BAL at the time of receipt. Examples of improperly handled samples include those with holding time exceedances, temperature exceedances, pH exceedances, improper container, evidence of improper sampling technique, improper handling (i.e., broken custody seal), improper documentation of samples on the chain-of-custody (COC) form, etc.

In the case of samples that have been collected or preserved improperly, the Project Manager immediately contacts the client to determine whether the client desires to continue with the analysis of samples. At the request of the client, BAL will perform analyses of samples even if they have non-conformance issues, but all such samples shall have their results qualified to indicate the non-conformance.

In the case of improperly documented samples, the Project Manager will contact the client to clarify any questions concerning the COC before samples are batched.

At all times, BAL reserves the right to refuse to accept samples at their sole discretion. Typically this would be reserved for samples that are deemed to be a threat to the health or safety of BAL personnel beyond what might reasonably be expected while working within an analytical laboratory.

Refer to the sample receiving SOP (BAL-2000) for specific information pertaining to BAL's sample acceptance policy.

7.0 Sample Custody

7.1 Field Custody

Formal custody requirements begin at BAL with the shipment of sampling equipment and containers to the field. Every shipment must be documented by BAL. With the exception of HDPE bottles sent for the collection of hydride, Se speciation, and all solid samples, a minimum of 10% of the bottles from 10% of the cases for each manufacturer lot # are tested for all applicable analytes prior to shipment. A certificate of analysis may be generated and supplied with all equipment at the request of the client. BAL provides Chain-of-Custody (COC) forms and two custody seals with each container shipment. Sample collection dates and times should be provided on the COC by the organization conducting the field sampling. If provided, the COC is used as documentation for sample collection dates and times. In addition, all sample container shipments are documented to track container information such as bottle cleaning batch numbers, quantity of containers shipped and their date of shipment.

While BAL does not typically provide field services, we do recommend that certain precautions be taken when collecting samples. Special consideration should be given to the procurement, transportation, preservation, and storage of samples to be analyzed. These procedures are intended to ensure that any analyte originally present in the sample matrix has not degraded and that contamination has not been introduced. For example, for mercury work, only rigorously acid-cleaned FEP, or pre-tested FLPE and glass containers with fluoropolymer lined lids may be used for water samples. See section 6.5 of this document for container, preservation, and holding time requirements for other analyses. Tissues and other solid matrices may be stored in FLPE containers, HDPE containers, glass containers with fluoropolymer lined lids, or plastic bags/wrap. Wet solid samples are preserved in the field by shipping on ice.

The courier is responsible for documenting the custody of the samples while the samples are in transit from the field to BAL.

7.2 Laboratory Custody

7.2.1 BAL Definition of Laboratory Custody

A sample is considered to be "in custody" in the laboratory if it meets any one of the following criteria:

- It is in the possession of a sample control specialist, a laboratory chemist, or a group leader.
- It was in the possession of a sample control specialist, a laboratory chemist, or a group leader, and then locked or sealed to prevent tampering.
- It is in a secure area (i.e., storage).

7.2.2 Sample Receipt

All samples delivered to BAL are received by a sample control specialist or designated alternate in the laboratory receiving area. Upon delivery of samples, the sample control specialist signs and dates the COC form (refer to SOP BAL-2001 for example of form).

Immediately after opening the cooler or other container, the sample control specialist confirms the presence of ice, measures sample temperatures (if required), and documents the condition of the samples (intact, broken, leaking, etc.). The sample control specialist also verifies that each container is properly labeled and sealed and compares the sample ID or field ID number against the COC form. The temperature of the samples at the time of receipt is determined by aiming a calibrated IR thermometer directly at a sample.

For all methods with temperature requirements, the thermal preservation of the samples must be maintained during the sample receiving to storage procedures. Therefore, samples with temperature requirements should be prioritized for login and placed in cold storage (walk-in refrigeration or freezer) if there is any doubt that temperatures might go out of criteria during the receiving procedure. Any movement of the samples must be documented. The laboratory must document if any required thermal preservation is not maintained during sample receipt and login. If thermal preservation is not maintained, the Project Manager must be notified immediately and the client shall be notified in writing (typically by email).

The sample control specialist is also responsible for ensuring that all samples are properly preserved. If any filtration or analysis of volatile mercury species is required, this should be performed before the preservation of samples. All samples must be preserved in accordance with the preservation instructions in each appropriate analytical methodology. If water samples that do require acidification are preserved in the field, the sample control specialist checks the pH and documents that it is less than 2. If it is not less than 2, additional preservation reagent is added to the sample(s) and the amount of acid required to adjust the pH to < 2 is documented on the Sample Receiving Log.

If the sample ID listed on the bottle label and the COC form do not match, the custody seals on any of the containers are broken, the temperature of the samples is above the method specified storage limit, or the samples are not properly preserved, the sample control specialist notes the problem directly in the LIMS “Work Order Comments” for the affected work order and notifies the project manager. The project manager then immediately notifies the client of any concerns.

If sample containers arrive with too little sample for analysis and this is noticed at receipt, then the sample control specialist logs in the sample for all analyses requested on the COC form, sets the status for the analyses on the affected sample to “cancelled”, and writes “insufficient sample for analysis” in the container comments field.

Refer to SOP BAL-2000 for a detailed description of BAL’s sample acceptance policy.

7.2.3 Sample Log In

When logging in samples, the sample control specialist must check the LIMS Project Information against the COC form received to ensure that the work has been authorized. If any discrepancies exist, the Project Manager is immediately notified. The Project Manager will confirm the contracted analyses and update either the LIMS or the original COC form prior to sample log-in.

All samples are given a unique sample identification number at the time of sample log in. This number consists of a work order number that is unique to each sample shipment received and a sample number for each sample within that particular shipment. Work order numbers consist of a 7-digit code (yyww###) where the first two numbers are associated with the year, the next two are associated with the week of the year, and the final three are associated with the number of shipments received in that week (e.g., the eleventh sample shipment received in the 8th week of 2016 is given the work order number 1608011). The samples within a shipment are then each identified by sequential numbering. For example, if three samples were received in the 1608011 shipment they would be given the sample numbers 1608011-01, 1608011-02, and 1608011-03. LIMS automatically generates a unique sample number for each client sample and the client's sample name is recorded. The maximum number of samples per work order number is 99; therefore, if more than 99 samples are received in a sample delivery group, then the shipment must be broken up into more than one work order. The unique BAL work order and sample numbers are referenced during all laboratory preparations and analyses.

When a bottle is removed from the zip-type bags in which it was sent, the exterior of the bottle should be rinsed with clean DIW (for low-level samples) and/or wiped with a clean cloth. Bottles are then labeled with the BAL sample number, BAL project number, client sample ID, matrix, date of sample receipt, receipt preservation, storage location, and list of analytes to be analyzed for. An example of a BAL sample label is as follows:

1608011-01 **CWP-MM008**

Client ID: Effluent A

Matrix: Water **02/25/2016**

Preservation: 5% HNO3

Home Location: Cabinet #7

As, TR Cd, TR Co, TR Fe, TR

After all of the log-in information is recorded in the LIMS, the BAL Sample Receipt Log (refer to SOP BAL-2000) is generated from the LIMS and is signed and dated by the sample control specialist.

Custody of the original samples is tracked in the LIMS by updating the storage location of the samples every time that they are moved.

7.2.4 Sample Storage

All samples are stored in a secure area. A secure area is defined as an area within the premises of BAL with restricted access. To satisfy these custody provisions, the laboratory implements the following procedures:

- Access doors to the laboratory facility are kept locked
- Visitors must sign in and are escorted while in the laboratory
- Samples remain in the secure area until they are removed for sample preparation or analysis

After the samples are logged in, the sample control specialist stores them, according to their specific holding requirements, in either the refrigerator, freezer, or ambient sample storage area.

Samples requiring refrigeration are stored in a walk in freezer in the center of the BAL facility or rarely a standalone fridge is used in certain instances. Samples requiring freezing are stored in a walk in freezer located within Prep Lab 2 or a standalone freezer located within Prep Lab 1. Samples requiring cryogenic freezing are placed in the cryogenic freezer located in Prep Lab 1. The samples are removed from the shipping cooler and stored in their original containers, unless damaged. Samples not requiring refrigeration are stored on shelves in the sample storage cabinets, which helps to protect samples from UV radiation. All standards and other chemicals used at BAL are stored separately from samples.

After the samples are stored, all sample information is placed in a folder. This information includes the original COC form(s), a copy of the BAL Sample Receipt Log, the shipping way bill (or a copy of it), and any other documents included with the shipment. The folder is labeled with the work order number, BAL project number, received date, and due date. The folder is given to the Project Manager/Project Coordinator who then reviews the information, signs and dates the BAL Sample Receipt Log, updates the status in LIMS from “received” to “available” and files the folder in the “Active Customer” file located in the Project Manager’s office.

Samples for all projects are assigned a due date. Each analytical Group Leader is responsible for ensuring that all due dates and sample turn-around times are met. This is done by checking the LIMS to see what deliverables are approaching the due dates and by checking the collection dates of samples with short holding times (less than 60 days). In addition, all Sample Processing Forms (SPF) have due dates recorded on them for each project.

The minimal duration of original sample storage at BAL is set at 60 days following the submittal of the final report, unless contractual requirements indicate a longer period of storage.

7.2.5 Sample Distribution and Tracking

The system for tracking samples through preparation and analysis consists of the LIMS, laboratory worksheets, laboratory notebooks, instrument operation logbooks, instrument printouts (raw data), and final analytical reports.

7.2.5.1 Sample Batching - After samples are set to "available" by the project manager, the samples are then batched by the analytical Group Leaders or their designee. There may be instances where the samples are batched when they are at "received" status, when this happens, the project manager must be notified. Batches are sequentially numbered starting with the letter B, then the last two digits of the year, followed by a four digit sequential number (e.g. the 805th batch in 2016 is numbered B160805. Samples are assigned to each batch in the LIMS. Sample custody is tracked electronically in the LIMS.

Original samples are batched according to the method by which they are to be prepared and/or analyzed. At the time of batching, the SPF is printed from the LIMS. The project manager should add any special QA requirements and/or pertinent information provided by the client concerning the sample preparation/analysis should be added to the project comments in the LIMS so that it will automatically appear on any SPF including samples for that project. If the project comments field is not large enough, additional notes can be made in the project notes field with instructions in the project comments field to "see project notes." Once batched, the status of the samples is automatically changed from "available" to "batched" in the LIMS.

7.2.5.2 Sample Preparation - The SPF is given to the chemist responsible for sample preparation. All sample preparation details must be documented on the SPF or sample preparation benchsheet. Copies of all preparation documentation, once complete, must accompany the SPF. In order to track both original samples and sample preparations, the chemist documents the removal of original samples from their primary storage location to the preparation location and back to their storage location in real time in the LIMS. For samples that aren't prepared in their original container, after the original samples are logged in as being returned to storage, the preparation technician changes the samples in the prep bench sheet to "extracts." These extracts can now be tracked separately from the original samples. The location of the extracts is entered as the preparation location. When sample preparation is finished and the extracts are moved to the lab or other storage location, this information is entered into the LIMS prep bench sheet. From then on, every time the extracts are moved, up to and including disposal, this information is updated in real time.

During sample preparation, any comments on unusual observances or deviations from the analytical method or SOP must be documented. (Note: Senior management must approve any deviations from the analytical method or SOP prior to the preparation of the samples.) Following sample preparation, the prepared samples are stored in a secure laboratory area. Once prepared, the status of the samples is changed from "batched" to "prepared" and the date/time of prep and chemist's initials are updated in the LIMS benchsheet.

Note:

If the sample has insufficient mass for sample preparation or is exhausted following preparation, then the technician should make a note on the benchsheet and send an email to the Group Leader and the affected Project Manager. The PM then adds a note to the container comments field for all

affected samples. Should the sample be inadvertently rebatched, this note will show up on any subsequent SPFs making it clear that the sample is already exhausted and cannot be reanalyzed. The Project manager should alert the other Group Leaders if any other analyses are affected and set the remaining analyses to "Cancelled".

7.2.5.3 Sample Analysis – When analyzing the samples, the chemist builds a sequence that contains the calibration and other sequence specific QC (ICVs, CCVs, CCBs) as well as all samples, including batch QC, from all of the batches analyzed as part of the sequence. Any comments on unusual observances or deviations from the analytical SOP must be documented and must be referenced on the SPF or the analytical benchsheet. As previously mentioned, movement of the batch throughout the lab is documented in the LIMS bench sheet.

7.2.5.4 Primary Data Review - The chemist uploads the data into the LIMS and then must review the data. Primary data review includes checking all LIMS entries in the prep bench sheet and the data upload for accuracy, determining whether criteria is met, and preparing analyst notes about the quality of the data. The chemist should also rebatch samples that need to be reprepared or reanalyzed and alert the Group Leader. The chemist then puts together the data package, which includes the analytical bench sheet, all SPFs, all sample preparation documentation, the data review checklist, and any chemist notes. Instrument raw data is saved electronically. Once the data has been primary reviewed, the status of the samples is changed from "prepared" to "reviewed – primary" in the LIMS and the SPF is signed and dated. Refer to SOP BAL-1501 for a complete discussion of primary data review.

7.2.5.5 Final Review - A member of the QA Group reviews the final data. After the final review, either the VP of Quality or his designee (QA Associate, QA Assistant) must sign and date the SPF and include comments on any unusual observations and/or deviations from the analytical method or SOP. The batch status in LIMS is updated from "reviewed – primary" to "reviewed – final." If any samples require rebatching, this is performed by a member of the QA Group prior to changing the status of the samples in the analyzed batch to "reviewed – final." Refer to SOP BAL-1502 for a complete discussion of final data review.

(Refer to section 12 of this document for additional discussion of the data reduction, validation, storage, and reporting process.)

7.2.5.6 Deviation Traceability - All documents are used to track any deviations from generally accepted handling of the samples. The main forms for tracking deviation are the SPF, preparation and analytical bench sheets, and analyst notes. These forms must contain any mention of unusual events or occurrences or deviations from SOPs and should list where this information can be found if relevant. Examples of possible entries include, but are not limited to the following:

- Samples not cold when removed from refrigeration-see instrument log book.
- Samples over distilled.

- Samples prepared differently from SOP.
- Out-of-Control calibration curve.

Each person is responsible for filling-out the appropriate information for the task performed. The next responsible person will not accept the data and SPF unless the data package is complete for what has been performed so far. In this way all necessary information concerning samples and all sample handling steps can be traced and noted in the report to the customer.

7.2.5.7 Subcontracting - Only in extremely rare occasions will BAL subcontract samples for analysis. This is done only with the prior consent of the client and the subcontractor laboratory must have an established and documented laboratory quality system that complies with all of the requirements of the original contract. For example, if a project has been contracted under DoD/DOE QSM requirements, then the subcontracted laboratory would have to meet the same requirements and also have DoD accreditation for all of the subcontracted analyses prior to analyzing any of the samples. In such a case, the documentation to transfer samples includes collection date and time (if available from the field samplers), the Field ID#, the BAL Lab ID #, the date of preparation (if extracts are transferred), and the requested analyses. Refer to section 5.4 for further discussion.

7.2.6 Sample Disposal

7.2.6.1 Sample Preparations – Unless otherwise specified in the contract, sample preparations may be disposed of once the preparations have been analyzed and the data has been reviewed and reported to the client. Additionally, sample preparations may be disposed of if the VP of Operations has determined that there is no further value in analyzing the preparations, such as water preparations for methyl mercury that are only stable for a maximum of two days. The disposal of each batch must be documented in the LIMS.

7.2.6.2 Original Samples – It is BAL policy to maintain all samples (aqueous and solid) for 60 days after reporting results unless previous arrangements have been made with the client. At least once a year, all refrigerators and freezers should be cleared of any samples for which results have been reported more than two months ago. Samples that require longer storage should be separated from those that may be disposed after the two month period. The location in the LIMS must be updated to indicate that the samples have been disposed.

7.2.6.3 Disposal Guidelines – The concentration of all elements of interest in each sample preparation and each original sample is calculated to determine the proper disposal method for each sample (Refer to BAL SOP BAL-2003 for disposal limits for specific elements). The method of disposal (routine verses high-level disposal) must be indicated on the appropriate form.

7.2.6.3.1 Routine Disposal

7.2.6.3.1.1 Water Samples and Acid Digestions - All water samples (including preparations) and acid digested solid samples that are not designated as being hazardous may be disposed down the sanitary sewer. All acidic samples must be neutralized prior to disposal.

7.2.6.3.1.2 Native Solid - All native solid samples (not sample preparations) that are not designated as hazardous may be discarded directly into the municipal waste.

7.2.6.3.2 High Level Disposal - All High Level metal sample waste (as well as other waste that is considered to be hazardous) must be disposed of appropriately. The total volume added to the High Level Waste containers must be logged in the "Waste Disposal Log."

7.2.6.3.2.1 Water Samples and Acid Digestions - All water samples (including preparations) and acid digested solid samples that are designated as hazardous are placed directly into the high level metals/corrosives waste storage container.

7.2.6.3.2.2 Native Solid Samples and Dry Weights - All native solid samples (not sample preparations) that are designated as hazardous are disposed of directly into the hazardous waste container.

7.2.6.3.2.3 All Solvent Extracts - All solvent extracts must be treated as hazardous waste. Solvent extracts may be consolidated in satellite containers, and then disposed of as hazardous waste.

7.2.6.3.3 Non-Routine Disposal - Samples that are designated by the client to be high level in an analyte not performed by BAL shall be considered hazardous and treated as hazardous waste upon release for disposal. In certain cases, BAL may contract with a client to analyze samples that are known to be hazardous beyond the scope of our analysis (such as samples containing a high level of organic contaminants or dioxins), these samples will be flagged as requiring special disposal (as per the VP of Operations) and disposed of through a licensed hazardous waste acceptance facility. BAL may also arrange with the client to return the remaining samples after analysis.

7.2.6.3.4 High-Level Metals Waste Transport and Ultimate Disposal – BAL schedules hazardous waste pick-up at least twice per year. It shall be the responsibility of the waste handling company to transport and dispose of the hazardous waste in a manner consistent with local and federal laws and regulations.

7.2.6.3.5 Low-level Radioactive Waste - All samples required to be disposed as low-level radioactive waste need to be in accordance with all local, state, and federal regulations regardless of the concentrations of other constituents.

7.2.6.4 Documentation – The LIMS is updated when necessary to both document disposal of samples (and sample preparations) and to initiate disposal or transfer of samples.

7.2.7 Catastrophic Failure of Storage Equipment

Refer to the Chemical Hygiene Plan in the event of a catastrophe (fire, loss of power, equipment failure) for the procedures to follow. If any samples are affected (go outside of holding temperature requirements or are otherwise damaged), it is the responsibility of the Project Manager to make sure that all affected samples are identified and documented in the associated Incident Report. The Project Manager is also responsible for informing the associated clients of all affected samples. All clients must be notified as soon as it is practical to do so.

8.0 Analytical Procedures

8.1 Methods

All methods applied by Brooks Applied Labs are designed and approved to provide the most representative data available for any trace metal application. Only validated methods are applied to client samples unless research and method development is necessitated by the nature of the project. Validated methods are identified by either of the following criteria: the method was a promulgated or draft method issued by the EPA or other recognized regulatory agency, or the method of validation conforms to BAL SOP BAL-7000. BAL prides itself on application of cutting edge science to generate real world solutions by investigating new methodologies on a continual basis. BAL offers clients the ability to custom tailor digestion, extraction, and analytical techniques to fit their needs without exceeding budgetary constraints.

All methods generated by BAL which are promulgated into an official SOP must identify if the method does not conform to US EPA standards. All reports which are generated using such methods must clarify that the results are not generated using a US EPA approved method. A modification to a method performed is typically accomplished by stating "mod" after the method number.

All BAL methods, standard operating procedures, inventions, ideas, processes, improvements, designs and techniques included or referred to therein, must be considered and treated as Proprietary Information, protected by the Washington State Trade Secret Act, RCW 19.108 et seq, and other laws. All Proprietary Information, written or implied, will not be distributed, copied, or altered in any fashion without prior written consent from BAL. All Proprietary Information (including originals, copies, summaries or other reproductions thereof) shall remain the property of BAL at all times and must be returned upon demand.

TABLE 8.1.1 – LIST OF ROUTINE METHODS AT BAL

Analyte	Method Description	Reference
Trace Metals	ICP-MS	EPA Methods 200.8 mod, 6020B mod, and 1638 mod
Mercury	CVAFS	EPA 1631E (Appendix for solids)
Methyl Mercury	CVAFS	EPA 1630 (mod for non-aqueous matrices)
As Speciation Analysis	HG-CT-AAS	EPA 1632A (mod for soils)
As Speciation Analysis	IC-ICP-MS	BAL-4100
Se Speciation Analysis	IC-ICP-MS	BAL-4200
Cr Speciation Analysis	IC-ICP-MS	BAL-4300
Total Cyanide	IC/PAD	BAL-4400

Refer to Appendix A for list of common abbreviations.

A complete list of BAL methods is found below.

TABLE 8.1.2 – LIST OF BAL STANDARD OPERATING PROCEDURES

SOP #	Abbreviated Title
BAL-0001	Ethics
BAL-0020	Maintenance – Support Equipment
BAL-0021	Maintaining Logbooks
BAL-0102	Preventing Contamination of Samples
BAL-0103	Element (LIMS) for Analysts
BAL-0104	Documentation of Reagents and Standards
BAL-0105	Security of Laboratory and Samples (Maintaining Logbooks)
BAL-0106	Training
BAL-0300	Sample Processing
BAL-0301	Filtration
BAL-0302	Filtration for Collection of Particulate
BAL-0303	Total Suspended Solids
BAL-0304	Homogenization – Sub-sampling
BAL-0305	Sample Extrusion from Sediment Cores
BAL-0308	Sample Storage and Disposal – Rad Materials
BAL-0501	Total Solids Determination
BAL-0502	pH of Solids
BAL-0600	Maintenance – Analytical Instruments
BAL-0700	Security of Electronic Data
BAL-0800	Purchase, Receipt, Storage of Consumables
BAL-1000	Writing, Reviewing SOPs
BAL-1001	Document Control
BAL-1002	Records of Client Samples
BAL-1003	Records of QC Results - Charts
BAL-1004	Internal Laboratory Audits
BAL-1005	Precision, Accuracy, Uncertainty
BAL-1006	Assessing Method Variability
BAL-1007	Identifying Systematic Errors
BAL-1008	Incident Report and Resolution

BAL-1009	MDL Studies, Validation, DOC
BAL-1500	Data Flow and Handling
BAL-1501	Primary Data Review
BAL-1502	Final Data Review
BAL-1503	Data Correction
BAL-1504	Acceptable Deviation from Protocol's
BAL-1700	Test Code Creation/Maintenance
BAL-2000	Receipt of Samples
BAL-2001	Sample Custody Maintenance and Tracking
BAL-2002	Sample and Client Identification
BAL-2003	Sample Storage and Disposal
BAL-2037	As(In) in Melarsamine by IC-ICP-MS
BAL-2300	Decontamination of Sample Prep Equip
BAL-2301	Decontamination of Sampling Equipment
BAL-2302	Decontamination of Glassware
BAL-3000	Manual Integration – AAS and AFS
BAL-3100	Hg in waters by EPA 1631E (CVAFS)
BAL-3101	Hg in Seds and Tissue by EPA 1631E with Appendix (CVAFS)
BAL-3200	MeHg in Waters, Seds, and Tissue (CVAFS)
BAL-3300	As speciation in Water, Seds and Tissue (HGAAS)
BAL-4000	Manual Integrations – IC-ICP-MS
BAL-4100	As Speciation by IC-ICP-MS for Waters and Tissues (Basic Anion Exchange)
BAL-4101	As Speciation of by IC-ICP-MS (Isocratic Anion Exchange)
BAL-4115	Alkaline Extraction for As Speciation in Environmental Tissues
BAL-4116	Organic Acid Extraction for As Speciation from Rice
BAL-4200	Se Speciation by IC-ICP-MS
BAL-4300	Cr^{6+} by IC-ICP-MS
BAL-4310	Extraction of Cr^{6+} from Soils and Seds (EPA 3060A mod.)
BAL-4500	Determination of Fe(II) and Fe(III) by Colorimetry
BAL-5000	Trace Elements Analysis (ICP-QQQ-MS)
BAL-5001	Trace Elements Analysis (ICP-MS) Perkin Elmer (back-up)
BAL-5002	Determination of Hardness by Calculation

BAL-5010	Total Recoverable Metals in Waters by Hotblock Sample Prep
BAL-5011	Total Recovery Metals digestion - Aqueous
BAL-5012	Total Metals–Aqueous–Closed Vessel Bomb
BAL-5020	RP Preparation of Saline Waters for Metals
BAL-5021	Column Chelation for Metals in Water
BAL-5030	Acid Digestion for Trace Metals in Solids - Seds and Biota
BAL-5032	Total Metals in Soils, Coal, Fly Ash
BAL-5040	MW Digestion Total Recoverable Metals - Food/Biota
BAL-6000	Generation of Level I Report
BAL-6001	Review of Request for Work & Contracts
BAL-6002	Customer Complaint
BAL-7000	Method Development
BAL-8000	Clean Sampling for TM (EPA 1669)
BAL-9001	Out-of-Specification Investigation (GMP)
BAL-9002	Qualification of Legacy Equipment (GMP)
BAL-9003	Change Control (GMP)

8.2 Method Modifications

Brooks Applied Labs is on the forefront of method development and improvement for trace metals and speciation. BAL has identified several modifications that improve the performance of standard EPA methods. All modifications are clearly stated in BAL standard operating procedures.

8.3 Laboratory Operations

8.3.1 Laboratory Containers Used for Preparation and Analysis

All containers for ultra-trace metals preparation and analysis must be rigorously cleaned and/or tested in order to minimize possible contamination. Refer to SOPs BAL-2300 through BAL-2302 for description of cleaning procedures for laboratory preparatory equipment.

8.3.2 Reagent Storage

All supplies (i.e., glassware, chemicals, and reagents) are of the highest possible quality to ensure quality assurance and to avoid contamination. Reagents purchased from commercial vendors are assigned a unique LIMS identification number and are labeled with the date received, the date opened, and the expiration date. Reagents used for stock and working standards are prepared from analytical reagent grade chemicals or higher purity grades, unless

such purity is not available. Reagent water is prepared by deionization of city water using reverse osmosis. Each prepared reagent is clearly labeled with the composition, concentration, date prepared, initials of preparer, expiration date, BAL lot # and special storage requirements, if any.

Reagent solutions are stored in appropriate glass or plastic containers under conditions designed to maintain their integrity (refrigerated, dark, etc.). Shelf life is listed on the label, and the reagent is removed from general lab use after it has expired. They may however still be used for research. Expired standards must be adequately segregated from working standards to prevent their accidental misuse. Reagent solutions are checked for contamination by testing reagent blanks before use. The expiration date of a standard or reagent may only be extended upon the approval of the Technical Director. Such approval must be indicated in the notes section of the LIMS entry.

Refer to BAL's Chemical Inventory list stored on the BAL server for a list of all chemicals used at BAL. The Chemical Hygiene Plan describes where and how these chemicals are stored at BAL. Safety and Data Sheets (SDS) are stored on the server as PDF files and should be consulted for detail concerning potential hazards associated with specific chemicals. SDSs are organized on the server by both chemical name and by CAS number. BAL-0104 describes the documentation of standards and reagents in the LIMS and the testing of standards and reagents.

8.3.3 Waste Disposal

Handling, storage, and disposal of laboratory-related hazardous wastes are subject to the regulations contained in the Resource Conservation and Recovery Act. BAL shall store, package, label, ship, and dispose of hazardous wastes in a manner which ensures compliance with all federal, state, and local laws. Potentially hazardous wastes include all standards, reagent solutions, process wastes, solvents, native samples, sample extracts, and digestates.

A waste is considered hazardous if:

1. The waste material is listed as hazardous in 40 CFR Part 261.30-261.33.
2. The material exhibits any of the characteristics of hazardous waste: ignitability, corrosiveness, reactivity, or extraction procedure toxicity.
3. The waste is listed in 1 or 2 above and is not excluded by any provisions under the Resource Conservation and Recovery Act.

A waste is considered an acute hazardous waste if it is identified in 40 CFR Part 261.31, 261.32, 261.33 (e) as an acute hazardous waste.

BAL is categorized as a Medium Quantity Generator. This category is defined as: A generator who generates 220 - 2200 kilograms of hazardous waste or < 1 kg of acute hazardous waste in a calendar month and stores all generated waste for no more than 180 days (40 CFR Part 261.5).

BAL shall ensure delivery of hazardous waste to a treatment, storage, or disposal facility, which is:

1. Permitted under 40 CFR Part 270
2. In the interim status under 40 CFR Parts 270 and 265
3. Authorized to manage hazardous waste by a state with a hazardous waste management program approved under Part 271; or
4. Permitted, licensed, or registered by a state to manage municipal or industrial solid waste (subject to local regulations).

Hazardous waste solvents as identified in the 40 CFR Part 261 may not be evaporated off in a fume hood. Solvents evaporated off during the extraction/testing process are exempt. Acidic and basic wastes may be neutralized and disposed of via the sanitary sewer if they are not hazardous due to the presence of other constituents (as subject to local regulations). Heavy metals may be precipitated from the liquid portion and disposed via the sanitary sewer (subject to local regulations).

Hazardous waste storage is limited to quantity and/or accumulation time and must comply with RCRA regulations as specified in the 40 CFR. These wastes should be packaged and separated according to the compatible groups (e.g. solvents, acids etc.).

Samples submitted to BAL for analysis are excluded from regulation as hazardous waste under 40 CFR Part 261.4(d) provided the samples are being transported to or from the laboratory, are being analyzed, are being held for analysis or are being maintained in custody for legal reasons. However, once the laboratory samples or extracts are removed from their original container and placed in a waste container, the exclusion provisions of 40 CFR Part 261.4(d) no longer apply. Samples that have been identified as hazardous may be either: 1) returned to the generator; or, 2) disposed of according to applicable Resource Conservation and Recovery Act (RCRA) regulations. Samples, which are determined to be non-hazardous, may be subject to local environmental regulations. A sample collector shipping sample to a laboratory and a laboratory returning samples to a sample collector must comply with U.S. Department of Transportation (DOT), U.S. Postal Service (USPS), or any other applicable shipping requirements.

Native samples and sample preparations must be disposed of in accordance with local, state, and federal regulations. Residual native samples must be separated by matrix (water, sediment/soil, and biota) and placed in the appropriate containers for disposal. Remaining sample preparation solutions must be separated by digestion type (acid, base, solvent extract) and placed in the appropriately labeled disposal containers.

8.3.4 Facility Description

BAL's research and analytical laboratory facility is located at 18804 North Creek Parkway, Suite 100, Bothell, Washington 98011. The location is close to Sea-Tac Airport, numerous colleges and universities, and state regulatory offices. The entire laboratory section of the facility is identified as a clean area, which is continually monitored for contamination through routine testing of the DIW system, air, and preparatory blanks. BAL has two designated sample preparation laboratories, a designated Inductively Coupled Plasma – Mass

Spectrometry (ICP-MS) laboratory, and a Cold Vapor Atomic Fluorescence Spectrophotometry (CVAFS) laboratory which shares space with Hydride Generation Atomic Absorption (HGAAS) isolated from the rest of the facility to maximize cleanliness. The facility also includes office space, as well as areas for consumables storage, bottle washing, sample receipt, sample storage, and waste neutralization/disposal. Clean room sticky mats are located at the entrance to each lab to minimize tracking in particles.

The CVAFS laboratory, sample preparation laboratories, receiving laboratory, bottle washing and sample storage areas are monitored monthly for atmospheric mercury levels to ensure that levels are suitably low for ultra-trace level mercury analysis. A warning level has been established at 15 ng/m^3 with a shutdown control level at 25 ng/m^3. Reagent water is tested for mercury each day prior to beginning analysis by testing blanks. The average blank must contain less than 10 pg Hg when analyzed on the MERX-T automated system.

The ICP-MS lab is where other metals analyses are conducted. Reagent water is tested by ICP-MS monthly.

All acid neutralized waste disposals are performed on a batch basis to eliminate the possibility of high level waste release. Disposal of all other toxic materials is carried out under contract with a licensed disposal company.

8.4 Exceptional Departures from Standard Operating Procedures (SOPs)

The Technical Director, VP of Quality, VP of Operations, or President/CEO must approve of any departure from BAL standard operating procedures. This approval must be documented in some way (Note on SPF, email, etc.). Documentation must include the reason for the departure. Under no circumstances is the Chemist or Group Leader authorized to alter any standard procedure without managerial approval. Such approval and the actual departure from BAL procedure must be fully documented and conveyed to the affected client. All quality control criteria must still be met for all reported data.

9.0 Calibration Procedures

9.1 Instrumentation

Refer to the Equipment List stored on the Brooks Applied Labs server for an up-to-date list of the equipment maintained at BAL.

9.2 Standard Receipt and Traceability

All stock standard solutions are received by the analytical laboratory and are documented in the LIMS. Information documented in the LIMS includes source, type of standard, date of receipt, lot number (if applicable), and expiration date. PDF copies of stock standard certificates are attached to the LIMS standard page for the standard.

All standard solutions are stored in a manner that is consistent with the manufacturers' recommendations.

Standards traceability is achieved by documenting all standard solution information in the LIMS. In addition to the previously mentioned documentation for stock standards, documentation for intermediate standard solutions must include: identification of primary (stock) standard used, the preparation date, method of preparation (specifically dilution information), the preparer's name, the concentration prepared, BAL lot #, and the expiration date. Documentation for working standards must include: identification of the stock and intermediate standards used, the preparation date, method of preparation (specifically dilution information), the preparer's name, the concentration prepared, BAL lot #, and the expiration date.

9.3 Standard Sources, Preparation, and Testing

All working standards are documented for traceability as discussed in section 9.2. All intermediate and working standards are made in accordance with the protocols of the specific procedure for which the standards shall be used. Refer to the analytical method SOP for the specific procedures followed for the preparation and holding time of any intermediate or working standard.

Any new standard must be tested prior to use. The acceptance criterion is that the average recovery of the new standard is within ± 5% of the average recovery of the previous standard. A minimum of three replicates of the old and the new standard must be analyzed for comparison. The RSD of the measurements of each standard may not exceed 5.0%. Only the Technical Director can authorize the use of any untested standard or standard that does not meet the testing criteria. Standards that are made daily are not tested against the old standard prior to use. Instead, they are verified against the second source standard as per method specific criteria.

Quality control reference materials are typically acquired from the National Resource Council of Canada (NRCC), the National Institute of Standards and Technology (NIST), the International Atomic Energy Agency (IAEA), RT Corp (RTC), or Community Bureau of Reference (BCR).

9.4 Analytical Instrument Calibration

The analytical methods or the SOP for the specific method specifies all calibration protocols, frequency and acceptance criteria. Full documentation for calibration is included with the sample data package. Each instrument used to analyze samples must pass the calibration criteria established in the appropriate method or operating procedure.

All standards used to prepare the calibration standard solution are obtained from accredited chemical suppliers and are tested for purity prior to use. Working standards are made from the stock standards at appropriate concentrations to cover the linear range of the calibration curve as outlined in the individual procedures. All laboratory analysis is documented by the analyst on the analytical bench sheets. All information concerning the calibration must be stored such that the calibration can be recreated if need be.

9.4.1 Instrument Calibration for CVAFS and HGAAS Methods

The instrument calibration consists of analyzing a minimum of three initial calibration blanks (used to correct all results, including those for the calibration standards, at the instrument) and a minimum of five standards. These standards should span the linear range of the instrument. Calibration coefficients are calculated for each concentration of the calibration and the average calibration coefficient is used to calculate results. For CVAFS the RSD of the calibration coefficients must be ≤ 15% and for HGAAS the RSD of the calibration coefficients must be ≤ 20%. Only calibration standards that meet recovery criteria as stated in the analytical methods are used in the calibration. Allowances for dropping calibration points are discussed in section 9.4.4.

9.4.2 Instrument Calibration for ICP-MS Methods

The instrument calibration consists of analyzing a minimum of four initial calibration blanks (used to blank offset the calibration) and a minimum of five standards (8 to 9 standards are routinely analyzed for ICP-MS analyses). These standards should span the linear range of the instrument. The calibration routinely used for ICP-MS analysis is linear, not forced through zero. The correlation coefficient (r) of the initial calibration for ICP-MS must be ≥ 0.995, but in general should be ≥ 0.998. If a weighted linear calibration forced through zero is used for ICP-MS analyses, then the r value must still be ≥ 0.995. All calibration standards must recover between 75 – 125% with the exception of the low calibration point that must recover between 70 – 130%. Since unweighted linear calibrations are heavily influenced by the standards at the high end of the calibration, calibration points near the bottom of the calibration will not significantly affect the linear regression and need not be removed even when failing to meet recovery criteria. However, the lowest calibration standard used to set the quantitation level of the analysis must recover within 70 -130% and all subsequent calibration standards must recover within the 75 – 125% acceptance criteria.

More than one calibration may be run during a single sequence. This is done for large analyte lists due to potential internal standard failures and instrument drift. In such cases, the best calibration may be used on an analyte-specific basis. This may only be done if the following is adhered to:

a. Both calibrations should be run at the beginning of the sequence and validated (acceptable ICV) prior to running client samples. NELAP/DoD/DOE standards require that calibration validation occurs before analysis of samples. If the calibration is going out of control due to failing internal standards due to column or cone issues, then the appropriate corrective action is to begin a new sequence with a new calibration and to upload the subsequent runs into the new sequence.
b. The calibration used for each analyte/isotope/condition must be clearly identified and only one calibration may be used per analyte/isotope/condition per sequence. All unused calibrations are to be set to not reportable in LIMS.

9.4.3 Instrument Calibration for Ion Chromatography – Inductively Coupled Plasma – Mass Spectrometry (IC-ICP-MS) and Ultraviolet Visible Spectroscopy (UV/Vis)

The instrument calibration consists of analyzing a minimum of four initial calibration blanks (used to blank offset the calibration) and a minimum of five standards. These standards should span the linear range of the instrument. Additionally, four replicates of the low calibration standard are typically analyzed. The average response is used for the low calibration standard. Additionally, the standard deviation of the low level standards may be used to estimate the batch specific MDL for procedures where the method blank results at the instrument are typically 0 µg/L. The calibration routinely used for metals speciation analysis is linear, forced through zero. The correlation coefficient (r) of the initial calibration for these methods must be ≥ 0.995, but in general should be ≥ 0.998. Additionally, if multiple species are being calibrated in an analysis, the RSD of the various slopes must be ≤ 10.0%. All calibration standards must recover between 75 – 125% with the exception of the low calibration point that must recover between 70 – 130%. Since unweighted linear calibrations are heavily influenced by the standards at the high end of the calibration, calibration points near the bottom of the calibration will not significantly affect the linear regression and need not be removed even when failing to meet recovery criteria. However, the lowest calibration standard used to set the quantitation level of the analysis must recover within 70 -130% and all subsequent calibration standards must recover within the 75 – 125% acceptance criteria.

9.4.4 Instrument Calibration for Ion Chromatography – Pulsed Amperometric Detection (IC-PAD) Methods

The instrument calibration consists of analyzing a minimum of four initial calibration blanks (used to blank offset the calibration) and a minimum of five standards. These standards should span the linear range of the instrument. Additionally, four replicates of the low calibration standard are typically analyzed. The average response is used for the low calibration standard. Additionally, the standard deviation of the low level standards may be used to estimate the batch specific MDL for procedures where the method blank results at the instrument are typically 0 µg/L. The calibration routinely used for cyanide analysis methods is analysis is a polynomial curve to the second order, forced through zero. The correlation coefficient (r) of the initial calibration for these methods must be ≥ 0.999. All calibration standards must recover between 75 – 125% with the exception of the low calibration point that must recover between 70 – 130%.

9.4.5 Allowances for Dropping Standards from the Calibration

When calibrating the instrument, the low calibration standard must be equal to or less than the method reporting limit (MRL). Standards may be removed from the bottom end or top end of the calibration when they do not meet acceptance criteria as long as at least three consecutive standards remain, however, this will result in a reduced range of quantitation. It is not permissible to drop a mid-level point from the calibration without also dropping either all of the points below or all of the points above it as well. The only exceptions for dropping a mid-point standard from a calibration is for a known misinjection or obvious spiking error. Even then, it is not allowable to drop two consecutive mid-point standards without the documented approval of the Technical Director.

9.4.6 Calibration Verification

The calibration is verified prior to the analysis of any client samples. The initial calibration check, consisting of one standard at the mid-point of the calibration curve and one calibration blank, is performed immediately following this calibration. The initial calibration verification (ICV) standard must be prepared from a source other than the one used to calibrate the instrument. Continuing calibration verification (CCV) standards are analyzed at the beginning and the end of each sequence and after every 10 client samples during the course of the analytical run. Initial and continuing calibration checks are used to establish whether ongoing instrument calibration is acceptable.

9.4.7 Results Outside of the Calibration

Results should not be reported if the instrument result for the sample is above the result for the high calibration standard. It is standard practice to dilute the high-level sample and reanalyze it such that the result at the instrument falls within the calibration. A result that is outside of the calibration range would not be reported without appropriate qualification or narration. An additional linearity check standard (high calibration check or HCV) that is above the calibration may be analyzed later in the run. The recovery of the HCV must be 90 – 110% for it to be used. If the HCV is acceptable, then a result that was above the calibration, but below the level of the HCV may be reported without qualification or narration. If this is done, results must be carefully evaluated to determine if there is any risk for potential carry over from the high sample or the HCV. Samples analyzed at dilution yielding results below the MRL should be reanalyzed at less dilution to be within the calibration curve.

9.4.8 Reanalysis of Samples and Calibration Procedures for All Automated Methods

When sample reanalysis is necessary, it must be performed on the most recent instrument calibration. If more than 48 hours have passed since the original calibration was last verified or if the instrument has been recalibrated since original sample analysis, then the original calibration must not be used and a new calibration must be performed. The new calibration must include all analytes of interest, as well as the instrument modes used in the original calibration (e.g. HEHe, H_2, O_2, etc.). If a new calibration is used the samples would be analyzed in a new sequence but would remain in the same batch. If the original calibration is to be re-used within 48 hours, the calibration must be re-verified by the analysis of instrument blanks and an ICV before sample reanalysis, and the samples must be bracketed with the

appropriate number of CCV/CCB sets. In this case, the samples remain in the same batch and sequence.

It is not necessary to re-analyze the entire batch or even all of the associated batch QC with the samples. When samples are reanalyzed, a 10% frequency of the associated MS/MSD sets (including native samples) must be reanalyzed as well to verify that elements have not precipitated out of the digestate and to verify that the analytical precision between the initial analysis and the reanalysis meets criteria for all samples that have a valid initial result. The acceptance criteria for analytical precision is equivalent to the analytical duplicate precision criteria listed in each method. If the reanalysis of the samples with valid initial results (including QC samples) do not meet precision criteria, the QA Officer and/or the Technical Director must be consulted to prescribe what corrective action is appropriate. Results from the reanalysis of the QC (that meet precision criteria) are not required to be reported to the client, but should be kept as validation data for the reanalyzed samples.

Other batch QC samples (DUPs blanks, SRMs, blank spikes, etc.) are not required to be reanalyzed unless there were specific issues with the initial analysis of these samples. If samples that do not have pre-prepared QC samples are reanalyzed (such as waters prepared with the closed-vessel original-container oven digestion), then the MS/MSD sets must be performed on the reanalyzed samples at a 10% frequency (not necessarily the samples that were initially spiked).

9.5 Periodic Calibration Procedures for Other Laboratory Equipment

Periodic calibration checks are performed for associated equipment such as balances, thermometers, pipettes, ovens, and refrigerators that are required in support of the preparation and analytical methods, but that are not routinely calibrated as part of the analytical procedure. All the calibration measurements are recorded in a laboratory log book as outlined in SOP BAL-0021.

BALANCES

Balances are calibrated annually by a contracted, certified professional. Balances are also checked with Class ASTM1 weights on a daily or as-used basis. At the beginning of each day that the balance is used, the analyst is required to perform at least one calibration check in the range of the material to be weighed. All calibration checks are documented in a laboratory log book. All weights used to calibrate balances on a daily basis are themselves calibrated at a minimum every 5 years against NIST traceable weights.

TABLE 9.5 - CRITERIA FOR BALANCE CALIBRATION CHECKS

4-POINT BALANCE

Class S / 1 Weight (g)	Warning Level (g)	Control Level (g)
0.0100	0.0099 - 0.0101	0.0098 - 0.0102
0.1000	0.0997 - 0.1003	0.0995 - 0.1005

1.0000	0.9995 - 1.0005	0.9990 - 1.0010
10.0000	9.9950 - 10.0050	9.9900 - 10.0100
100.0000	99.9500 - 100.0500	99.9000 - 100.1000

3-POINT BALANCE

Class S / 1 Weight (g)	Warning Level (g)	Control Level (g)
0.100	0.099 - 0.101	0.098 - 0.102
1.000	0.995 - 1.005	0.990 - 1.010
10.000	9.950 - 10.050	9.900 - 10.100
100.000	99.750 - 100.250	99.500 - 100.500

TOP LOADING BALANCES

Class S / 1 Weight (g)	Warning Level (g)	Control Level (g)
1.00	0.99 - 1.01	0.98 - 1.02
10.00	9.95 - 10.05	9.90 - 10.10
100.00	99.5 - 100.50	99.00 - 101.00
200.00	199.00 - 201.00	198.00 - 202.00

PIPETTES

All pipettes are calibrated monthly. Pipette performance is monitored by gravimetrically measuring the volume of DIW dispensed by each pipette over the range of its use with the assumption that the density of the water in the laboratory is 1.000 g/mL (± 0.003 g/mL). If the pipette does not meet criteria, it should be adjusted. After adjustment, the pipette check procedure should be performed. Both the pre-adjustment and post-adjustment measurements are maintained in a laboratory log book. Bias and precision are measured for all new pipettes prior to being put into service and then quarterly thereafter. Bias and precision are based on 10 measurements. The criteria are that the average measurement must be within ± 2% of the measured volume and the RSD of the measurements must be ≤ 1%.

OVENS, HOTPLATES, SAND BATHS, WATER BATHS, REFRIGERATORS, AND FREEZERS

Temperatures are checked with calibrated thermometers and necessary adjustments to the temperature settings are made as required. Refrigerators and freezers are checked on a daily basis and all ovens, hotplates, sand baths, and water baths are checked at least once during each use. Refrigerator and freezer temperatures are recorded on temperature chart recorders or in laboratory logs that are maintained by the Sample Control Group Lead. Oven, hotplate, sand bath, and water bath temperature are recorded in the sample preparation logs.

THERMOMETERS

The performance of each thermometer is compared annually to a certified NIST-grade thermometer and correction factors and the calibration due date are posted on each thermometer.

10.0 Preventative Maintenance

10.1 Routine Maintenance Measures

Refer to SOPs BAL-0020 and BAL-0600 (Preventative Maintenance of Support Equipment and Analytical Instruments) for greater detail of all required preventative maintenance.

TABLE 10.1 - PREVENTATIVE MAINTENANCE

Instrument	Activity	Frequency
ICP-MS	Inspect and/or change all tubing	Daily
	Clean sampler and skimmer cones	As needed
	Inspect torch and injector	Daily
	Check gas and coolant levels	Daily
	Change roughing pump oil	Monthly*
	Update mass calibration (tuning)	Monthly
	Replace quartz torch and injector	Semi-Annually*
	Replace sampler and skimmer cones	Semi-Annually*
	Replace RF coil	Semi-Annually*
	Replace chiller air filter	Annually*
	Replace ion lens	Annually*
	Schedule manufacturer maintenance	As needed
IC (for IEC)	Change eluents	Daily
	Inspect lines for leaks and obstructions	Daily
	Clean and test analytical column	Monthly*
CVAFS (Manual Total Hg)	Prepare new soda lime pre-traps	Daily
	Condition bubblers and blank traps	Daily
	Check all fittings	Daily
	Soak bubblers for 15 min. in 1% KOH and then over the weekend with 10% HCl	Weekly*
	Inspect and/or change all tubing	Monthly*
	Make, test, and change out traps	Quarterly*
	Change lamp	Semi-Annually*
	Blank traps on incoming gas lines	Semi-Annually*
	Clean/change quartz cell	Annually*
	Check Electronics	Annually

* or as needed
** Daily, prior to use, at the nominal volume for all DoD and DOE analyses

TABLE 10.1 - PREVENTATIVE MAINTENANCE (CONTINUED)

Instrument	Activity	Frequency
CVAFS	Prepare new soda lime pre-traps	Daily
(MERX-T)	Check all tubing and clear any liquid in tubing	Daily
	Inspect and/or change all tubing	Monthly*
	Change Traps	Semi-Annually*
	Change lamp	Semi-Annually*
	Change traps on incoming gas lines	Semi-Annually*
	Clean/change quartz cell	Annually*
CVAFS	Inspect and/or change all tubing	Monthly*
(MERX-M MeHg)	Condition GC column at 80 ºC overnight	Quarterly*
	Change lamp	Semi-Annually*
	Check and change out traps	Semi-Annually*
	Blank traps on incoming gas lines	Semi-Annually*
	Clean/change quartz cell and replace GC column	Annually*
AA - Flame	Check tubing, pump and lamps	Daily
(As Speciation)	Rinse water removal trap with DIW	Daily*
	Clean spectrophotometer windows	Weekly
	Soak bubblers over weekend in 30% nitric acid	Monthly*
	Inspect and/or change all tubing	Monthly*
	Clean nebulizer	Semi-Annually
	Fine tune the instrument wavelength	Annually*
	Check instrument optics	Annually*
Colorimetric /	Clean sample compartment	Daily
Spectrophotometer	Windows cleaned	Monthly
	Check Electronics and lamp alignment	Annually
pH Meter	Clean; 2 pt. Calibration	After each use
Balances	Clean pans and compartment	Before/after every use
	3 to 4 pt. Calibration check	Before every use
	Certified Calibration	Annually
Pipettes	Check calibration	Monthly**
	Check bias and precision	Quarterly*
Conductivity Meter	Check batteries and probe cables	Weekly*
Refrigerator /	Check temperature	Daily
Freezers	Clean interior	Monthly*
	Check temp. against NIST cert. thermometer	Annually

10.1.1 Air Testing

The mercury lab, the sample preparation labs, the sample receiving laboratory, and bottle washing are monitored monthly for atmospheric mercury levels to ensure that these levels are sufficiently low for ultra-trace level mercury analysis. Air from each lab is pumped through a soda lime pre-trap and onto gold-coated substrate trap at a flow rate of 1 L/min until at least 20 L of air have been collected per trap. A warning level has been established at 15 ng Hg/m^3 with a shutdown control level at 25 ng Hg/m^3. Results from the monthly air tests are saved electronically on the server.

10.1.2 Water Testing

Reagent water is monitored for Hg on a daily basis when calibration blanks are analyzed. A minimum of four 25 mL aliquot of fresh reagent water, each with 0.2 mL $NH_2OH \cdot HCl$ and 0.2 mL of $SnCl_2$, are analyzed at the beginning of the run sequence. The average results must be < 5 pg Hg with a standard deviation < 2.5 pg Hg. A high level of mercury detected in the reagent water analysis may also be attributed to the MERX system itself, the reagents, or the soda lime pre-traps. Regardless of the source, all analysis is stopped until the source of contamination is determined and the problem is corrected. The results are stored with each batch.

Reagent water is tested for trace metals by ICP-MS at a minimum of once per month when instrument water blanks collected from every sink used to clean equipment, prepare reagents/samples, or analyze samples are analyzed. Specific elements are tested for with each batch. Currently, water blanks must be less than the element specific MRL or client specific requirements. Results for water testing are stored on the server in Excel© spreadsheets.

10.1.3 Equipment and Reagent Testing

All reagents (acids, standards, etc.) and equipment (bottles, vials, etc.) are tested prior to use. The acceptance criteria for specific reagents and equipment are specified in the individual SOPs describing the use of the reagents or the decontamination of equipment. In all cases, contract specified requirements take precedence over BAL acceptance criteria.

10.2 Documentation

Instrument logbooks are maintained for all equipment. These logbooks contain a complete history of past performance and maintenance. Analytical instrument logbooks document instrument usage, routine maintenance, and non-routine repairs.

10.3 Contingency Plans

10.3.1 Major Equipment Failure

For major equipment failure of CVAFS instruments, the laboratory has backup instrumentation. BAL's sister company, Brooks Rand Instruments, is an instrument manufacturer specializing in ultra-trace level mercury analyzers; therefore, a stock of replacement parts and complete analyzers exist and expert service personnel are readily available.

For flame AA's, rental equipment is locally available in the case of a major equipment failure while instrumentation is being repaired.

For the ICP-MS, BAL has nine instruments that can be used for total metals and speciation analysis giving the lab plenty of redundancy.

BAL currently has an excess of balances and refrigerators/freezers. If any of this equipment fails backup equipment is immediately available. Other equipment such as the conductivity meter and the pH meter are relatively inexpensive and will be purchased immediately if major equipment failure is determined.

10.3.2 Loss of Power

BAL has a backup generator to reduce the risk of loss of power to critical instrumentation and key support equipment. However, if there is a total or partial loss of power to the lab, then the procedures outlined in Appendix C of the Chemical Hygiene Plan must be followed.

10.3.3 Invalidation of work

Results for all sample analyses affected by equipment failure may be ruled invalid depending upon the circumstances. When QC criteria are not met during analysis, all instrumentation is thoroughly checked and appropriate maintenance action is taken. Subsequent reanalysis of the affected samples is then initiated after the instrumentation is proven to be functioning properly. The Technical Director, BAL President/CEO, VP of Quality, and the VP of Operations have the authority to stop work whenever there is evidence of non-conforming work. Once work is stopped, corrective action must take place and be documented. Permission to restart work must be granted by the Technical Director, BAL President/CEO, VP of Quality, or the VP of Operations.

11.0 Quality Control Checks and Routines to Assess Precision and Accuracy and the Calculation of Method Detection Limits

The laboratory uses quality control samples to assist in assessing the validity of the analytical results of field samples. The use of quality control samples helps to assess analytical accuracy and precision in the laboratory. Quality control samples are analyzed in the same manner as field samples at a frequency described either in the individual procedures or in the contract with the client. If the quality control sample results fall within acceptable criteria (also detailed in the method), then the field sample data are considered to be valid or acceptable as is. However, it is important to keep in mind that errors made during sample collection can seriously affect the analytical results of field samples. In other words, the quality or validity of the field sample data is only partially supported by the laboratory quality control sample results. Field quality control samples are the other necessary component for the validity of field sample results.

Laboratory quality control (QC) samples include method blanks, calibration checks, replicates, spiked samples, and standard or certified reference materials (SRM/CRM). The specific frequency and type of QC samples analyzed are described in the individual analytical method, SOP, or client-specific Statement of Work (SOW). In some cases, contracts may specify additional or more stringent QC requirements beyond what the method requires. In these cases the contract specific QC requirements are followed. In addition to these routine QC samples, performance evaluation samples required for certification are analyzed semi-annually.

11.1 Quality Control Checks

11.1.1 Field QC Checks

Brooks Applied Labs is rarely involved in field sampling. The client is responsible for field sampling activities and therefore mandates the requirements for field QC checks. However, BAL suggests that the following field QC be collected.

11.1.1.1 Trip Blanks

Trip blanks are used to demonstrate that sampling equipment and collected samples have not been contaminated during transit. Trip blanks consist of laboratory reagent water collected into a sampling container at the laboratory. The trip blank is then double bagged (as per sampling containers for use in the field) and affixed with a custody seal to indicate if it has been tampered with. The trip blank is then shipped with the sampling kit to and from the field. The trip blank must not be opened again until it has returned to the laboratory.

When collected and analyzed, the level of the analyte of interest in the trip blank should be less than the reporting limits or less than 10% of any affected sample results. If criteria are not met, then the client must be notified and every effort should be made to determine the source of the contamination and to eliminate it if possible.

11.1.1.2 Field Blanks

Field blanks are used to demonstrate that the samples were not contaminated during the collection procedure or while in transit (Note: The analysis of trip blanks in conjunction with field blanks can better pinpoint the source of contamination). Field blanks are collected in the field, typically using lab-supplied reagent water, and simulating the collection of actual samples as well as can be done. Once collected, the field blank is treated in every way as an actual sample.

When collected and analyzed, the level of the analyte of interest in the field blank should be less than the reporting limits or less than 10% of any affected sample results. If criteria are not met, then the client must be notified and every effort should be made to determine the source of the contamination and to eliminate it if possible.

Many methods require that field blanks be collected and analyzed if results are to be reported for regulatory purposes. While BAL does not require that clients provide field blanks for analysis, BAL does inform clients of this regulatory requirement in the quote signed by the client prior to any work performed as well as in any case narrative that includes relevant results.

11.1.1.3 Field Duplicates

Field duplicates are used to assess precision in the collection procedures. When collected, the field duplicate relative percent difference (RPD) should be no greater than that allowed for method duplicates by the specific analytical method, the SOP, or the SOW. If the RPD is greater than the acceptance criterion, then the sampling team should be notified. When analyzed in conjunction with method duplicates (Section 11.1.2.7), field duplicates will aid in determining the source of any imprecision.

11.1.2 Lab QC Checks

Laboratory QC samples are analyzed with every batch and sequence to validate method performance. The number of QC checks analyzed will depend on batch size and the number of analyses associated with the sequence. Batches at BAL may contain more than 20 client samples as long as QC frequency requirements are met. For instance, most EPA methods only require one method blank per batch of 20 samples. BAL routinely prepares four method blanks; therefore, a batch could contain up to 80 samples as long as SRM, BS, DUP, and MS/MSD frequency requirements were also met.

11.1.2.1 Method Blanks

A method blank is a sample of reagents or reagent water treated as a sample such that it is prepared in conjunction with and undergoes the same analytical processes (i.e. same reagents added at time of sample preparation, digested in the same type container (if available) at the same temperatures/times, etc.) as the corresponding field samples. Method blanks are used to monitor laboratory performance and contamination introduced during sample preparation and analysis. The method blank acceptance criteria are method specific (Refer to the specific analytical method, the SOP, or the contractual requirements).

In cases where a sufficient number of method blanks (minimum of four) have been prepared and analyzed with the batch to characterize the nature of the blanks and the potential for any reagent or spot contamination, one blank may be rejected as a Grubb's Outlier if it meets the criteria for doing so at the 5% or less risk of false rejection level (refer to Section 11.2.3 for further discussion on how the Grubb's Test for Outliers is applied to data). If a method blank is rejected as a Grubb's Outlier, then its value is not used to calculate the mean or the standard deviation of the method blanks used to blank-correct the batch data or calculate any batch specific reporting limits. However, the data should still be evaluated against the method blank considered to be a Grubb's Outlier. If spot contamination is suspected, then any data point not ≥ 10 times the rejected method blank would require reanalysis or appropriate qualification.

There are some circumstances that do allow for the removal of a method blank from the batch upload, but only when it can be shown that the removed method blank does not impact the data quality. Examples include a misinjected method blank or a method blank that is elevated due to carry over from a previously analyzed high-level sample. For blank corrected data, the Grubb's Outlier method blank must be removed from the LIMS upload in order for the sample results to be appropriately corrected.

The discarding of any data point as a Grubb's Outlier and the potential effect on overall data quality must be narrated to the client. Current LIMS limitations do not allow method blanks rejected as Grubb's Outliers to appear on the "Method Blanks & Reporting Limits" page of the report. Therefore, the value of any rejected method blank must be reported in the case narrative section of the data report. Grubb's Outliers may never be discarded for non-method blank corrected data except for reasons noted above. Refer to Section 12.6 (Data Reporting) for specific instructions on how method blanks are evaluated and reported for uncorrected results.

11.1.2.2 Matrix Spikes

Matrix spikes are routinely included in the analytical batch as they are required for most methods utilized at BAL. Method-specific or client-specific frequency and recovery requirements are variable and available in the method, the SOP, or the SOW, whichever is applicable. Matrix spikes are typically analyzed at a frequency of one per every ten client samples. Although not a requirement, if a batch contains samples of different submatrices, matrix spikes should be prepared and analyzed for each submatrix type to ensure that there is no matrix-specific interference. It is up to the client to request additional matrix spikes on their samples if they suspect matrix issues. The native sample should always be run at the same dilution as the spikes to check for suppression, but may be reported from a different dilution if necessary to obtain results that are above the MRL. At any time, a post-spike sample may be prepared and analyzed at the instrument. The recovery criteria for analytical spikes is typically equivalent to the recovery criteria for the associated CCVs.

11.1.2.3 Blank Spikes

If reference materials are unavailable, it is BAL policy to prepare a Blank Spike (BS) at a frequency of at least 5% per batch of samples. The BS is typically spiked at approximately

10 – 20 times the MRL with default acceptance criteria of 75-125% if an appropriate SRM.

11.1.2.4 Performance Evaluation (PE) Samples

Performance Evaluation samples are analyzed as blind samples and are analyzed at a minimum of semi-annually. BAL purchases PE samples from Environmental Resource Associates (ERA). All PE studies utilize samples that are blind not only to the analyst but also the entire laboratory staff until after the results have been submitted to the appropriate agency and the final report for the study is issued. ERA PE results are forwarded directly to BAL and all appropriate accrediting bodies.

Additionally, BAL routinely participates in laboratory intercomparison studies offered by such institutes as MAPEP (U.S. Department of Energy's Mixed Analyte Performance Evaluation Program), the International Atomic Energy Agency (IAEA), the United States Geological Survey (USGS), etc. Laboratory intercomparison studies such as these allow BAL the opportunity to evaluate our performance on more non-traditional matrices not typically available from PE providers.

11.1.2.5 Calibration Verification

Independent Calibration Verification (ICV) standards are standards that are from a different source than the working standards. The ICV is analyzed once immediately following the calibration. Verification standards made directly from the working standards are also used throughout the analysis to check the continuing accuracy of the calibration. They are often referred to as Continuing Calibration Verification (CCV) standards. For most methods utilized at BAL, the CCV samples must be analyzed at the beginning and the end of an analytical batch after every 10 client samples throughout the analysis. For most analyses, Continuing Check Blanks (CCB) are analyzed after each CCV sample to ensure that there is no carry-over of analyte to the field sample analysis. Additional requirements may be specified in the specific analytical method, SOP, or contractual requirements.

11.1.2.6 Quality Control Samples

Quality control (QC) samples are additional QC checks for evaluating the accuracy of the analysis. These samples may be prepared by BAL (as with Blank Spikes) or purchased from an outside source (as with SRMs) depending upon their availability. Frequency and recovery criteria for QC samples are method specific. Refer to the specific analytical method, the SOP, or the SOW for specific frequency and recovery requirements.

11.1.2.7 Duplicates (Method Duplicates or Matrix Spike Duplicates)

Duplicate samples and/or matrix spike duplicates must be analyzed at a minimum frequency of 10% per analytical batch and should be performed on each matrix in the batch for all analytical methods employed at BAL. Refer to the specific analytical method, the SOP, or the SOW for specific frequency and precision requirements.

11.1.2.8 Reagents and Standards Purity Checks

All reagents used in the preservation, preparation or analysis of samples must be checked for the appropriate parameters prior to use.

Likewise, all standards (except those made daily) are tested against previously tested, non-expired standards prior to use to ensure that they are acceptable for use as calibration, calibration verification, or spiking standards.

11.2 Routine Methods Used to Assess Precision and Accuracy

11.2.1 Accuracy and Precision

11.2.1.1 Precision

Precision from two replicates is expressed as % Relative Percent Difference (% RPD). Precision from more than two replicates is expressed as % Relative Standard Deviation (or % RSD) and shall be calculated from the following formulae:

$$RPD = \left(\frac{|a - b|}{\bar{x}} \right) \times 100$$

Where: a = result a from native sample, or for matrix spike samples, result from the matrix spike (native + spike concentration) sample
b = result b from native sample duplicate, or for matrix spike samples, result from the matrix spike duplicate (native + spike duplicate concentration) sample
$\bar{x}$ = Mean (average) of the two results

$$\%RSD = \left(\frac{s}{\bar{x}} \right) \times 100$$

Where: $\bar{x}$ = Mean (average) of the data points
s = Standard deviation calculated as:

$$s = \sqrt{\frac{\sum_{i=1}^{n} \left(x_i - \bar{x} \right)^2}{n - 1}}$$

Where: x_i = the individual data point for each n
n = the total number of data points

11.2.1.2 Accuracy from Spiked Samples

The accuracy of a measurement shall be determined by the recovery of a known amount of analyte in a real sample as:

$$\%\,R = \left(\frac{Cs - Cu}{S} \right) \times 100$$

Where: C_s = concentration of spiked sample
C_u = concentration in non-spiked sample (can be 0 for results < MDL)
S = expected concentration (spiking level)
%R = percent recovery

11.2.1.3 Accuracy from Known Concentrations
The accuracy of a measurement based on known concentrations shall be calculated as:

$$\%\,R = \left(\frac{\text{Sample concentration}}{\text{Reported True Value}}\right) \times 100$$

11.2.1.4 Upper and Lower Warning and Control Limits for Acceptance Criteria
When not method based, Upper and Lower Warning Limits (WL) and Control Limits (CL) for determining acceptance criteria shall be calculated as follows:

$$CL = P_{av} \pm 3s$$

where: CL = Control Limit (upper and/or lower)
P_{av} = Mean of P (percent recovery or RPD)
s = standard deviation of the mean of P

and

$$WL = P_{av} \pm 2s$$

where: WL = Warning Limit (upper and/or lower)

11.2.2 Quality Control Charts

Quality Control charts are used to determine acceptance criteria for in-house developed methods and review the relevance of QA criteria parameters used in each analytical method. Separate quality control charts should be established for each analytical method, for each parameter or analyte, and for each matrix type, both for precision and for accuracy. Control charts are automatically updated for all test codes in the LIMS as data is uploaded.

Control charts for SRMs or blank spikes are reviewed quarterly. However, control charts can be constructed and used to monitor laboratory and method performance for other several other parameters such as spike recoveries, duplicate analyses, calibration verification standard recoveries, and blank analyses. Control charts use both the mean and standard deviation in order to identify out-of-control events as per Standard Methods, 21st Edition, Section 1020 B.

The LIMS automatically generates control charts, where the mean and the standard deviation, warning limits, and control limits are automatically calculated and updated. Control charts can be determined by method, who prepared the samples, who analyzed the samples, which instrument was used to analyze the samples, and over what dates the samples were analyzed.

The individual data points are plotted against the mean and the ± 2 (warning limit), ± 3 (control limit), and ± 4 standard deviations.

Refer to SOP BAL-1003 for further discussion of the use of control charts for identifying systematic errors.

11.2.3 Grubb's Outlier

An outlier is an extreme value, high or low, that has questionable validity as a member of the measurement set with which it is associated. Outliers are **not** used in assembling the quality control charts for purposes of setting acceptance limits or determining reporting limits. Outliers may be rejected from the data set for the following reasons:

- A known experimental aberration occurred, such as instrument failure or inconsistency in the procedure or technique

- The T value for the data is larger than the tabulated values using the Grubb's test for outliers (Table 12.1). Outliers at BAL are determined with a 95% confidence level (or 5% risk of false rejection). The T value is calculated using the following equation:

$$T = \frac{|X_0 - \overline{X}|}{SD}$$

where: X_0 is the extreme value being measured
$\overline{X}$ is the mean of the measurement set for *n* observations including X_0
SD is the standard deviation associated with X including X_0

If a value is rejected, the mean and standard deviation are recalculated using the remaining data. This procedure can be reiterated using the next extreme value until no outliers remain.

TABLE 11.2 - GRUBB'S TEST FOR OUTLIERS

Number of	Risk of False Rejection				
Data Points	0.1%	0.5%	1%	**5%**	10%
3	1.155	1.155	1.155	**1.153**	1.148
4	1.496	1.496	1.492	**1.463**	1.425
5	1.780	1.764	1.749	**1.672**	1.602
6	2.011	1.973	1.944	**1.822**	1.729
7	2.201	2.139	2.097	**1.938**	1.828
8	2.358	2.274	2.221	**2.032**	1.909
9	2.492	2.387	2.323	**2.110**	1.977
10	2.606	2.482	2.410	**2.176**	2.036
15	2.997	2.806	2.705	**2.409**	2.247
20	3.230	3.001	2.884	**2.557**	2.385

25	3.389	3.135	3.009	**2.663**	2.486
50	3.789	3.483	3.336	**2.956**	2.768
100	4.084	3.754	3.600	**3.207**	3.017

Tabulated values obtained from Quality Assurance of Chemical Measurements by John Keenan Taylor, 1987.

11.3 Method Detection Limits and Reporting Limits

11.3.1 Method Detection Limits for Routine Environmental Analyses

Note: The procedure for the acceptable determination of a method detection limit (MDL) is currently being reviewed and revised by the EPA. As new requirements are accepted by the EPA, they may be incorporated by BAL.

The MDL is the minimum concentration of an analyte of interest that can be measured and reported with 95 percent confidence that the value is above zero. MDLs for accredited methods are determined by replicate analysis of a sample or BS that is one to five times the estimated detection limit for the analyte of concern or up to 10 times the level for multi-analyte tests. Sample aliquots may be used from a native sample or a representative matrix that has sufficient analyte (present or spiked) to make the concentration one to five times the estimated MDL. If a sample low enough in the analyte of interest is not available, then blank spikes may be spiked at the appropriate level for the MDL study. A minimum of seven aliquots (BAL routinely prepares and analyzes eight MDL aliquots) must be analyzed for the determination of the MDL. As long as seven aliquots remain, one MDL aliquot may be discarded, but only if there is a defensible reason for doing so (e.g. the auto sampler did not sample the cup, sample was not spiked, etc.). The reason for the abnormal result must be known and it must be shown that no other results could have been affected. The MDL is then calculated as the standard deviation of the replicate analysis multiplied by the "student's t" value for the number of replicates analyzed (3.143 for seven replicates; 2.998 for eight replicates). When evaluating a MDL, the aliquots must be carried through the entire method as per client samples. If, for a particular method of analysis, the concentration in the aliquots is below the MDL, then they cannot be used to calculate the MDL. In such a case, the MDL study is repeated with appropriately spiked MDL aliquots or the MDL could be lowered if acceptance criteria is met. More specific information on the MDL procedure and calculation are found in the EPA in "Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11", 40 CFR 136, Appendix B.

MDL studies are performed annually for accredited aqueous and solids methods. More frequent MDL studies may be required by accrediting bodies or at the discretion of BAL management. Additionally, the MDL must be verified any time major changes in a procedure are made. Major changes include changes in instrumentation, procedural changes to either the preparation or the analysis of samples, etc. The MDL study is intended to represent the capabilities of BAL and; therefore, should only be performed by experienced analysts.

As part of the annual MDL study for accredited analytes, a Limit of Detection (LOD) validation check must be performed either during or immediately following the study (within one week and prior to analyzing any samples using the new MDL) on all instruments that might be used for the analysis. The LOD validation check consists of a BS sample spiked at 2

– 3 times the determined MDL for single analyte tests and 1 – 4 times the MDL for multi-analyte tests. The response produced during the analysis of the LOD validation check must be greater than 3 times above the instrument's noise level to be acceptable. If the LOD validation analysis fails to meet the acceptance criterion, then additional LOD validation checks must be performed at a higher level to set a higher MDL or the MDL study must be re-conducted. For some accreditations, such as DoD and DOE, the MDL verification check must be analyzed quarterly on each instrument used to analyze samples for each analyte for which DoD or DOE accreditation is maintained. As a rule, the MDL verification is prepared and analyzed at levels of 1, 2, and 3 times the MDL when first establishing the MDL/LOD and then at 2 to 3 times the MDL quarterly. It is BAL policy to always set the LOD at a level of at least 2 times the MDL and no more than 3 (single analyte) or 4 (multi-analyte) times the MDL.

BAL currently defines the different units for the MDL validation test as such.

- **Background Noise**: The average response of the method blanks during a single analytical run (sequence).
- **Instrument Noise**: The variation of the individual method blanks around the background noise during the sequence. To be as conservative as possible, this is measured for any single sequence as the absolute difference between the response of the lowest and the highest method blanks.
- **MDL Verification Response**: This is measured as the instrument response for the analysis of the MDL verification corrected for the background noise.

The method reporting limit (MRL) is based on the level of the low standard used in the instrument calibration and the volumes/weights used in the analysis of samples. The MRL cannot be less than the MDL and is typically 3 to 10 times the MDL, but can be as low as 2 times the MDL. The Limit of Quantitation (LOQ) is the concentration of an analyte of interest where the relative confidence in the measured value is ± 30% at the 95% confidence level. LOQs are estimated as 9 to 12 times the standard deviation from the MDL determination. In general, BAL uses the terms MRL and LOQ interchangeably in reporting.

The validity of the LOQ/MRL is confirmed annually (quarterly for DoD/DOE) for each instrument used with the analysis of four laboratory fortified blank samples spiked at 1 – 2 times the level of the LOQ/MRL. Recovery of the LOQ/MRL BS is judged against the established method acceptance criteria or client data quality objectives, whichever are more stringent, for precision and accuracy.

The instrument detection limit (IDL) is determined at initial set-up of the instrument and after any significant change (such as change in equipment or reagents used, carrier gases, gas pressures, etc.). Additionally, an IDL study may be used as part of an analyst's demonstration of capability as long as their ability to prepare samples is not being evaluated. A minimum of seven analytical spikes prepared at the level of the low calibration standard are analyzed and the IDL is then calculated as the standard deviation of the replicate analysis multiplied by the Student t value ($t_{.99}$) for the number of replicates analyzed (3.143 for seven replicates; 2.998 for eight replicates). The calculated IDL must be less than the MDL for all methods analyzed on the instrument. Additionally, a minimum of four analytical spikes prepared at

approximately 10 times the MRL are analyzed. The recovery and standard deviation of these QCS samples must meet sample specific requirements of the method being performed.

All MDL and IDL studies are documented and maintained by the Quality Assurance Group. Documentation includes the date of the study, the name of the analyst conducting the study, the analytical method(s), the analyte of interest, preparation notes, and all raw data from analysis.

11.3.2 MDLs and MRLs for Non-Routine Environmental Analyses, Food Testing, and R&D

For non-routine environmental analyses (anything that does not fall under TNI or DoD/DOE scopes of accreditation) that are not being used for regulatory compliance purposes, there is more flexibility in how the MDL and MRL are determined.

For methods where an MDL study does not exist or for which BAL is not accredited, the MDL can be determined on a batch specific basis. MDLs may be estimated from the standard deviation of the method blanks. If done, the MDL is estimated as 3 times the standard deviation of the method blanks for method blank corrected data and as the average of the method blanks + 3 times the standard deviation for uncorrected data. For methods where method blanks typically yield a result of 0 at the instrument, the 3 times the standard deviation of replicate analyses of the low calibration standard are used to estimate the MDL. The MRL is set at the lowest calibration standard or at a minimum of 2 times the estimated MDL, whichever is greater. Under no circumstances may the reported MDL be less than 10% the level of the MRL.

For food testing, the MDL can be determined as described in section 11.3.1, the AOAC Method 2015.01, or in a similar fashion, as long as the procedure for determining the MDL is documented.

MDLs can also be calculated using historic data for method blanks. Data for at least 20 method blanks should be available in the LIMS control charts before calculating an MDL by this procedure. For uncorrected data, the MDL is determined as the average of the method blanks plus the standard deviation of the method blanks multiplied by the Students' T value for the number of replicates at the 99% confidence interval. For method blank corrected data, the MDL would just be the standard deviation of the method blanks multiplied by the Students' T value for the number of replicates at the 99% confidence interval. As before, the MDL may not be less than 10% the level of the usable MRL for the method.

11.4 Initial and Continuing Demonstration of Capability

11.4.1 Initial Demonstration of Capability (IDOC)

Every analyst must perform an IDOC study prior to independently analyzing or preparing samples by each method. For sample preparation, the IDOC is the preparation of an MDL study. For analysis, an MDL study can be part of an IDOC, but is not required since it will also be affected by the preparation of the samples and isn't fully indicative of the analyst's ability to properly analyze sample preparations. If an MDL is not part of the IDOC, then an

IDL study is performed where a minimum of seven analytical spikes prepared at the level of the low calibration standard by the analyst at the instrument must be analyzed. These samples are analyzed as per an MDL study and the resulting detection limit must be less than the MDL for the analytical method. In addition, a minimum of four QCS (typically 4 replicates of the ICV) samples are prepared as per the specific analytical method requirements and analyzed. The average recovery and the RSD yielded by the analysis of the QCS samples must meet the specific requirements of the analytical method being performed. Specific requirements for a passing IDOC are outlined in BAL SOP BAL-1009.

11.4.2 Continuing Demonstration of Capability (CDOC)

In addition to performing an IDOC prior to performing analysis, each analyst must demonstrate that s/he is continuously capable of performing the analysis. This capability is judged annually by reviewing control charts for the methods performed by the analyst, looking at four consecutive batches performed by the analyst to ensure that all batch specific QA was met, or by having the analyst perform another IDOC or MDL study to demonstrate that the analyst is still capable of obtaining accurate and precise results. The full analyte list need not be reviewed for multi-analyte analyses to show continuing capability. At a minimum, 6 analytes will be reviewed. Specific requirements for a passing CDOC are outlined in BAL SOP BAL-1009. If the analyst has not demonstrated continuing capability for a method, then the VP of Operations will determine if additional training is required and the analyst must successfully perform an IDOC prior to analyzing any further client samples.

11.4.3 Documentation

All raw data, including preparation logs, analytical bench sheets, and instrument printouts, used to perform the IDOC or CDOC study are scanned and attached to the relevant sequence in the LIMS and saved for no less than seven years. All IDOC and CDOC studies must be reviewed by the QA Department and the VP of Quality must annually certify that each analyst is capable of performing their respective duties by completing a Demonstration of Capability Certification Statement form for each method an analyst performs. The VP of Quality's signature (or Technical Director's signature in the absence of the VP of Quality) and date on the Demonstration of Capability Certification Statement form indicates authorization by management for the specified person to perform the indicated laboratory procedure. These forms are kept in the employee training records.

11.5 General QC Requirement Statement

The QC requirements previously listed are general requirements only. Specific methods or client-specific Statements of Work may have more stringent requirements that take precedence.

12.0 Data Reduction, Validation, Reporting and Storage

Prior to release of analytical results, all unknown sample and associated quality control data are subjected to the full review process briefly described below. Refer to BAL SOPs BAL-1500, BAL-1501, and BAL-1502 for a detailed description of the data review procedure.

12.1 Analytical Integration

Analytical instrumentation signal output is integrated by manufacturer specific software (i.e., Guru™, MassHunter, Perkin Elmer ELAN, or Total Chrome software). Analytical runs are stored electronically. Integration software is verified by the QA samples. Any integration software related problem that affects samples would also affect QA samples; therefore, as long as QA criteria are met, the software is assumed to be operating properly. The IT department at BAL maintains all documentation of integration software upgrades.

12.2 Data Entry

The preparation technician or analyst is responsible for entering all sample masses/volumes and preparation volumes into the bench sheet in the LIMS, as well as any batch specific QC information. The analyst is responsible for checking this information, entering all analytical specific information into the instrument software, and uploading all of the instrument results into the LIMS. The analyst ensures that all data is present. All final results are automatically calculated in the LIMS using formulas specific to the analytical method used.

12.3 Data Reduction

The analyst or other data uploader is responsible for uploading all instrument data into the LIMS and performing primary validation of the data. Initial data reduction is performed by the instrument software to obtain initial results in units of measured pg, measured ng, or µg/L. This information, along with volumes/masses used in the preparation/analysis of the samples is either uploaded or hand entered into the LIMS where final results are calculated according to the method used to analyze the samples. The following documentation must be present with every data package: preparation notes, SPFs, lab bench sheets, Analysis bench sheet, and analyst's notes. All instrument printouts are stored electronically as PDF files and must have the analytical sequence recorded on them and the sample ID for each instrument response.

12.4 Primary Data Review

After the data has been acquired and any necessary calculations performed, the primary data review is performed by the analyst. Items to be reviewed include correct upload of the data, sample identity, instrument calibration, QC samples, detection limits, numerical computations, accuracy of transcriptions, sample preparation logs, instrument/analytical logs, and compliance with the individual method.

12.5 Final Data Review and Validation

Following the analyst's review, the raw data and calculations undergo final review by the QA Group. The QA Group also reviews comments about analytical conditions as well as any interpretations made by the analyst. Additionally, the QA Group examines the QC sample data and ensures that the analytical results meet or exceed the acceptance criteria for frequency, accuracy, and precision.

After final data review and validation is complete, the QA Group applies any necessary data qualifiers, sets data to be reported to "reportable," and signs-off on the sample processing form. Only the Technical Director, VP of Quality or delegate and, in extreme cases, the VP of Operations or BAL President/CEO have authority to change the reportability of data after final data review. All changes in the status of data (e.g. batched, prepared, reviewed – primary, reviewed – final, reportable versus non-reportable, etc.) is tracked and updated automatically in the LIMS with a time stamp and identity of the person that made the change.

12.6 Data Reporting

Prior to data reporting, the Project Manager responsible for the report reviews that data a final time for any discrepancies. The final client report is generated only when the Project Manager is satisfied that the data is valid and all project specific requirements have been met. Any Level IV report or reports where issues require atypical narration goes through secondary review by another Project Manager or Project Coordinator who then signs-off on the report as well. Only then is the report sent to the client.

Typically at BAL, results are reported down to the MDL. Results ≤ the MDL are reported at the MDL and qualified "U" as non-detectable. Results at or below the MRL but above the MDL are reported as the calculated result and qualified "B" as an estimate. Results above the MRL are reported as the calculated result without qualification. For Department of Defense (DoD) work, results are either reported only down to the LOQ or by the rules defined in the DoD/DOE QSM. All sample results are reported to three significant figures except for percent total solids results and results for QC samples, which are both reported up to four significant figures. Sediment and soil results are typically reported on a dry-weight basis by dividing the wet weight result by the percent total solids result. Biota results are typically reported on a wet-weight basis. However, upon request, biota results may be reported on a dry-weight basis as well.

Any sample that yields a non-detectable result and shows <30% recovery of the matrix spike cannot be reported unqualified. The sample is qualified "R" to indicate that all generated results for the sample are unusable and no result for the sample is reported. Refer to section 11.3 for differences in how the limits of detection are defined between work performed under the TNI standard and work performed under the DoD/DOE QSM and how this affects how results are reported by BAL.

If contamination is suspected due to elevated method blanks, then results may require special qualification. The criterion for acceptable elevated method blanks is that the value of the highest method blank concentration for any detectable (> MDL) method blank must be ≤ 10% of any

quantifiable (> LOQ) result. Any quantifiable result not ≥ 10 times the absolute value of the highest detectable method blank concentration is qualified "X" and narrated as being an estimate due to elevated method blanks with a high bias. Results below the LOQ will already be qualified as estimates and do not require additional qualification due to the elevated method blanks.

Generally, data are reported in a format generated by the BAL LIMS with a case narrative or a cover letter attached. All of the data, including standard spike recoveries, control samples, duplicate analyses, and results from blank analyses, are reported along with the sample results. Data quality issues are addressed in the cover letter or case narrative, which discusses each batch and sequence associated with the work order being reported. Final reports are submitted to all required parties (project dependent). A copy of each report is stored electronically as a PDF file for BAL's internal records (see 12.7 data storage). All laboratory report forms and reporting formats shall be in compliance with the reporting requirements of the applicable project for which they are generated. A specific statement clearly identifying any results that do not meet the specific project requirements (i.e. non-NELAP accredited or non-DoD accredited work performed by BAL) is included, if applicable, in the final report.

Every reasonable effort is made to report data with acceptable associated quality assurance sample (QC) results. However, barring this, data is qualified appropriately to indicate when batch QC does not meet specific acceptance criteria. A list of all data qualifiers and their definitions is included with every data report. While BAL has its own in-house data qualifiers that are defined on the "Report Information" page of the report, the use of project or accreditation specific qualifiers always take precedence over BAL qualifiers. In such instances, the project specific qualifier definitions would over-ride the BAL qualifier definitions on the "Reporting Information" page of the final report and discrepancies would be narrated.

Electronic files may be transferred to a client via electronic data deliverable (EDD) or by email with the following statement:

CONFIDENTIAL

This electronic message transmission (including any attachments) is intended only for use by the addressee(s) named herein; it contains legally privileged and confidential information. If you are not the intended recipient, you are hereby notified that any dissemination, distribution, printing, or copying is strictly prohibited. If you have received this e-mail in error, please notify the sender and permanently delete any copies thereof.

Electronic signatures as pictures of actual signatures are used for signing many legal documents, including PDF copies of data reports, at BAL. The original JPEG or TIF is maintained by the user and only the user and the IT Manager have access to the original copy. Use of another person's signature file without the express permission of the signatures originator is grounds for disciplinary action.

Disclosing information about client results or contracts to any party outside of BAL without prior permission from the client and BAL and without following all reporting policies stated in the BAL Comprehensive Quality Assurance Plan and associated standard operating procedures is forbidden by all personnel. The term "reporting" refers to any electronic, written, or spoken discussion of client data or other confidential and proprietary information. To protect the client's

proprietary rights, data must never be reported over the phone. Additionally, data can only be reported directly to the client with whom BAL has a legal contract to perform work, unless BAL has written permission from the client to release the data or report to a third party.

12.7 Data Storage

For all data generated by BAL, data packets and electronic summaries are kept for a minimum of 7 years, longer if contract specific requirements call for longer storage. All computer files are stored both on computer hard drive and on backup disks. Computer files of client reports are organized by sample tracking number, batch spreadsheets are organized by batch number, and all project information is organized by project numbers. All sequence specific data is also scanned and stored electronically, this includes: SPFs, analyst notes, handwritten benchsheets, and Excel® results spreadsheets. The original hardcopy data packet is stored for at least 6 months in the file cabinets in the main office before being shredded.

The Project Manager is responsible for maintaining all client specific files. All client reports are stored electronically as a PDF. These reports contain copies of the original SRL, as well as any information provided by the client including chain-of-custody forms, analysis request forms, airbills (full reports only), etc.

All MDL study documentation and other QA documentation are scanned and attached to the relevant sequence in the LIMS. Hard copies of the original data are filed by the QA Department by date and stored for at least one year before being shredded. Scanned data is kept for a minimum of 7 years.

All data generated is scanned and stored electronically for a minimum of 7 years. After five years, electronic data may be removed from the server, but is backed up to two separate external hard drives that are stored at separate, secure locations. Any paper work that has not been scanned for any reason will be stored for a minimum of 7 years. All hard copies of any documents that could be traced directly to a client are destroyed by shredding prior to disposal. All employees at BAL are authorized to access electronic information. No levels of accessibility for employees exist. The IT Manager monitors the upkeep of computer files.

13.0 Document Control Policies

13.1 SOPs, Manuals, Handbooks, and Plans

All documents important to the internal operations of BAL go through formal procedures as to their writing, approval, implementation, retirement, and sharing.

13.1.1 Writing and Approval of SOPs, Manuals, Handbooks, and Plans

Once it has been determined that a new policy or procedure is required at BAL, the most appropriate employee(s) (i.e., whoever has the most knowledge or experience in the given area) is/are delegated to write a document detailing the policy or procedure. Once the document has been written, it must pass up through a chain of approval specific to the type of document being written.

All SOPs begin with the appropriate person writing the procedure. The Group Leader (if applicable), the VP of Quality, and the VP of Operations then must approve the SOP. All analytic and technical SOPs must be approved by the Technical Director. The CQAP must be approved by the VP of Quality, VP of Operations, the Technical Director, and the President/CEO of BAL. BAL Handbooks and Plans follow their own specific chain-of-approval processes, with final approval coming from the VP of Operations or BAL President/CEO.

If an error is discovered during any portion of the approval process, the person who found the error makes a note of it and sends the document back to the original writer. It is the writer's responsibility to address the error and then reinitiate the approval process from the very beginning.

Upon approval, each person in the chain of approval must sign and date the document. Only upon final approval is the document considered to be in force and all procedures within the document from that date forth are enforced until the document is retired (see section 13.1.3).

13.1.2 Annual Review of SOPs, Manuals, Handbooks, and Plans

All BAL SOPs and Plans are reviewed within each calendar year. If no changes in the procedure are required, the reviewer signs and dates the document as being reviewed. If changes are required, the appropriate employee is designated to make the required revisions. The new revision of the document must then go through the same chain of approval it went through for its initial writing. Upon final approval, the new revision is considered in force, and the old revision is retired. Refer to SOP BAL-1000 for specific procedures to follow when revising an SOP.

13.1.3 Retirement of SOPs, Manuals, Handbooks, and Plans

When a BAL document is retired, the original is clearly labeled "Retired" and the date of its retirement is also clearly indicated. All copies of the retired document are either destroyed or also clearly labeled as being outdated. The original is then archived as a historical record (either electronically as a PDF file or as a hardcopy) for no less than 7 years.

13.1.4 Proprietary Information

Many of the analytical methods used at BAL have been developed in-house and are considered proprietary information. Clients or other organizations requesting particular SOPs are required to first sign an "Agreement for Confidential Disclosure and Restricted Use of Proprietary Information." Whenever possible, "client ready" SOPs, where all proprietary information has been removed, are given to clients instead of full SOPs. The permission of the President/CEO must be obtained prior to sending any non-redacted SOP containing proprietary information.

13.1.5 Uncontrolled Documents

Uncontrolled documents are defined as any document (CQAP, SOP, "cheat sheet", password, etc.) or portion thereof that has not been signed and dated as being approved for use in the laboratory and is not under the direct control of the VP of Quality. No such document is allowed to be posted or used in the laboratory and must be immediately removed upon detection. When referencing the CQAP or an SOP, the current approved version should be opened directly from the server from the SOP & other DOCs folder. All documents in this folder are PDF versions with signed and dated cover pages. If "cheat sheets" or isolated pages from SOPs would be of value in the lab, then these must be approved by the VP of Quality who then assigns the printed out pages a unique document ID, signs and dates the printout, and is responsible for ensuring that it is updated as needed.

13.1.6 Controlled Documents

The VP of Quality is responsible for ensuring that all controlled documents are tracked through the laboratory. At a minimum, all controlled documents must contain the following:

- Title of controlled document
- Revision number
- Approval signatures
- Date put into service
- Date retired (if applicable)

13.2 Client Records

All client reports, records of results, and correspondences are maintained by BAL for a period of no less than 7 years. All project information is electronically stored on the server for a minimum of 7 years. All "Active Client" specific files are maintained by the Project Manager. All client data generated following October 15, 2006 is scanned and stored electronically for a minimum of 7 years.

In the event that BAL should go out of business, it is BAL's stated policy that every attempt will be made to notify all clients (past and present) and ask them how they would wish to have their records maintained or transferred. In the advent of a change in ownership, it is BAL's policy that all records become the property of the new owner unless specifically requested otherwise by the client. All reasonable demands of the client shall be met and no client information shall be removed from BAL premises without the client's written consent.

13.3 Employee Records

All employee records, including resumes, training, IDOC and MDL studies, are maintained by BAL for a period of no less than 7 years following the departure of the employee.

14.0 Information Systems

14.1 Hardware

A local area network (LAN) connects staff computers and printers for local access, as well as providing external email, faxes, and Internet access. Two server computers provide secure electronic file access via the LAN. Server hardware consists of one Lenovo ThinkServer TS440 running Microsoft® Windows Server 2012 R2 configured as a primary domain controller and print server for networked printers. The second server is a Dell PowerEdge 2950 running Microsoft® Windows Server 2012 Standard edition. This server is configured as a backup domain controller. Both are equipped with multiple hard disk drives and employ RAID configurations to prevent data loss in the event of a single hard drive failure.

A dedicated server runs the LIMS application and is accessible via the LAN. The hardware is a Dell PowerEdge R715 Server. It has four hard disk drives configure in a RAID 1+0, and a fifth HDD is maintained as a hot swappable disk. The OS is Microsoft® Windows Server 2008. The LIMS program runs on SQL Server 2005 application.

A remote desktop server is configured to provide remote access for offsite employees. The server is a Dell PowerEdge R710 running Microsoft® Windows Server 2012 Standard edition and Remote Desktop Services.

14.2 System Backup

Backup software, Retrospect version 7.0, provides scheduling, automation and monitoring of backup for both server and workstation files. The software is run on a networked spare workstation. All data files located on the server are backed up daily. These include the LIMS file (Microsoft® SQL Server), instrument data, and client-related files. Other selected files on the workstation are also backed up weekly.

Multiple external hard drives are used to perform the backups. Hard drives connected to the backup workstation remain so for two to three weeks, after which they are removed to an offsite location and other external hard drives with the oldest backed up data are then connected to the backup PC.

14.3 Security

A Dell SonicWall NSA 220W router/firewall protects the LAN from the public internet. Workstation access is available to all authorized employees via domain logon. Shared data is available throughout the local network. Specific directories or files may be protected from access using Group Policy security settings if the data owner considers it necessary. Data loss is safeguarded through redundancy. Redundancy is accomplished via backups as mentioned and secure storage of data in hard copy.

All computer accounts are password protected so that unauthorized access is not allowed. Individuals are required to logoff of LIMS when they leave the workstation. Additionally,

individuals should logoff or lock their workstation when they leave unless that work station is running an instrument that would be disrupted by logging off. Security is maintained by working in a secure facility and having a small, tight-knit staff. Any unknown entities would be immediately recognized.

15.0 Corrective Action

15.1 Corrective Action

The laboratory has a corrective action system to identify any situations that may adversely affect data quality. These situations include, but are not limited to:

- Results outside of quality control criteria as outlined in individual SOPs
- Statistically out-of-control-events
- Deviations from normally expected results
- Suspect data
- Deviations from the method
- Special sample handling requirements

Corrective action may also be initiated as a result of other QA activities, such as performance or system audits.

Once a requirement for corrective action has been identified, the VP of Operations and/or the VP of Quality must be notified immediately. A verbal notification may be initially made; however, written documentation of the problem is required typically using an incident report form (Refer to BAL SOP BAL-1008). The VP of Quality, VP of Operations, or the Technical Director is responsible for evaluating the situation and determining the appropriate corrective action. The VP of Quality, VP of Operations, Technical Director, and President/CEO of BAL have the authority to stop work whenever a nonconformance issue may threaten the quality of data produced by BAL. Corrective action steps include, but are not limited to:

- Problem identification
- Investigation to determine the root cause of the condition
- Action to eliminate the problem
- Increased monitoring to evaluate the effectiveness of the corrective action
- Verification that the problem has been eliminated

Documentation of problems requiring corrective action is important to overall laboratory management. Any lab personnel may initiate a corrective action, but it is the VP of Quality who is responsible for ensuring that the action is documented. The VP of Quality is also responsible for verifying that initial action has taken place and appears effective and, after an appropriate time, for checking to see if the problem has been fully resolved.

15.2 Reporting Improper Laboratory Practices

In addition to documenting laboratory incidents, determining root cause, and developing effective corrective action through the incident report and resolution procedure, BAL is required to report any incident that includes improper laboratory practices as defined in the current DoD/DOE QSM. Examples of prohibited practices that require notification of our DoD accrediting body (ANAB) include:

- Fabrication, falsification, or misrepresentation of data
- Improper clock setting (time traveling) or improper date/time recording
- Unwarranted manipulation of samples, software, or analytical conditions
- Misrepresenting or misreporting QC samples
- Improper calibration
- Concealing a known analytical or sample problem
- Failing to report the occurrence of a prohibited practice or known improper or unethical act to the appropriate laboratory, contact representative, or government official.

When an improper laboratory practice is discovered by the lab, BAL must report the incident and submit the associated corrective action directly to ANAB.

15.3 Client Communication and Complaints

Brooks Applied Labs is committed to providing the best laboratory services available in the industry. To this end it is vital that good and proper communication is always maintained with our clients. Clients' opinions of the services provided by BAL are very important to us. All client comments, whether positive or negative, are taken seriously. If a client has a complaint, it is recorded and kept on file by the Client Services Manager. Complaints may encompass any aspect of the services provided by BAL, including analytical services, technical services, or quality assurance.

Once a complaint has been recorded, the BAL manager who is most responsible for the service to which the complaint is directed shall handle the matter with the client. If necessary, the BAL manager will initiate a corrective action to deal with any legitimate deficiencies brought to our attention by clients. The resolution of all complaints shall be recorded along with the initial complaint.

Any events that cast doubt on the validity of any test results already reported must be conveyed to the affected client within one business day of when the events become evident to BAL management. BAL is commonly contracted to do research or method validation for clients, due to the variable nature of this work, clients are typically contacted within a month of finalizing the R&D report. In addition to any phone messages, the client must also be promptly notified in writing. This is typically done in the form of an email. If more formal documentation is required, then a signed letter may be provided, as well as copies of any associated corrective actions.

Both negative and positive feedback from clients are reviewed at the end of the year as part of the Managerial Review in an effort to constantly improve the quality system and products and services provided by BAL.

15.4 External Audits

Corrective action may also be initiated by external audits by regulatory agencies or clients. BAL considers audits as an opportunity to improve upon our services. Any deficiencies discovered during external audits are documented and corrective actions are initiated to address them. All root cause analyses and corrective actions are documented and maintained by the VP of Quality.

16.0 Performance and System Audits

16.1 System Audits

16.1.1 Internal Systems Audits

BAL conducts specific function audits on an annual basis. The laboratory is audited against the most current DoD or DOE checklist to ensure that BAL remains compliant with all accreditation requirements.

Additionally, laboratory walk-through audits are performed monthly throughout the laboratory. Each lab group is audited separately. Laboratory walk-through audits are not as thorough as the annual audit, but serve to ensure that quality assurance procedures are being performed routinely before issues arise. The findings from monthly walk-through audits and any necessary corrective actions are presented in monthly QA reports.

16.1.2 External Systems Audits

BAL has occasional audits from various clients and accrediting agencies. The principal organizations that conduct audits of BAL's facilities and operations are the Washington State Department of Ecology, DOECAP as part of the Department of Energy audit program, and ANAB as part of Department of Defense, ISO 17025, and TNI (on behalf of Florida DOH) accreditations. BAL views external audits as an excellent tool for evaluating our quality and for finding areas for improvement. BAL always welcomes any client (current or potential) or government agency to conduct on-site audits.

16.2 Performance Evaluation

Performance Evaluation must be conducted at least biannually and may consist of blind samples, split samples with another laboratory (interlaboratory comparison study), QC samples (unknown to the analyst), performance test samples, and/or blind spiked samples. BAL frequently participates in assisting agencies to certify reference materials. Any chemist may analyze these performance evaluation samples as long as they have successfully completed training for the affected analysis. The Project Manager and VP of Quality are responsible for overseeing BAL's participation in each study, and all associated documentation, reporting, and record keeping.

External Performance Evaluations are as follows:

Agency	Study Title	Frequency
ERA	Blind PE samples*	Semi-Annually**
RTC	Blind PE samples*	Semi-Annually**

* As a better indication of overall laboratory performance, PE samples are treated like all other received samples in terms of receipt, preparation, quality control, and analysis.

** Participation in additional PE studies may be required as part of corrective action.

16.3 Annual Management Review of the Quality Systems

Brooks Applied Labs management conducts an annual review of the quality systems to ensure that they are still effective. All reports by managerial personnel, the outcome from all recent internal and external audits, the results from PE studies and interlaboratory comparisons, changes in the volume and type of work performed, feedback from clients, and corrective actions are taken into account during the managerial review. This review also looks ahead to anticipated issues for the coming year(s). The ultimate purpose of this review is to ensure the continued effectiveness and improvement of the quality systems in place at BAL. The summary of management review for the previous year is typically completed during the first quarter of the year. This summary includes a corrective action plan for any outstanding findings that come out of the management review.

APPENDIX A – Common Abbreviations

AA – Atomic Absorption

BLK – Method Blank

BAL – Brooks Applied Labs

BS – Blank Spike

CCB – Continuing Calibration Blank

CCV – Continuing Calibration Verification

CDOC – Continuing Demonstration of Capability

CGMP – Current Good Manufacturing Practices

CLIA – Clinical Laboratory Improvement Amendments

COC – Chain of Custody

CQAP – Comprehensive Quality Assurance Plan

CRM – Certified Reference Material

CVAFS – Cold Vapor Atomic Fluorescence Spectrophotometry

DoD – Department of Defense

DoD/DOE QSM – DoD/DOE Consolidated Quality Systems Manual for Environmental Laboratories

DOE – Department of Energy

DUP – Method Duplicate

EDD – Electronic Data Deliverables

EPA – Environmental Protection Agency

ERA – Environmental Resource Associates

FEP – Fluorinated Ethylene Propylene (Teflon™)

FLPE – Fluorinated High-Density Polyethylene

HDPE – High-Density Polyethylene

HEPA – High Efficiency Particulate Air

HGAAS – Hydride Generation Atomic Absorption Spectrometry

IDOC – Initial Demonstration of Capability

IC – Ion Chromatography

IC-PAD – Ion Chromatography with Pulsed Amperometric Detection

ICP-MS – Inductively Coupled Plasma – Mass Spectrometry

IC-ICP-MS – Ion Chromatography – Inductively Coupled Plasma – Mass Spectrometry

ICV – Initial Calibration Verification

IT – Information Technology

LCS – Laboratory Control Sample (typically a BS or SRM)

LIMS – Laboratory Information Management System

LOD – Limit of Detection

LOQ – Limit of Quantification

MDL – Method Detection Limit

MRL – Method Reporting Limit

MS/MSD – Matrix Spike / Matrix Spike Duplicate

NELAC – National Environmental Laboratory Accreditation Conference

NELAP – National Environmental Laboratory Accreditation Program

PM – Project Manager

QA – Quality Assurance

QC – Quality Control

QCS – Quality Control Standard

RPD – Relative Percent Difference

RSD – Relative Standard Deviation

SPF – Sample Processing Form

SOP – Standard Operating Procedure

SOW – Statement of Work

SRM – Standard Reference Material

TNI – The NELAC Institute

VP – Vice President

SOP #BAL-3101

BAL Procedure for EPA Method 1631, Appendix to (1/01): Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation by Cold Vapor Atomic Fluorescence Spectrophotometry (CVAFS)

Brooks Applied Labs

Revision 003
Written 2/11/16
Revised 6/1/16

Reviewed _____
_____
_____
_____

VP of Operations	Date 6/2/16
VP of Quality	Date 6/2/16
Hg Group Leader	Date 6/2/16

BAL Procedure for EPA Method 1631, Appendix to (1/01): Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation by Cold Vapor Atomic Fluorescence Spectrophotometry (CVAFS)

1.0 SCOPE AND APPLICATION

1.1. Method BAL-3101 is modification of Appendix to EPA Method 1631 and is based on peer-reviewed, published articles for the determination of total mercury in a wide range of biological and geological matrices. All samples must be subject to an appropriate digestion step prior to analysis.

2.0 SUMMARY OF METHOD

2.1. Prior to analysis, the solid samples must be acid digested to break down the sample matrix and oxidized to convert all mercury species to mercuric ions.

2.2. Method BAL-3101 is a cold vapor atomic fluorescence technique, based upon the fluorescence of 253.7 nm radiation by excited elemental mercury (Hg^0) atoms in an inert gas stream. Mercuric ions in the oxidized sample are reduced to Hg^0 using stannous chloride ($SnCl_2$), and then purged onto gold amalgamation traps using nitrogen gas as a means of preconcentration. Mercury vapor is thermally desorbed into the fluorescence cell. Fluorescence intensity is measured as a function of total mercury collected, which is converted to concentration by the size of the aliquot purged.

2.3. The actual detection limits for this method will be dependent upon the specific techniques used to prepare the samples. Current detection limits as determined by Brooks Applied Labs (BAL) are found in Table 1 of this document.

3.0 DEFINITIONS

3.1. A partial list of definitions is presented below. Most definitions can be found for specific terms in the sections where they are first mentioned. Many of the definitions mentioned in this method are based on those found in the glossary for EPA Method 1631e. Please refer directly to this method for a more detailed list.

3.2. May: This action is allowed, but not required.

3.3. May Not: This action is prohibited.

3.4. Must: This action is required.

3.5. Shall: This action is required.

3.6. Should: This action is suggested, but is not required.

4.0 INTERFERENCES

4.1. Due to the strong oxidation step there are no observed interferences with this method.

4.2. The potential exists for destruction of the gold traps (and consequently, low recoveries) if free halogens are purged onto them. When these instructions are followed accurately, this outcome is unlikely.

4.3. Water vapor may collect in the gold traps, and be released into the fluorescence cell where it condenses, giving a false peak due to scattering of the excitation radiation. This can be minimized with the use of a soda lime pre-trap.

4.4. As always with atomic fluorescence, the fluorescent intensity is strongly dependent upon the inertness of the carrier gas. Using only ultrapure gases minimizes the possibility of quenching due to trace gases, but it still remains the analyst's responsibility to ensure high purity inert carrier gas is used and that the analytical train is leak-free.

5.0 SAFETY

5.1. Refer to EPA Method 1631E, section 5.0, for safety issues associated with the use of this method.

5.2. Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability, and nervousness. Organo-mercurials may cause permanent brain damage. Because of this, only highly trained personnel familiar with the dangers and precautions to take when working with mercury compounds should ever handle standards and/or high level samples.

5.3. Material safety data sheets (MSDSs) are maintained for all chemicals used in this method. The MSDS sheets are stored in the mercury laboratory in appropriately marked binders.

5.4. Refer to the latest revision of the Chemical Hygiene Plan (CHP) for additional safety precautions and required protective equipment.

6.0 APPARATUS AND MATERIALS

6.1. Routine preventative maintenance for the equipment used in this procedure is described in detail in the standard operating procedure BAL-0600.

6.2. Specific equipment used at BAL is listed below. Any modifications to EPA Method 1631E are described and explained. Figure 1 of this document shows the setup of the single-trap amalgamation system used at BAL and Figure 2 shows the setup of the automated system.

6.1.1. Brooks Rand Instruments (BRI) Model III Atomic Fluorescence Spectrophotometer: To achieve the low detection levels and small, interference-free sample aliquots claimed by this method, a very sensitive CVAFS detector is required. Such systems are built at BRI (Model III) based on the principals discussed in the literature. Refer to the "Brooks Rand Instruments LLC Model III Operations Manual" for instrument operating instructions. The initial setup of the instrument is governed by the range of the display of the signal height (0-2 million). The offset is set at about 10000 for the MERX-T system and about 5000 for the manual setup.

6.1.2. BRI MERX Total Purge and Trap Unit: The purge and trap module controls all of the gas flows throughout the system. It contains the gold amalgamation traps and controls their heating and cooling. It contains the soda lime trap, which is responsible for purge gas drying and conditioning.

6.1.3. Autosampler: An AI Scientific septum piercing autosampler with 168 slots is used at BAL. Other autosamplers may be used if they are compatible with the Guru software, have septum piercing capabilities, and can hold the 40 mL glass vials.

6.1.4. BRI Amalgamation Control Module: Controls the heating of the gold-coated bead traps to 450 ºC and then the cooling of the traps following desorption of Hg from the trap.

6.1.5. BRI flow meter/needle valve: Capable of controlling and measuring gas flow to the purge vessel at 200-500 $mL \cdot min^{-1}$.

6.1.6. Teflon® adapters (BRI part #08401 through #08404) and tubing (BRI part #08405 through #08407): Connections between components and traps are made using 3.2-mm OD pre-cleaned Teflon® FEP tubing and Teflon® friction-fit or threaded tubing connectors.

6.1.7. Soda lime pre-trap (BRI part #03410): For preventing acid-fumes and moisture from degrading the gold-coated sand or gold wire traps. A 10 cm x 0.9 cm (diameter) Teflon® tube containing 2-3 grams of commercially available reagent grade, non-indicating, 6-12 mesh soda lime chunks, packed in between plugs of silanized glass wool. This trap is purged of Hg by placing it on the output of a clean cold vapor generator, partially filled with deionized water (DIW) and 500 µL stannous chloride ($SnCl_2$), and purging for a minimum of 15 minutes with nitrogen (N_2) at 400 mL/min. Bubbler blanks are analyzed first so that any indication of unclean pre-traps would be evident immediately.

6.1.8. Cold vapor generator (BRI part #03200): A 220-mL flask with a standard taper 24/40 neck, fitted with a sparging stopper having a coarse glass frit that extends to within 0.2 cm of the flask bottom.

6.1.9. Teflon® split bottle: Used for measurement and pre-reduction of original oxidized samples prior to analysis. A 125-mL blank tested Teflon® bottle with a unique identifier is permanently associated with a corresponding cold vapor generator.

6.1.10. Gold-Coated Glass Bead traps or equivalent (BRI part #03030): Used for trapping gaseous elemental mercury ($Hg^{0)}$. Gold-coated glass bead traps made with gold-coated glass beads are used to analyze solid samples. Both gold-coated sand traps are used to analyze air samples.

6.1.11. Recorder: BAL uses direct data acquisition with the BAL Guru integration software instead of a chart recorder or integrator as described in EPA Method 1631E, section 6.6. The BAL Model III comes complete with the Hg Guru™ integrating software. Refer to the "Brooks Rand Instruments LLC Model III Operations Manual" for Hg Guru™ software/integrator operating instructions. Hg Guru™ software requires an IBM compatible computer (minimum requirements are a Pentium II® processor running at 400 MHz, a CD-ROM Drive, 128 MB RAM, and 50 MB free space on the hard-drive) and runs MS Windows® 98 or higher. Use of this integration software is faster, eliminates the expense of chart recorders and/or integrators, allows for storage of data in diskette form, and eliminates possible transcription errors.

6.1.12. Pipettes: All plastic pneumatic fixed volume and variable pipettes in the range of 10 µL to 5.0 mL.

6.1.13. Nichrome wire coil (BAL part #08300) with plug (#08301): Used for heating the gold trap to thermally desorb the mercury.

6.1.14. Refluxing digestion flask: 20.5 mL (small mass) or 40 mL glass VOA vials with fluoropolymer lined lids. Acid-cleaned 1-inch diameter glass marbles are used over the vial mouth as pressure relief valves.

6.1.15. Cold digestion vials: 40 mL glass VOA vials with fluoropolymer lined lids.

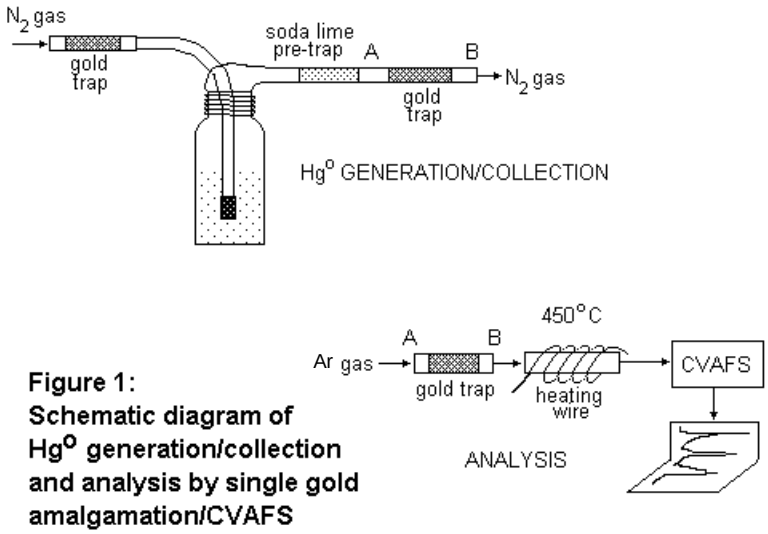
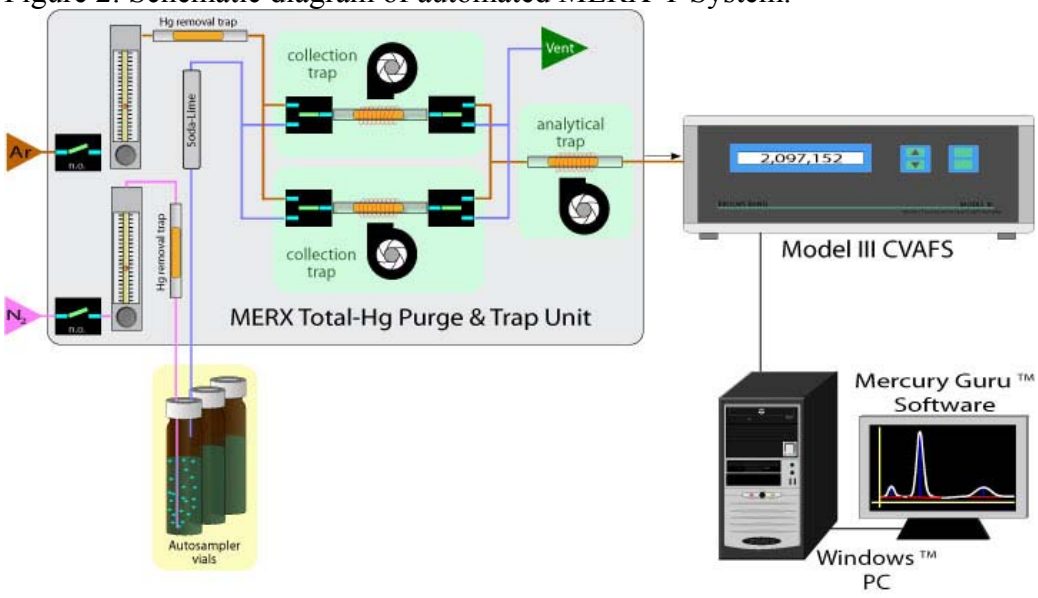


Figure 1: Schematic diagram of Hg^0 generation/collection and analysis by single gold amalgamation/CVAFS

Figure 2: Schematic diagram of automated MERX-T System.



7.0 REAGENTS

Document standard or reagent preparation in the standards and reagents log in the LIMS. Record the standard or reagent type, identification number, preparation date, lot number, expiration date, and analyst name in the appropriate fields. Record the standard or reagent type, identification number, preparation date, analyst's initials and expiration date on the container.

7.1. Water: Reagent water is monitored for Hg on a daily basis when calibration blanks are analyzed. A minimum of four 25 mL aliquot of fresh reagent water, each with 0.2 mL NH_2OH•HCl and 0.2 mL of $SnCl_2$, are analyzed at the beginning of the run sequence. They each must be < 10 pg Hg. A high level of mercury detected in the reagent water analysis may also be attributed to the bubbler itself, NH_2OH•HCl, $SnCl_2$, or the soda lime pre-traps. Regardless of the source, all analysis is stopped until the source of contamination is determined and the problem is corrected. The results are stored with each batch.

7.2. Air: It is vital that the laboratory air be low in both particulate and gaseous mercury in order to reduce the risk of contamination. Sticky mats are located at the entrance as an precautionary measure. The mercury lab, the sample preparation labs, the shipping and receiving labs and the sample processing lab are monitored monthly for atmospheric mercury levels to ensure that these levels are sufficiently low for ultra-trace level mercury analysis. Air from each lab is pumped through a soda lime pre-trap and onto either a gold wire or gold-coated sand trap at a flow rate of 1 L/min until at least 20 L of air have been collected per trap. A warning level has been established at 15 ng Hg/m^3 with a shutdown control level at 25 ng Hg/m^3. Results from the monthly air tests are stored electronically on the BAL computer server.

7.3. Nitric acid (HNO_3): Trace-metal reagent grade is purchased and analyzed for Hg before use. In general, it is possible to obtain acid containing less than 5 pg/mL Hg. When a lot number meeting this specification is found, several cases are purchased. Generally, lower values can be obtained in this manner than by re-distilling acid in the laboratory. Only "trace-metal' grade acid should be used. Acids labeled as "ULTRA-PURIFIED" have historically had higher concentrations of mercury and should be avoided.

7.4. Sulfuric acid (H_2SO_4): Trace-metal reagent grade is purchased and analyzed for Hg before use. In general, it is possible to obtain acid containing less than 5 pg/mL Hg. When a lot number meeting this specification is found, several cases are purchased. Generally, lower values can be obtained in this manner than by re-distilling acid in the laboratory. Only "trace-metal' grade acid should be used. Acids labeled as "ULTRA-PURIFIED" have historically had higher concentrations of mercury and should be avoided.

7.5. Hydrochloric acid (HCl): Trace-metal reagent grade is purchased and analyzed for Hg before use. In general, it is possible to obtain acid containing less than 5 pg/mL Hg. When a lot number meeting this specification is found, several cases are purchased. Generally, lower values can be obtained in this manner than by re-distilling acid in the laboratory. Only "trace-metal' grade acid should be used. Acids labeled as "ULTRA-PURIFIED" have historically had higher concentrations of mercury and should be avoided.

7.6. Hydroxylamine hydrochloride (NH_2OH•HCl), stannous chloride ($SnCl_2$), and bromine monochloride (BrCl): Each of these reagents is prepared according to instructions listed in section 7.0 of Method 1631E. Reagent blanks must be analyzed each time that BrCl

is made anew. The SnCl2 and NH_2OH•HCl are tested daily whenever calibration blanks are analyzed.

Note: BrCl is tested whenever a new bottle is prepared prior to use with samples. During initial testing, reagent blanks are prepared in 40mL glass vials at four different concentrations of BrCl (1.0%, 2.0%, 5.0%, and 10%). The BrCl is pre-reduced in the sample vial and then stannous chloride is added and purged using MERX Total HG Purge & Trap Module. The amount of Hg per reagent unit (reagents used in a 1.0% BrCl method blank) must be ≤ 10 pg.

7.7. Stock mercury standard: A commercially available 1000 $mg \cdot L^{-1}$ mercury atomic absorption standard that is traceable to NIST is used. This stock standard should be replaced by the manufacturer's expiration date. Store this standard in an appropriate location separate from the working standards.

7.8. Intermediate mercury standard solution: 0.250 mL of the stock solution is diluted to 250.0 mL with ultrapure deionized water and 5.0 mL of BrCl. This solution contains 1000 ng/mL Hg, and must be kept refrigerated in a tightly closed Teflon® bottle. This solution should be replaced bi-annually.

7.9. Mercury working standards: 2.50 mL of the intermediate mercury standard solution is diluted to 250 mL with high purity water containing 2.50 mL BrCl solution, to make a 10.0 ng/mL working solution (as discussed in EPA Method 1631E, section 7.9). A 1.00 ng/mL working standard should be made by diluting 0.250 mL of the intermediate mercury standard to 250 mL with DIW containing 2.50 mL BrCl solution. These working standards of 10.0 ng/mL and 1.00 ng/mL are added in appropriate aliquots to reagent water and analyzed to create the calibration curve and CCV samples. These solutions should be replaced monthly.

7.10. Independent Calibration Verification (ICV) Standard: 0.250 mL of the standard reference material (SRM) NIST 1641d (mercury in water) is added 247.25 mL DIW and 2.5 mL BrCl for a final volume of 250 mL. After adjusting for the density of the SRM, the final value for this standard is 15.68 ng/L. NIST 1641d is purchased directly from the National Institute of Standards and Technology.

7.11. Nitrogen: Grade 4.8 (99.999% purity) nitrogen that has been further purified by the removal of Hg using a gold-coated sand trap.

7.12. Argon or Helium: Grade 4.8 (99.999% purity) inert gas that has been further purified by the removal of Hg using a gold-coated sand trap.

7.13. Soda Lime: for soda lime canister. 4-8 mesh. Fisher AA36596A4 or equivalent.

7.14. Silanized Glass Wool: for soda lime canister. Supelco 2-0410 or equivalent.

8.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

8.1. Samples should be collected into glass, polyethylene, or fluoropolymer jars. Polyethylene bags are also acceptable for all but very low level and/or very wet solid samples. Dry samples such as coal and ores may be collected and stored in heavy gauge paper pouches.

8.2. Dry samples such as ores, coal, paper, and wood may be stored indefinitely in a secure, cool, low in mercury, atmosphere.

8.3. Samples containing biota (i.e. wet or dry sludge), and all wet sediment samples are shipped to the laboratory at 0-4 ºC and stored at < 4 ºC for up to 1 year. Dry samples such as ores, paper, and wood may be shipped unrefrigerated and stored indefinitely in a cool, dry location low in mercury.

8.4. Biota samples are to be frozen at < -15 °C (standard freezer on coldest setting) until use. Samples may be stored for a maximum holding time of 1 year.

8.5. Freezing and thawing of sediment samples may adversely affect their homogeneity. Ideally, sediment samples will not be frozen, however, projects often require multiple analyses on the same sample including speciation analysis which requires the samples to stay frozen until analysis. To reduce the impact of freezing on the sample, aliquots for all analyses are taken at the same time to reduce the freeze/thaw cycles. If the client requests it, the sediment samples can be aliquoted and weighed at the laboratory prior to freezing to further negate the impact of freezing on homogeneity. If wet sediment samples have been frozen prior to preparation, they must be sequentially homogenized into smaller aliquots as follows. First the whole sample must be emptied into a clean weigh boat and thoroughly homogenized. Then half of the sample is transferred back into the original container. The sample remaining in the weigh boat is thoroughly homogenized before half is transferred back into the original container again. The procedure is continued until the appropriate sample preparation weight is left and is then placed the sample preparation vial. Refer to BAL-0304 for further discussion of sample homogenization. Additionally, any other associated sample preparations to be performed with the sample (such as percent solids analysis) should homogenized and aliquoted at the same time to ensure that the aliquots are similar in sample characteristic. All remaining sample is stored in the original sample container at < -15 ºC for up to 1 year.

8.6. To better assure homogeneity, large particles such as rocks and sticks can be removed by screening the samples through a 2.0 mm sieve. Though to prevent contamination as much as possible, the typical practice at BAL is to pick out large particles prior to aliquoting for sample prep.

8.7. All dissection, homogenization, and other handling of the samples are to occur by clean room gloved personnel in an environment free of mercury contamination.

9.0 CALIBRATION AND STANDARDIZATION

9.1. Refer to EPA Method 1631E, section 10.0, for a detailed description of instrument calibration.

9.2. Instrument Calibration: BAL has adopted the following procedure. There are two different analytical systems that may be used for analysis of samples. They can be analyzed by manual analysis or by using the MERX-T automated total mercury system. Both systems use analogous techniques with some minor changes at sample analysis. The AFS detectors used are the same for either instrument. The AFS detectors built by BAL are capable of achieving extremely low detection limits.

It is required that a calibration curve of at least 5 points be prepared prior to the analysis of samples to verify linearity and quantify sample concentrations. Sections 9.0 and 10.0 of EPA Method 1631E do not state a frequency requirement for calibration. BAL performs a new calibration each day and when it is observed that the instrument is no longer in control. Additionally, analysis of the ICV standard must also meet the criterion in Table 2 for the calibration to be validated.

9.2.1. For manual analysis, the instrument is calibrated down to 25 pg. The standards typically used to calibrate the instruments for total mercury at BAL are 25 pg, 100 pg, 500 pg, 2500 pg, and 10,000 pg. The standards are added to the split bottle that contains approximately 100 mL of DI water, pre-reduced with $NH_2OH \cdot HCl$, and then poured into the bubbler. The calibration is based on the measured picograms of mercury in the bubbler and not on concentration.

9.2.2. For automated analysis on the MERX-T, the instrument is calibrated down to 10 pg. The standards typically used to calibrate the instruments for total mercury are 10 pg, 25 pg, 100 pg, 500 pg, 2,500 pg and 10,000 pg. The standards are added to a 40 mL septa top vial that contains approximately 25 mL of DI water, pre-reduced with $NH_2OH \cdot HCl$. Stannous chloride is added to the vial which is then capped and placed on the autosampler.

9.2.3. Section 9.4.1.3 of EPA Method 1631E suggests that the mean peak area for all bubbler blanks (including those analyzed during the analytical sequence to ensure that no carryover occurs from high-level samples) should be subtracted from all raw data before results are calculated. This does not allow for the continuous determination of whether QA results are in control, thereby forcing the analyst to analyze all samples prior to determining if all QA criteria are met. BAL subtracts the average peak area measurement of the first four bubbler blanks (or one blank for each split bottle and bubbler used in the analysis of samples) analyzed at the beginning of the analytical run from all raw data for result calculations. The acceptance criterion for the initial bubbler blanks for manual analysis (referred to as calibration blanks) is each ≤ 40 pg, average ≤ 20 pg, and standard deviation ≤ 7.5 pg. The criterion for subsequent bubbler blank checks is that each blank must be ≤ 40 pg and ± 20 pg from the

calibration blank mean. For automated analysis, the acceptance criterion for the initial bubbler blanks is each ≤10 pg. The criterion for subsequent bubbler blank checks is that each blank must be ≤10 pg and ± 5 pg from the calibration blank mean.

9.2.4. BAL uses the following equation to calculate the calibration factor (CF_x) for Hg in each of the standards:

$$CF_x = (C_x) / (A_x - A_{CB})$$

Where:
C_x = mass of Hg in standard analyzed (pg)
A_x = peak area for Hg in standard
A_{CB} = mean peak area for Hg in calibration blanks

This calculation differs from the calculation given in 1631E in that the numerator and denominator are reversed.

10.0 PROCEDURE

10.1. Sample Preparation

10.1.1. General considerations: Dissect and/or homogenize the sample with clean stainless steel tools. Clean the stainless steel tools with ultrapure deionized water and a mild detergent (such as Alconox®) between sample aliquots. Refer to SOP BAL-0304 for instructions in the homogenization of various sample matrices.

10.1.2. All method blanks are prepared by just adding reagents to the sample preparation vial unless otherwise specified in this SOP. No "reference matrix material" or additional DIW is added to the method blanks. This is allows for method blank correction.

10.1.3. Hot re-fluxing HNO_3/H_2SO_4 digestion: This procedure is used for **biota**, wood, paper, tissue, sludge, or other soils high in organic content. An aliquot of homogenized sample of 0 [redacted] is weighed directly into [redacted] glass vial with a fluoropolymer-lined lid. To prep homogenization blanks, pipette [redacted] of the homogenization blank water into a vial then prep as normal.

Glass vials are purchased from BRI and BRI provides a COA certifying that the vials are not a potential source of mercury contamination. Cleaned and tested fluoropolymer vials may also be used. If necessary, up to 2-3 mL of DIW may be used to rinse the sample down to the bottom [redacted] of H_2SO_4 and [redacted] of HNO_3 are pipetted into the sample, and the preparation is swirled.

Caution: This mixture gets hot and emits caustic fumes!

Acid washed fluoropolymer cones or glass marbles are placed on each glass vial to allow refluxing of the preparation. Samples are next placed on a hotplate, and brought up to a refluxing boil in temperature increments to avoid excessive foaming, especially common with tissue samples. At BAL a hotplate is used, the samples are digested at a temperature [redacted] ºC for [redacted] and then refluxed at a hotplate temperature of [redacted] ºC for [redacted]. The samples are allowed to cool prior to removal from the hotplate. 0.50 mL of BrCl is added to each sample, and then the samples are diluted to the [redacted] mark with deionized water. Experience and numerous intercalibration studies show that undigested rock material or animal fat does not affect the accuracy of this digestion for Hg, because these fractions are both very low in initial Hg content, and are effectively leached by the boiling acid.

Alternative method: *If fluoropolymer vials are used, the caps should be tightened so that the threads make good contact. If a sand bath is used, then samples are digested [redacted] °C for [redacted] and then refluxed at a sand bath temperature of [redacted] °C for [redacted], or until [redacted]*

As a substitute, [redacted] volumetric flasks may be used. After addition of the HNO_3/H_2SO_4 mixture, [redacted], and the samples are allowed to predigest at [redacted]. Samples are then heated in the same manner as previously described. After digestion the flasks should be allowed to cool, [redacted] of BrCl is added and the volume brought [redacted] with ultrapure deionized water [redacted]

10.1.4. Coal samples, and other samples high in elemental carbon, are prepared following the biota preparation method described above with a few modifications. An aliquot of [redacted] is weighed directly into a [redacted] glass vial with a [redacted] lid. Continue to follow the biota preparation method. After samples are allowed to cool add [redacted] BrCl and dilute the digestate to [redacted] with deionized water, shake vigorously and allow to sit for at least [redacted] prior to analysis.

10.1.5. Alternative [redacted] aqua regia digestion: This procedure is for geological media such as, ores, sediments, and soils. Since the matrix is leached rather than dissolved, the sample must [redacted] if the total mercury content is desired.

Weigh to the nearest mg [redacted] into [redacted] vial. In a fume hood, add [redacted] of concentrated HCl, swirl, and add [redacted] of concentrated HNO_3. [redacted] The preparation should then be allowed to digest at [redacted]

For all samples add [redacted] BrCl and dilute the digestate to [redacted] with deionized water, shake vigorously, and allow to fully settle prior to analysis.

Note: More organic samples may require higher levels of BrCl. BAL typically adds [redacted] of BrCl to most aqua regia preparations, but more organic samples may require higher levels of BrCl [redacted]. Additionally, reagent water without BrCl may be used if the samples are appropriate to such preparation and very low method blanks are required.

10.1.6. Preparation of **Capillary Collected Blood** Samples at Micro Volumes: The following method is for blood that is collected in capillary tubes only. If the blood is collected in a vial with adequate volume, the standard biota prep should be used unless otherwise specified.

10.1.6.1. Pipette [redacted] of DIW into a [redacted] vial. If the blood is not already in a capillary tube, use a pre-cleaned capillary tube to draw up an aliquot of the blood sample. Weigh the tube with the blood aliquot to the nearest 0.1 mg. Place one end of the capillary tube just below the surface of the DIW and using a modified pipette tip, force the blood into the water. Due to the high viscosity of the blood, it may be necessary to rinse out the capillary tube several times. Each time ensure that all of the liquid has been forced out of the capillary tube by observing bubbles in the solution. It is important to remove the capillary tube from the liquid while bubbles are still forming to prevent any sample being drawn back into the tube. The tube is then reweighed and the sample mass is calculated by difference. A final sample mass of ~20 mg is typical.

10.1.6.2. Then [redacted] of a [redacted] HNO_3:H_2SO_4 mixture is added to each vial, the vial is covered with a clean marble or fluoropolymer cone, and the samples are heated at 100 ºC for an hour. Then the temperature is raised to [redacted] ºC and the samples are heated [redacted]. The samples are then removed from the heat and allowed to cool to room temperature. [redacted] of BrCl is added and each sample preparation is made up [redacted] with deionized water.

10.2. Instrumental Analysis:

10.2.1. BAL has adopted the following modifications from EPA Method 1631E for manual analysis of mercury.

10.2.1.1. Samples and standards are purged [redacted] at [redacted]. However, longer purge times may be necessary if the lab and/or sample temperature is [redacted] °C. Lab temperature is maintained between [redacted], so longer purging times are not generally necessary.

10.2.1.2. Four [redacted] aliquots of water are poured into each of the bubblers prior to calibration. [redacted] of $SnCl_2$ is added to each bubbler and the aliquots are used to condition the soda-lime pre-[redacted]. Following this pre-trap conditioning step, the purged water is discarded and [redacted] aliquots of DIW along [redacted] $NH_2OH•HCl$ are poured from the four split bottles into their associated bubblers[redacted] $SnCl_2$ is added to this water, which is then purged [redacted] onto a gold amalgamation trap and analyzed for Hg. A high level of mercury in the calibration blanks may be attributed to the split bottle, the bubbler, the reagents, or the soda lime pre-traps. Regardless of the source, all analysis using the contaminated bubbler/split bottle/trap combination is stopped until the source of contamination is determined and the problem is corrected. Use of all uncontaminated bubblers, split bottles, and traps may continue during this process.

10.2.1.3. All calibration standards and QC samples are added [redacted] DIW in a thoroughly rinsed split bottle prior to transfer to a bubbler for analysis.

10.2.1.4. Aliquots of the digestate, usually in the range [redacted] is added [redacted] mL of deionized water in a split bottle. [redacted] of $NH_2OH•HCl$ is added directly to the split bottle and allowed to react for at least 5 minutes to ensure that no trace halogens remain prior to transfer to the bubbler for purging onto a gold amalgamation trap for analysis. PURGING OF FREE HALOGENS ONTO THE GOLD TRAPS WILL RESULT IN DAMAGE, AND LOW IRREPRODUCIBLE RESULTS THEREAFTER. Split bottles and bubblers are rinsed a minimum of three times with DIW in between sample aliquots. The contents of the split bottle are poured into a bubbler and approximately [redacted] of $SnCl_2$ is pipetted into the bubbler. The caps are replaced and secured to the vessel with a bubbler clamp, and the sample is purged [redacted]. The instant that $SnCl_2$ and the sample aliquot are combined, reduction of mercuric ions to volatile Hg^0 begins. The analyst should minimize the time between sample reduction and the start of sample collection. In order to best minimize potential loss of the volatile Hg^0, the analyst must have the gold trap in place prior to adding $SnCl_2$ to the purge vessel and connects the N_2 purge gas after the bubbler top is clamped in place.

Note: $NH_2OH•HCl$ and $SnCl_2$ are added in excess to the samples such that the measurement of an exact volume is not necessary. However, the repipettors used to dispense these reagents must still be checked daily when DoD client samples are analyzed.

10.2.1.5. As discussed in Method 1631E and in section 12.6 of this SOP, follow the attached analytical sequence (Table 2). Generally, analysts should follow the suggestion in Method 1631E that samples suspected to contain the lowest concentration of mercury (i.e., known blank samples) should be analyzed first followed by samples containing potentially higher levels (i.e., known influent samples).

10.2.1.6. As discussed in section 12.6 of this SOP, the analyst may choose to analyze CCV samples more frequently than required by Method 1631E. A CCV must be analyzed after every 10 client samples (not counting quality control samples) to verify ongoing control of the system.

10.2.1.7. The PMT and offset are recorded on the bench sheet at the beginning of each day after auto zeroing the instrument previous to measuring the noise. When the baseline shifts more than 200-300 units, the systems should be adjusted in the following manner. Auto zero the Model III. Compare the offset to the original offset for the day. If it is more than 100 units different than the original reading, adjust the PMT to obtain an offset that is within 100 units of the original reading. The PMT and offset must be recorded every time the instrument is auto zeroed regardless of if the PMT was adjusted.

10.2.1.8. BAL has found that checking for mercury carryover in a bubbler by analyzing a bubbler blank is not necessary unless an unusually high level sample has been purged. Carryover tests, performed using spikes of 10,000 pg, 20,000 pg, 40,000 pg, and 100,000 pg, resulted in carryover of less than 50 pg Hg from the bubbler and trap. To avoid carryover from the bubbler or split bottle, the analyst should rinse each bubbler and split bottle three times with DIW between all samples. To avoid carryover from the trap, the analyst should heat the mid- and downstream sections of any traps associated with a higher-level sample (such as any sample above the calibration range) for an additional [redacted] following sample desorption. If a purged sample contains > 20,000 pg Hg, the analyst must follow the above corrective actions and analyze a bubbler blank and split bottle blank check using fresh DIW and the associated bubbler, split bottle and trap. The analysis of the split bottle blank should be performed on a different bubbler to facilitate proper contamination identification. Using previously purged DIW is not necessary for the bubbler blank. If any bubbler or split bottle blank fails (> 40 pg Hg and/or > ± 20 pg from average calibration blank on associated bubbler and trap), the analyst must identify and correct the source of contamination, and demonstrate that the bubbler and trap pass the blank criterion before sample analysis can continue using that bubbler, split bottle, and trap.

10.2.1.9. Any samples run in a bubbler, split bottle and/or on a trap associated with a carryover sample (e.g., a sample with > 20,000 pg Hg) must be reanalyzed, if sufficient sample volume exists.

10.2.1.10. After analysis is complete, split bottles and bubblers are rinsed with DIW three times and filled with DIW. Split bottles are stored tightly capped until further use. During the workweek, all bubblers are filled with DIW overnight and thoroughly rinsed prior to use in the morning. At the end of each week, the bubblers are purged with [redacted] This procedure should be done more frequently if the bubbler starts to appear dirty before the end of the week.

10.2.2. BAL has adopted the following modifications from EPA Method 1631E for the automated analysis of mercury using the MERX-T.

10.2.2.1. Samples and standards are purged for [redacted]. However, longer purge times may be necessary if the lab and/or sample temperature is [redacted] Lab temperature is maintained [redacted], so longer purging times are not generally necessary.

10.2.2.2. For the MERX-T, each [redacted] becomes the samples' bubbler. See the MERX-T Manual for an instrument description and overview of how it functions. Approximately [redacted] of DI water is added to each vial that is for blanks or standards. For samples, the appropriate amount of sample [redacted] added to each vial and the vial is brought up to approximately [redacted] with DI water. If spikes are needed, the correct amount of standard is pipetted into the appropriate vial [redacted] of hydroxylamine-HCl solution is added to each vial for pre-reduction of the BrCl[redacted] of stannous chloride solution is added to each vial shortly after which the vial is capped and shaken before being placed on the autosampler.

10.2.2.3. All calibration standards and QC samples are added [redacted] aliquots of DIW in a [redacted] top vial for analysis.

10.2.2.4. Aliquots of the digestate, usually in the range of 0.10-1.0 mL [redacted] is added [redacted] of deionized water in a 40-mL septa top vials. 0.2 mL of NH_2OH•HCl is added directly to the vial and allowed to react prior to the addition of 0.2 mL of $SnCl_2$. Shortly after which the vial is capped and shaken before being placed on the autosampler.

10.2.2.5. As discussed in Method 1631E and in section 12.6 of this SOP, follow the attached analytical sequence (Table 4). Generally, analysts should follow the suggestion in Method 1631E that samples suspected to contain the lowest concentration of mercury (i.e., known blank samples) should be analyzed first followed by samples containing potentially higher levels (i.e., known influent samples).

10.2.2.6. As discussed in section 12.6 of this SOP, the analyst may choose to analyze CCV samples more frequently than required by Method 1631E. A CCV is analyzed after every 10 sample injections to verify ongoing control of the system.

10.2.2.7. The PMT and offset are recorded on the bench sheet at the beginning of each day after auto zeroing the instrument previous to measuring the noise. Since the analysis is automated and may take place while the analyst is not present, the PMT is not adjusted during an analytical run.

10.2.2.8. Since the MERX-T utilizes the individual vials as bubblers, "bubbler" carryover is not a concern. Carryover can occur on the run just after a high sample (subsequent run carryover) and/or on the second run after a high sample (trap carryover).

BAL has found that checking for mercury carryover by analyzing a carryover blank is not necessary unless an unusually high level sample has been purged. Carryover tests, performed using spikes of 10,000 pg, 20,000 pg, 40,000 pg, and 100,000 pg, resulted in carryover of less than 12.5 pg Hg. If a purged sample contains > 20,000 pg Hg, the analyst must reanalyze the high sample (at an appropriate dilution) and the two samples that immediately follow it to check that the samples are not biased high due to carryover.

10.2.2.9. New vials are used for every analysis to reduce the possibility of cross contamination. If the autosampler needle needs cleaning, it can be placed in a vial containing [redacted] HNO_3 and sonicated [redacted].

10.2.3. Gold traps should be tracked by unique identifiers so that any trap producing poor results can be quickly recognized and discarded. Occasionally due to inadvertent contact with halogen fumes, bubbler solution, organic fumes, or overheating, a sampling trap will become damaged, giving low and/or irreproducible results. Suspect traps should be checked with at least two consecutive standard runs before continued use. Traps should be replaced quarterly or as soon as possible after quality control results indicate their degradation. Additionally, traps should be replaced whenever integration peaks become abnormally shaped (no longer symmetrical with steep slopes).

10.2.3.1. The MERX-T uses dual amalgamation and contains only 2 sample collection traps and 1 analytical trap. Guru automatically records the sample collection trap used for analysis (X or Y). The agreement between the two traps should be assessed during the calibration. If significant inconsistencies are noted between the X and Y trap, then the problem should be addressed and fixed and the system should be recalibrated before analyzing client samples. When replacing traps, all three should be replaced at the same time.

10.2.4. Section 11.3.2 of Method 1631E states that before each trap is analyzed, argon should be passed through the trap for approximately two minutes to drive off condensed water vapor prior to heating and desorbing for three minutes. Historically, BAL has not experienced significant analytical problems associated with water vapor on traps. However, if the analyst observes peaks that desorb prior to the mercury peak, this technique may be employed.

11.0. DATA ANALYSIS AND CALCULATIONS

11.1. All instrument peaks are integrated using Hg Guru™ software. This integration is automated; however, there are occasions when Guru™ will not properly integrate a peak. This is most common with small peaks, abnormally shaped peaks, or peaks with excessive tailing. When a peak is not properly integrated by Guru™, the analyst may manually integrate or adjust the integration of the peak using the Guru™ software (refer to the Guru™ user manual). When manually integrating or adjusting the integration of a peak, the analyst must note that the peak was manually integrated and the reason for the manual integration. This is done on the analytical benchsheet at the time of the manual integration. The final data reviewer is authorized to accept or reject the manual integrations. If they are accepted, the manually integrated peaks are printed and signed and dated as approved by the final data reviewer. The final peak integrations are used to calculate the reported results.

11.2. The following equations are used at BAL to calculate sample results.

11.2.1. To calculate the amount of mercury measured during an analytical run (P), employ the following formula:

P_S = measured Hg (in pg) = $CF_m(A_S - A_{BB})$

Where:
CF_m = mean calibration factor
A_S = measured peak area for the analyzed sample
A_{BB} = mean peak area for Hg in calibration bubbler blanks

11.2.2. To determine the amount of total mercury from the preparation of the method blanks (P_{MB}), use the following formula for each method blank:

$P_{MB} = [(A_{MB} - A_{BB}) CF_M \cdot V_D]/ V_A$

Where:
A_{MB} = peak area of the method blank
A_{BB} = mean peak area for Hg in calibration bubbler blanks
CF_m = mean calibration factor
V_D = final dilution volume of the method blank
V_A = volume of the method blank analyzed

11.2.3. To determine the concentration of total mercury in a sample, the calculation is performed as follows:

$$Hg \text{ (in ng/g or ppb)} = [(P_S/V_A)V_D - P_{MB}]/M$$

Where:
P_S = Hg (pg) from equation in section 11.2
V_A = volume of digestate analyzed in mL
V_D = final dilution volume (mL) of the sample preparation
M = digested sample mass in mg
P_{MB} = the mean total Hg (pg) from the preparation of the method blanks
Note: If a customer does not desire blank correction, then P_{MB} is not subtracted.

11.3. It is BAL's policy to method blank correct sample results unless specifically requested not to do so by the client.

11.4. Method 1631E states that results below the ML should be reported as less than the level of the ML or as required by the regulatory authority, and that field blank results below the ML but above the MDL should be reported to 2 significant digits. Because BAL is not always aware of the original source of a sample or the specific needs or requirements of our clients, all results above the BAL determined MDL are reported to 3 significant digits.

12.0. QUALITY CONTROL

12.1. Refer to EPA Method 1631E, section 9.0, for a detailed description of the quality control procedures employed at BAL for this method. Consult Section 17, Table 1 of this SOP for the current MDL (method detection limit) and ML (minimum limit) determined at BAL for the analysis of mercury using Method 1631E (Table 1). The ML is sometimes referred to as the method reporting limit (MRL). Acceptance criteria and corrective action procedures are listed in Table 2.

12.2. For easy reference for QC criteria refer to Table 2, which outline typical run sequences and required QA samples and Table 3, which describes all required QA frequency requirements and QA acceptance criteria along with corrective actions for failed QA.

12.3. All quality control data should be maintained and available for easy reference and/or inspection.

12.4. Samples containing high analyte concentrations should be analyzed at a reduced volume. For all quantified results, peak areas obtained for samples must ultimately fall below the peak area obtained from the highest standard analyzed and above the peak area obtained from the lowest standard analyzed in the calibration curve.

12.5. Analysts who have not performed EPA Method 1631E previously at BAL must complete an initial demonstration of capability (IDOC) study, which includes the analysis of samples for MDL determination. Refer to Table 3 in Section 18 of this SOP for the general analytical sequence for the IDOC.

12.6. When analyzing client samples, BAL will follow the general analytical sequence found in Table 2 of Section 17 of this SOP. Note that in order to avoid potential carryover from high-level samples, samples projected to have only low levels of mercury (field blanks, method blanks, and samples otherwise expected to be low based on historical data or small amounts of particulate matter) should be analyzed at the beginning of the run sequence. Although EPA Method 1631E suggests (in section 9.1.7) that method blanks may be analyzed intermittently throughout the analysis, BAL analysts should analyze all method blanks at the beginning to avoid potential carryover and to verify that all method blanks meet criteria before proceeding with sample analysis. In addition, whereas Method 1631E only requires analysis of two CCV samples (at the start and at the end of the run sequence), it is BAL policy to run additional CCV samples to ensure ongoing control of the system.

12.7. Matrix spike (MS) and matrix spike duplicate (MSD) recoveries are analyzed at a minimal frequency of one per every 10 client samples. At least one matrix spike sample and matrix spike duplicate sample set must be analyzed per batch and at least two must be analyzed if more than 10 client samples are in a batch. Criterion for MS/MSD analysis is recoveries of 70-130% with a relative percent difference ≤ 30% for sediment and biota and 65-135% with a relative percent difference of ≤ 35% for blood or other samples using the micro method. The target spiking concentration is 2 – 5 times the level of the native sample or 5 times the sample specific MRL, whichever is greater. If there is no historic data on which to base the spike concentration, then the following default spiking levels should be used:

Sediment Samples:	1000 ng/g
Biota Samples:	1000 ng/g
Other Biota Samples:	1000 ng/g

12.8. A minimum of 3 method blanks per batch must be run. The criterion for the method blank is average method blank less than two times the MDL and standard deviation less than 0.67 times the MDL or less than $1/10^{th}$ of the associated client samples. Brooks Applied routinely prepares and analyzes 4 method blanks to allow for the possible outlier.

13.0. METHOD PERFORMANCE

13.1. Refer to the Appendix to EPA Method, section 13.0, for information regarding the verification of this method.

13.2. The detection limits reported in Table 1 were achieved by performing a full MDL study as described in 40 CFR 136, Appendix B. The quality control acceptance limits reported

in Table 3 are developed from the EPA methods and are validated as achievable at BAL in the control charts maintained for the method.

14.0. POLLUTION PREVENTION

14.1. Refer to EPA Method 1631E, section 14.0, for EPA recommendations regarding pollution prevention techniques.

14.2. Whenever feasible, lab personnel should use pollution prevention techniques to limit waste generation. The cost involved in purifying acids makes such recycling unpractical at BAL. Instead, every effort is made to reduce volumes necessary to still produce the best possible results. When making standards, they should be prepared in volumes consistent with their use in the laboratory to minimize the volume of expired standards to be disposed.

15.0 WASTE MANAGEMENT

15.1. Refer to EPA Method 1631E, section 15.0, for information and references related to managing waste produced by application of this method.

15.2. All waste is disposed of in accordance with state and federal regulations either by sewer disposal (only if concentrations are below the King County sewer limits) or through a licensed and bonded hazardous waste disposal facility.

16.0. REFERENCES

16.1. Bloom, N.S. and Crecelius, E.A. (1983). "Determination of Mercury in Seawater at Subnanogram per Liter Levels." *Mar. Chem.* 14:49.

16.2. Bloom, N.S. and Fitzgerald, W.F. (1988). "Determination of Volatile Mercury Species at the Picogram Level by Low-Temperature Gas Chromatography with Cold-Vapour Atomic Fluorescence Detection." *Anal. Chim. Acta.* 208:151.

16.3. EPA Appendix to Method 1631. (2001). "Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation."

16.4. EPA Method 1631.E. (8/02). "Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry."

16.5. Fitzgerald, W.F., and Gill, G.A. (1979). "Sub-Nanogram Determination of Mercury by Two-Stage Gold Amalgamation and Gas Phase Detection Applied to Atmospheric Analysis." *Anal. Chem.* 15:1714.

16.6. Liang, L., and Bloom, N.S. (1992). "Determination of Total Hg by Single-Stage Gold Amalgamation with Cold Vapor Atomic Spectrometric Detection." *JAAS.* June 8:001

17.0. TABLES AND BENCHSHEETS

Table 1. Current Method Detection Limits and Minimum Levels Determined at BAL for the Analysis of Total Mercury in Solids Using EPA Method 1631, Appendix

Matrix	Preparation Method	Default Mass/ Volume	Default Final Volume	Default Analysis Volume	Method Detection Limit (MDL)[1,2]	Limit of Quantitation (LOQ)
Sediment	Aqua regia [redacted] digestion [redacted] BrCl)	1 g (wet) 0.5 g (dry)	40 mL	10 mL (manual) 1 mL (MERX-T)	0.05 ng/g	0.15 ng/g
Coal (samples high in elemental carbon)	HNO_3/H_2SO_4 hot digestion [redacted] BrCl)	0.5 g	40 mL	10 mL (manual) 1 mL (MERX-T)	0.60 ng/g	2.00 ng/g
Biota/Sludge	HNO_3/H_2SO_4 hot digestion [redacted] BrCl)	1 g (wet) < 0.5 g (dry)	40 mL	10 mL (manual) 1 mL (MERX-T)	0.04 ng/g	0.10 ng/g
Biota – Blood in Capillary tubes	HNO_3/H_2SO_4 hot digestion [redacted] BrCl)	20 mg	21 mL	21 mL	1.5 ng/g	4.5 ng/g
Hair (w/washing step)	HNO_3/H_2SO_4 hot digestion [redacted] BrCl)	100 mg	21 mL	5 mL	0.80 ng/g	2.50 ng/g

NOTES:

1. MDL as determined by the procedure 40 CFR Part 136, Appendix B.
2. All MDLs are calculated using a default mass of 1000 mg (wet weight)

Table 2a. Quality Control Acceptance Criteria and General Analytical Run Sequence for the Analysis of Total Mercury on the manual system.

Run	Run Name	Section Name	Analyze	Requirements
01 02 03 04	Calib. Blank Calib. Blank Calib. Blank Calib. Blank	Calibration Blanks	IBL IBL IBL IBL	each CB <40 pg Ave. <20 pg StDev <7.5 pg
05 06 07 08 09	25 pg std 100 pg std 500 pg std 2500 pg std 10000 pg std	Calibration*	25 pg Hg 100 pg Hg 500 pg Hg 2500 pg Hg 10000 pg std	RSD<15% Rec. Low Std. = 75-125%
10	ICV (1568 pg std)	Independent Calibration Verification	1568 pg std, NIST 1641d	Recovery 85-115%
11 12	Method Blank Method Blank	Method Blank	BLK BLK	See Below
13	Cont. Calib. Blank	Carryover Check for High Std	CCB	< 40 pg, ± 20 pg from ave.
14 15	Method Blank Method Blank	Method Blanks	BLK BLK	Average < 2 x MDL and St. Dev. < 2/3rd of MDL or High MB < 1/10th sample
16	SRM-1	Certified Reference Materials	CRM	Recovery = 75-125% Blood CRM Rec. = 65-135%
17	BS	Laboratory Fortified Blank	Blank Matrix Spiked at 4.0 ng	Recovery = 70-130% (use only if no CRM available)
18 19	Sample 01 Sample 01DUP1	Sample Analysis Duplicate Analysis	Sample 01 Native Sample 01 Duplicate	RPD≤30% or ±2xMRL if results ≤5xMRL
20 21	Sample 01MS1 Sample 01MSD1	Matrix Spike Analysis Spike Duplicate Analysis	01 + Spike 01 + Spike	Recovery = 70-130% RPD≤30%**
22 23 24 25 26 27 28 29 30	Sample 02 Sample 03 Sample 04 Sample 05 Sample 06 Sample 07 Sample 08 Sample 09 Sample 10	Sample Analysis	Sample 02 Sample 03 Sample 04 Sample 05 Sample 06 Sample 07 Sample 08 Sample 09 Sample 10	
32	CCV (500 pg std)	Continuing Calibration Verification	5.0 ng·L^{-1} std	Recovery 77-123%
33 34	Sample 11 Sample 11DUP2	Sample Analysis Duplicate Analysis	Sample 11 Native Sample 11 Duplicate	RPD≤30% or ±2xMRL if results ≤5xMRL
35 36	Sample 11MS2 Sample 11MSD2	Matrix Spike Analysis Spike Duplicate Analysis	11 + Spike 11 + Spike	Recovery = 70-130% RPD≤30%**
37 38 39 40 41 42 43 44 45	Sample 12 Sample 13 Sample 14 Sample 15 Sample 16 Sample 17 Sample 18 Sample 19 Sample 20	Sample Analysis	Sample 12 Sample13 Sample 14 Sample 15 Sample 16 Sample 17 Sample 18 Sample 19 Sample 20	
46	CCV (500 pg std)	Continuing Calibration Verification	5.0 ng·L^{-1} std	Recovery 77-123%

Table 2b. Quality Control Acceptance Criteria and General Analytical Run Sequence for the Analysis of Total Mercury on the automated MERX-T system

Run	Run Name	Section Name	Analyze	Requirements
1 2	Rinse Rinse	Blanking Equipment	DIW + analytical reagents	n/a
3 4 5 6	IBL1 IBL2 IBL3 IBL4	Calibration Blank	Analytical Blanks	< 10 pg
7 8 9 10 11 12 13	CAL1 CAL2 CAL3 CAL4 CAL5 CAL6 CAL7	Calibration	5 pg Hg 10 pg Hg 25 pg Hg 100 pg Hg 500 pg Hg 2500 pg Hg 10000 pg Hg	RSD of CF < 15%; recovery of low standard = 75-125%
14	ICV1	Initial Calibration Verification	1568 pg Hg	Recovery 85-115%,
15 16 17	CCB1 CCB2 CCB3	Carryover Blank	DIW + analytical reagents	< 10 pg
18 19 20 21	BLK1 BLK2 BLK3 BLK4	Method Blank	BLK	Average < 2 x MDL and St. Dev. < 2/3rd of MDL or High MB < 1/10th sample
22	SRM-1	Certified Reference Materials	CRM	Recovery = 75-125% Blood CRM Rec. = 65-135%
23	BS	Laboratory Fortified Blank	Blank Matrix Spiked at 4.0 ng	Recovery = 70-130% (use only if no CRM available)
24 25	Sample 01 Sample 01DUP1	Sample Analysis Duplicate Analysis	Sample 01 Native Sample 01 Duplicate	RPD≤30% or ±2xMRL if results ≤5xMRL
26 27	Sample 01MS1 Sample 01MSD1	Matrix Spike Analysis Spike Duplicate Analysis	01 + Spike 01 + Spike	Recovery = 70-130% RPD≤30%**
28	CCV (500 pg std)	Continuing Calibration Verification	5.0 ng·L^{-1} std	Recovery 77-123%
29	CCB	Carryover Blank	DIW + analytical reagents	< 10 pg
Next	10 Injections	Sample Analysis	Samples	
Last	CCV (500 pg std)	Continuing Calibration Verification	5.0 ng·L^{-1} std	Recovery 77-123%
Next	CCB	Carryover Blank	DIW + analytical reagents	< 10 pg

* Calibration Curve may be adjusted depending on expected concentration range of samples and on the linear range due to instrumentation.

** Matrix spike / spike duplicate acceptance criteria for blood and small mass samples is recovery = 65-135% with an RPD ≤ 35%.

Table 3a. QC Criteria and CAPs for the Analysis of Total Mercury by CVAFS

QC Sample	Measure	Minimum Frequency	Criteria	Corrective Action
Bubbler Blank (IBL)	Contamination from bubblers	1 per bubbler used prior to analysis	Manual each ≤ 40 pg avg ≤ 20 pg std ≤7.5 pg MERX-T each ≤ 10 pg	Clean and test bubblers until criteria met prior to any analysis
Calibration Standards (CAL)	Acceptability of the Calibration Curve	Each day prior to analyzing samples and whenever CCV/CRM analysis fails	RSD of response factors ≤ 15%; Recovery of Low Standard = 75 – 125%	Reanalyze suspect calibration standard. If criteria still not met, then remake standards and recalibrate the instrument
Independent Calibration Verification (ICV)	Test of the entire analytical system	1 per batch following the calibration (following calibration blanks if verifying past calibration)	Recovery = 85 – 115%	Correct problem prior to continuing analysis, recalibrate system if required
Continuing Calibration Verification (CCV)	Accuracy	2 per batch (one at the beginning and one at the end of each batch)	Recovery = 77 – 123%	Correct problem and reanalyze CCV. If criteria met, reanalyze samples backwards until 2 consecutive results w/RPD ≤ 20%
Carryover Check Bubbler Blank (CCB)	Contamination due to carryover in the bubbler/trap	On same bubbler/trap following any result exceeding ½ the carryover threshold of 20,000 pg	Manual ≤ 40 pg and within ± 20 pg of avg bubbler blank MERX-T ≤ 10 pg or < 1/10th of associated samples	Clean and continue to test bubbler/trap combo until criteria met prior to further use. Samples analyzed following a result ≥ ½ the carryover threshold must be reanalyzed
Method Blank (BLK)	Contamination from reagents, lab ware, etc.	3 per batch	Avg < 2 x MDL StDev < 2/3rd of MDL or High MB < 1/10th of associated samples	Correct problem until criteria met. All samples associated with a contaminated method blank must be reanalyzed.
Certified Reference Material (CRM)	Accuracy	1 per batch	Recovery = 75 – 125%; Capillary Blood CRM Rec. = 65 – 135%	Correct problem prior to continuing analysis
Matrix Spike/Spike Duplicate (MS/MSD)	Accuracy and Precision within a given matrix	1 per 10 client samples	Recovery = 70 – 130%; RPD ≤ 30% Blood and Small Mass Criteria Recovery = 65 – 135%; RPD ≤ 35%	If **recoveries** similar but fail recovery criteria, interference may be present in the sample and the result must be qualified. If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.

Table 3a continued

QC Sample	Measure	Minimum Frequency	Criteria	Corrective Action
Method Duplicate (DUP)	Precision within a given matrix	In conjunction with MS/MSD samples and when deemed necessary	RPD ≤ 30% Blood and Small Mass Criteria RPD ≤ 35% or for both, results < 5x the MRL and ±2x the MRL of each other	If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.
Analytical Duplicate	Precision at the instrument	When deemed necessary to validate results	RPD ≤ 24% or results < 5x the MRL and ± the MRL of each other	If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.

Brooks Rand Labs

THg Sed/Soil Prep Benchsheet Page

SOP / Rev #: BR-0002 Rev _____

Prepped By: _____ Batch: _____

Preparation Start Date/Time*: _____ Preparation End Date/Time**: _____

* Time is when the first reagents are added. ** Time is when the last sample is brought upto volume

Sample ID	Sample Mass (g)	Sample ID	Sample Mass (g)	Sample ID	Sample Mass (g)

Batch QC ID	Sample Source	Spike vol (uL)	Spike conc (ng/mL)	Spike/CRM ID	Spike Witness

Reagent	ID		0.5 mL BrCl	
8 mL HCl			Final Dilution Vo	
2 mL HNO3				
Balance ID				

(Example Log)

Brooks Rand Labs

THg Biota Prep Benchsheet

SOP / Revision #: BR-0002 Rev _____

Prepped By: _____ Batch: _____

Preparation Start Date/Time*: _____ Preparation End Date/Time**: _____

* Time is when the first reagents are added.

** Time is when the last sample is brought upto volume

Sample ID	Sample Mass (g)	Sample ID	Sample Mass (g)	Sample ID	Sample Mass (g)

Batch QC ID	Sample Source	Spike vol (uL)	Spike conc (ng/mL)	Spike/CRM ID	Spike Wit-ness

Target Temp/Time 1:	70 C/1 hour
Target Temp/Time 2:	90-100 C/3 hrs
Temp/Time 1 (measured / corrected):	/
Temp/Time 2 (measured / corrected):	/
Balance ID:	
Thermometer ID:	
Final Dilution Vol:	

Reagent	ID
7 mL HNO_3	
3 mL H_2SO_4	
0.5 mL BrCl	

Comments:

(Example Log) **Hg Analysis Sheet: T-Hg / Other: _____** Page 1 of ___

Sequence:_____ Batch(es):_____

Analyst:_____ Date:_____ Instrument ID:_____

10ng/mL std ID:_____ 1ng/mL std ID: _____ ICV std ID:_____

$NH_2OH \cdot HCl$ #:_____ $SnCl_2$ #:_____

Initial offset: _____ Initial PMT: _____

Run #	Split Bottle	Trap	Bubb.	Brooks Applied Sample ID	Analy. Vol. (mL)	Dilution Factor	Analysis comments / For spiked QC: Source sample, standard ID, and spiked volume (mL)
1				SEQ-IBL1	---		
2				SEQ-IBL2	---		
3				SEQ-IBL3	---		
4				SEQ-IBL4	---		
5				SEQ-CAL1	0.025		1ng/mL
6				SEQ-CAL2	0.100		1ng/mL
7				SEQ-CAL3	0.050		10ng/mL
8				SEQ-CAL4	0.250		10ng/mL
9				SEQ-CAL5	1.00		10ng/mL
10				SEQ-ICV1	1.00		NIST 1641d
				SEQ-CCB1	---		

Comments:

_____

_____

_____

(Example Log) **Hg Analysis Sheet: T-Hg / Other: ____** Page ____ of ____

Sequence:____________________ Analyst:____________ Date:______________

Run #	Split Bottle	Trap	Bubb.	Brooks Applied Sample ID	Analy. Vol. (mL)	Dilution Factor	Analysis comments / For spiked QC: Source sample, standard ID, and spiked volume (mL)

Comments:

____

____

(Example Log)

(Example Log) **Hg Analysis Sheet : T-Hg MERX-T:_____** Page 1 of____

Sequence:_____ Batch(es):_____

Analyst:_____ Date:_____ Instrument ID:_____

10ng/mL std ID:_____ 1ng/mL std ID: _____ ICV std ID:_____

$NH_2OH \cdot HCl$ #:_____ $SnCl_2$ #:_____

Initial offset: _____ Initial PMT: _____

	Run #/ Pos #	Brooks Applied Sample ID	Analy. Vol. (mL)	Dilution Factor	Analysis comments / For spiked QC: Source sample, standard ID, and spiked volume (mL)
	1	Rinse	---		
	2	Rinse	---		
	3	SEQ-IBL1	---		
	4	SEQ-IBL2	---		
	5	SEQ-IBL3	---		
	6	SEQ-IBL4	---		
	7	SEQ-CAL1	0.010		1ng/mL
	8	SEQ-CAL2	0.025		1ng/mL
	9	SEQ-CAL3	0.100		1ng/mL
	10	SEQ-CAL4	0.050		10ng/mL
	11	SEQ-CAL5	0.250		10ng/mL
	12	SEQ-CAL6	1.000		10ng/mL
	13	SEQ-ICV1	1.000		NIST 1641d
	14	SEQ-CCB	---		
	15	SEQ-CCB	---		
	16	SEQ-CCB	---		

Comments:

_____

_____

Balance ID: _____

(Example Log)

Hg Analysis Sheet : T-Hg / Other:_____ Page____of____

Sequence:_____ Analyst:_____ Date:_____

	Run #/ Pos #	Brooks Applied Sample ID	Analy. Vol. (mL)	Dilution Factor	Analysis comments / For spiked QC: Source sample, standard ID, and spiked volume (mL)

Comments:

_____

_____

_____

_____

_____

Appendix A to BR-0002: Modifications to EPA Method 1631, Appendix

EPA Method 1631		BAL SOP BAL-3101	
SECTION	**DESCRIPTION**	**SECTION**	**DESCRIPTION**
A1.2	These conditions allow determination of Hg at concentrations ranging from 1.0 to 5000 ng/g in solid and semi-solid matrices.	**10.2**	These conditions allow measurements of Hg concentrations in the range of 0.03 – 16,000 ng/g. Dilutions can be made at the time of analysis to extend the range if necessary.
A1.3	The MDL for Hg has been determined to be in the range of 0.24 to 0.48 ng/g when no interferences present. The MRL has been established as 1.0 ng/g. These levels assume a sample size of 0.5 g.	**10.1 & 17, Table 1**	Sample size is [redacted] MDL=0.03-1.5, MRL=0.1 – 4.5 ng/g depending on matrix and prep type.
A4.4	The use of hydroxylamine hydrochloride to remove free halogens is not needed for solid sample digestates.	**10.2**	[redacted] of hydroxylamine hydrochloride is added to the samples at analysis.
A8.3.3	Wet sediment samples must be aliquotted and weighed at the laboratory and prior to freezing. Wet sediment samples may be held for 1 year if frozen at <-15 C.	**8.2, 8.4**	Wet sediment samples are refrigerated at <4 C until analysis. If speciation is needed on the same sample, the sample is stored frozen until analysis then thawed and homogenized prior to analysis.
A9.3.1	Spike and analyze 1 out of every 10 samples of the same matrix type, in duplicate, at a concentration 2 - 5 times the background concentration of Hg in the unspiked sample or at the concentration in the IPR (Appendix Section A9.2.2), whichever is greater. Calculate the percent recovery in each aliquot and the RPD between the aliquots. The individual recoveries and the RPD shall meet the MS/MSD recovery acceptance criteria in Table A1. If either recovery or the RPD does not meet the acceptance criteria, correct the problem and repeat the test according to the procedures in Sections 9.3.4 and/or 9.3.5 of Method 1631B.		Spiking levels for each MS/MSD are 2-5 times any historical data available for each client. Otherwise default spiking levels are 1000ng/g for sediment or biota samples and 500ng/g for invertebrates or plant samples.
A9.4.2	Method blank—For each batch of 20 samples (Section 9.1.7 of Method 1631B), digest and analyze a method blank using the most appropriate reference matrix (Appendix Section A7.1). The laboratory may process a greater number of method blanks, if desired, and average the results. The method blank must include all sample processing steps; e.g., homogenization (Appendix Section A8.3.2.1).	**12.8**	4 Method Blanks are prepped with each batch of samples; a minimum of three should be used to calculate the average. Homogenization blanks are prepared separately if the sample needs more homogenization than stirring with a spatula. No reference material is included in Method Blanks. All method blanks are analyzed at the beginning of each batch to reduce carryover.
A9.5	A9.5 Ongoing precision and recovery (OPR; see Section 9.5 of Method 1631B)—The OPR (laboratory control sample) for solid and semi-solid samples is test of the entire analytical system and includes		No Blank Spike (OPR) is prepared, instead one SRM is prepared per 20 samples in each batch and is taken through the entire analytical process.

	all sample processing procedures; e.g., homogenization (Appendix Section A8.3.2.1) and digestion (Appendix Section A11.1 or A11.2).		
A9.5.1	Analyze an aliquot of the appropriate reference matrix (see Appendix Section A7.1), spiked at the concentration in the IPR.	**12**	A certified reference material is prepared and analyzed with each batch. They are not spiked and the concentration will not necessarily be at the IPR level.
A11.2.2	Cap the vessel with a clean glass marble or inverted fluoropolymer cone.	**10.1.3**	For sediments, the vial caps are loosely placed on top of the vials during digestion.
A12.1	Pipette a 0.01- to 5.0-mL volume of diluted digestate (Appendix Section A11.1.4, A11.2.3, or A11.2.4) directly into a bubbler containing approximately 100 mL of pre-purged SnCl2-containing water.	**10.2**	For analysis on the MERX-T, a maximum of [redacted] added to [redacted] septum topped vial containing [redacted] of DIW [redacted] hydroxylamine hydrochloride then [redacted] stannous chloride is added and the vial is placed in the autosampler for analysis. For analysis on the manual system, the maximum aliquot is [redacted] The digestate is added [redacted] of DIW in a split bottle with [redacted] hydroxylamine hydrochloride. The entire contents of the split bottle are added to the bubbler [redacted] stannous chloride is added.
A12.2	Purge the solution onto a gold trap for 20 minutes. These conditions allow measurement of Hg concentrations in the range of 1 – 5,000 ng/g (parts per billion).	**10.2**	For analysis on the MERX-T, samples are purged for at [redacted] and allows measurements of Hg in the range from 10 to 10,000 pg. For analysis on the manual system, the samples are purged in the bubblers for [redacted] These conditions allow measurements of Hg concentrations in the range of 25 to 10,000 pg.
A12.3	Change the SnCl2-containing water in the bubbler after a total of 10 mL of digestate has been added. Water must be placed in the bubbler and purged for a minimum of 10 minutes prior to addition of another digestate aliquot.	**10.2**	The bubblers are emptied, rinsed and filled with fresh DIW between every sample. The DIW used is sufficiently low in ambient Hg and does not need to be purged prior to use.

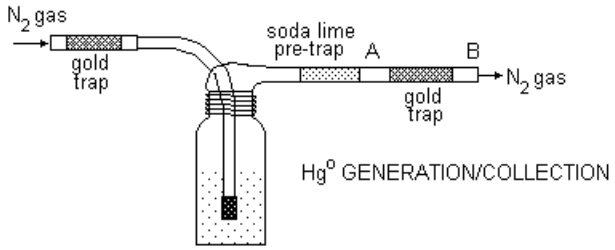
[See Appendix B: Differences between EPA Method 1631e and BAL-3100 for any analysis modifications, as they may also be applicable to BAL-3101.]

Appendix to Brooks Applied Labs Procedure for EPA Method 1631, Revision E (02/05)

DIFFERENCES BETWEEN EPA METHOD 1631, REVISION E, AND BROOKS APPLIED LABS SOP #BAL-3100

The following differences exist between EPA Method 1631, Revision E (with relevant sections marked in boldface), and the practices employed by Brooks Applied Labs (SOP #BAL-3100):

- **Figures 1 and 2** (referenced within **sections 2.6 and 2.7**) give schematic diagrams of the bubbler, purge and trap, CVAFS analytical system. In contrast, for the manual system Brooks Applied Labs (BAL) purges samples with nitrogen in bubblers onto sample traps. After samples are purged, the sample traps are disconnected from the bubbler system and desorbed using argon, as shown below:



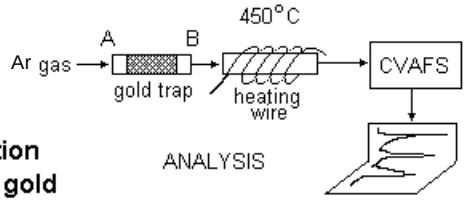


Figure 1:
Schematic diagram of Hg^0 generation/collection and analysis by single gold amalgamation/CVAFS

- **Section 6.4.3** specifies the use of acid fume pretraps consisting of 8-14 mesh soda lime chunks and which are purged [redacted] with nitrogen. For the manual system, BAL uses pretraps consisting of [redacted] lime chunks and which are purged for [redacted] with nitrogen.

- BAL uses direct data acquisition with the BAL "Hg Guru" integration software instead of a chart recorder or integrator as described in **section 6.6**.

- Method 1631E identifies two working standards at 10.0 ng Hg/mL and 0.10 ng Hg/mL (**sections 7.9 and 7.10**) used for instrument calibration. Instead of the 0.10 ng Hg/mL working standard, BAL uses a 1.0 ng Hg/mL standard, along with the 10 ng Hg/mL standard, for calibration.

- **Section 8.5.1 and 11.1.1** states the option of oxidizing the sample with BrCl in the original container. BAL follows that optional protocol.

- **Section 9.3.1** states that the concentration of the spike for matrix spike (MS) and matrix spike duplicate (MSD) samples shall be at 1-5 times the background level of the native sample. BAL accepts a broader spiking level.

- **Section 9.4.1.3** specifies that the mean result for all bubbler blanks analyzed during an analytical batch should be subtracted from all raw data before results are calculated. Instead, BAL subtracts the average peak area measurement of the first four blanks (IBL) analyzed prior to the calibration from all raw data. Split bottles are used for the measurement and/or transfer of all standards, client samples, and quality control samples to the bubblers for manual analysis.

- **Section 9.4.7** specifies that 5% of any lot of bottles should be tested. BAL has found that there is little variation within any given lot of FLPE bottles, glass bottles, or glass vials.

Therefore, BAL randomly tests only 10% of the bottles or vials from the first opened case from each lot before accepting or rejecting the lot, up to a maximum of 10 cases per lot number. A minimum of 10% of the cases of any given lot number are tested. BAL uses the tighter acceptance criterion of THg ≤ 0.2 ng/L, as opposed to ≤ 0.5 ng/L, for passing a lot number of bottles. In addition, four method blanks are prepared with every batch (> 10% of any given batch) using the same bottles that the samples are collected and prepared in, which serves as a further indicator of any potential contamination due to the bottles.

- **Section 10.1** does not state a frequency requirement for calibration. BAL performs a new calibration at the beginning of each analytical day and whenever CCV recovery fails to meet the acceptance criteria as outlined in 1631E, Table 2.

- **Section 10.2.2.1** lists calibration points of 50 pg, 500 pg, 2500 pg, 5000 pg, and 10000 pg Hg created from aliquots of working standards discussed above. BAL uses a calibration curve with points of 10pg, 25 pg, 100 pg, 500 pg, 2500 pg, and 10000 pg. These points are produced by adding 0.010mL, 0.025 mL and 0.100 mL of a 1.0 ng Hg/mL standard (10pg, 25 pg and 100 pg respectively) and by adding 0.050 mL, 0.250 mL, and 1.0 mL of a 10.0 ng Hg/mL standard (500 pg, 2500 pg, and 10000 pg respectively). For Manual Analysis, BAL does not add aliquots of the working standard directly to the bubblers; instead aliquots of standards are added to approximately 100 mL of DI water in a split bottle prior to transfer to the bubbler.

- **Section 11.2.1.2** states that samples are purged with nitrogen for 20 minutes at gas flow rate of 300-400 mL/min. For manual analysis, BAL purges all samples [redacted] High-level standard recoveries and subsequent bubbler blanks indicate [redacted] is an adequate purge time to volatilize and collect mercury. For automated anlaysis, samples are purged for [redacted].

- BAL has not found the step described in section **11.3.2** (passing argon through the sample trap prior to desorption) to be necessary to eliminate condensed water vapor prior to trap desorption.

Figure 3: Schematic Diagram of the Flow-Injection, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System. This system is not employed by BAL.

SOP #BAL-3100

Procedure for EPA Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

Brooks Applied Labs

Supersedes BR-0006 rev 004h

Revision 001

Written 2/12/16

Reviewed _____
_____
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_____

_____	3/1/16
VP of Operations	Date
_____	3/1/16
VP of Quality	Date
_____	3/2/16
Hg Group Leader	Date

Procedure for EPA Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

1.0. SCOPE AND APPLICATION

1.1. Method BAL-3100 is the performance-based procedure followed at Brooks Applied Labs (BAL) as a modification of EPA Method 1631E. BAL has been performing mercury analysis by cold vapor atomic fluorescence since 1989, and during this time has identified several modifications to EPA Method 1631E that improve the quality of the data and the efficiency of the analytical process. These improvements and specific information about the equipment and forms used are detailed within this SOP, following the organizational format of Method 1631E. Unless specifically stated otherwise in this document, all apparatus, materials, reagents, standards, and procedures as stated in EPA Method 1631E are used at BAL.

1.2. Though EPA Method 1631E was written specifically for water samples, this method may be modified, as described in this SOP, for the analysis of many types of aqueous samples, including (but not limited to) chemicals, wastes, and biomonitoring samples (urine and plasma).

2.0. SUMMARY OF METHOD

2.1. Prior to instrumental analysis, the aqueous samples must be prepared according to the procedure discussed in EPA Method 1631E.

2.2. Refer to EPA Method 1631E, section 2.0, for the summary of the method employed at Brooks Applied Labs.

2.3. Refer to the Appendix at the end of this document for a summary of differences between EPA Method 1631, Revision E, and BAL SOP #BAL-3100.

3.0. DEFINITIONS

3.1. A partial list of definitions is presented below. Most definitions can be found for specific terms in the sections where they are first mentioned. Many of the definitions mentioned in this method are based on those found in the glossary for EPA Method 1631E. Please refer directly to this method for a more detailed list.

3.2. May: This action is allowed, but not required.

3.3. May Not: This action is prohibited.

3.4. Must: This action is required.

3.5. Shall: This action is required.

3.6. Should: This action is suggested, but is not required.

4.0. CONTAMINATION AND INTERFERENCES

4.1. Refer to EPA Method 1631E, section 4.0, for a detailed account of possible contamination routes and interferences that may be encountered during the analysis, and descriptions of how these may be avoided or minimized at BAL.

5.0. SAFETY

5.1. Refer to EPA Method 1631E, section 5.0, for safety issues associated with the use of this method.

5.2. Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability, and nervousness. Organo-mercurials may cause permanent brain damage. Because of this, only highly trained personnel familiar with the dangers and precautions to take when working with mercury compounds should ever handle standards and/or high level samples.

5.3. Material safety data sheets (MSDSs) are maintained for all chemicals used in this method. The MSDS sheets are stored in the sample receiving laboratory in appropriately marked binders.

5.4. Refer to the latest revision of the Chemical Hygiene Plan (CHP) for additional safety precautions and required protective equipment.

6.0. APPARATUS AND MATERIALS

6.1. Refer to EPA Method 1631E, section 6.0, for a list of materials used in this method.

6.2. Routine preventative maintenance for the equipment used in this procedure is described in detail in the standard operating procedure BAL-0600 (Maintenance – Analytical Instruments).

6.3. Detailed instructions for the decontamination of bottles and other equipment are described in BAL SOPs #BAL-2300 (Decontamination of Sample Prep Equip.) and #BAL-0102 (Preventing Contamination of Samples).

6.4. Specific equipment used at BAL is listed below. Any modifications to EPA Method 1631E are described and explained. Figure 1 of this document shows the setup of the single-

trap amalgamation system used at BAL and Figure 2 shows the setup of the automated system.

6.4.1. Brooks Rand Instruments (BRI) Model III Atomic Fluorescence Spectrophotometer: To achieve the low detection levels and small, interference-free sample aliquots claimed by this method, a very sensitive CVAFS detector is required. Such systems are built at BRI (Model III) based on the principals discussed in the literature. Refer to the "Brooks Rand Instruments LLC Model III Operations Manual" for instrument operating instructions. [redacted]

6.4.2. BRI MERX Total Purge and Trap Unit: The purge and trap module controls all of the gas flows throughout the system. It contains the gold amalgamation traps and controls their heating and cooling. It contains the soda lime trap, which is responsible for purge gas drying and conditioning.

6.4.3. Autosampler: An AI Scientific septum piercing autosampler with 168 slots is used at BAL. Other autosamplers may be used if they are compatible with the Guru software, have septum piercing capabilities, and can hold the 40 mL glass vials.

6.4.4. BRI Amalgamation Control Module: Controls the heating of the gold-coated bead traps to [redacted] and then the cooling of the traps following desorption of Hg from the trap.

6.4.5. BRI flow meter/needle valve: Capable of controlling and measuring gas flow to the purge vessel at 200-500 $mL \cdot min^{-1}$.

6.4.6. Teflon® adapters (BRI part #08401 through #08404) and tubing (BRI part #08405 through #08407): Connections between components and traps are made using 3.2-mm OD pre-cleaned Teflon® FEP tubing and Teflon® friction-fit or threaded tubing connectors.

6.4.7. Soda lime pre-trap (BRI part #03410): For preventing acid-fumes and moisture from degrading the gold-coated sand or gold wire traps. A 10 cm x 0.9 cm (diameter) Teflon® tube containing 2-3 grams of commercially available reagent grade, non-indicating, [redacted] soda lime chunks, packed in between plugs of silanized glass wool. This trap is purged of Hg by placing it on the output of a clean cold vapor generator, partially filled with deionized water (DIW) and [redacted] stannous chloride ($SnCl_2$), and purging for a [redacted] with nitrogen (N_2) at [redacted] Bubbler blanks are analyzed first so that any indication of unclean pre-traps would be evident immediately.

6.4.8. Cold vapor generator (BRI part #03200): A 220-mL flask with a standard taper 24/40 neck, fitted with a sparging stopper having a coarse glass frit that extends to within 0.2 cm of the flask bottom.

6.4.9. Teflon® split bottle: Used for measurement and pre-reduction of original oxidized samples prior to analysis. A 125-mL blank tested Teflon® bottle with a unique identifier is permanently associated with a corresponding cold vapor generator.

6.4.10. Gold-Coated Glass Bead traps or equivalent (BRI part #03030): Used for trapping gaseous elemental mercury ($Hg^{0)}$. Gold-coated glass bead traps made with gold-coated glass beads are used to analyze solid samples. Both gold-coated sand traps are used to analyze air samples.

6.4.11. Recorder: BAL uses direct data acquisition with the BAL Guru integration software instead of a chart recorder or integrator as described in EPA Method 1631E, section 6.6. The BAL Model III comes complete with the Hg Guru™ integrating software. Refer to the "Brooks Rand Instruments LLC Model III Operations Manual" for Hg Guru™ software/integrator operating instructions. Hg Guru™ software requires an IBM compatible computer (minimum requirements are a Pentium II® processor running at 400 MHz, a CD-ROM Drive, 128 MB RAM, and 50 MB free space on the hard-drive) and runs MS Windows® 98 or higher. Use of this integration software is faster, eliminates the expense of chart recorders and/or integrators, allows for storage of data in diskette form, and eliminates possible transcription errors.

6.4.12. Pipettes: All plastic pneumatic fixed volume and variable pipettes in the range of 10 µL to 5.0 mL.

6.4.13. Sampling Bottles: BAL uses clean and blank tested glass and fluoropolymer lined (FLPE) bottles for sample collection and preparation. For further information on the cleaning (or decontamination) and testing of bottles refer to SOP #BAL-2300.

6.4.14. Nichrome wire coil (BAL part #08300) with plug (#08301): Used for heating the gold trap to thermally desorb the mercury.

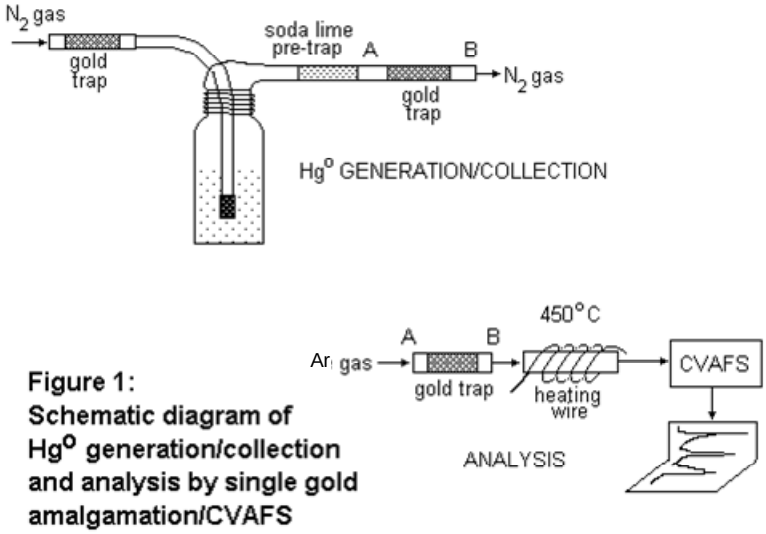
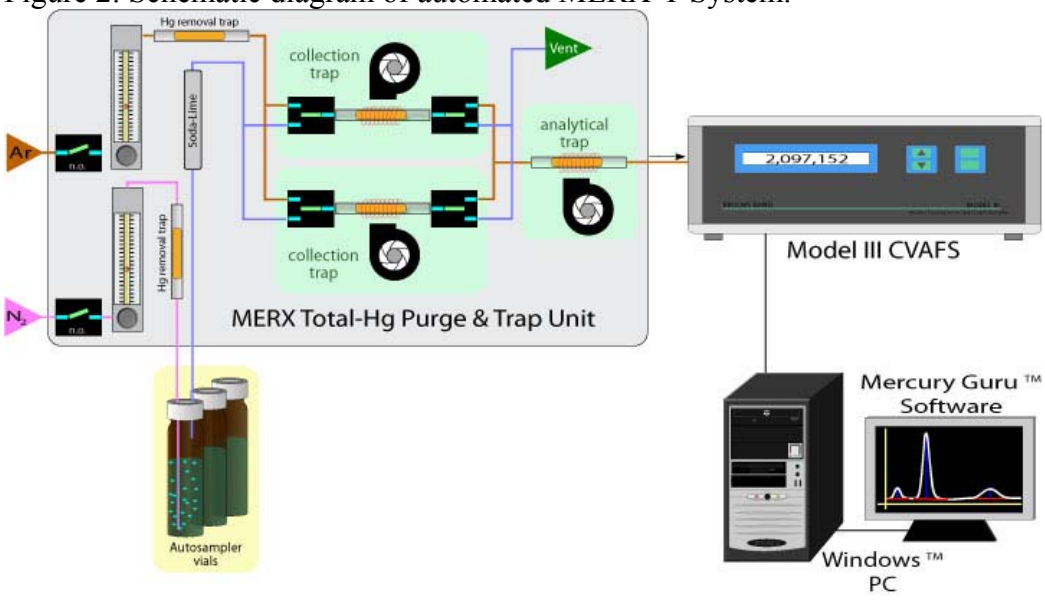


Figure 1: Schematic diagram of Hg^0 generation/collection and analysis by single gold amalgamation/CVAFS

Figure 2: Schematic diagram of automated MERX-T System.



7.0. REAGENTS AND STANDARDS

Document standard or reagent preparation in the standards and reagents log in the LIMS. Record the standard or reagent type, identification number, preparation date, lot number, expiration date, and analyst name in the appropriate fields. Record the standard or reagent type, identification number, preparation date, analyst's initials and expiration date on the container.

7.1. Water: Reagent water is monitored for Hg on a daily basis when calibration blanks are analyzed. A minimum of four 25 mL aliquot of fresh reagent water, each with [redacted] NH_2OH•HCl and [redacted] of $SnCl_2$, are analyzed at the beginning of the run sequence. They

each must be < 10 pg Hg. A high level of mercury detected in the reagent water analysis may also be attributed to the bubbler itself, the $SnCl_2$, or the soda lime pre-traps. Regardless of the source, all analysis is stopped until the source of contamination is determined and the problem is corrected. The results are stored with each batch.

7.2. Air: It is vital that the laboratory air be low in both particulate and gaseous mercury in order to reduce the risk of contamination. Sticky mats are located at the entrance as an precautionary measure. The mercury lab, the sample preparation labs, the shipping and receiving labs and the sample processing lab are monitored monthly for atmospheric mercury levels to ensure that these levels are sufficiently low for ultra-trace level mercury analysis. Air from each lab is pumped through a soda lime pre-trap and onto either a gold wire or gold-coated sand trap at a flow rate of 1 L/min until at least 20 L of air have been collected per trap. A warning level has been established at 15 ng Hg/m^3 with a shutdown control level at 25 ng Hg/m^3. Results from the monthly air tests are stored electronically on the BAL computer server.

7.3. Hydrochloric acid: Trace-metal purified reagent HCl is purchased and analyzed for Hg before use. In general, it is possible to obtain acid containing less than 5 pg/mL Hg. When a lot number meeting this specification is found, several cases are purchased. Generally, lower values can be obtained in this manner than by re-distilling acid in the laboratory. Only "trace-metal' grade acid should be used. Acids labeled as "ULTRA-PURIFIED" have historically had higher concentrations of mercury and should be avoided.

7.4. Hydroxylamine hydrochloride ($NH_2OH•HCl$), stannous chloride ($SnCl_2$), and bromine monochloride (BrCl): Each of these reagents is prepared according to instructions listed in section 7.0 of Method 1631E. Reagent blanks must be analyzed each time that BrCl is made anew. The SnCl2 and $NH_2OH•HCl$ are tested daily whenever calibration blanks are analyzed.

Note: BrCl is tested whenever a new bottle is prepared prior to use with samples. During initial testing, reagent blanks are prepared in 40mL glass vials at four different concentrations of BrCl (1.0%, 2.0%, 5.0%, and 10%). The BrCl is pre-reduced in the sample vial and then stannous chloride is added and purged using MERX Total HG Purge & Trap Module. The amount of Hg per reagent unit (reagents used in a 1.0% BrCl method blank) must be ≤ 10 pg.

7.5. Stock mercury standard: A commercially available 1000 µg/mL mercury standard that is traceable to NIST is used. This stock standard should be replaced by the manufacturer's expiration date.

7.6. Intermediate mercury standard solution: 0.250 mL of the stock solution is diluted to 250.0 mL with ultrapure deionized water and 5.0 mL of BrCl. This solution contains 1000 ng/mL Hg, and must be kept refrigerated in a tightly closed Teflon® bottle. This solution should be replaced bi-annually.

7.7. Mercury working standards: 2.50 mL of the intermediate mercury standard solution is diluted to 250 mL with high purity water containing 2.50 mL BrCl solution, to make a

10.0 ng/mL working solution (as discussed in EPA Method 1631E, section 7.9). A 1.00 ng/mL working standard should be made by diluting 0.250 mL of the intermediate mercury standard to 250 mL with DIW containing 2.50 mL BrCl solution. These working standards of 10.0 ng/mL and 1.00 ng/mL are added in appropriate aliquots to reagent water and analyzed to create the calibration curve and CCV samples.

7.8. Independent Calibration Verification (ICV) Standard: 0.250 mL of the standard reference material (SRM) NIST 1641d (mercury in water) is added 247.25 mL DIW and 2.5 mL BrCl for a final volume of 250 mL. After adjusting for the density of the SRM, the final value for this standard is 15.68 ng/L. NIST 1641d is purchased directly from the National Institute of Standards and Technology.

7.9. Nitrogen: Grade 4.8 (99.999% purity) minimum nitrogen that has been further purified by the removal of Hg using a gold coated sand trap.

7.10. Helium or Argon: Grade 4.8 (99.999% purity) minimum inert gas which has been further purified by the removal of Hg using a gold-coated sand trap.

7.11. Soda Lime: for soda lime canister. 4-8 mesh. Fisher AA36596A4 or equivalent.

7.12. Silanized Glass Wool: for soda lime canister. Supelco 2-0410 or equivalent.

8.0. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

8.1. Refer to EPA Method 1631E, section 8.0, and EPA Method 1669 (*Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels*) for a detailed description of sample collection, preservation, and storage methods.

9.0. QUALITY CONTROL

9.1. Refer to EPA Method 1631E, section 9.0, for a detailed description of the quality control procedures employed at BAL for this method. Consult Section 18 of this SOP for the current MDL (method detection limit) and ML (minimum limit) determined at BAL for the analysis of mercury using Method 1631E (Table 1). The ML is sometimes referred to as the method reporting limit (MRL). Acceptance criteria and corrective action procedures are listed in Table 2.

9.2. All quality control data should be maintained and available for easy reference and/or inspection.

9.3. Samples containing high analyte concentrations should be analyzed at a reduced volume. For all quantified results, peak areas obtained for samples must ultimately fall below the peak area obtained from the highest standard analyzed and above the peak area obtained from the lowest standard analyzed in the calibration curve.

9.4. Analysts who have not performed EPA Method 1631E previously at BAL must complete an initial demonstration of capability (IDOC) study, which includes the analysis of samples for MDL determination. Refer to Table 3 in Section 18 of this SOP for the general analytical sequence for the IDOC.

9.5. When analyzing client samples, BAL will follow the general analytical sequence found in Table 4 of Section 18 of this SOP. Note that in order to avoid potential carryover from high-level samples, samples projected to have only low levels of mercury (field blanks, method blanks, and samples otherwise expected to be low based on historical data or small amounts of particulate matter) should be analyzed at the beginning of the run sequence. Although EPA Method 1631E suggests (in section 9.1.7) that method blanks may be analyzed intermittently throughout the analysis, BAL analysts should analyze all method blanks at the beginning to avoid potential carryover and to verify that all method blanks meet criteria before proceeding with sample analysis. In addition, whereas Method 1631E only requires analysis of two CCV samples (at the start and at the end of the run sequence), it is BAL policy to run additional CCV samples (one after every 10 client samples) to ensure ongoing control of the system.

9.6. EPA Method 1631E states that spiking levels of the MS/MSD shall be equal to the regulatory compliance limit or 1-5 times the background concentration of the sample, whichever is greater. Typically 1-20 times the background concentration is considered and acceptable spike level.

Note: Since it is known that there is a possibility of mercury sticking to bottle walls, BAL oxidizes samples in their original bottle in order to labilize any mercury that may be sticking to the bottle walls. This does not allow for representative aliquots to be taken prior to prep and so MS/MSDs are not prepared at the time of prep. BAL prepares MS/MSDs at the time of analysis.

9.7. A minimum of 3 method blanks per batch must be run or 1 per every 20 client samples, whichever is greater. Method blanks must be prepared with the same lot of BrCl used to prepare the samples. Section 12.3 describes how the method blanks are used to correct results for samples prepared at BrCl concentrations other than 1.0%. BAL routinely prepares and analyzes 4 method blanks to allow for the possible outlier.

In the event that samples need to be added to a batch following the initial preparation of method blanks (such as quick turn-around-samples), a single check method blank must be prepared using the same reagents as those used to prepare the method blanks for the batch. The check method blank is uploaded as a SEQ-ICB and is evaluated against the method blanks used to correct the data. The check blank must be less than 2 times the MDL or be within 3 standard deviations of the average of the method blanks used to correct the sample results. If the criteria are not met, then the sample(s) prepared at the same time as the check method blank are evaluated against the result for the check method blank and qualified J if not greater than 10 times the result of the check method blank. Under no circumstances can samples be added to a previously prepared batch if they cannot be prepared with the exact same reagent lots.

10.0. CALIBRATION AND STANDARDIZATION

10.1. Refer to EPA Method 1631E, section 10.0, for a detailed description of instrument calibration.

10.2. Instrument Calibration: BAL has adopted the following procedure. There are two different analytical systems that may be used for analysis of samples. They can be analyzed by manual analysis or by using the MERX-T automated total mercury system. Both systems use analogous techniques with some minor changes at sample analysis. The AFS detectors used are the same for either instrument. The AFS detectors built by BAL are capable of achieving extremely low detection limits.

It is required that a calibration curve of at least 5 points be prepared prior to the analysis of samples to verify linearity and quantify sample concentrations. Sections 9.0 and 10.0 of EPA Method 1631E do not state a frequency requirement for calibration. BAL performs a new calibration each day and when it is observed that the instrument is no longer in control. Additionally, analysis of the ICV standard must also meet the criterion in Table 2 for the calibration to be validated

10.2.1. For manual analysis, the instrument is calibrated down to 25 pg. The standards typically used to calibrate the instruments for total mercury at BAL are 25 pg, 100 pg, 500 pg, 2500 pg, and 10,000 pg. The standards are added to the split bottle that contains approximately 100 mL of DI water, pre-reduced with $NH_2OH \cdot HCl$, and then poured into the bubbler. The calibration is based on the measured picograms of mercury in the bubbler and not on concentration.

10.2.2. For automated analysis on the MERX-T, the instrument is calibrated down to 10 pg. The standards typically used to calibrate the instruments for total mercury are 10 pg, 25 pg, 100 pg, 500 pg, 2,500 pg and 10,000 pg. The standards are added to a 40 mL septa top vial that contains approximately 25 mL of DI water, pre-reduced with $NH_2OH \cdot HCl$. Stannous chloride is added to the vial which is then capped and placed on the autosampler.

10.2.3. Section 9.4.1.3 of EPA Method 1631E suggests that the mean peak area for all bubbler blanks (including those analyzed during the analytical sequence to ensure that no carryover occurs from high-level samples) should be subtracted from all raw data before results are calculated. This does not allow for the continuous determination of whether QA results are in control, thereby forcing the analyst to analyze all samples prior to determining if all QA criteria are met. BAL subtracts the average peak area measurement of the first four bubbler blanks (or one blank for each split bottle and bubbler used in the analysis of samples) analyzed at the beginning of the analytical run from all raw data for result calculations. The acceptance criterion for the initial bubbler blanks for manual analysis (referred to as calibration blanks) is each $\leq$ 40 pg, average $\leq$ 20 pg, and standard deviation $\leq$ 7.5 pg. The criterion for subsequent bubbler blank checks is that each blank must be $\leq$ 40 pg and $\pm$ 20 pg from the calibration blank mean. For automated analysis,

the acceptance criterion for the initial bubbler blanks is each ≤10 pg. The criterion for subsequent bubbler blank checks is that each blank must be ≤10 pg and ± 5 pg from the calibration blank mean.

10.2.4. BAL uses the following equation to calculate the calibration factor (CF_x) for Hg in each of the standards:

$$CF_x = (C_x) / (A_x - A_{CB})$$

Where:
C_x = mass of Hg in standard analyzed (pg)
A_x = peak area for Hg in standard
A_{CB} = mean peak area for Hg in calibration blanks

This calculation differs from the calculation given in 1631E in that the numerator and denominator are reversed.

11.0. PROCEDURE

11.1. Sample Preparation: Refer to EPA Method 1631E, section 11.0, for more detailed guidelines regarding sample preparation and analysis. Practices employed specifically by BAL are outlined below and in the appendix to this SOP.

11.1.1. Four method blanks are prepared with each batch of samples. All method blanks are preserved to approximately [redacted] BrCl in DIW. This is allowable because BAL tests each new batch of BrCl made to prove that it maintains linearity with increased concentration.

The samples are then preserved to a percentage of BrCl that is dependent upon amount of particulate in sample. A clear sample would [redacted]; a fairly clear sample with some particulate would get [redacted] BrCl; a sample with lots of particulate would get [redacted]. If a sample has a yellow tinge to it, a yellow sticker is placed on top of the bottle to indicate that the coloration is due to the matrix and not necessarily due to any BrCl added to the sample during preparation. After the addition of BrCl, the samples must be ovened for a minimum of 4 hours at 65ºC.

NOTE: [redacted]

11.1.2. Total mercury samples are oxidized in their original container in accordance with the allowance in EPA Method 1631e section 8.5.1 and 11.1.1. Since this does not allow matrix spikes or matrix spike duplicates (MS/MSD) to be prepared at the time of preservation. MS/MSD are prepared at the time of analysis as stated in section 9.6 of this SOP.

11.1.3. All batches are placed in an oven for 4 hours at approximately 65ºC. Prior to analysis an oxidation check is performed. The oxidation check consists of looking at each sample individually to check that the sample is still yellow, which indicates the BrCl was added in excess. Any sample that is suspected of not having excess BrCl, or a sample with a yellow sticker on top is tested with starch iodide indicator paper. If the paper turns purple, then the sample is presumed to be adequately preserved. If the paper stays white, more BrCl is added to the sample and the sample is re-ovened. If additional BrCl was added to any of the samples in a batch, a subsequent oxidation check is required.

11.1.4. Urine samples should be oxidized with [redacted] BrCl by default. They must be checked for excess BrCl using starch iodide paper as the BrCl color will be indistinguishable from the color of the urine itself.

11.2. Instrumental Analysis Manual System: BAL has adopted the following modifications from EPA Method 1631E.

[redacted] Samples and standards are purged for [redacted]

11.2.2. Four 100-mL aliquots of water are poured into each of the bubblers prior to calibration. [redacted] of $SnCl_2$ is added to each bubbler and the aliquots are used to condition the soda-lime pre-traps for [redacted]. Following this pre-trap conditioning step, the purged water is discarded and 100 mL aliquots of DIW along with [redacted] NH_2OH•HCl are poured from the four split bottles into their associated bubblers. [redacted] $SnCl_2$ is added to this water, which is then purged for [redacted] onto a gold amalgamation trap and analyzed for Hg. A high level of mercury in the calibration blanks may be attributed to the split bottle, the bubbler, the reagents, or the soda lime pre-traps. Regardless of the source, all analysis using the contaminated bubbler/split bottle/trap combination is stopped until the source of contamination is determined and the problem is corrected. Use of all uncontaminated bubblers, split bottles, and traps may continue during this process.

11.2.3. All calibration standards and QC samples are added to 100 mL aliquots of DIW in a thoroughly rinsed split bottle prior to transfer to a bubbler for analysis.

11.2.4. Aliquots of the original oxidized sample of approximately 100 mL are weighed into split bottles.

Note: Samples suspected or known to be significantly denser than freshwater, such as brine water and FGD samples should either be pipetted or have a quick density check performed on them (pipette 1 mL of sample and weigh it to calculate g/mL). The density check must be recorded, and if necessary, the volume entered into LIMS must be density corrected. A density check need not be performed if the sample is pipetted. If pipetted, a

note must be made on the benchsheet stating that the value recorded is a measured volume and not a mass. The I.D. of the pipette used must be recorded on the benchsheet. Sample volumes may also be measured using 50-mL Environmental Express tubes.

[redacted] of NH_2OH•HCl is added directly to the split bottle and allowed to react for at least [redacted] prior to transfer to the bubbler for purging onto a gold amalgamation trap for analysis. Split bottles and bubblers are rinsed a minimum of three times with DIW in between sample aliquots.

11.2.5. Urine samples should be pipetted into split bottles, as the density of urine is not equivalent to the density of water. A maximum of 25 mL will be used (giving an MRL/MDL 4x higher than for water samples). Urine samples can be analyzed in the same batch as water samples but must have QC performed on at least 1 urine sample.

11.2.6. As discussed in Method 1631E and in section 9.5 of this SOP, follow the attached analytical sequence (Table 4). Generally, analysts should follow the suggestion in Method 1631E that samples suspected to contain the lowest concentration of mercury (i.e., known blank samples) should be analyzed first followed by samples containing potentially higher levels (i.e., known influent samples).

11.2.7. As discussed in section 9.5 of this SOP, the analyst may choose to analyze CCV samples more frequently than required by Method 1631E. A CCV must be analyzed after every 10 client samples (not counting quality control samples) to verify ongoing control of the system.

[redacted] The PMT and offset are recorded on the bench sheet at the beginning of each day after auto zeroing the instrument previous to measuring the noise. [redacted]

11.2.9. BAL has found that checking for mercury carryover in a bubbler by analyzing a bubbler blank is not necessary unless an unusually high level sample has been purged. Carryover tests, performed using spikes of 10,000 pg, 20,000 pg, 40,000 pg, and 100,000 pg, resulted in carryover of less than 50 pg Hg from the bubbler and trap. To avoid carryover from the bubbler or split bottle, the analyst should rinse each bubbler and split bottle three times with DIW between all samples. To avoid carryover from the trap, the analyst should heat the mid- and downstream sections of any traps associated with a higher-level sample (such as any sample above the calibration range) for an additional 3 minutes following sample desorption. If a purged sample contains > 20,000 pg Hg, the analyst must

follow the above corrective actions and analyze a bubbler blank and split bottle blank check using fresh DIW and the associated bubbler, split bottle and trap. The analysis of the split bottle blank should be performed on a different bubbler to facilitate proper contamination identification. Using previously purged DIW is not necessary for the bubbler blank. If any bubbler or split bottle blank fails (> 40 pg Hg and/or > ± 20 pg from average calibration blank on associated bubbler and trap), the analyst must identify and correct the source of contamination, and demonstrate that the bubbler and trap pass the blank criterion before sample analysis can continue using that bubbler, split bottle, and trap.

11.2.10. Any samples run in a bubbler, split bottle and/or on a trap associated with a carryover sample (e.g., a sample with > 20,000 pg Hg) must be reanalyzed, if sufficient sample volume exists.

11.2.11. After analysis is complete, split bottles and bubblers are rinsed with DIW three times and filled with DIW. [redacted]

11.3. BAL has adopted the following modifications from EPA Method 1631E for the automated analysis of mercury using the MERX-T.

11.3.1. Samples and standards are purged for 7 minutes at 400 mL/min. However, longer purge times may be necessary if the lab and/or sample temperature is < 16 °C. Lab temperature is maintained between 20 ºC to 25 ºC, so longer purging times are not generally necessary.

11.3.2. For the MERX-T, each 40-mL septa top vial becomes the samples' bubbler. See the MERX-T Manual for an instrument description and overview of how it functions. Approximately 25 mL of DI water is added to each vial that is for blanks or standards. For samples, the appropriate amount of sample (max 25 mL) is added to each vial and if it is less than 25 mL, the vial is brought up to approximately 25 mL with DI water. If spikes are needed, the correct amount of standard is pipetted into the appropriate vial and 0.200 mL of hydroxylamine-HCl solution is added to each vial for pre-reduction of the BrCl. 0.200 mL of stannous chloride solution is added to each vial shortly after which the vial is capped and shaken before being placed on the autosampler.

11.3.3. All calibration standards and QC samples are added to 25 mL aliquots of DIW in a 40-mL setpa top vial for analysis.

11.3.4. Aliquots of the digestate, usually in the range of 1-25 mL (maximum of 25 mL due to necessary space needed for purging in the vial), is added to 0-25 mL of deionized water in a 40-mL septa top vials. 0.2 mL of NH_2OH•HCl is added directly to the vial and allowed to react prior to the addition of 0.2 mL of $SnCl_2$. Shortly after which the vial is capped and shaken before being placed on the autosampler.

11.3.5. As discussed in Method 1631E and in section 9.5 of this SOP, follow the attached analytical sequence (Table 4). Generally, analysts should follow the suggestion in Method 1631E that samples suspected to contain the lowest concentration of mercury (i.e., known blank samples) should be analyzed first followed by samples containing potentially higher levels (i.e., known influent samples).

11.3.6. As discussed in section 9.5 of this SOP, the analyst may choose to analyze CCV samples more frequently than required by Method 1631E. A CCV is analyzed after every 10 sample injections to verify ongoing control of the system.

11.3.7. The PMT and offset are recorded on the bench sheet at the beginning of each day after auto zeroing the instrument previous to measuring the noise. Since the analysis is automated and may take place while the analyst is not present, the PMT is not adjusted during an analytical run.

11.3.8. Since the MERX-T utilizes the individual vials as bubblers, "bubbler" carryover is not a concern. Carryover can occur on the run just after a high sample (subsequent run carryover) and/or on the second run after a high sample (trap carryover).

BAL has found that checking for mercury carryover by analyzing a carryover blank is not necessary unless an unusually high level sample has been purged. Carryover tests, performed using spikes of 10,000 pg, 20,000 pg, 40,000 pg, and 100,000 pg, resulted in carryover of less than 12.5 pg Hg. If a purged sample contains > 20,000 pg Hg, the analyst must reanalyze the high sample (at an appropriate dilution) and the two samples that immediately follow it to check that the samples are not biased high due to carryover.

11.3.9. New vials are used for every analysis to reduce the possibility of cross contamination. If the autosampler needle needs cleaning, it can be placed in a vial containing [redacted].

11.4. Gold traps should be tracked by unique identifiers so that any trap producing poor results can be quickly recognized and discarded. Occasionally due to inadvertent contact with halogen fumes, bubbler solution, organic fumes, or overheating, a sampling trap will become damaged, giving low and/or irreproducible results. Suspect traps should be checked with at least two consecutive standard runs before continued use. Traps should be replaced quarterly or as soon as possible after quality control results indicate their degradation. Additionally, traps should be replaced whenever integration peaks become abnormally shaped (no longer symmetrical with steep slopes).

11.4.1. The MERX-T uses dual amalgamation and contains only 2 sample collection traps and 1 analytical trap. Guru automatically records the sample collection trap used for analysis (X or Y). The agreement between the two traps should be assessed during the calibration. If significant inconsistencies are noted between the X and Y trap, then the problem should be addressed and fixed and the system should be recalibrated before analyzing client samples. When replacing traps, all three should be replaced at the same time.

11.4.2. Section 11.3.2 of Method 1631E states that before each trap is analyzed, argon should be passed through the trap for approximately two minutes to drive off condensed water vapor prior to heating and desorbing for three minutes. Historically, BAL has not experienced significant analytical problems associated with water vapor on traps. However, if the analyst observes peaks that desorb prior to the mercury peak, this technique may be employed.

12.0. DATA ANALYSIS AND CALCULATIONS

12.1. All instrument peaks are integrated using Hg Guru™ software. This integration is automated; however, there are occasions when Guru™ will not properly integrate a peak. This is most common with small peaks, abnormally shaped peaks, or peaks with excessive tailing. When a peak is not properly integrated by Guru™, the analyst may manually integrate or adjust the integration of the peak using the Guru™ software (refer to the Guru™ user manual). When manually integrating or adjusting the integration of a peak, the analyst must note that the peak was manually integrated and the reason for the manual integration. This is done on the analytical benchsheet at the time of the manual integration. The final data reviewer is authorized to accept or reject the manual integrations. If they are accepted, the manually integrated peaks are printed and signed and dated as approved by the final data reviewer. The final peak integrations are used to calculate the reported results.

12.2. The following equations are used at BAL to calculate sample results.

12.2.1. To calculate the amount of mercury measured during an analytical run (P), employ the following formula:

$$P = Hg\ (pg) = CF_m\ (A_S - A_{CB})$$

Where:
CF_m = mean calibration factor
A_S = gross peak area measured in sample analysis
A_{CB} = mean peak area for Hg in calibration bubbler blanks

12.2.2. To determine the concentration of total mercury in a sample, the calculation is performed as follows:

$$\text{Hg (in ng/L or ppt)} = \{[(P/V_A)*V_D]\text{-MB}\}/\ V_O$$

Where:
P = Hg (pg) from equation in section 12.2
V_A = volume (mL) of the sample preparation that was analyzed
V_D = final dilution volume (mL) of the sample preparation
V_O = volume (mL) of the original sample used in the preparation
MB = multiple of the average result (in total pg Hg) for the 0.5% BrCl method blanks.

Note: The multiplier of the average 1.0% BrCl method blank result is based on the concentration of BrCl in the prepared sample. A multiplier of 1 is applied if the sample was prepared at 1.0% BrCl, 2 if the sample was prepared at 2.0% BrCl, 5 if the sample was prepared at 5.0% BrCl, etc.

12.3. It is BAL's policy to method blank correct sample results unless specifically requested not to do so by the client.

12.4. Method 1631E states that results below the ML should be reported as less than the level of the ML or as required by the regulatory authority, and that field blank results below the ML but above the MDL should be reported to 2 significant digits. Because BAL is not always aware of the original source of a sample or the specific needs or requirements of our clients, all results above the BAL determined MDL are reported to 3 significant digits.

13.0. METHOD PERFORMANCE

13.1. Refer to EPA Method 1631E, section 13.0, for information regarding the verification of this method.

13.2. The detection limits reported in Table 1 were achieved by performing a full MDL study as described in 40 CFR 136, Appendix B. The quality control acceptance limits reported in Table 2 are developed from the EPA methods and are validated as achievable at BAL in the control charts maintained for the method.

14.0. POLLUTION PREVENTION

14.1 Refer to EPA Method 1631E, section 14.0, for EPA recommendations regarding pollution prevention techniques.

14.2 Whenever feasible, lab personnel should use pollution prevention techniques to limit waste generation. The cost involved in purifying acids makes such recycling unpractical at BAL. Instead, every effort is made to reduce volumes necessary to still produce the best possible results. This analysis requires small amounts of acid to be used in the preparation of the samples [redacted]. When making

standards, they should be prepared in volumes consistent with their use in the laboratory to minimize the volume of expired standards to be disposed.

15.0. WASTE MANAGEMENT

15.1. Refer to EPA Method 1631E, section 15.0, for information and references related to managing waste produced by application of this method.

15.2. All waste is disposed of in accordance with state and federal regulations either by sewer disposal (only if concentrations are below the King County sewer limits) or through a licensed and bonded hazardous waste disposal facility.

16.0. REFERENCES

16.1. EPA Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, August 2002 (including references cited in section 16.0).

16.2. Model III (CVAFS Mercury Analyzer) Operating Manual, September 2004. Brooks Rand Instruments LLC, 3958 6th Ave NW, Seattle, WA.

16.3. EPA Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels, July 1996.

17.0. GLOSSARY

17.1. Refer to EPA Method 1631E, section 17.0, for additional definitions of terms used throughout the text of 1631E.

18.0. FIGURES (1631E), TABLES, AND BENCHSHEETS

Table 1. Current Method Detection Limits and Minimum Levels Determined at Brooks Applied Labs for the Analysis of Total Mercury in Water Using EPA Method 1631

Matrix	Preparation Method	Method Detection Limit (MDL)[1]	Minimum Level (ML)[2]
Water	Digestion	0.10 ng/L	0.40 ng/L

NOTES:
1. MDL as determined by the procedure 40 CFR Part 136, Appendix B. MDL and ML reported here are for method blank corrected results.
2. At BRL the ML is often referred to as the method reporting limit (MRL).

Table 2. Quality Control Criteria and Corrective Action Procedures for the Analysis of Total Mercury by CVAFS

QC Sample	Measure	Minimum Frequency	Criteria	Corrective Action
Calibration Blanks / Bubbler Blank	Contamination from split bottles / bubblers	1 per split bottle / bubbler used	Manual each ≤ 40 pg avg ≤ 20 pg std ≤ 7.5 pg MERX-T ≤ 10 pg	Clean and test split bottles / bubblers until criteria met prior to any further analysis
Calibration Standards	Acceptability of the calibration curve	Daily (first batch of the day) or when ICV/CCV fail	RSD of response factors ≤ 15%; Recovery of Low Standard = 75 – 125%	Reanalyze suspect calib stand w/diff trap/bubbler. If criteria still not met, then remake standards and recalibrate the instrument.
Continuing Calibration Verification (CCV)	Accuracy	1 immediately after calibration followed by 1 every 10 client samples and 1 at the end of each batch)	Recovery = 77 – 123%	For manual system - Correct problem and reanalyze CCV. If criteria met, reanalyze samples backwards until 2 consecutive results w/RPD ≤ 20% or recalibrate. For MERX-T – reanalyze samples bracketed by failing CCV unless QA Manager accepts a passing spike instead.
Carryover Check Bubbler Blank	Contamination due to carryover in the bubbler/trap	Perform on same bubbler/trap combination following any measured result ≥ 20,000 pg	Manual ≤ 40 pg and within ± 20 pg of avg bubbler blank MERX-T ≤ 10 pg or <1/10th of associated samples	Remake and condition the soda-lime trap. Clean and continue to test bubbler/trap combo until criteria met prior to further use. Samples analyzed using same bubbler and/or trap following a result ≥ 20,000 pg must be reanalyzed.
Method Blank ([redacted] BrCl in reagent water)	Contamination from reagents, lab ware, etc.	3 per batch	Each MB ≤ 0.5 ng/L and StDev ≤ 0.1 ng/L or highest MB < 0.1 times the lowest reported result.	Correct problem until criteria met. All samples affected by high method blanks (sample < 10x the highest MB) must be qualified accordingly.
Independent Calibration Verification (ICV)	Independent check of system performance	1 per batch	Recovery = 85 – 115%	Correct problem prior to continuing analysis. Otherwise, recalibrate system.
Matrix Spike / Matrix Spike Duplicate	Accuracy and Precision within a given matrix	1 per 10 client samples	Recovery = 71 – 125%; RPD ≤ 24%	If **recoveries** similar but fail recovery criteria, an interference is present in the sample and the result must be qualified. If **RPD** criteria not met, then the MS/MSD should be reanalyzed.
Method Duplicate	Precision within a given matrix	As per client request	RPD ≤ 24% or if results < 5x the MRL then ± the MRL of one another	If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.

Table 3. Quality Control Acceptance Criteria and General Analytical Run Sequence for the Analysis of Total Mercury using the MERX-T System.

Run	Run Name	Section Name	Analyze	Requirements
1 2	Rinse Rinse	Rinses	Rinse DIW	N/A
3 4 5 6	IBL IBL IBL IBL	Calibration Blanks	Split bottle blank Split bottle blank Split bottle blank Split bottle blank	< 10 pg
7 8 9 10 11 12	10 pg std 25 pg std 100 pg std 500 pg std 2500 pg std 10000 pg std	Calibration	10 pg Hg 25 pg Hg 100 pg Hg 500 pg Hg 2500 pg Hg 10000 pg Hg	StDev < 15% of Ave. RF Recovery of 10 pg std = 75-125%
13	ICV (1568pg std)	Independent Calibration Verification	1568 pg (NIST 1641d)	Recovery 85-115%
14	CCB	Continuing Calibration Blank	Same trap associated with the 10000 pg std.	Each < 10 pg
15	CCV (500pg std)	Continuing Calibration Verification	5.0 ng/L	Recovery 77-123%
16 17 18	CCB CCB CCB	Continuing Calibration Blank	DIW and reagents	Each < 10 pg
19 20 21 22	Method Blank 01 Method Blank 02 Method Blank 03 Method Blank 04	Method Blank	[redacted] BrCl MB	Each ≤ 0.40 ng/L StDev ≤ 0.10 ng/L
Next	Field, Equipment, or Bottle Blanks	Field, Equipment, or Bottle Blanks	Field, Equipment, or Bottle Blanks	< ML or < 1/5th of associated sample conc.
Next	1st Half of Samples (including MS1 and MSD1)	Sample Analyses Matrix Spike Matrix Spike Duplicate	Sample X X + spike X + dup. Spike Other Samples	Recovery 71-125%; RPD < 24% Recovery 71-125%; RPD < 24%
Next	CCV (500pg std)	Continuing Calibration Verification	5.0 ng/L	Recovery 77-123%
Next	CCB	Continuing Calibration Blank	DIW and reagents	Each < 10 pg
Next	2nd Half of Samples (including MS2 and MSD2)	Sample Analyses Matrix Spike Matrix Spike Duplicate	Sample Y Y + spike Y + dup. Spike Other Samples	Recovery 71-125%; RPD < 24% Recovery 71-125%; RPD < 24%
Next	CCV (500pg std)	Continuing Calibration Verification	500 pg Hg	Recovery 77-123%
Last	CCB	Continuing Calibration Blank	DIW and reagents	Each < 10 pg

Notes

1. All standards and samples are corrected for mean calibration blank.
2. All samples prepared with one level of BrCl are corrected by multiplication of the [redacted] BrCl MB result by the factor required to produce a result equivalent to that of the BrCl level used to oxidize the sample.
3. Field and equipment blanks are only analyzed if provided by clients. Bottle blanks are analyzed for the bottles provided by Brooks Applied Labs.
4. A carry-over blank is run following any high-level sample (≥20,000 pg measured). The carry-over blank is analyzed using the same split bottle, bubbler, and trap associated with the high-level sample.
5. Field blanks and other potentially low-level samples should be analyzed at the beginning of the analytical run; samples suspected to contain comparatively high levels of mercury should be run at the end of the sequence.
6. Reagent blanks must be analyzed when new BrCl is made and must average less than 10 pg of Hg per blank unit.
7. Equipment blanks are analyzed only on a project-specific basis.

Table 4. Quality Control Acceptance Criteria and General Analytical Run Sequence for the Analysis of Total Mercury using the Manual System.

Run	Run Name	Section Name	Analyze	Requirements
1 2 3 4	IBL IBL IBL IBL	Calibration Blanks	Split bottle blank Split bottle blank Split bottle blank Split bottle blank	Each < 40 pg; Ave. < 20 pg; StDev < 7.5 pg
5 6 7 8 9	25 pg std 100 pg std 500 pg std 2500 pg std 10000 pg std	Calibration	25 pg Hg 100 pg Hg 500 pg Hg 2500 pg Hg 10000 pg Hg	StDev < 15% of Ave. RF Recovery of 25 pg std = 75-125%
10	ICV (1568pg std)	Independent Calibration Verification	1568 pg (NIST 1641d)	Recovery 85-115%
11	CCV (500pg std)	Continuing Calibration Verification	5.0 ng/L	Recovery 77-123%
12 13	Method Blank 01 Method Blank 02	Method Blank	[redacted] BrCl MB	All MB ≤ 0.5 ng/L
14	CCB	Continuing Calibration Blank	Same split bottle and bubbler associated w/the 10000 pg std.	< 40 pg and within ± 20 pg of the average CB
15 16	Method Blank 03 Method Blank 04	Method Blank	[redacted] BrCl MB [redacted] BrCl MB	Each ≤ 0.5 ng/L StDev ≤ 0.07 ng/L
Next	Field, Equipment, or Bottle Blanks	Field, Equipment, or Bottle Blanks	Field, Equipment, or Bottle Blanks	< ML or < 1/5th of associated sample conc.
Next	1st Half of Samples (including MS1 and MSD1)	Sample Analyses Matrix Spike Matrix Spike Duplicate	Sample X X + spike X + dup. Spike Other Samples	 Recovery 71-125%; RPD < 24% Recovery 71-125%; RPD < 24%
Next	CCV (500pg std)	Continuing Calibration Verification	5.0 ng/L	Recovery 77-123%
Next	2nd Half of Samples (including MS2 and MSD2)	Sample Analyses Matrix Spike Matrix Spike Duplicate	Sample Y Y + spike Y + dup. Spike Other Samples	 Recovery 71-125%; RPD < 24% Recovery 71-125%; RPD < 24%
Last	CCV (500pg std)	Continuing Calibration Verification	500 pg Hg	Recovery 77-123%

Notes

8. All standards and samples are corrected for mean calibration blank.
9. All samples prepared with one level of BrCl are corrected by multiplication of the [redacted] MB result by the factor required to produce a result equivalent to that of the BrCl level used to oxidize the sample.
10. Field and equipment blanks are only analyzed if provided by clients. Bottle blanks are analyzed for the bottles provided by Brooks Applied Labs.
11. A carry-over blank is run following any high-level sample (≥20,000 pg measured). The carry-over blank is analyzed using the same split bottle, bubbler, and trap associated with the high-level sample.
12. Field blanks and other potentially low-level samples should be analyzed at the beginning of the analytical run; samples suspected to contain comparatively high levels of mercury should be run at the end of the sequence.
13. Reagent blanks must be analyzed when new BrCl is made and must average less than 20 pg of Hg per blank unit.
14. Equipment blanks are analyzed only on a project-specific basis.

(Example Log)

Brooks Rand Labs

THg Water Prep Benchsheet

SOP / Rev #: BR-0006 Rev __

Prepped By:		Batch:		1st - 24 hr Check Date/Time:	
		BrCl ID:		2nd - 24 hr Check Date/Time:	
Preparation Start Date/Time*:		Preparation End Date/Time**:			

* Time is when the first reagents are added.

** Time is when the last sample is brought upto volume

Sample ID	Sample Aliquot (mL)	BrCl added (mL)	%BrCl	24 hr Check (initials)	only fill out if additional BrCl is added: Additional BrCl Added (mL)	2nd - 24 hr Check (initials)	Adjusted %BrCl

Time in:		Oven Temp (measured / corrected):	/
Time out:		Oven Temp (measured / corrected):	/
		Thermometer ID:	
NOTES:			

(Example Log) **Hg Analysis Sheet: T-Hg / Other: _____** Page 1 of ___

Sequence:_____ Batch(es):_____

Analyst:_____ Date:_____ Instrument ID:_____

10ng/mL std ID:_____ 1ng/mL std ID: _____ ICV std ID:_____

$NH_2OH \cdot HCl$ #:_____ $SnCl_2$ #:_____

Initial offset: _____ Initial PMT: _____

Run #	Split Bottle	Trap	Bubb.	Brooks Applied Sample ID	Analy. Vol. (mL)	Dilution Factor	Analysis comments / For spiked QC: Source sample, standard ID, and spiked volume (mL)
1				SEQ-IBL1	---		
2				SEQ-IBL2	---		
3				SEQ-IBL3	---		
4				SEQ-IBL4	---		
5				SEQ-CAL1	0.025		1ng/mL
6				SEQ-CAL2	0.100		1ng/mL
7				SEQ-CAL3	0.050		10ng/mL
8				SEQ-CAL4	0.250		10ng/mL
9				SEQ-CAL5	1.00		10ng/mL
10				SEQ-ICV1	1.00		NIST 1641d
				SEQ-CCB1	---		

Comments:

_____

_____

_____

(Example Log) **Hg Analysis Sheet: T-Hg / Other: ____** Page ____ of ____

Sequence:______________________ Analyst:____________ Date:______________

Run #	Split Bottle	Trap	Bubb.	Brooks Applied Sample ID	Analy. Vol. (mL)	Dilution Factor	Analysis comments / For spiked QC: Source sample, standard ID, and spiked volume (mL)

Comments:

____

____

(Example Log)

(Example Log) **Hg Analysis Sheet : T-Hg MERX-T:_____** Page 1 of___

Sequence:_____ Batch(es):_____
Analyst:_____ Date:_____ Instrument ID:_____

10ng/mL std ID:_____ 1ng/mL std ID: _____ ICV std ID:_____
$NH_2OH \cdot HCl$ #:_____ $SnCl_2$ #:_____
Initial offset: _____ Initial PMT: _____

	Run #/ Pos #	Brooks Applied Sample ID	Analy. Vol. (mL)	Dilution Factor	Analysis comments / For spiked QC: Source sample, standard ID, and spiked volume (mL)
	1	Rinse	---		
	2	Rinse	---		
	3	SEQ-IBL1	---		
	4	SEQ-IBL2	---		
	5	SEQ-IBL3	---		
	6	SEQ-IBL4	---		
	7	SEQ-CAL1	0.010		1ng/mL
	8	SEQ-CAL2	0.025		1ng/mL
	9	SEQ-CAL3	0.100		1ng/mL
	10	SEQ-CAL4	0.050		10ng/mL
	11	SEQ-CAL5	0.250		10ng/mL
	12	SEQ-CAL6	1.000		10ng/mL
	13	SEQ-ICV1	1.000		NIST 1641d
	14	SEQ-CCB	---		
	15	SEQ-CCB	---		
	16	SEQ-CCB	---		

Comments:_____

_____

Balance ID: _____

(Example Log)

Hg Analysis Sheet: T-Hg / Other:____ Page____of____

Sequence:_____ Analyst:_____ Date:_____

	Run #/ Pos #	Brooks Applied Sample ID	Analy. Vol. (mL)	Dilution Factor	Analysis comments / For spiked QC: Source sample, standard ID, and spiked volume (mL)

Comments:_____

_____

_____

_____

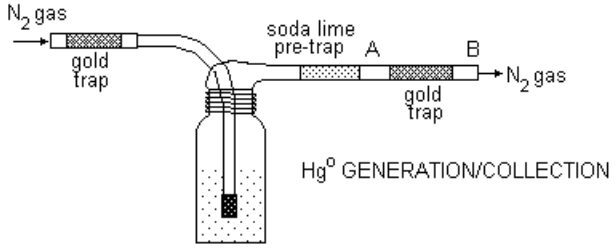
_____

Appendix to Brooks Applied Labs Procedure for EPA Method 1631, Revision E (02/05)

Differences between EPA Method 1631, Revision E, and Brooks Applied Labs SOP #BAL-3100

The following differences exist between EPA Method 1631, Revision E (with relevant sections marked in boldface), and the practices employed by Brooks Applied Labs (SOP #BAL-3100):

- **Figures 1 and 2** (referenced within **sections 2.6 and 2.7**) give schematic diagrams of the bubbler, purge and trap, CVAFS analytical system. In contrast, for the manual system, Brooks Applied Labs (BAL) purges samples with nitrogen in bubblers onto sample traps. After samples are purged, the sample traps are disconnected from the bubbler system and desorbed using argon, as shown below:



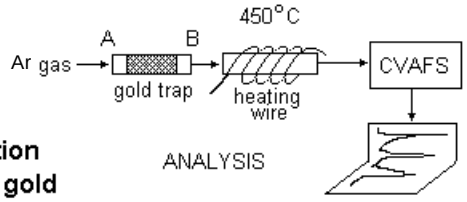


Figure 1: Schematic diagram of Hg^0 generation/collection and analysis by single gold amalgamation/CVAFS

- **Section 6.4.3** specifies the use of acid fume pretraps consisting of 8-14 mesh soda lime chunks and which are purged for 1 hour with nitrogen. BAL uses pretraps consisting [redacted] soda lime chunks and which are purged [redacted] with nitrogen.

- BAL uses direct data acquisition with the BAL "Hg Guru" integration software instead of a chart recorder or integrator as described in **section 6.6**.

- Method 1631E identifies two working standards at 10.0 ng Hg/mL and 0.10 ng Hg/mL (**sections 7.9 and 7.10**) used for instrument calibration. Instead of the 0.10 ng Hg/mL working standard, BAL uses a 1.0 ng Hg/mL standard, along with the 10 ng Hg/mL standard, for calibration.

- **Section 8.5.1 and 11.1.1** states the option of oxidizing the sample with BrCl in the original container. BAL follows that optional protocol.

- **Section 9.3.1** states that the concentration of the spike for matrix spike (MS) and matrix spike duplicate (MSD) samples shall be at 1-5 times the background level of the native sample. BAL accepts a broader spiking level.

- **Section 9.4.1.3** specifies that the mean result for all bubbler blanks analyzed during an analytical batch should be subtracted from all raw data before results are calculated. Instead, BAL subtracts the average peak area measurement of the first four blanks (IBL) analyzed prior to the calibration from all raw data. Split bottles are used for the measurement and/or transfer of all standards, client samples, and quality control samples to the bubblers for manual anlaysis.

- **Section 9.4.7** specifies that 5% of any lot of bottles should be tested. BAL has found that there is little variation within any given lot of FLPE bottles, glass bottles, or glass vials. Therefore, BAL randomly tests only 10% of the bottles or vials from the first opened case from each lot before accepting or rejecting the lot, up to a maximum of 10 cases per lot number. A minimum of 10% of the cases of any given lot number are tested. BAL uses the tighter acceptance criterion of THg ≤ 0.2 ng/L, as opposed to ≤ 0.5 ng/L, for passing a lot number of bottles. In addition, four method blanks are prepared with every batch (> 10% of any given batch) using the same bottles that the samples are collected and prepared in, which serves as a further indicator of any potential contamination due to the bottles.

- **Section 10.1** does not state a frequency requirement for calibration. BAL performs a new calibration at the beginning of each analytical day and whenever CCV recovery fails to meet the acceptance criteria as outlined in 1631E, Table 2.

- **Section 10.2.2.1** lists calibration points of 50 pg, 500 pg, 2500 pg, 5000 pg, and 10000 pg Hg created from aliquots of working standards discussed above. BAL uses a calibration curve with points of 10pg, 25 pg, 100 pg, 500 pg, 2500 pg, and 10000 pg. These points are produced by adding 0.010mL, 0.025 mL and 0.100 mL of a 1.0 ng Hg/mL standard (10pg, 25 pg and 100 pg respectively) and by adding 0.050 mL, 0.250 mL, and 1.0 mL of a 10.0 ng Hg/mL standard (500 pg, 2500 pg, and 10000 pg respectively). For Manual Analysis, BAL does not add aliquots of the working standard directly to the bubblers; instead aliquots of standards are added to approximately 100 mL of DI water in a split bottle prior to transfer to the bubbler.

- **Section 11.2.1.2** states that samples are purged with nitrogen for 20 minutes at gas flow rate of 300-400 mL/min. For manual analysis, BAL purges [redacted]. High-level standard recoveries and subsequent bubbler blanks indicate that [redacted] is an adequate purge time to volatilize and collect mercury. For automated anlaysis, samples are purged [redacted].

- BAL has not found the step described in section **11.3.2** (passing argon through the sample trap prior to desorption) to be necessary to eliminate condensed water vapor prior to trap desorption.

Figure 3: Schematic Diagram of the Flow-Injection, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System. This system is not employed by BAL.

SOP #BAL-3200

Determination of Methyl Mercury by Aqueous Phase Ethylation, Trap Pre-Collection, Isothermal GC Separation, and CVAFS Detection: BAL Procedure for EPA Method 1630 (Aqueous Samples) and EPA Method 1630, Modified (Solid Samples)

Brooks Applied Labs

Supersedes BR-0011 rev 013h

Revision 001

Written 2/12/16

Reviewed _____
_____
_____
_____

BAL VP of Operations	Date 3/1/16
BAL VP of Quality	Date 3/1/16
Hg Group Leader	Date 3/2/16

Determination of Monomethylmercury by Aqueous Phase Ethylation, Pre-Concentration, Isothermal GC Separation, and CVAFS Detection: BAL Procedure for EPA Method 1630 (Waters) and EPA Method 1630, Modified (Solids)

1.0 Scope and Applications

1.1 Method BAL-3200 is the performance based procedure followed at Brooks Applied Labs (BAL) as EPA Draft Method 1630. Unless specifically stated otherwise in this document, all apparatus, materials, reagents, standards and procedures as stated in EPA Method 1630 are used at BAL.

1.2 EPA Draft Method 1630 is for the determination of methyl mercury only in filtered and unfiltered aqueous samples. BAL Method BAL-3200 is additionally used for the determination of methyl mercury in sediment, biota, and biomonitoring samples. BAL has developed specific sample preparation methods for these matrices. With the exception of the maximum volumes analyzed, the procedures followed for the analysis of sediment and biota preparations are identical to the procedures followed for aqueous preparations.

1.3 EPA Draft Method 1630 describes manual analysis of samples for monomethylmercury (MeHg) determination. Since its publication, Brooks Rand Instruments has developed MERX, an automated system for the determination of MeHg. The analytical method in this SOP will refer to use of MERX unless otherwise stated.

2.0 Summary of Method

2.1 Prior to instrumental analysis, all samples are prepared in a way to reduce matrix interference and preserve the MeHg that is present.

2.1.1 Aqueous samples are prepared by distillation at approximately 138°C under N_2 according to the procedure discussed in EPA Draft Method 1630, section 11.

2.1.2 Sediment samples are prepared by [redacted] extraction.

2.1.3 Biota samples are prepared by [redacted] digestion in [redacted] and oven digestion [redacted], which can then be followed [redacted] if a lower detection limit is required.

2.2 MeHg is determined by an improved method (Liang, Bloom, and Horvat 1994). The prepared sample is buffered to pH 4.5-5 [redacted] The ethylation reagent adds an ethyl group to the methyl mercury compound (CH_3Hg^+ $^-C_2H_5$) making volatile methylethyl mercury. It also adds two ethyl groups to the Hg^{2+} making volatile diethyl mercury. The volatile mercury species are liberated from the liquid and collected by purging with dry, Hg free nitrogen into a pyrex tube filled with Tenax™. The ethyl mercury

derivatives are then thermally desorbed with Hg free argon and transferred to a GC column held in an oven at a temperature determined by BRI. BRI tests each GC column and the best temperature determined is supplied to BAL along with the GC column. The GC column separates the species chromatographically by mass. The ethylated Hg compounds are pyrolized at [redacted] to Hg (0), then quantified by a cold vapor atomic fluorescence spectrophotometer (CVAFS). This method can by applied for the determination of MeHg in a variety of sample matrices and has been demonstrated as being very sensitive, precise, and accurate. Good agreement was obtained for the determination of MeHg in standards, SRMs and real samples in comparison to the manual system used at Brooks Applied Labs and less variability was seen in the automated system.

3.0 Definitions

3.1 A partial list of definitions is presented below. Most definitions can be found for specific terms in the sections where they are first mentioned. Many of the definitions mentioned in this method are based on those found in the glossary for EPA Method 1630. Please refer directly to this method for a more detailed list.

3.1.1 May: This action is allowed but not required.

3.1.2 May Not: This action is prohibited.

3.1.3 Must: This action is required.

3.1.4 Shall: This action is required.

3.1.5 Should: This action is suggested, but is not required.

4.0 Interferences

4.1 If properly applied, the distillation procedure will remove most, if not all, of the significant interferences. However, the concentration of hydrochloric acid (HCl) in the solution will affect the distillation of methyl mercury from the solution. Too little HCl could cause the distillation of methyl mercury to not be quantitative while too much HCl could cause the co-distillation of HCl fumes, which according to EPA Method 1630 can interfere with the ethylation process. This interference was investigated at BAL. See section 9.5.1.1 for more details.

4.1.1 EPA Method 1630 dictates that fresh water samples must be preserved with between 0.3% to 0.5% (v/v) 11.6 M HCl (BAL preserves fresh water samples with 0.4% (v/v) 11.6 M HCl) and that salt water samples must be preserved with between 0.1% to 0.2% (v/v) 18 M sulfuric acid (H_2SO_4) (BAL preserves salt water samples with 0.2% (v/v) 18 M H_2SO_4).

4.2 Samples must not be preserved with nitric acid as it may cause partial decomposition of the analyte during distillation.

4.3 Positive artifact is possible with the distillation of samples that are high in inorganic mercury. Ambient organic matter may methylate 0.01% to 0.05% of the ambient inorganic mercury during distillation. In inorganic mercury contaminated waters this can significantly affect the results for methyl mercury. Solvent extraction may be preferable to distillation in samples that are high in divalent mercury (Hg(II)).

[redacted]

4.7 Refer to EPA Method 1630, Section 4.0, for a detailed account of possible contamination routes, interferences to the analysis, and how these are avoided or minimized at BAL.

5.0 Safety

5.1 Refer to EPA Method 1630, section 5.0, for safety issues associated with the use of this method.

5.2 Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability, and nervousness. Organo-mercurials may cause permanent brain damage. Because of this, only highly trained personnel familiar with the dangers and precautions to take when working with mercury compounds should ever handle standards and/or high level samples.

5.3 Material safety data sheets (MSDSs) are maintained for all chemicals used in this method. Scanned MSDS sheets are stored on the server and can be located by either chemical name or by CAS number.

5.4 Refer to the latest revision of the Chemical Hygiene Plan (CHP) for additional safety precautions and required protective equipment.

6.0 Apparatus and Materials Used at BAL

6.1 Refer to EPA Method 1630, Section 6.0 for a list of materials used in the method employed at BAL.

6.2 Routine preventative maintenance for the equipment used in this procedure is described in detail in the standard operating procedure BAL-0600 (Maintenance – Analytical Instruments).

6.3 Detailed instructions for the decontamination of bottles and other equipment are described in BAL SOPs BAL-2300 and BAL-0102.

6.4 Specific equipment used at BAL is listed below. Any modifications to EPA Method 1630 are described and explained.

6.4.1 Autosampler: An AI Scientific septum piercing autosampler with 168 slots is used at BAL. Other autosamplers may be used if they are compatible with the Guru software, have septum piercing capabilities, and can hold the 40 mL glass vials.

6.4.1.1 Reaction vessels: A 40 mL flat bottomed amber glass vial with a Teflon lined septum cap is used as the reaction vessel.

6.4.2 MERX Methylmercury Autoanalyzer: The MERX methylmercury autoanalyzer is designed and manufactured by BRI. For a diagram, see Figure 1.

6.4.2.1 The Purge and Trap Module:

6.4.2.1.1 A purge vessel, a 50 mL glass tube with a port coming out of the top and another port coming off the base is used as the purge vessel. Mercury free nitrogen is purged into the reaction vessel forcing the sample into purge vessel from the port at the bottom. The nitrogen continues to pass through both the reaction vessel and the purge vessel during the entire purge time. As the sample is purged, the volatile mercury species are swept from the sample onto the preconcetration trap via the port at the top of the purge vessel.

6.4.2.1.2 The preconcentration trap is a Tenax trap [redacted] that is kinked at both ends and used for the collection of purged organomercury species.

6.4.2.2 The GC and Pyro Module: Isothermal gas chromatography system consisting of a GC column, GC oven, and pyrolitic column. For a diagram of the system see Figure 1.

6.4.2.2.1 Gas Chromatography (GC) Column: A GC column [redacted]. Under a flow of high purity argon, organomercury species desorbed from the

preconcetraion trap are carried by argon gas through the GC column where they are separated by mass.

6.4.2.2.2 Pyrolitic Column: The pyrolitic column is a quartz tube that [redacted] After GC separation, species pass through the pyrolitic column where they are thermally decomposed to elemental mercury before going through the detector.

6.4.3 Atomic fluorescence spectrophotometer: CVAFS systems are built by BRI (Model III). Refer to the "Brooks Rand Instruments, LLC Model III Operations Manual" for instrument operating instructions.

6.4.4 Recorder: BAL uses direct data acquisition with the Mercury Guru™ integration software instead of a chart recorder or integrator as described in EPA Method 1631E, section 6.6. Refer to the "Brooks Rand Instuments LLC Model III Operations Manual" for Guru™ software/integrator operating instructions and system requirements. Use of this integration software is faster, eliminates the expense of chart recorders and/or integrators, allows for storage of data in electronic form, and eliminates possible transcription errors.

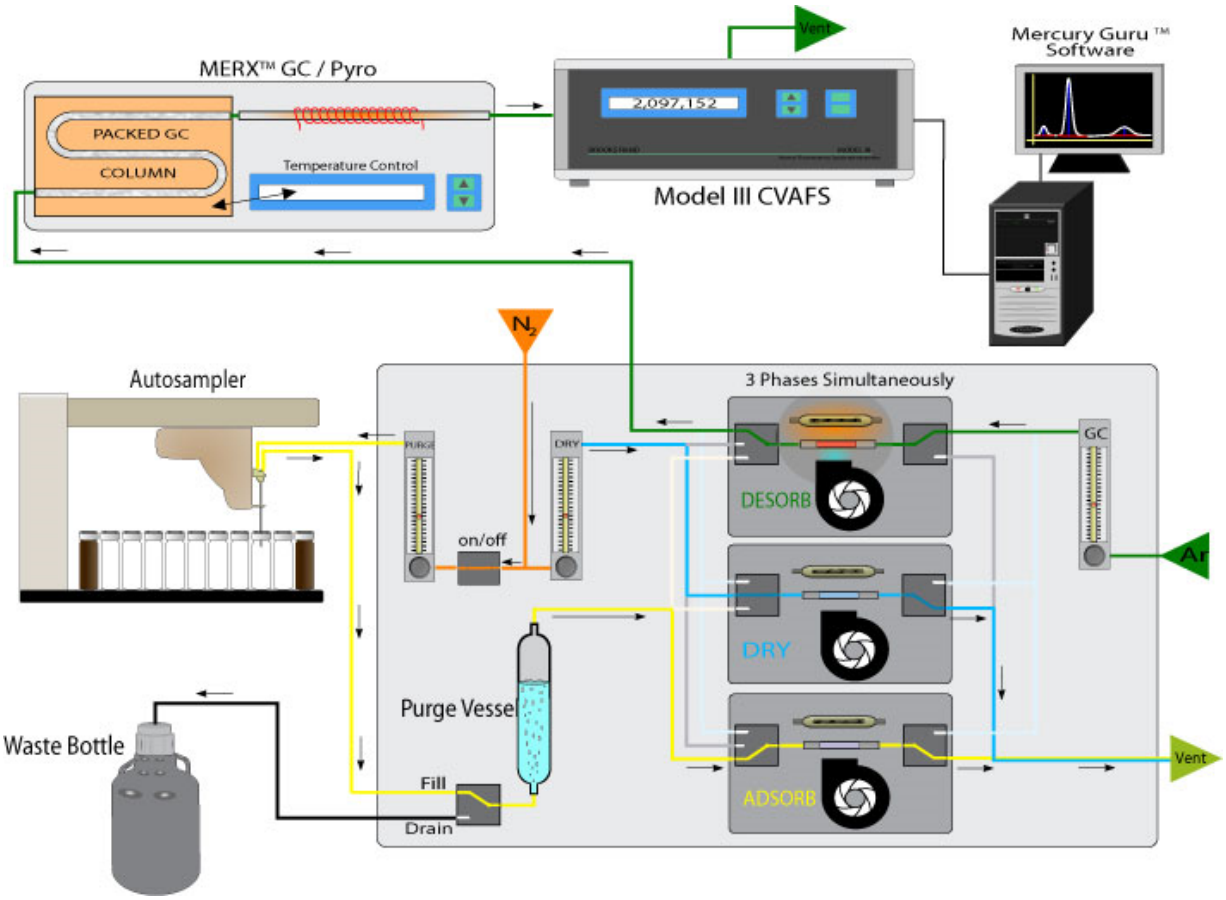


Figure 1. Diagram of sample flow path and connections for MERX MeHg analytical system.

7.0 Standards and Reagents

7.1 Refer to EPA Method 1630, Section 7.0 for a list of standards and reagents employed at BAL.

7.2 Water: Deionized water (DIW) is monitored on a daily basis during calibration of the instrument as part of the calibration blanks and the recoveries of the calibration standards.

7.3 MeHg Standard Solutions: Since MeHg is susceptible to photo oxidation, all standards should be stored in dark areas and minimally exposed to direct light during the working day.

7.3.1 Standard stock solution: 1 µg/mL MeHg OH is purchased from a known, accredited vendor. This standard is used to prepare the calibration standards. This solution should be kept refrigerated and it is verified by in-house testing before being put into use.

7.3.2 Working standard: 10 ng/mL MeHg. Dilute 1.00 mL of 1 µg/mL stock solution, 0.2 mL of HCl, and 0.5 mL of HOAc with DIW to a final volume of 100 mL. This solution may be kept at room temperature and expires after one month.

7.3.3 Working standard: 1 ng/mL MeHg. Dilute 0.10 mL of 1 µg/mL stock solution, 0.2 mL of HCl, and 0.5 mL of HOAc with DIW to a final volume of 100 mL. This solution may be kept at room temperature and expires after one month.

7.3.4 Daily standard: 0.01 ng/mL MeHg. Dilute 0.05 mL of 1ng/mL working standard to 5 mL with DIW. This solution expires at the end of the day.

7.3.5 Daily standard: 0.1 ng/mL MeHg. Dilute 0.5 mL of 1ng/mL working standard to 5 mL with DIW. This solution expires at the end of the day.

7.3.6 Independent calibration verification (ICV) stock standard: 1 µg/mL MeHg Cl stock standard is purchased from a known, accredited vendor. This solution should be kept refrigerated and it is verified by in-house testing before being put into use.

7.3.7 Independent calibration verification (ICV) standard: Dilute 0.1mL of 1 µg/mL MeHg Cl stock standard, 0.2mL of HCl, and 0.5mL HOAc with DIW to a final volume of 100mL. This solution may be kept at room temperature and expires after one month.

7.4 Sodium tetraethylborate ($NaBEt_4$) solution: [redacted]

[redacted]

7.5 Sodium acetate buffer: [redacted]

7.6 Methanolic potassium hydroxide solution [redacted] The solution is stored in a fluoropolymer bottle and shall be remade every six months or as needed.

7.7 Gases: All gasses are passed through a gold-coated sand trap to remove traces of mercury prior to use.

7.7.1 Argon used as the carrier gas from trap desorption, through the GC and pyrolitic column to the detector and is from cryogenic bleed off.

7.7.2 Nitrogen used as a purge gas for sweeping derivatives from a bubbler is plumbed from cryogenic bleed-off.

7.8 [redacted] potassium chloride (KCl) [redacted] L-Cysteine solution: [redacted]. This solution shall be remade every six months or as needed, such as when crystals begin to form.

7.9 11.6 M hydrochloric acid (HCl): Used for the preservation of freshwater samples.

7.10 18 M sulfuric acid (H_2SO_4): Concentrated form used to preserve saltwater samples. This acid is also used to prepare 9 M H_2SO_4.

7.10.1 9M H_2SO_4: Mix equal parts DIW and pre-tested, concentrated H_2SO_4. Introduce the reagents slowly as this procedure generates a great deal of heat. Allow complete cooling before capping tightly. This solution shall be remade every six months or as needed.

7.11 Potassium bromide/sulfuric acid solution (KBr/H_2SO_4): [redacted] This solution shall be remade every six months or as needed.

7.12 1 M copper sulfate solution ($CuSO_4$): [redacted] This solution shall be remade every six months or as needed.

7.13 DCM (methylene chloride): DCM (HPLC Grade) may be purchased from an authorized vendor.

8.0 Sample Collection, Preservation, and Handling

8.1 Refer to EPA Method 1630, Section 8.0 and EPA Method 1669 (*Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels*) for a detailed description of sample collection, preservation, and storage methods.

8.2 Aqueous samples may be collected in fluoropolymer, FLPE, or glass containers with fluoropolymer lined lids. Aqueous samples should be maintained at 0 – 4 ºC from the time of collection until preservation and must be preserved within 2 days of collection. Freshwater samples are preserved with 4 mL/L of pre-tested concentrated HCl while saline samples (salinity > 10 ppt) and urine samples are preserved with 2 mL/L of pre-tested 18 M H_2SO_4. Preserved samples are stable for up to 6 months if kept in the dark and cool. BAL defines "cool" as ≤ 12 °C.

As long as a sample is field preserved and received at ambient temperature, then no qualification of the data is necessary. A holding time study has been conducted by BAL (R&D Study Hg-38) showing that preserved samples are stable at ambient room temperature (up to 25 ºC) when stored in the dark for at least 1 month. Therefore, though the BAL policy is to store all aqueous samples for MeHg at 0 – 4 ºC, results for preserved samples stored at ambient room temperature in the dark will not be qualified as long as the samples are prepared within 1 month of receipt.

8.3 Biota and sediment samples may be collected in glass or HDPE containers. Solid samples should be frozen as quickly as possible, ideally within 2 days and must be frozen within 7 days of collection. Samples can be maintained for up to 7 days at 0 – 4 °C from the time of collection until receipt by BAL. Upon receipt at BAL, solid samples are moved to freezers for storage. Solid samples may be held for at least 1 year if kept in the dark at < -15 °C.

> *NOTE: Due to uncertainty inherent in accurately measuring sample temperatures, it is understood that the measured temperature may be as much as ±2 °C from the actual temperature. Therefore, as long as biota and sediment samples remain unfrozen, ±2 °C are allowed beyond the above ranges without requiring qualification of the data.*

9.0 Sample Preparation

9.1 Refer to EPA Method 1630, Section 11.0 for a detailed description of the preparation of samples. Depending on the purposes and definitions of investigations of mercury biogeochemistry cycling, samples are prepared in the following methods prior to analysis.

9.2 Preparation of aqueous samples for MeHg analysis: Two isolation methods, distillation and solvent extraction, have been used at BAL for the determination of MeHg in aqueous samples. Good agreement was obtained in the comparison of the two methods for most water samples studied: For organic rich and/or high level sulfide containing samples, the distillation showed some advantages over the solvent extraction method with higher recoveries (85 ± 4%, Horvat, Bloom, and Liang, 1993). In addition, extraction consumes large quantities of organic solvent, which can result in environmental contamination. Therefore, if preparation of aqueous samples is needed, distillation is the preferred preparation method for aqueous samples at BAL.

9.2.1 Distillation of aqueous samples:

Reagents KCl L-Cysteine, H_2SO_4

Distillation devices: Vials and caps for distillation and distillate collection are made of fluoropolymer obtained by Savillex Corporation, USA. Caps have 1/8" ports for friction fit 1/8" fluoropolymer tubing.

Distillation procedures: An aliquot of water sample, typically 50 ml, is transferred into a 60 mL fluoropolymer vial (for high MeHg concentration samples, smaller sample sizes should be used, but bring the final volumes to a known volume near 50 mL with 0.4% HCl solution Urine samples can be batched with other water samples but at least one urine sample should have QC performed.

All blanks, including blank spikes (BS), should be prepared by aliquoting 50 mL of a 0.4% HCl solution then spiked if appropriate. Add 0.2 mL of the 20% KCl/0.2% L-Cysteine solution and 0.5 mL of 9 M H_2SO_4 (Start the distillation after addition of reagents

The distillate is collected in a 60 mL fluoropolymer vial containing 15 mL of DIW Bring the final volume of the receiving vial to the 58 mL mark with DIW. Depending on its MeHg concentration, transfer an aliquot of the distillate into the methylation reaction vessel for analysis as described in section 10.3.

NOTE:

Special considerations to take when distilling samples:

- *Be especially careful to not touch the rinsed tubing nor let any potentially contaminated object touch the tubing that is to be inserted into the receiving vial.*
- *When measuring out the sample aliquot, it is acceptable to weigh out 50 mL (± 1 mL). Each vial is engraved with a line at 50mL and it is also acceptable to measure out the sample aliquot by pouring up to this line.*
- *Measure out the 15 mL of DIW for the receiving vial by pouring into a pre-cleaned 15-mL centrifuge tube.*

Besides the typical distillation of aqueous samples, BAL also offers "ultra low-level" distillation Refer to the bottle washing SOP (BAL-2300) for a full description of the cleaning procedures for equipment used in this method.

9.3 Preparation of biological materials for MeHg.

digestion of biota samples: Weigh biological material (wet, homogenous) into a mL fluoropolymer vial. Add solution, cap the vial tightly and shake the sample to suspend the material. Digest the sample in an oven A maximum of of the sample preparation may be analyzed due to the content. The digestion may be scaled up if larger volumes are required.

[redacted]

9.3.2 Distillation (following alkaline digestion): If there is matrix interference or if a lower detection limit is desired, the [redacted] digestate can be [redacted] (Horvat, Bloom, and Liang, 1993). [redacted] significantly reducing the detection limits for the analysis of MeHg in biota samples.

[redacted]

Note: Brooks Applied Labs routinely [redacted] of the [redacted] digestate unless otherwise specified on the Sample Processing Form.

9.4 Preparation of sediments and soils for MeHg.

[redacted] Solvent extraction for sediments: [redacted] Approximately [redacted] of sediment sample is accurately weighed into a clean 30 mL fluoropolymer vial. [redacted] solution (Section 7.13) and [redacted] solution (Section 7.14) are added to the sample. [redacted] of [redacted] are accurately weighed and added. [redacted] samples are centrifuged [redacted] assist in the separation of the aqueous layer from the organic layer. [redacted] The organic layer is collected directly to a [redacted] 125 mL Teflon® bottle. [redacted] 50 mL of DIW is added to the [redacted]. The sample is then heated [redacted] until the [redacted] layer has evaporated off. [redacted]

NOTE: [redacted]

The sample is then diluted to 100 mL with additional DIW. [redacted] The extracted sample is analyzed following the same procedure used for the analysis of water distillations.

MeHg Sediment Prep step by step instructions

- *Take samples out the night before to give them time to thaw. (On the weekends, place in the fridge, not in the lab)*
- *Rinse funnels and vials from the acid vat (4BLK, 1SRM (at 0.500g) per every 20 samples, Samples+ 1 sets of DUP/MS/MSD per 10 samples)*
- *Decant the samples-if needed and not previously decanted.*
- *Weigh out of samples.*
- *Spike and add reagents*
- *Weigh your vials (with cap on) and record it in the Original column of your Prep sheet.*
- *Add (methylene chloride).*
- *Weigh the vials and record on the 2nd column of your Prep sheet. As you weigh, shake the vials!*
- *label the 125mL With the caps on and label on, weigh the bottles and record on the prep sheet. Triple rinse and fill 2 500mL bottles with DIW and place on hot plate.*
- *Centrifuge for*
- *Grab all the equipment and set it up in the hood. (Styrofoam bottle holder, 125mL bottles, funnels, filters, garbage can, dirty bin, waste bucket with no soda ash!)*
- *Put the bottles in the holder, funnel on, and filter paper on. Filter the samples. Dispose the waste (this waste will appear greenish) into the waste bucket**, throw the filter paper into the garbage can and put the funnel into dirty bin with the empty vials. Close caps to 125mL bottle, reweigh and record.*
- *Add a 50mL DIW. (Use EE tube to measure out 50mL) and place on the hot plate . Bring it up to 100mL final volume,*

*** The waste bucket with the waste must be transferred to the proper satellite waste container before leaving for the day.*

9.5 Holding times for sample preparations.

9.5.1 Distillations: It is BAL policy to try to analyze all sample distillates within a 48 hour time frame. However, water and sediment preps and distillates of biota digestates have been shown to be stable for up to 72 hours if stored at room temperature and in the dark (BAL R&D Study Hg-16). Distillates must not be refrigerated or frozen.

9.5.1.1 EPA Method 1630 specifies that distillates with a pH <3.5 must be discarded. BAL has found that good recoveries can still be obtained for these samples if an adequate pH is obtained prior to ethylation. [redacted] The following procedure was instated to ensure proper pH is attained before analysis.

9.5.1.1.1 The pH of all samples is tested using pH strips after prep. The pH of each distillate is recorded on the prep log.

9.5.1.1.2 If the pH is 2.5 or less, [redacted] of acetate buffer should be added to the sample after it is weighed out into the analytical reaction vial.

9.5.1.1.3 The buffered sample should be capped and shaken then 0.050 mL removed for pH testing (via 4-6 pH strip).

9.5.1.1.3.1 [redacted]

9.5.1.1.3.2 [redacted]

9.5.1.1.4 The analyst must record on the bench sheet the amount of acetate buffer added to the sample if it is more than 0.300 mL.

9.5.2 Extractions: Water and sediment extractions (once back extracted into water) are stable for up to 72 hours if stored at room temperature in the dark. However, it is Brooks Applied Labs policy to try to analyze all samples within a 48 hour time frame.

9.5.2.1 The holding time limit for intermediate organic extraction (prior to back extraction) has been examined and was shown to be stable for up to one week. Currently, Brooks Applied Labs performs the entire extraction procedure within one day unless explicit approval is given in advance by the VP of Operations.

9.5.3 Digestions: Biological digestates are more stable than distillates and may be stored up to 30 days prior to analysis. [redacted]

9.5.4 The 72 hour hold times are counted from the end of prep (as recorded on the benchsheet) to the addition of the sodium tetraethyl borate solution to an aliquot at analysis. Once the sodium tetraethyl borate solution is added and the sample is capped, the sample is now stable for an additional 4 days. It should be noted that LIMS will flag samples red for exceeding prep holding times incorrectly because it uses the prep start time and the analysis time as its basis and it should always be double checked at data review.

10.0 Instrument Calibration and Sample Analysis

10.1 Refer to EPA Method 1630, Sections 10.0, 11.0, and 12.0 for a detailed description of the analysis of samples and the calculation of results.

10.2 Instrument Calibration: BAL follows EPA method 1630, Section 10.0 for the instrument calibration with the same exceptions as for sample analysis. Standards, typically 0.5, 1, 2, 10, 50, 250, and 1000 pg MeHg, are added into reaction vessels containing 40 mL of DIW and buffer ([redacted] of acetate buffer). Only the ethylation blanks analyzed prior to the calibration standards are used for blank correcting the calibration and sample results. Any other ethylation blanks analyzed following the calibration standards are used only to check for carryover or other potential system contamination.

10.3 Instrumental Analysis: BAL has adopted the following modifications.

For samples, add appropriate sample volumes plus DIW as necessary for a final volume of 40 mL (for sample preparation see Section 9 of this SOP). Some acid can be carried over during distillation causing some distillates to have a low pH (< 4). In that event, follow instructions in section 9.5.1.1 to bring the final pH to 4 – 5.5, which is the optimum pH for ethylation. An aliquot of [redacted] of $NaBEt_4$ is added, a squirt bottle of DIW is used to fill the vial until there is a convex meniscus, the sample is tightly capped, shaken, then placed in the appropriate position on the autoanalyzer.

Sample Prep Description	Maximum Analysis volumes
Water - Distillation	30 mL
Water - Low Level Distillation	40 mL
Biota - Digest/Distill	40 mL
Biota – Digest	0.030 mL
Sediment – Back Extraction	5 mL

Note: Based on a study conducted at BAL, it has been determined that samples can be analyzed up to 4 days after ethylation and yield acceptable recoveries.

Place the samples in the autosampler. Under the "Automation" tab in Guru, make sure all settings are correct then press "Start Batch". It will commence a 10 minute trap blanking cycle then begin to take up sample.

Peak integration is begun in conjunction with the heating of the Tenax trap. The typical heating time for the MERX system is between 9 and 9.9 seconds. The organomercury species are desorbed and carried through the GC column that is held in an oven set to the specified temperature. The species elute in order of increasing molecular weight, pass through the pyrolitic column, [redacted] at which point all organomercury species are converted into Hg(0), and detected by CVAFS. The reaction vessels are used only once then discarded.

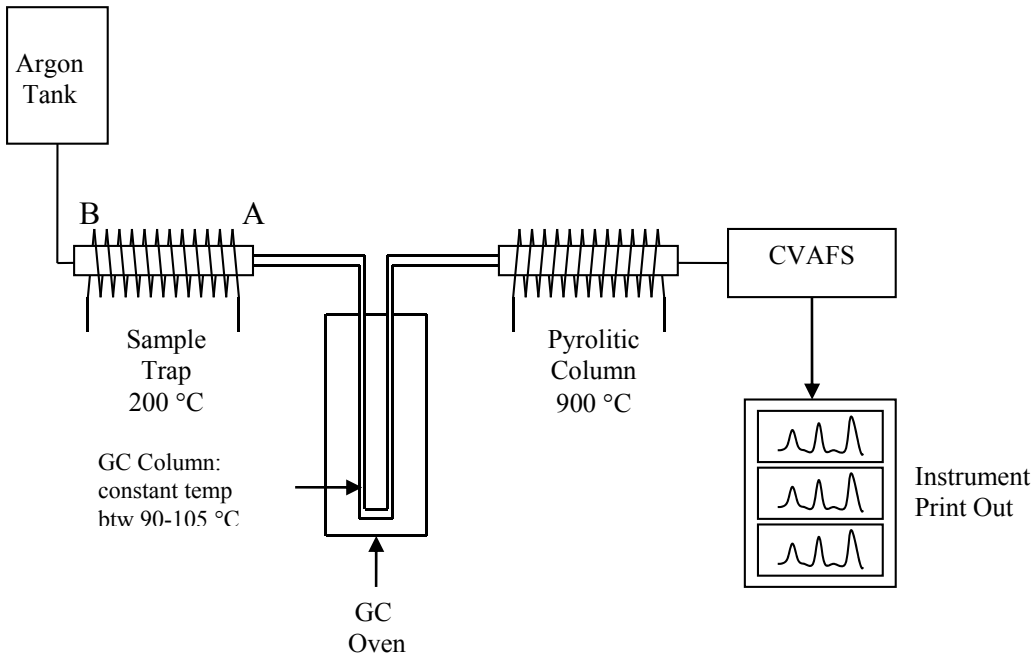


Figure 2: A schematic diagram of the isothermal Gas chromatograph system

Quick MeHg MERX Analysis Guide

Setting Up MERX before Analysis

- Open Guru Template – It is suggested a template is created with the typical setting as well as the typical calibration curve entered in.

- Save as Data (File → Save As Data)
- Ensure purge and trap, GC and pyro, AFS, and autosampler are powered on.
    - **NOTE**: the detector should stay on all of the time or be turned on 24-48 hours before using the instrument.
- Connect instruments to computer (Instrument → Connect; then press Accept)
- Turn gases on at regulator to 17 psi
    - In the Automation tab, there is a set of 3 check boxes to turn the gases on, click those boxes and check the gas flows at the rotometers. It should be at typical settings of:
        - Purge: 65
        - Dry: 60
        - GC: 35

- Auto Zero the detector
- Measure Noise (Instrument → Measure Noise)
- Check run duration (5 min), purge duration(5 min), drying duration (3 min) and heating time (9.9 seconds) to make sure they are at the desired settings. (In the Batch Information tab, times listed)
    - For samples with surrogate n-prHg spikes, a run duration of 7 minutes and a purge duration of 6 minutes should be used.
- Set the number of vials to be analyzed (this can be adjusted while the system is analyzing). Also ensure that the first vial position number is correct (default is position 1).
- Put your prepared vials on the autosampler. **see instructions below.
- Press Start Batch.
- At this point you can continue to prepare samples to put on the autosampler or update the Run Information in Guru.

Preparing vials for MeHg analysis on MERX

<u>Water samples prepared via Distillation</u>

- Add sample to vial using balance after shaking (record exact amount of sample added, max volume of 30mL)
- Add [redacted] of Acetate Buffer or appropriate amount for samples
- Add [redacted] of NaBEt4
- Bring up to volume with Reagent Water (use a squirt bottle)
- Cap tightly
- Invert rapidly to mix contents

<u>Biota or sediment samples</u>

- Add approximately 40 mL of Reagent Water to vial (exact amount doesn't matter)

- Add sample to vial (shake sediment samples prior to aliquoting) (record exact amount of sample added)
- Add [redacted] of Acetate Buffer or appropriate amount for samples
- Add [redacted] of NaBEt4
- Bring up to volume with Reagent Water (use a squirt bottle)
- Cap tightly
- Invert rapidly to mix contents

11.0 Calculations

11.1 All instrument peaks are integrated using Hg Guru™ software. This integration is automated; however, there are occasions when Guru™ will not properly integrate a peak. This is most common with small peaks, abnormally shaped peaks, or peaks with excessive tailing. When a peak is not properly integrated by Guru™, the analyst may manually integrate or adjust the integration of the peak using the Guru™ software (refer to the Guru™ user manual). When manually integrating or adjusting the integration of a peak, the analyst must note that the peak was manually integrated and the reason for the manual integration. This is done on the analytical benchsheet at the time of the manual integration. The final data reviewer is authorized to accept or reject the manual integrations. If they are accepted, the manually integrated peaks are printed and signed and dated as approved by the final data reviewer. The final peak integrations are used to calculate the reported results.

11.2 BAL uses the following formulas for the calculation of monomethyl mercury in a given sample.

11.2.1 Mean Calibration Coefficient:

A calibration coefficient (CF) is calculated for each standard used in the calibration as follows:

$$CF = CS_{pgMeHg} / (CS_{PH} - EB_{PH})$$

Where CS_{pgMeHg} is the calibration standard measured in picograms of methyl mercury, CS_{PH} is the peak height obtained during the analysis of the standard, and EB_{PH} is the mean peak height obtained during the analyses of all of the ethylation blanks that were analyzed prior to the calibration standards. The mean calibration coefficient (CF_{avg}) is then calculated for all of the standards used in the calibration.

11.2.2 Measured methyl mercury in the sample preparation:

The amount of methyl mercury present in the analyzed volume of the sample preparation is calculated using the equation:

$$MeHg_{measured\ pg} = (A_{PH} - EB_{PH}) \cdot CF_{avg}$$

Where A_{PH} is the peak height obtained during the analysis of the sample preparation.

11.2.3 Total methyl mercury in the sample preparation:

The total amount of methyl mercury present in the sample preparation is calculated using the equation:

$$MeHg_{total\ pg} = [(MeHg_{measured\ pg}) / V_A] \cdot V_D$$

Where V_D is the final dilution volume of the sample preparation in mL and V_A is the volume analyzed of the sample preparation in mL.

11.2.4 Concentration of methyl mercury in the sample:

The final concentration of methyl mercury in the sample is calculated using the equation:

$$MeHg_{conc} = (MeHg_{total\ pg} - MB_{total\ pg}) / V_o$$

Where $MB_{total\ pg}$ is the average total picograms of methyl mercury present in the method blanks and V_o is either the volume of the prepared sample measured in mL (aqueous samples) or the weight of the prepared sample measured in mg (solid samples). Therefore, the final concentration of methyl mercury in the sample is reported in units of ng/L for aqueous samples and in units of ng/g for solid samples.

NOTE: The total picograms of methylmercury present in each method blank is calculated using the same formula used to calculate the total picograms of methyl mercury in the sample preparation.

Sediment and soil results are typically dry weight corrected by dividing the wet weight result by the percent total solids result. Biota results are typically reported on a wet weight basis, but can be reported dry weight corrected upon request.

11.2.5 Empirically derived correction factor

BAL routinely recovery corrects results for distilled samples, as per EPA Draft Method 1630, to account for the fact that the distillation procedure is not 100% efficient in recovering methyl mercury. Results are multiplied by an empirically derived correction factor that is based on the average recovery of the appropriate quality control sample. If an appropriate QCS sample is not available, the correction factor is based on the average recovery of the spikes made to samples with a similar matrix to the sample of concern.

The correction factor is calculated using the following equation:

$$F = 100 / R$$

Where F is the empirically derived correction factor and R is the running mean of the recoveries of the last 30 quality control samples or matrix spikes. The empirically derived correction factor is updated quarterly or any time that there is a significant change in performance.

BAL does not use the IPR and CCV samples to calculate the correction factor since, unlike the client samples and quality control samples, these samples are not distilled.

12.0 Quality Control

12.1 Refer to EPA Method 1630, Section 9.0 for a detailed description of the quality control procedures employed at BAL for this method.

12.2 All quality control data should be maintained and available for easy reference and/or inspection.

12.3 Each analyst must perform an initial demonstration of capability (IDOC) for the analysis of methyl mercury prior to the analysis of any client samples. The IDOC consists of an initial precision and recovery (IPR) study following the procedure in EPA Draft Method 1630, Section 9.2.2. The acceptance criteria and run sequence for the IDOC can be found in Table 3 in Section 17 of this SOP.

12.4 Calibration data must be composed of a minimum of 1 ethylation blank (BAL analyzes 3 ethylation blanks prior to analyzing the calibration standards) and a minimum of 5, preferably 7, standards (See Section 7.3 and 10.2 of this SOP for the standards used to calibrate instruments at BAL). Such a calibration should be run daily, prior to analysis, or whenever stock standards have been remade, conditions have changed, or initial calibration check (ICV) or ongoing precision and recovery (CCV) as defined in Section 12.5 do not yield acceptable recoveries.

12.5 The CCV solution prepared by spiking the reaction vessel with 25 pg methyl mercury using the calibration standard and followed by an ethylation blank must be analyzed after every 10 client samples and at the end of the analysis of each analytical batch. Additionally, BAL analyzes an independent calibration check (ICV) solution (see sections 7.3.6 and 7.3.7 for the preparation of this solution) by spiking this solution directly into the reaction vessel prior to the analysis of each analytical batch. The criterion for the recovery of the CCV solution is 67-133% and the recovery criterion for the recovery of the ICV solution is 80-120%. All ethylation blanks must contain no more than the level of methyl mercury found in the low calibration standard.

12.6 Matrix spike/matrix spike duplicate (MS/MSD) analysis should be performed once per every 10 client samples or once per batch, whichever is greater. The target spiking

concentration is 2 – 5 times the level of the native sample or 5 times the sample specific MRL, whichever is greater. If there is no historic data on which to base the spike concentration, then the following default spiking levels should be used:

Aqueous Samples:	1 ng/L
Sediment Samples:	2 ng/g
Fish Samples:	750 ng/g
Other Biota:	200 ng/g

The matrix spike sample is processed through the entire preparation and analytical procedure. Bias is then determined by calculating the percent recovery of the known amount using the following formula:

Percent Recovery = 100 ∗ (spiked sample result (conc.) - sample result (conc.)) / (amount spiked)

The criterion for spike recovery is determined by control charts and is different for each matrix type. The specific matrix spike recovery criteria for each matrix type and preparation procedure can be found in Tables 5 and 6 in Section 17 of this SOP.

The relative percent difference between the MS and the MSD is calculated using the following formula:

$$RPD = 200 \cdot (\left| MS\text{-}MSD \right|) / (MS + MSD)$$

The RPD for the MS/MSD pair must meet the criterion for each of the matrix types found in Tables 5 and 6 in Section 17 of this SOP.

12.7 Method duplicates are prepared and analyzed upon client request. For solid matrices method duplicates should be performed in conjunction with the MS/MSD samples (once per every 10 client samples or once per batch, whichever is greater) and whenever the heterogeneity of a sample is deemed great enough that it may cause problems with the analysis of the sample. The relative percent difference (RPD) between duplicate samples is calculated using the same formula as used to calculate the RPD between the MS and MSD samples. The specific RPD criteria for each matrix type and preparation procedure can be found in Tables 5 and 6 in Section 17 of this SOP. If the acceptance criterion for duplicate analysis is not met for either samples or matrix spike samples, then the associated samples must be qualified or the problem must be corrected and the samples reanalyzed.

12.8 Field duplicates are analyzed at the client's discretion. The acceptance criterion for field duplicate analysis is the same as that used for method duplicate analysis. The client must be notified immediately anytime that the acceptance criterion for field duplicates is not met.

12.9 Four method blanks (MBLK) should be prepared and analyzed with each batch. For water distillation batches, the method blanks are prepared using reagent water acidified to 0.4% HCl in order to simulate the preservation of the samples. For sediment and biota

batches, the method blanks are prepared just using the reagents. All method blank results must meet the acceptance criteria set forth in Tables 5 and 6 of Section 17.

12.10 Blank spikes (BS) are prepared and analyzed with each distillation batch at a frequency of one per every 20 client samples or once per batch, whichever is greater. BSs are prepared by spiking a method blank sample with the calibration standard at a concentration of approximately 1.0 ng/L. The BS is then distilled as per an aqueous sample. BSs must meet the acceptance criteria set forth in Tables 5 and 6 of Section 17.

12.11 Appropriate standard reference materials (SRM) for MeHg are prepared for all batches containing tissue or sediment samples. It is BAL policy to prepare one SRM per every 20 client samples or once per batch, whichever is greater. Two entirely different SRMs may be prepared if different matrix types are analyzed together. Criteria for SRM recoveries are determined by control charts. If control charts are not available then SRM results should be within 35% of the certified value (following recovery correction) for the analysis to be considered valid. SRM accuracy results not meeting this criterion shall be reprepared and reanalyzed or qualified at the discretion of the Laboratory Director. Currently, there are not any water based SRMs available.

13.0 Method Performance

13.1 Refer to EPA Method 1630, section 13.0, for information regarding the verification of this method.

13.2 The detection limits reported in Table 1 were achieved by performing a full MDL study as described in 40 CFR 136, Appendix B. The warning and control limits listed in Table 2 show the limits achievable at BAL.

14.0 Pollution Prevention

14.1 Refer to EPA Method 1630, section 14.0, for EPA recommendations regarding pollution prevention techniques.

14.2 Whenever feasible, lab personnel should use pollution prevention techniques to limit waste generation. The cost involved in purifying acids makes such recycling unpractical at BAL. Instead, every effort is made to reduce volumes necessary to still produce the best possible results. When making standards, they should be prepared in volumes consistent with their use in the laboratory to minimize the volume of expired standards to be disposed.

15.0 Waste Management

15.1 Refer to EPA Method 1630, section 15.0, for information and references related to managing waste produced by application of this method.

15.2 All waste is disposed of in accordance with state and federal regulations either by sewer disposal (only if concentrations are below the King County sewer limits) or through a licensed and bonded hazardous waste disposal facility.

16.0 References

16.1 Liang, L.; Bloom, N.S.; and Horvat, M. (1994) "Simultaneous Determination of Mercury Speciation in Biological Materials by GC/CVAFS After Ethylation and Room-Temperature Precollection." *Clin. Chem.* 40/4: 602-607.

16.2 Bloom, N.S.; Colman, J.A.; and Barber, Lee. (1997) "Artifact Formation of Methyl Mercury during Aqueous Distillation and Alternative Techniques for the Extraction of Methyl Mercury from Environmental Samples." *Journal of Analytical Chemistry* 358:371-377.

16.3 Bloom, N.S. (1989) "Determination of Picogram Levels of Methylmercury by Aqueous Phase Ethylation, Followed by Cryogenic Gas Chromatography with Cold Vapor Atomic Fluorescence Detection." *Canadian Journal of Fisheries and Aquatic Sciences.*

16.4 Long, S.J.; Scott, D.R.; and Thompson, R.J. (1973) "Atomic Absorption Determination of Elemental Mercury Collected from Ambient Air on Silver Wool." *Anal Chem*. 45: 2227-2233.

16.5 Horvat, M.; Bloom, N.; and Liang, L. (1993) "A Comparison of Distillation with Other Current Isolation Methods for the Determination of Mercury Compounds in Low Level Environmental Samples, Part I: Sediments." *Analytica Chimica Acta* 281:135-152.

16.6 Horvat, M.; Liang, L.; and Bloom, N. (1993) "Comparison of Distillation with Other Current Isolation Methods for the Determination of Mercury Compounds in Low Level Environmental Samples., Part II: Waters." *Analytica Chimica Acta* 282:153-168.

16.7 Horvat, M.; May, K.; Stoeppler, M.; and Byrne, A.R. (1988) *Appl. Organomet. Chem.* 2: 515.

16.8 EPA Draft Method 1630 (January 2001) "Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS."

16.9 EPA Method 1669 (April 1995) "Sampling Ambient Water for Trace Metals At EPA Water Quality Criteria Levels."

17.0 Tables and Benchsheets

Table 1 Current method detection limits and minimum levels determined at BAL for the analysis of methyl mercury using EPA Method 1630

Matrix	Preparation Method	Default Mass/ Volume	Default Final Volume	Default Analysis Volume	Method Detection Limit (MDL)[1]	Limit of Quantitation (LOQ)
Water	Distillation	50 mL	58 mL	30 mL	0.020 ng/L	0.050 ng/L
Water (ultra-low)	Distillation	50 mL	58 mL	40 mL	0.010 ng/L	0.025 ng/L
Sediment/ Sludge	Extraction	2.5 g	100 mL	5 mL	0.008 ng/g	0.025 ng/g
Biota	Digestion	100 mg	2.5 mL	30 µL	1.0 ng/g	3.0 ng/g
Biota	Digestion/ Distillation	100 mg	58 mL	40 mL	0.07 ng/g	0.20 ng/g

NOTES:
1. MDL as determined by the procedure 40 CFR Part 136, Appendix B. MDL and LOQ reported here for distillations are for recovery corrected results.
2. The ultra-low method requires rigorously cleaned distillation vials and a lower calibration achievable only on the auto-analyzer system.

Table 2 Summary of recent control chart data ending April 2014 (approximately 30 data points used for each chart) for the analysis of methyl mercury using EPA Method 1630

QA Sample	Matrix	Mean[1] Recovery (%)	Warning Limit (%) ± 2 StDev[2]	Control Limit (%) ± 3 StDev[2]
ICV	ALL	99	7	10
CCV	ALL	102	10	15
Matrix Spikes	Water	105	18	27
Matrix Spikes[3]	Sed/Sludge	90	34	52
Matrix Spike	Biota	109	22	33
BS	Water	99	22	33
SRM[3,4]	Sed/Sludge	89	20	30
SRM[5]	Biota	82	14	21
QA Sample	**Matrix**	**Mean RPD**	**Warning Limit (%) Mean ± 2 StDev**	**Control Limit (%) Mean ± 3 StDev**
Duplicates	Water	10	17	25
Duplicates[2]	Sed/Sludge	16	19	28
Duplicates	Biota	8	15	23

NOTES:
1. Recoveries for distillations (water samples) have been recovery corrected using an empirically derived correction factor.
2. Warning and control limits are expressed around the mean.
3. Control limits for sediments prepared by DCM extraction.
4. SQC-1238 (Sediment) is the SRM used for sediments. Use of this SRM began in August 2010. Control charts were generated from that time up to April 2011.
5. DORM-3 (Dogfish Muscle) is the SRM used for most biota samples.

Table 3 Quality Control Acceptance Criteria and General Analytical Run Sequence for the Initial Demonstration of Capability for the Analysis of Methyl Mercury

Run	Run Name	Section Name	Analyze	Requirements
1 2 3	Rinse Rinse Rinse	Blanking Equipment	DIW + analytical reagents	n/a
4 5 6	IBL IBL IBL	Calibration	Ethylation Blank Ethylation Blank Ethylation Blank	≤ the MRL
7 8 9 10 11 12 13	0.5 pg std 1 pg std 2 pg std 10 pg std 50 pg std 250 pg std 1000 pg std	Calibration[1]	0.5 pg std 1 pg std 2 pg std 10 pg std 50 pg std 250 pg std 1000 pg std	RSD of Avg. CF ≤ 15% Recovery of Low Standard 65-135%
14	ICV	Independent Calibration Verification	~100 pg	Recovery 80-120%
15	Ethylation Blank	Contamination Check	Ethylation Blank	≤ the MRL
16	CCV std (25pg)	Continuing Calibration Verification	25 pg	Recovery 67-133%
17 18 19	Ethylation Blank Ethylation Blank Ethylation Blank	Contamination Check	Ethylation Blank Ethylation Blank Ethylation Blank	≤ the MRL
 20 212 223 242 526 27 	MDL sample MDL sample MDL sample MDL sample MDL sample MDL sample MDL sample MDL sample	Method Detection Limit[2,3]	Appropriate matrix spiked at a level of 1 – 5 times the expected MDL	Calculated MDL no greater than 5 times the spike level
28	CCV std (25pg)	Continuing Calibration Verification	25 pg	Recovery 67-133%
29	Ethylation Blank	Contamination Check	Ethylation Blank	≤ the MRL
30 31 32 33	IPR std IPR std IPR std IPR std	Initial Precision and Recovery	ICV solution	Ave. recovery 69-131%, RSD ≤ 31%
34	CCV std (25pg)	Continuing Calibration Verification	25 pg	Recovery 67-133%
35	Ethylation Blank	Contamination Check	Ethylation Blank	≤ the MRL

NOTES:
1. All standards and samples are corrected for mean ethylation blank. Lower calibrations can be achieved as long as ethylation blank criteria are still met.
2. All samples are corrected for mean method blank.
3. Distilled samples are recovery corrected prior to calculating the MDL.

Table 4 Quality Control Acceptance Criteria and General Analytical Run Sequence for the Analysis of Methyl Mercury

RUN	Analyze	Description	Requirements
1 2 3	Rinse Rinse Rinse	DIW + analytical reagents	n/a
4 5 6	IBL IBL IBL	Contamination Check	≤ the MRL
7 8 9 10 11 12 13	0.5 pg std 1 pg std 2 pg std 10 pg std 50 pg std 250 pg std 1000 pg std	Calibration Curve[1]	RSD of Avg. CF ≤ 15% Recovery of Low Standard 65-135%
14	ICV (independent calib. verific.) (prepared with NIST 1946; around 30 pg MeHg)	Precision and Recovery	80 – 120% recovery
15	Ethylation Blank	Contamination Check	≤ the MRL
16	CCV std (25 pg)	Continuing Calibration Check	67 – 133% recovery
17 18 19	Ethylation Blank Ethylation Blank Ethylation Blank	Contamination Check	≤ the MRL
20 21 22 23	Method Blank 1 (MB-1) Method Blank 2 (MB-2) Method Blank 3 (MB-3) Method Blank 4 (MB-4)	Contamination Check	Refer to specific water and solid criteria found in Tables 5 and 6.
Next	Known Blanks	Trip, Field, or Equipment Blanks	Result < ML or < 1/10th associated sample results
Next	BS or SRM	Precision and Recovery	Rec = 67 – 133%[3] for aqueous, Rec = 65 – 135% for sediment samples, and Rec = 65-135% for biota samples
Next	Sample 01[2] Sample 01MD	Native Sample Duplicate Sample	RPD ≤ 35% or ±MRL for aqueous samples and ±2xMRL for solids if results are ≤5xMRL
Next	Sample 01MS Sample 01MSD	Matrix Spike Matrix Spike Duplicate	Rec = 65 – 135%[3] for aqueous; Rec = 65 – 135% for sediment and biota samples; RPD ≤ 35%
Next	Sample 02	Client Sample	
Next	CCV std (25 pg)	Continuing Calibration Check	67 – 133% recovery
Next	Ethylation Blank	Contamination Check	≤ the MRL
Next	Sample 03 through Sample 13	Client Sample	
Last	CCV std (25 pg)	Continuing Calibration Check	67 – 133% recovery
Next	Ethylation Blank	Contamination Check	≤ the MRL

NOTES:

1. The calibration curve may be adjusted depending on the expected range of samples (i.e. seds and biota 10pg-5000pg). Lower calibrations can be achieved as long as ethylation blank criteria are still met.
2. Any known field or equipment blanks should not be spiked and should be analyzed prior to other samples. The acceptance criterion for these samples is a result < the ML.
3. Recovery corrected.

Table 5 Quality Control Acceptance Criteria and Corrective Action Guidelines for the Analysis of Methyl Mercury in Aqueous Samples by Distillation

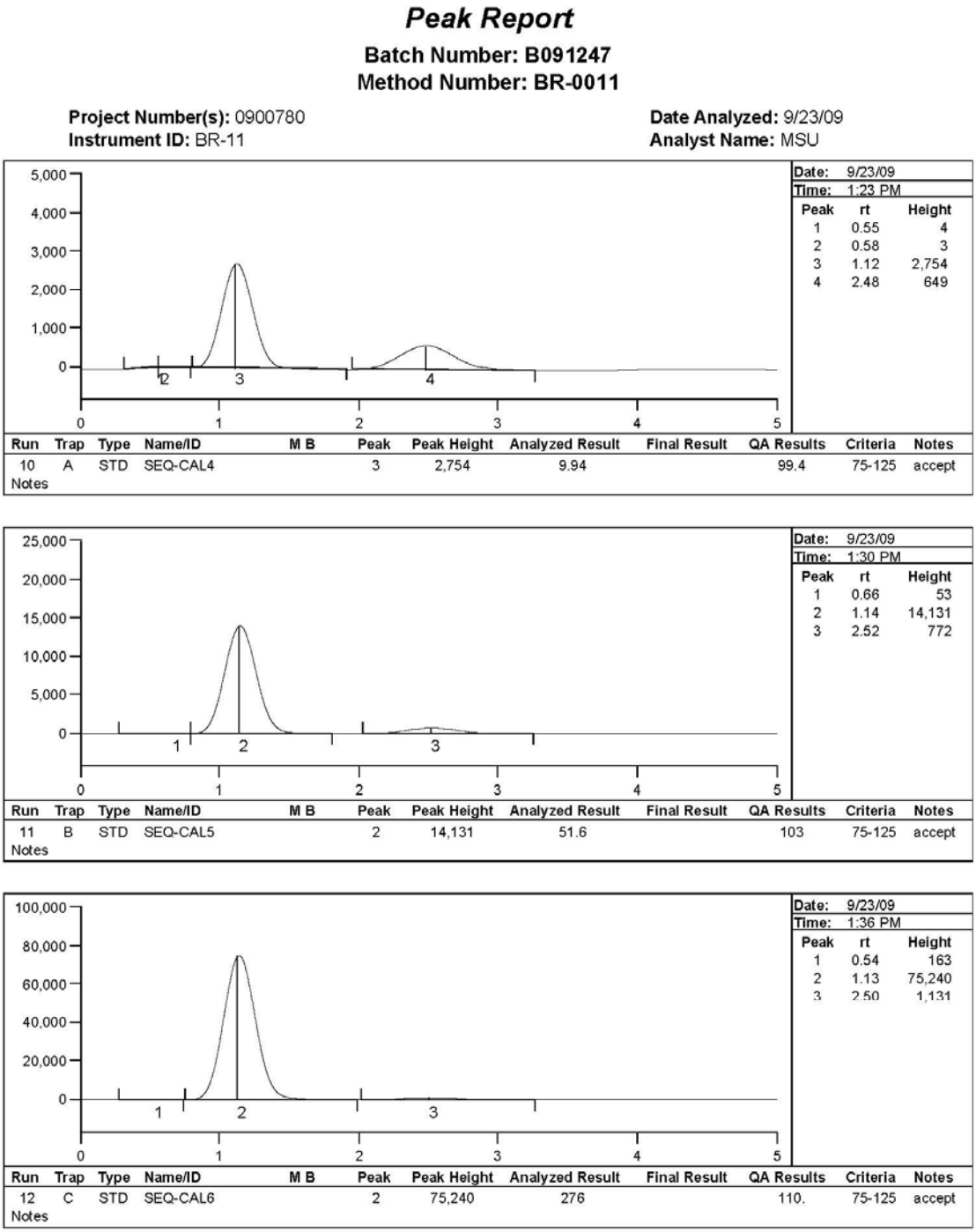
QC Sample	Measure	Minimum Frequency	Criteria	Corrective Action
Ethylation Blank	Contamination from bubblers	4 prior to calib.; following calib. and end of batch	≤ the MRL	Clean and test bubblers until criteria met prior to any analysis
Calibration Standards	Acceptability of the Calibration Curve	Daily, prior to analysis of samples or whenever the CCV fails	RSD of response factors ≤ 15%; Recovery of Low Standard = 65 – 135%	Reanalyze suspect calibration standard. If criteria still not met, then remake standards and recalibrate the instrument
Independent Calib. Ver. (ICV) Continuing Calib. Ver. (CCV)	Accuracy	Following Cal.; Beginning and end and 1 per 10 sample preparations	ICV Rec. = 80-120% CCV Rec. = 67-133%	Correct problem (recalibrate, remake standard, etc.) and reanalyze ICV/CCV. If criteria met, reanalyze samples backwards (if possible) until 2 consecutive results with RPD ≤ 20%
Carryover Check Ethylation Blank	Contamination due to carryover in the bubbler/trap	Following any unusually high result and CCV. Currently ≥ 2x the high standard	≤ the MRL	Clean and continue to test bubbler/trap combo until criteria met prior to further use. Reanalyze samples that were analyzed in same bubbler/trap following high result
Method Blank	Contamination from reagents, lab ware, etc.	4 per batch	Avg ≤ 0.045 ng/L StDev ≤ 0.015 ng/L or < $1/10^{th}$ of associated samples	Correct problem. All samples associated with a contaminated method blank must be reanalyzed.
Blank Spike (BS)	Accuracy	1 per 20 samples	Recovery = 67 – 133%*	Reanalyze remaining volume. Correct problem prior to continuing analysis
Matrix Spike/Spike Duplicate	Accuracy and Precision within a given matrix	1 per 10 client samples	Recovery = 65 – 135%*; RPD ≤ 35%	If **recoveries** similar but fail recovery criteria, an interference is present in the sample and the result must be qualified. If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.
Method Duplicates	Precision	Per client request	RPD ≤ 35% or ± MRL if sample < 5x MRL	Correct problem and reanalyze all associated samples.

* Recovery Criteria for Matrix Spikes and BS samples are based on recovery corrected results.

Table 6 Quality Control Acceptance Criteria and Corrective Action Guidelines for the Analysis of Methyl Mercury in Solid Samples by Distillation, Extraction, and/or Digestion

QC Sample	Measure	Minimum Frequency	Criteria	Corrective Action
Ethylation Blank	Contamination from bubblers	4 prior to calib.; following calib. and end of batch	≤ the MRL	Clean and test bubblers until criteria met prior to any analysis
Calibration Standards	Acceptability of the Calibration Curve	Daily, prior to analysis of samples or whenever the CCV fails	RSD of response factors ≤ 15%; Recovery of Low Standard = 65 – 135%	Reanalyze suspect calibration standard. If criteria still not met, then remake standards and recalibrate the instrument
Independent Calib. Ver. (ICV) Continuing Calib. Ver. (CCV)	Accuracy	Following Cal.; Beginning and end and 1 per 10 sample preparations	ICV Rec. = 80-120% CCV Rec. = 67-133%	Correct problem (recalibrate, remake standard, etc.) and reanalyze ICV/CCV. If criteria met, reanalyze samples backwards (if possible) until 2 consecutive results with RPD ≤ 20%
Carryover Check Ethylation Blank	Contamination due to carryover in the bubbler/trap	Following any unusually high result. Currently ≥ 2x the high standard	≤ the MRL before further analysis of samples on same bubbler/trap	Clean and continue to test bubbler/trap combo until criteria met prior to further use. Reanalyze samples that were analyzed in same bubbler/trap following high result
Method Blank	Contamination from reagents, lab ware, etc.	4 per batch	Avg ≤ 2 x MDL StD ≤ 2/3rd MDL or < 1/10th of associated samples	Correct problem. All samples associated with a contaminated method blank must be reanalyzed.
Standard Reference Material (SRM)	Accuracy	1 per 20 samples	Soil Rec=65-135% Biota Rec=65-135%*	Correct problem prior to continuing analysis
Matrix Spike/Spike Duplicate	Accuracy and Precision within a given matrix	1 per 10 client samples	Soil Rec=65-135%; RPD ≤ 35% Biota Rec=65-135%*; RPD ≤ 35%	If **recoveries** similar but fail recovery criteria, an interference is present in the sample and the result must be qualified. If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.
Method Duplicate	Precision within a given matrix	In association with MS/MSD	RPD ≤ 35% or ± 2x MRL if sample < 5x MRL	If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.

* Recovery Criteria for Distilled Matrix Spikes and SRM samples are based on recovery corrected results.



Peak Report

Batch Number: B091247
Method Number: BR-0011

Project Number(s): 0900780
Instrument ID: BR-11

Date Analyzed: 9/23/09
Analyst Name: MSU

Page 4 of 25 (Peak Report)

Mercury Guru ver 4.0 © 1995-2008 Brooks Rand LLC

Figure 3 Examples of Typical Chromatograms Generated by the Mercury Guru Software During the Analysis of Methyl Mercury Using EPA Method 1630

Example Log

Brooks Rand Labs

MMHg Water Prep Benchsheet

Prepped By: _____ SOP/Rev #: BR-0011 / _____

Prep Date: _____ Batch: _____

Sample ID	Sample Mass (g)	Slot #	Time On	Time Off	pH	Sample ID	Sample Mass (g)	Slot #	Time On	Time Off	pH

Batch QC ID	Source	Spike vol (uL)	Spike conc (ng/mL)	Spike ID	Spike Witness	Reagent	ID
						0.5 mL H2SO4	
						0.2 mL KCL/ L-cysteine	
						HCl	
						Hot Block Temp	
						Final Dilution Vol	
Comments:						Balance ID	

Example Log

Brooks Rand Labs

MMHg Biota Prep Benchsheet

SOP/Rev BR-0011 Rev _____

Prepped By: Batch: _____

Preparation Start Date/Time*: Preparation End Date/Time**:

* Time is when the first reagents are added.

** Time is when the last sample is brought upto volume

Sample ID	Sample Mass (g)	Sample ID	Sample Mass (g)	Sample ID	Sample Mass (g)

Batch QC ID	Sample Source	Spike vol (uL)	Spike conc (ng/mL)	Spike/CRM ID	Spike Wit-ness

Target Temp/Time:	**65 C/4 hours**
Oven ID:	
Oven Temp (measured / corrected):	/
time on:	
time off:	
Balance ID:	
Thermometer ID:	
Volume:	

Reagent	ID
1 mL KOH/Me	
Methanol	

Comments:

Example Log

Brooks Rand Labs

MMHg Sed/Soil Prep Benchsheet

SOP / Revision #: BR-0011 Rev __________

Prepped By: ______________ **Batch:** ______________

Preparation Start Date/Time*: ______________ Preparation End Date/Time**: ______________

* Time is when the first reagents are added. ** Time is when the last sample is brought upto volume

Sample ID	Sample mass (g)	Original vial (g)	vial plus DCM (g)	**EXINFO 1** EXTRACT. DCM (g)	**EXINFO 2** Empty Bottle (g)	**EXINFO 3** Bottle + DCM (g)	Recovered DCM (for LIMS calcs)	Rec. DCM/ext. DCM
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
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				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!

Batch QC ID	Sample Source	Spike vol (uL)	Spike conc (ng/mL)	Spike/CRM ID	Spike Witness
BS1		63	1.0		
MS/MSD1					
MS/MSD2					
MS/MSD3					
SRM1/2	SQC-1238				

	Reagent ID
CuSO4	
KBr/H2SO4	
DCM	
Balance ID:	
Thermometer ID: (measured / corrected):	/
Date/Time On:	
Date/Time Off:	
Final Dilution Vol:	

MeHg Analysis Benchsheet: MeHg MERX-M

Sequence : _____	Batches: _____
Analyst:	Date: Instrument ID: BR-09

1 ng/mL std ID:_____	$NaBEt_4$ ID:_____
0.01 ng/mL std ID:_____	Acetate Buffer ID:_____
1ng/mL ICV std ID:_____	
Pipette ID (if used for sample aliquoting): MeHg Pipette Set	Balance ID:_____

*distillates are weighed out for analysis since the distillate has a density of 1 g/mL

	Run# / Pos #	BRL Sample ID	Analyze Vol (mL)*	Dilution Factor	Analysis Comments / for spiked QC: Source ID, standard ID, and spike volume
	1	Rinse	--		
	2	Rinse	--		
	3	Rinse	--		
	4	SEQ-IBL1	--		
	5	SEQ-IBL2	--		
	6	SEQ-IBL3	--		
	7	SEQ-CAL1	0.05		0.01 ng/mL
	8	SEQ-CAL2	0.1		0.01 ng/mL
	9	SEQ-CAL3	0.2		0.01 ng/mL
	10	SEQ-CAL4	1		0.01 ng/mL
	11	SEQ-CAL5	0.05		1 ng/mL
	12	SEQ-CAL6	0.25		1 ng/mL
	13	SEQ-CAL7	1		1 ng/mL
	14	SEQ-CCB	--		
	15	SEQ-ICV1	0.1		1ng/mL ICV standard
	16	SEQ-CCB	--		
	17	SEQ-CCV	0.025		1 ng/mL
	18	SEQ-CCB	--		
	19	SEQ-CCB	--		
	20	SEQ-CCB	--		
	21				
	22				
	23				
	24				

Appendix A: Brooks Applied Labs' Procedure for EPA Draft Method 1630 (01/01)

Differences Between EPA Method 1630 and Brooks Applied Labs SOP #BAL-3200

The following differences exist between EPA Method 1630 (with relevant sections marked in boldface) and the practices employed by Brooks Applied Labs (SOP #BAL-3200):

- **Section 1.1** of EPA Draft Method 1630 (hitherto referred to as simply 1630) states that the scope of the method is for the determination of methyl mercury in aqueous samples. Brooks Applied Labs has made use of several peer reviewed papers to expand the scope of the method to include sediment and biota. Therefore, any description of the preparation or analysis of sediment or biota samples found in this SOP will NOT be found in 1630 and should be considered a difference.

- According to 1630, Carbotraps® are heated for 45 seconds at a temperature of 450-500 ºC (**Section 6.5.4**). Brooks Applied Labs uses Tenax traps instead of Carbotraps®. The Tenax traps are heated for 30 seconds at a temperature of 200 ºC.

- 1630 describes the GC column as being 1 meter long (**Section 6.5.6.1**); whereas, the GC column used at Brooks Applied Labs is 50 cm long.

- **Section 6.5.6.4** of 1630 states that the GC column should be heated to a temperature of 110 ºC (± 2 ºC). [redacted]

- **Section 7.8** of 1630 states that frozen ethylation reagent ($NaBEt_4$) is good for one week. Brooks Applied Labs has found frozen $NaBEt_4$ to be stable for up to one month as long as it has not been thawed.

- **Sections 9.4.1, 10.1.1.2, 11.2.1, and 11.2.2** of 1630 state that the bubblers with ethylation blanks, standards, and samples should have 300 µL of acetate buffer and 40 µL of $NaBEt_4$ added to them prior to purging. Brooks Applied Labs adds the 300 µL of acetate buffer, [redacted]

- The calibration standards used in 1630 are listed, in order of analysis, as 5 pg, 50 pg, 100 pg, 200 pg, and 1000 pg (**Section 10.1.1.2**). Brooks Applied Labs calibrates the instrument using the following standards: 2 pg, 10 pg, 50 pg, 250 pg, and 1000 pg.

- **Section 11.1.1** of 1630 makes no mention of adding additional H_2SO_4 to the sample prior to distillation. Brooks Applied Labs routinely adds 0.5 mL of 9M H_2SO_4 to each distillation vial prior to beginning the distillation.

- **Section 11.1.1** of 1630 states that 200 µL of 1% APDC solution is used as the chelator and is added to the distillation vial just prior to beginning the distillation. [redacted]

[redacted]

- **Section 11.1.7** of 1630 states that distillates should not be stored longer than 48 hours prior to analysis. BAL has performed stability tests that indicate that water and sediment distillates and extractions can be stored in the dark for up to 72 hours without affecting the analysis of the samples. Refer to BAL Research and Development Study Hg-16.

- 1630 states that once the sample preparation and all of the reagents have been added to the reaction vessel (bubbler), they should be allowed to react for 17 minutes so that all of the methyl mercury is converted to volatile methylethyl mercury prior to purging (**Sections 10.1.1.2 and 11.2.4**). Brooks Applied Labs has found that 15 minutes are sufficient for this process.

- 1630 states that the bubbler contents should be purged onto the trap for 17 minutes (**Section 11.2.5**). Brooks Applied Labs has found that 15 minutes are sufficient for this process.

- Following purging, 1630 states that any adsorbed water should be removed from the Carbotraps® by drying the trap for 7 minutes with N_2 gas (**Section 11.2.6**). Brooks Applied Labs has found that 5 minutes is sufficient for drying the Tenax traps.

SOP #BAL 0304

Sample Homogenization

Brooks Applied Labs

Supersedes BR-0106

Written 2/10/2016
Revised 2/10/16
Revision 001

Reviewed _____

_____

_____

_____

VP of Operations	Date
VP of Quality	Date
SC Group Lead	Date

Sample Homogenization

1.0 SCOPE AND APPLICATION

1.1 Method BAL 0304 describes the procedures employed at Brooks Applied Labs (BAL) to ensure that all sample matrices are sufficiently homogenized prior to any aliquot being removed for preparation. This method is applicable to all sample matrices including aqueous, soil/sediment, and biota, and all of their associated submatrices.

2.0 SUMMARY OF METHOD

2.1 Prior to sample preparation, each sample is homogenized by the procedure appropriate to its specific matrix and submatrix type. The following procedures are standard at BAL, unless specific instructions to do otherwise are received from the client.

2.2 Aqueous samples:

2.2.1 Thoroughly shake aqueous samples prior to the removal of any sample from the original collection container.

2.3 Soils and sediments samples:

2.3.1 Pour off all overlying water upon receipt unless sediments for speciation are received frozen. If this is not done for any reason, it must be documented on the sample receiving log. If decantation is not done at receipt, then overlying water is poured off prior to sample preparation.

2.3.2 If necessary, remove inhomogeneous material, such as detritus, using forceps.

2.3.3 If necessary, sieve sample through a #10 sieve (pore size of 2.0 mm). This is only done if the sample is very dry with a lot of rocks and only with project manager (PM) approval

2.3.4 Final homogenization is performed by successively mixing and quartering the sample until the desired aliquot mass is obtained. Samples that are already well homogenized can be mixed in and samples directly from the sample container.

2.4 Biota samples:

2.4.1 Biota samples are homogenized by various techniques, depending upon their submatrix. Dissection, chopping, cutting, grinding, and other techniques may be required to initially homogenize the sample.

2.4.2 Final homogenization of the sample should be performed through successive mixing and quartering as per soil samples. Samples that are already well homogenized can be mixed directly from the sample container.

3.0 DIFFICULTIES

3.1 The presence of cinnabar (mercury sulfide) in mineral samples to be analyzed for mercury can make sample homogenization difficult. Small particles of this material can be so much higher in mercury content than the surrounding sample matrix that even a slight difference between aliquots can greatly affect the final results. Other minerals may have similar effects in samples prepared for other analyses. For this reason, all soils and sediments suspected of containing cinnabar should be subsampled by successive mixing and quartering.

3.2 High water content in solid samples can make obtaining representative aliquots more difficult. For this reason, overlying water is removed from the sample prior to subsampling, whenever possible and unless otherwise requested by the client.

3.3 Heterogeneous material, such as detritus in aqueous and soil samples, rocks in soil samples, or bone and shell material in biota samples can make obtaining representative samples difficult. When practical and when it would not cause sample contamination, heterogeneous material should be removed from the sample.

3.4 Freezing and thawing of wet sediments samples can change sample matrix consistency. Therefore, samples should be homogenized and subsampled for all analyses at the same time following any thawing of the samples.

4.0 APPARATUS AND MATERIALS

4.1 Size #10 stainless steel sieve: Sieve with a 2.0 mm pore size and 3” diameter.

4.2 Stainless steel and plastic forceps

4.3 Stainless steel, rubber, and Teflon spatulas and/or microspatulas

4.4 Clean plastic weigh boats

4.4 Stainless steel kitchen knives and cleavers

4.5 Dissecting tools

4.6 Stainless steel scissors or garden shears

4.7 Plastic or glass cutting boards

4.8 Mortar and pestle

4.9 Magic Bullet® with extra cups

4.10 Meat Grinder: TS-108 Mincer with attachment to grind biota samples

4.12 409®

4.13 Ninja Blender

4.14 Blentec Blender

4.15 Cuisenart Blender

4.16 Dewer filled with liquid nitrogen

4.17 Jars, 4oz-64oz size, depending upon samples size

4.18 Stainless steel hammer

5.0 PROCEDURE

5.1 Water Sample Homogenization:

5.1.1 Any aqueous sample that is to be prepared in another container besides the original sampling container may only be split under the following conditions: The samples must be acidified to pH < 2 for a minimum of 3 hours before splitting into separate aliquots. Samples to be filtered may not be acidified prior to filtration. Therefore, most samples where a portion of the sample is to be filtered must be received cold (0-4º C) and split within 48 hours of collection. Refer to BRL standard operating procedure BR-0104 for specific sample filtration guidelines.

5.1.2 All water samples, including samples that appear to be free of any particulate material, must be thoroughly shaken prior to pouring off any aliquot. Samples that are high in particulate may require repeated mixing during splitting to prevent any material from settling while pouring off aliquots.

5.1.2.1 Multiphase samples, such as mixes of water and oil, will require special attention. Upon receipt of an aqueous sample that is multiphasic, the sample custodian must immediately notify the project manager. The client must then be consulted as to how they want the sample handled (e.g. analyze just the aqueous layer or analyze the entire mixed sample). The agreed upon method for handling the multiphasic samples should be recorded in the project notes. If a single layer is to be analyzed, the layer

of interest is removed from the other layer(s) by carefully pipetting it into a separate container. If the whole sample is to be analyzed, repeated vigorous shaking during pour offs will be necessary to ensure that the mixture is as homogenous as possible. Use of a sonicator may be necessary to ensure complete homogenization.

5.2 Soil and Sediment Sample Homogenization:

5.2.1 It is preferable that soil and sediment samples (unless they are received freeze dried) are homogenized and subsampled prior to freezing for storage. Freezing and thawing of wet samples can cause changes to the matrix consistency that may make it difficult to obtain similar aliquots between freezing and thawing events. If homogenization is not possible prior to freezing, then all aliquots for all analyses should be collected on the same day without refreezing the samples

5.2.2 Unless requested not to do so by the client, any overlying water should be poured off from soil or sediment samples as they are received and prior to freezing. Care must be exercised to avoid accidental loss of solid material. If for any reason the overlying water is not poured off at the time of receipt, it must be noted on the sample receiving log. If not at receipt, then overlying water should be poured off prior to sample preparation.

5.2.3 Again, unless otherwise requested not to do so by the client, unwanted detritus may be removed using stainless steel or plastic tweezers. Tweezers are cleaned using 409® and are triple rinsed with deionized water (DIW). Tweezers must be thoroughly cleaned between each use and any time possible contamination is suspected (i.e. tweezers dropped or handled without gloves, etc.)

5.2.4 Samples are further homogenized in one of two ways depending on the apparent homogeneity of the sample.

5.2.4.1 If the sample appears somewhat homogenous and there is space available in the sample container, the sample is mixed well with a spatula in its original container and the appropriate aliquot is removed and weighed into the sample preparation container.

5.2.4.2 If samples appear inhomogenous or if the sample container is too full to mix the sample, then samples are further homogenized by successive mixing and quartering of the sample. For this procedure, the sample is thoroughly mixed with a clean spatula in a new weigh boat. (Again, the spatula must be cleaned between each use and any time possible contamination is suspected.) Approximately 75% of the sample is returned to the original collection container. The remaining 25% is thoroughly mixed in the weigh boat and again 75% of the remaining

sample is returned to the original collection container. The appropriate aliquot is removed and weighed into the sample preparation container.

5.2.5 Mineral samples that require crushing and pulverizing are subcontracted out for this service. The returned material is then treated like any other soil or sediment sample for homogenization purposes. An equipment blank should be requested for each group of samples subcontracted out for any homogenization step.

5.2.6 Multi-Increment Sub-Sample Collection - If the client requires that sub-sampling be performed using the incremental sampling method (ISM), then this procedure must be followed, unless otherwise agreed to with the clieent. This procedure is not appropriate for wet sediments, samples with very low concentrations (due to the risk of bias due to contamination), or samples to be analyzed for volatile compounds (e.g., elemental mercury)

5.2.6.1 Pour the field sample into a large plastic tray and air dry the sample in a clean location until the sample is dry.

5.2.6.2 If necessary, sieve the sample to < 2 mm. If sieved, spread the sample back out onto the large plastic tray following sieving.

5.2.6.3 Ensure sample is spread evenly and relatively compact in the tray, and then take 30 sub-samples from the sample with a small plastic spoon or scoop. The size of each increment should be based on the total mass needed for all sample analyses to be performed. Composite the increments into one small vial or jar, appropriate for the analyses to be performed.

5.2.6.4 If this procedure is used, a total solids analysis is not required for reporting results on a dry-weight basis, as the sample is dried prior to weighing out for sample digestion.

5.3 Biota Sample Homogenization: There are several different biota sub-matrices, each with its own unique challenges to homogenization. The major biota types analyzed at Brooks Rand are covered below. Some sub-matrices may require additional procedures. The Laboratory Manager should be consulted whenever there is any question as to how a sample should be handled.

Note: Samples which require speciation analysis may require a different homogenization approach depending on the chemistry of the target molecules. Lyophilization or cryo-grinding may be more appropriate to prevent species transformation during the homogenization procedure.

The necessary time, effort, and applicable equipment required to homogenize tissue samples are dependent on the sample size, target elements/molecules, and nature of tissue. Although a sample may be small in mass it may be necessary to homogenize it using the blender due to the hardness of the material (e.g. bone, teeth). Homogeneity is also a subjective variable as it relies on the visual acuity of the technician. In general, samples should be processed for homogenization until the consistency and color of the material is uniform throughout. If there is any question regarding the uniformity or capacity to homogenize a specific sample matrix the VP of Operations or Technical Director should be consulted.

5.3.1 Large Tissue: (i.e. fish, amphibian, mammal tissue)

NOTE: Not all tissues will require every procedure discussed.

5.3.1.1 Dissection: Depending on the client's instructions, some samples may require dissection to remove specific tissues. Only 409® cleaned stainless steel dissection tools should be used. Special care must be taken to avoid cross contamination from undesired tissues. The dissection tools must be cleaned with 409® and triple rinsed with DIW between samples and any time possible contamination is suspected (i.e. blade dropped on the floor, scissors laid down on surface without clean bench liner, nut crackers handled without gloves, etc.).

5.3.1.2 Filleting: All fish filleting should be performed on a glass or plastic cutting board using stainless steel paring knives. Fillets should be removed from the lateral area of the fish behind the head and pectoral fin to before the tail fin. If requested by the client, prior to collecting the fillet, the skin should be removed from the fish. Clients may also request the fish be de-scaled prior to filleting or homogenization. When removing the fillet, care should be taken to avoid all bones and do not cut into the gut cavity as it may contaminate the fillet tissue. Gloves must be changed between handling each fish sample and all cutting utensils and cutting boards must be 409® cleaned and triple rinsed with DIW between samples and any time possible contamination is suspected.

5.3.1.2 Chopping,cutting, smashing: Tissues too large to fit in the blender cups or meat grinder will require chopping into smaller portions. This is accomplished using a clean stainless steel knife, cleaver, or scissors and a plastic or glass cutting board. If a sample is so dense that it requires smashing, a stainless steel hammer, covered in a plastic bag may be used to smash the sample (contained within a plastic bag). All cutting

utensils and cutting boards must be 409® cleaned and triple rinsed with DIW between samples and any time possible contamination is suspected.

5.3.1.3 Grinding: Large tissues may require grinding. A TS 108 Meat Grinder is used to perform this. Any homogenized sample that becomes lodged in the screw must be removed. The screw, grinder plate, holding tray and feed tube must be removed and cleaned with 409® and triple rinsed with DIW after each sample. If a homogenization blank is required, it must be collected by running DIW through the grinder and collecting it into a bottle appropriate for the analysis to be performed (refer to section 6).

5.3.1.4 Blending using Magic Bullet®, Ninja Blender, Cuisenart Blander, Blendtec Blender: Small to medium amounts of biota may be blended using the Magic Bullet® ,Ninja Blender, Cuisenart Blender, or Blendtech Blender-depending upon the type or size of the samples. One blender cup should be used per homogenization batch and cleaned with 409® and triple rinsed with DIW after each sample. If a homogenization blank is required, it must be collected by blending DIW in the cup or pitcher and pouring it into a bottle that is appropriate for the analysis to be performed. 5.3.1.5 Final homogenization: Aliquots from homogenized samples should still be subsampled using the successive mixing and quartering method as described for soils and sediments (Section 5.2.4).

5.3.2 Shellfish: (i.e. clams, oysters, etc.)

Shellfish will typically require removal from the shell prior to homogenization. If the shellfish are fresh, then discard any excess liquid from inside of the shell. If the shellfish have been frozen, then collect the excess liquid as part of the sample since some cells may have lysed and released material from the tissues. If all of the tissue is to be homogenized, then care should be taken to remove the entire mantle from the shell as some toxins and metals will accumulate at higher levels in the mantle tissue than other tissues. Once removed from the shell, all of the tissue should be homogenized as per large tissues (Section 5.3.1). A client may request that the shell (such as from a crab) be included in the sample homogenization. If this is the case, the sample should be blended using the Blendtec Blender as it has the strongest motor.

5.3.3 Small Invertebrates: (i.e. insects, worms, plankton)

Micro invertebrates and plankton samples can typically be homogenized within their collection containers using a clean spatula or microspatula. A porcelain mortar and pestle can also be used. Additional cutting and blending may be required for larger invertebrate samples. If necessary, follow the guidelines found in Section 5.3.1.

5.3.4 Blood

Blood samples should never be shaken or stirred to mix as this may cause frothing of the sample. Instead, the vial containing the blood sample should be gently inverted to mix the sample. Blood samples should only remain thawed and exposed to the atmosphere for the minimum amount of time necessary for sampling in order to avoid clotting.

5.3.5 Teeth

Tooth samples are typically too small to split into separate aliquots prior to preparation. For these instances, the whole tooth is digested in a strong nitric acid solution. This solution is thoroughly mixed and aliquots are taken for the various analyses or method duplicate samples.

When there is sufficient sample to allow for it, the sample should be ground using a clean mortar and pestle and thoroughly mixed as per sediments prior to removing any aliquots.

5.3.6 Miscellaneous: (i.e. feathers, hair, leaves)

Material not easily ground should be cut into small portions with clean stainless steel scissors. These samples can also be frozen by pouring liquid nitrogen over them into a mortar, then using the pestle to homogenize. This will require 2-3 rounds of pouring liquid nitrogen and homogenizing. These are then mixed and subsampled.

It is important that these samples are properly homogenized as some elements that bioaccumulate in these samples will be more concentrated in different portions of the sample depending on when exposure to the element occurred.

5.4 Cleaning Homogenization Equipment: It is the responsibility of the sample control specialist to ensure that all equipment is cleaned properly prior to homogenization and cleaned and stored following sample homogenization.

5.4.1 All sample equipment must be scrubbed using 409®,a plastic dish scrub brush, and DIW and then triple rinsed with copious amounts of DIW.

5.4.2 Homogenization equipment should either be dried on clean bench liners in a clean air hood or dried on a counter with clean bench liners both beneath and covering the equipment.

5.4.3 Once dried, the homogenization equipment that comes into contact with the samples must be placed into zip style plastic bags. They are then stored in the appropriate drawer, cabinet or closet until further use.

6.0 QUALITY ASSURANCE

6.1 Documentation: Homogenization of samples must be fully documented. Initial treatment of a sample upon receipt, such as pouring off excess water from sediments, must be documented in the sample receiving log (SRL) and in appropriate logs according to SOPBAL 2000. Further homogenization of biota samples must be noted on the Sample Homogenization Bench sheet. There are two different Homogenization Bench Sheet tabs in this spreadsheet: The "Simplified prep sheet" is to be used when samples submitted weigh over 10g(Exhibit A). No sample pre or post weights will be recorded on this bench sheet. The "Expanded prep sheet" (Exhibit B) is to be used if samples weigh less than 10g and/or the client requests that it be used. Pre and post sample weights shall be recorded on this sheet. The sample control specialist performing the homogenization should check with the Project Manager regarding which bench sheet to use for homogenization. Additional required information such as sex, species, etc. should be recorded under the "comments" section of either bench sheet. For biota samples that aren't completely homogenous due to bone, cartilage, scales, etc., additional notes should be made on the Sample Characteristics Log for biota (Exhibit B) if requested by Project Manager. Every soil and sediment sample requiring homogenization must be described in detail using the Sample Characteristics Log for soils and sediments (Exhibit C).

6.2 Homogenization blanks:

6.2.1 Blanks will be collected on any new piece of homogenization equipment, and whenever a new technician is homogenizing samples., and will be taken to the appropriate lab for analysis with the other water blanks (tubing, bottle, etc).

Analyst specific homogenization blanks must be obtained by having the new technician performing the homogenization thoroughly clean the homogenization equipment being used, then run DIW through the piece of equipment and collect the DIW into the appropriate container for testing. Blanks should be collectedfor both mercury and trace metals. Results of the specialist's homogenization blanks should be recorded in the Lot Testing 20XX spreadsheet under the Homog Blank Testing 20XX tab. Each piece of equipment will be named and labeled appropriately, for example: "Magic Bullet #2". New equipment specific homogenization blanks are also collected when the piece of equipment is brand new and thoroughly cleaned. These should also be documented under the Homog Blank Testing 20XX tab in the Lot Testing 20XX spreadsheet.. When either type of homogenization blank (New technician or piece of equipment) is collected, the blank must be included in the homogenization bench sheet pertaining to the particular batch the blanks were collected in.

Blanks are collected by running DIW over or through the cleaned equipment and collecting the water in a container appropriate for the analysis to be performed. Only bottles known to be clean for the element(s) of interest may be used to collect the blank. For equipment such as blenders, DIW should be added to the blender cup and "blended" for a period of time similar to the time taken to homogenize a sample.

6.2.2 If the blank was requested by the client, then it is logged-in to the appropriate work order and batched for analysis of the client's analyte list with the client's samples. If client-requested homogenization blanks are required, then the PM must ensure this is incated on the homogenization SPF. A homogenization blank should be collected after the first sample, but prior to homogenizing the final sample. Homogenization blanks are always prepared after the equipment has been cleaned and before use with the next sample. Homogenization blanks must be preserved and prepared as per the analytical method for the element(s) of interest.

For client requested blanks, acceptance limits will depend on the element of interest and the sample matrix type. The minimum acceptance criterion is that the homogenization equipment must contribute less than $1/10^{th}$ to the total element of interest found in the sample. Since homogenization blanks will be measured in aqueous units (i.e. ng/L) and the affected samples will typically be measured in solid units (i.e. ng/g), blank results should be compared to sample results by looking at the total mass (pg or ng) of analyte present in the homogenization blank compared to the total mass of analyte present in the sample when it was homogenized. This can be estimated by multiplying the sample result by the estimated sample mass that was homogenized using the equipment.

6.2.3 To ensure that the ongoing cleanliness of the larger blending equipment, a monthly homog blank should be collected from each blender cup and blade. The blanks should be collected for mercury and trace metals and submitted to the appropriate lab for analysis. Results should be recorded in the 20XX Lot Testing Spreadsheet in the "Monthly Homog Blank" tab. If it is found that a blender is contaminated, is should either be labeled to include which analytes is should not be used for, or removed from circulation and discarded.

7.0 REFERENCES

"Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual." EPA-823-B-01-002. (11/01).

8.0 BENCHSHEETS

Homogenization benchsheets are printed from LIMS from the batch bench sheet using the "print" button. Depending on the type of homoginaztion to be performed, the analyst chooses either the "bch_SC_Homog_Expanded.rpt" or the "bch_SC_Homog_Simple.rpt".

Examples of sample characteristics logs are provided on the following pages.

Exhibit A

Example
Basic Homogenization Log

Batch Homogenization Log Sheet (BAL-0304 Rev _____)

Batch: B160366

Date: _____ **Start Time:** _____

Initials: _____ **End Time:** _____

Sample ID	Equipment	Comments (length, sex, # of organisms, etc)
1608016-01		
1608016-02		
1608016-03		
1608016-04		
1608016-05		
1608016-06		
1608016-07		
1608016-08		
1608016-09		
1608016-10		
1608016-11		
1608016-12		
1608016-13		
1608016-14		

Additional Notes:

Example
Expanded Homogenization Log

Batch: B160366

Date: _____ **Start Time:** _____

Initials: _____ **End Time:** _____

Sample ID	Pre Mass	Post Mass	Equipment	Comments (length, sex, # of organisms, etc)
1608016-01				
1608016-02				
1608016-03				
1608016-04				
1608016-05				
1608016-06				
1608016-07				
1608016-08				
1608016-09				
1608016-10				
1608016-11				
1608016-12				
1608016-13				
1608016-14				

Additional Notes:

Exhibit B

Example

Sample Characteristics Log (Biota)

Prep Technician:_____ Date:_____

Client ID(s):_____

Sample ID	Matrix/Submatrix	Physical Characteristics

Additional Notes:_____

_____

_____

_____

_____

Exhibit C

Example

Sample Characteristics Log (Soil/Sediment)

Batch:_____ Initials:_____ Date:_____

Key: Rock = Rk, Sand = Sd, Silt = St, Clay = Cl, Organic Matter = OM

Write a 1 – 10 (indicating approximate percentage of constituents) after each descriptor, the numbers should always add up to 10. For example Rk 2, Sd 8

Sample ID	Decanted	**Matrix (1-10)**					**Comments** Color, odor, homogeneity, rock size, etc.
		Rk	**Sd**	**St**	**Cl**	**OM**	

Comments: