SOP #BR-0002

BRL 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 Rand Labs

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Lab Manager	6-9-11 Date
QA Manager	6/8/11 Date
Scientist (if applicable)	06.06.11 Date

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BRL 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 BR-0002 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 BR-0002 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 Rand Labs (BRL) 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.

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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 BR-1205 (Preventative Maintenance).

6.2. Specific equipment used at BRL is listed below. Any modifications to EPA Method 1631E are described and explained.

6.2.1. Equipment needed for analysis of mercury using BRL manual system

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6.2.1.1. Brooks Rand Labs Amalgamation Control Module (BRL part #02100): Controls the heating of the gold-coated sand traps to 450 ºC and then the cooling of the traps following desorption of Hg from the trap.

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

6.2.1.3. Brooks Rand Labs flow meter/needle valve (BRL part #08510): Capable of controlling and measuring gas flow to the purge vessel at 200-500 $mL \cdot min^{-1}$.

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

6.2.1.5. Soda lime pre-trap (BRL 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 port of a clean cold vapor generator, partially filled with deionized water (DIW) and 500 µL stannous chloride ($SnCl_2$), and purging for at minimum of 15 minutes with nitrogen (N_2) at 400 $mL \cdot min^{-1}$. Bubbler blanks are analyzed first so that any indication of unclean pre-traps would be evident immediately.

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

6.2.1.7. 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.

6.2.1.8. Gold wire traps or gold-coated sand traps (BRL part #03010 or #03020): Used for trapping gaseous elemental mercury (Hg^0). Gold-coated sand traps made with gold-coated 40/100 mesh quartz sand are used for analyze solid samples. Both gold-coated sand traps and gold wire traps are used to analyze air samples. Gold wire traps are made by forming 300mg of 0.001 inch diameter gold wire into a plug that is inserted into quartz tubing. Figure 1 of this document shows the setup of the single-trap amalgamation system used at BRL.

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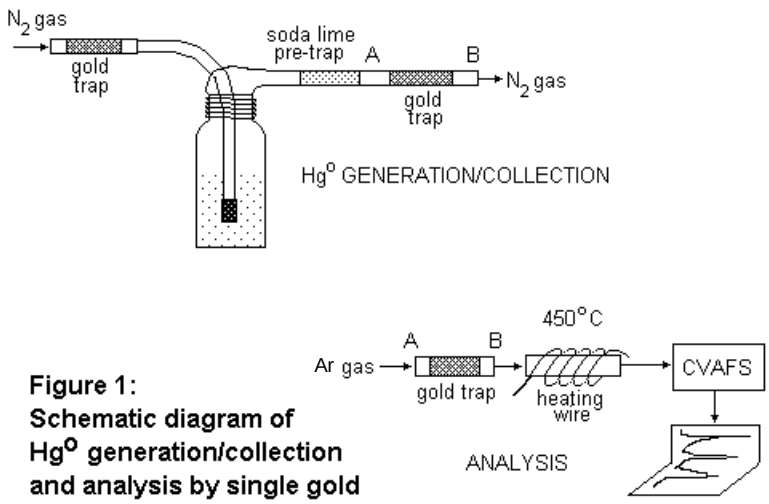

Figure 1: Schematic diagram of Hg° generation/collection and analysis by single gold amalgamation/CVAFS

6.2.2. General equipment needed for analysis for either the manual or the automated MERX-T system.

6.2.2.1. Brooks Rand Labs Model III Atomic Fluorescence Spectrophotometer (BRL part #07002): To achieve the low detection levels a very sensitive CVAFS detector is required. Such systems are built at BRL (BRL Model III) based on the principles discussed in the literature. Refer to the "Brooks Rand, LLC Model III Operation Manual" for instrument operating instructions.

6.2.2.2. Recorder: BRL uses direct data acquisition with the BRL Guru integration software instead of a chart recorder or integrator as described in EPA Method 1631E, section 6.6. The BRL Model III comes complete with the Hg Guru™ integrating software. Refer to the "Brooks Rand 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.

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

6.2.2.4. Bottle-top dispenser: Fixed volume pipettor in the range of 1.0 mL to 10 mL.

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6.2.2.5. 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.

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

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

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 100 mL aliquot of fresh reagent water, each with 0.5 mL $NH_2OH{\bullet}HCl$ and 0.5 mL of $SnCl_2$, are analyzed at the beginning of the run sequence. The average results must be < 20 pg Hg with a standard deviation < 7.5 pg Hg. A high level of mercury detected in the reagent water analysis may also be attributed to the bubbler itself, $NH_2OH{\bullet}HCl$, $SnCl_2$, 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. The BRL sample preparation lab clean room is equipped with a laminar flow hood that provides incoming air to the lab. Outside air, which is very low in Hg, is filtered through a particulate filter and then through HEPA filters before entering the mercury lab. Positive pressure is maintained to ensure that there is no incoming air through routes other than the laminar flow hoods. Sticky mats are located at the entrance as an additional precautionary measure. The mercury lab, the sample preparation lab, and the shipping and receiving area 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 BRL 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.

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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. All open cases of acids for trace metal work are stored in the locked storage area located in the north mezzanine level of the building.

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. All open cases of acids for trace metal work are stored in the locked storage area located in the north mezzanine level of the building.

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. All open cases of acids for trace metal work are stored in the locked storage area located in the north mezzanine level of the building.

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 fluorinated polyethylene (FLPE) bottles at five different concentrations of BrCl (1.0%, 2.0%, 5.0%, and 10%). The BrCl is prereduced in the split bottle and then added to the bubbler with stannous chloride. A linear regression is performed to determine the level of contamination due to the bottles (b = y intercept) and the true concentration of Hg in the reagents. The acceptance criterion for the linear regression is an r^2 value ≤ 0.9 (If 2 or more of the points are less than the low standard, 25 pg Hg, then r^2 criteria may not be relevant and the lot of BrCl must be approved by QA). The amount of Hg per reagent unit (reagents used in a 1.0% BrCl method blank) must be ≤ 20 pg. If BrCl is prepared using the same lot of KBr and KBrO3 as a lot of BrCl already tested for linearity, then only two reagent blanks need be made at 10% BrCl to ensure the continued purity of the reagents. All lots of HCl are pretested and must already be shown to be pure prior to any use in reagents.

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As such, linearity testing of new BrCl is not required if only the HCl lot has changed.

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 such as the "high level standards and samples" cabinet located on the south wall of the shop.

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 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.

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8.2. 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.

8.3. 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.

8.4. 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 SOP BR-0106 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.

8.5. 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 BRL is to pick out large particles prior to aliquoting for sample prep.

8.6. 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: BRL 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.

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The AFS detectors used are the same for either instrument. The AFS detectors built by BRL are capable of achieving extremely low detection limits.

9.3. 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. BRL 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.

9.3.1. For manual analysis, the instrument is calibrated down to 25 pg. The standards typically used to calibrate the instruments for total mercury at BRL 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.

9.3.2. 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.

9.3.3. 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. BRL 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, average ≤ 5 pg and standard deviation ≤ 3.3 pg. The criterion for subsequent bubbler blank checks is that each blank must be ≤10 pg and ± 5 pg from the calibration blank mean.

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

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

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 BR-0106 for instructions in the homogenization of various sample matrices.

10.1.2. 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 0.8 to 1.2 g (0.2 to 0.4 g for dry material such as wood, paper, freeze dried samples, etc.) is weighed directly into a 40 mL glass vial with a fluoropolymer-lined lid. To prep homogenization blanks, pipette 10 mL of the homogenization blank water into a vial then prep as normal.

Glass vials are tested by lot number prior to use to ensure that they are not a potential source of mercury contamination. Glass vials are triple rinsed with DIW prior to use in the preparation of samples. 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. 3.0 mL of H_2SO_4 and 7.0 mL 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 BRL a hotplate is used, the samples are digested at a temperature of 70 ºC for 1 hour and then refluxed at a hotplate temperature of 90-100 ºC for 3 hours. 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 40 mL 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

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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 at 100 °C for 1 hour and then refluxed at a sand bath temperature of 150 °C for 2 hours, or until all organic matter is dissolved, the solution looks substantially colorless or light yellow, and the brown gas above the liquid has almost disappeared.*

As a substitute, 100 mL volumetric flasks may be used. After addition of the HNO_3/H_2SO_4 mixture, a clean glass marble is placed over the mouth, and the samples are allowed to predigest at room temperature for 1 hour. Samples are then heated in the same manner as previously described. After digestion the flasks should be allowed to cool, and 1.0 mL of BrCl is added and the volume brought up to the 100 mL mark with ultrapure deionized water. A section of parafilm is secured over the flask opening, and the samples are thoroughly homogenized by gentle agitation prior to analysis.

10.1.3. Alternative cold aqua regia digestion: This procedure is for geological media such as coal, ores, **sediments, and soils**. Since the matrix is leached rather than dissolved, the sample must be pulverized prior to digestion if the total mercury content is desired.

Weigh to the nearest mg approximately 1.0 g (~0.5 g for dry material such as dust, coal, etc.) into a 40 mL vial. In a fume hood, add 8.0 mL of concentrated HCl, swirl, and add 2.0 mL of concentrated HNO_3. The vial may be loosely capped (tightening the lid may cause the vial to explode). The preparation should then be allowed to digest at room temperature overnight.

For all samples other than those high in elemental carbon (i.e. coal), add 0.5 mL BrCl and dilute the digestate to 40 mL with deionized water, shake vigorously, and allow to fully settle prior to analysis. For coal samples, and other samples high in elemental carbon, dilute the digestate to 40 mL with up to 0.07 N (35%) BrCl solution to prevent any mercury from re-adsorbing to the carbon particles. Shake the preparation vigorously and allow it to settle overnight.

Note: More organic samples may require higher levels of BrCl. BRL typically adds 0.5 mL of BrCl to most aqua regia preparations, but more organic samples may require higher levels of BrCl (levels between 1.25% and 35% BrCl). Additionally, reagent water without BrCl may be used if the samples are appropriate to such preparation and very low method blanks are required.

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10.1.4. 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.

10.1.4.1. Pipette 1 mL of DIW into a 20.5 mL glass 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.

10.1.4.2. Then 1.0 mL of a 7:3 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 150 ºC and the samples are heated for an additional two hours. The samples are then removed from the heat and allowed to cool to room temperature. 250 µL of BrCl is added and each sample preparation is made up to 20.5 mL with deionized water.

Additional Notes: The SRM used for blood analysis (SRM-966) comes in two levels of trace metals. Only Level 2 is certified for mercury. Only Level 2 should be used as an SRM for this analysis. The level of mercury in this SRM is high enough that 20 mg of the SRM is sufficient. The SRM should be weighed out in the same manner as the samples.

10.2. Instrumental Analysis:

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

10.2.1.1. Samples and standards are purged for 15 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.

10.2.1.2. Four 100-mL aliquots of water are poured into each of the bubblers prior to calibration. 0.5 mL of $SnCl_2$ is added to each bubbler and the aliquots are used to condition the soda-lime pre-traps for a minimum of 15 minutes, as

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noted in 6.2.1.5 of this SOP. Following this pre-trap conditioning step, the purged water is discarded and 100 mL aliquots of DIW along with 0.5 mL $NH_2OH•HCl$ are poured from the four split bottles into their associated bubblers. 0.5 mL $SnCl_2$ is added to this water, which is then purged for 15 minutes 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 to 100 mL aliquots of 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 of 0.10-1.0 mL (maximum of 10 mL due to trap degradation by acid fumes), is added to 70-100 mL of deionized water in a split bottle. 0.5 mL 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 0.5 mL 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 for 15 minutes. 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.

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

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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. BRL 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.

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 10% (w/v) KOH in DIW for approximately 30 minutes or until all visible residue is removed and are then triple rinsed and filled with 10% HCl. This procedure should be done

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more frequently if the bubbler starts to appear dirty before the end of the week.

10.2.2. BRL 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 6 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.

10.2.2.2. 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 10 mL) is added to each vial and 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.

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

10.2.2.4. Aliquots of the digestate, usually in the range of 0.10-1.0 mL (maximum of 10 mL due to trap degradation by acid fumes), is added to 15-25 mL of deionized water in a 40-mL septa top vials. 0.2 mL of $NH_2OH{\bullet}HCl$ is added directly to the vial and allowed to react for at least 5 minutes prior addition of 0.2 mL of $SnCl_2$, capping, and placing on the autosampler for purging onto a gold amalgamation trap for analysis.

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 must be analyzed after every 10 client samples (not counting quality control samples) to verify ongoing control of the system.

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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).

BRL 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 15% HNO_3 and sonicated for 15 minutes.

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, BRL has not experienced significant analytical problems associated

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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. The following equations are used at BRL to calculate sample results.

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

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

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

11.3. 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

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

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

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.

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

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11.6. 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 BRL is not always aware of the original source of a sample or the specific needs or requirements of our clients, all results above the BRL 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 BRL for this method. Consult Section 17, Table 1 of this SOP for the current MDL (method detection limit) and ML (minimum limit) determined at BRL 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 BRL 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, BRL 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, BRL 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 BRL policy to run additional CCV samples (one after every 10 client samples) to ensure ongoing control of the system.

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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. 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 Rand 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 BRL 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 BRL. 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

All Brooks Rand Labs (BRL) SOPs are Proprietary Information and protected by WA state law. Proprietary Information shall be kept in the strictest confidence & shall not be used or appropriated to benefit any party without prior written consent from BRL.

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 such as Philips Services.

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

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17.0. TABLES AND BENCHSHEETS

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

Matrix	Preparation Method	Method Detection Limit (MDL)[1,2]	Minimum Level (ML)
Sediment/Sludge	Aqua regia cold digestion (1.25% BrCl)	0.05 ng/g	0.15 ng/g
Coal (samples high in elemental carbon)	Aqua regia cold digestion (diluted w/35% BrCl)	0.20 ng/g	0.50 ng/g
Biota/Sediment	HNO_3/H_2SO_4 hot digestion (1.25% BrCl)	0.04 ng/g	0.10 ng/g
Biota – Blood in Capillary tubes	HNO_3/H_2SO_4 hot digestion (1.25% BrCl)	1.5 ng/g	4.5 ng/g
Hair (w/washing step)	HNO_3/H_2SO_4 hot digestion (1.25% BrCl)	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)

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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 $ng \cdot L^{-1}$ 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 $ng \cdot L^{-1}$ std	Recovery 77-123%

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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; ave. < 5 pg; stdev < 2.5 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; ave. < 5 pg; stdev < 2.5 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 $ng \cdot L^{-1}$ std	Recovery 77-123%
29	CCB	Carryover Blank	DIW + analytical reagents	< 10 pg; ave. < 5 pg; stdev < 2.5 pg
Next	10 Injections	Sample Analysis	Samples	
Last	CCV (500 pg std)	Continuing Calibration Verification	5.0 $ng \cdot L^{-1}$ std	Recovery 77-123%
Next	CCB	Carryover Blank	DIW + analytical reagents	< 10 pg; ave. < 5 pg; stdev < 2.5 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%.

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Table 3a. Quality Control Criteria and Corrective Action Procedures for the Manual 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	each ≤ 40 pg avg ≤ 20 pg std ≤7.5 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	≤ 40 pg and within ± 20 pg of avg bubbler blank	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 <u>Mass Criteria</u> 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.
Method Duplicate (DUP)	Precision within a given matrix	In conjunction with MS/MSD samples and when deemed necessary	RPD ≤ 30% Blood and Small <u>Mass Criteria</u> RPD ≤ 35%	If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.

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Table 3b. Quality Control Criteria and Corrective Action Procedures for the Automated Analysis of Total Mercury by CVAFS using the MERX-T.

QC Sample	Measure	Minimum Frequency	Criteria	Corrective Action
Calibration Blanks / Bubbler Blank	Contamination from instrument, vials, or reagents	4 – 2 per trap	each ≤ 10 pg avg ≤ 5 pg std ≤ 2 pg	Clean reanalyze until criteria met prior to any further analysis. Clean instrument and/or remake reagents if needed.
Calibration Standards	Acceptability of the calibration curve	Daily (first batch of the day) or if CCV/ICV fail.	RSD of response factors ≤ 15%; Recovery of Low Standard = 75 – 125%	Reanalyze suspect calib stand. 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 samples and 1 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%. Otherwise, recalibrate system.
Carryover Check Bubbler Blank	Contamination due to carryover in the instrument/trap	Perform 2 following any measured result ≥ 20,000 pg	≤ 10 pg (within ± 5 pg of avg bubbler blank before used for additional analyses)	Remake and condition the soda-lime trap. Clean and continue to test blanks until criteria met prior to further use. The two samples analyzed following a result ≥ 20,000 pg must be reanalyzed.
Method Blank (1% BrCl in reagent water)	Contamination from reagents, lab ware, etc.	3 per batch (BRL usually does 4)	Each MB ≤ 0.5 ng/L and StDev ≤ 2/3 the MDL 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 = 70 – 130%; RPD ≤ 30% Blood and Small <u>Mass Criteria</u> 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.
Method Duplicate	Precision within a given matrix	In conjunction with MS/MSD samples and when deemed necessary	RPD ≤ 30% Blood and Small <u>Mass Criteria</u> RPD ≤ 35%	If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.

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Table 4. Certified Reference Materials Applicable to this SOP and in Stock at BRL

Agency	**Name (ID)**	**Matrix**	**Certified Level of Hg**
National Institute of Standards and Technology			
NIST	966 (Level 2)	Bovine Blood	31.4 $\mu g \cdot L^{-1}$ (29.8 $\mu g \cdot kg^{-1}$)
	2976	Mussel Tissue	0.061 $mg \cdot kg^{-1}$
NIST	1946	Lake Fish Tissue	0.433 $mg \cdot kg^{-1}$
Institute for Reference Materials and Measurements			
IRMM	BCR-580	Marine Sediment	132 $mg \cdot kg^{-1}$
National Resource Council of Canada			
NRCC	DORM-3	Fish Homogenate	0.382 $mg \cdot kg^{-1}$
	DOLT-4	Fish Liver	2.58 $mg \cdot kg^{-1}$
	TORT-2	Lobster Hepatopancreas	0.270 $mg \cdot kg^{-1}$
	PACS-2	Marine Sediment	3.04 $mg \cdot kg^{-1}$
	MESS-3	Marine Sediment	0.091 $mg \cdot kg^{-1}$
GBW	GBW 07604	Poplar Leaves	0.026 $mg \cdot kg^{-1}$
International Atomic Energy Agency			
IAEA	IAEA-085	Human Hair	23.2 $mg \cdot kg^{-1}$
	IAEA-086	Human Hair	0.573 $mg \cdot kg^{-1}$
	IAEA-407	Fish Tissue	0.222 $mg \cdot kg^{-1}$

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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				

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(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:

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(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 Rand 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:_____

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

Sequence:______________________ Analyst:____________ Date:_______________

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

Comments:____

(Example Log)

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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 Rand 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: _____

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(Example Log)

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

Sequence:_____ Analyst:_____ Date:_____

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

Comments:_____

_____

_____

_____

_____

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

EPA Method 1631		BRL SOP BR-0002	
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 ~1 g, 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**	0.5 mL 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.
A9.4.2	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.
A9.5.1	Analyze an aliquot of the appropriate reference matrix (see Appendix Section A7.1), spiked at the concentration in the IPR.	**12**	Blank spikes at the MRL are prepared and analyzed, but there is no matrix added to them.
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**	The maximum aliquot is 10 mL. The digestate is added to ~100 mL of DIW in a split bottle with 0.5 mL hydroxylamine hydrochloride. The entire contents of the split bottle are added to the bubbler then 0.5 mL 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**	The samples are purged in the bubblers for 15 minutes. These conditions allow measurements of Hg concentrations in the range of 0.03 – 16,000 ng/g.
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 BR-0006 for any analysis modifications, as they may also be applicable to BRL-0002.]

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Appendix B: Brooks Rand Labs Procedure for EPA Method 1631, Revision E (02/05)

Differences between EPA Method 1631, Revision E, and Brooks Rand Labs SOP #BR-0006

The following differences exist between EPA Method 1631, Revision E (with relevant sections marked in boldface), and the practices employed by Brooks Rand Labs (SOP #BR-0006):

● **Figures 1** (referenced within **sections 6.2.1.8**) give schematic diagrams of the bubbler, purge and trap, CVAFS analytical system. In contrast, Brooks Rand Labs (BRL) 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:

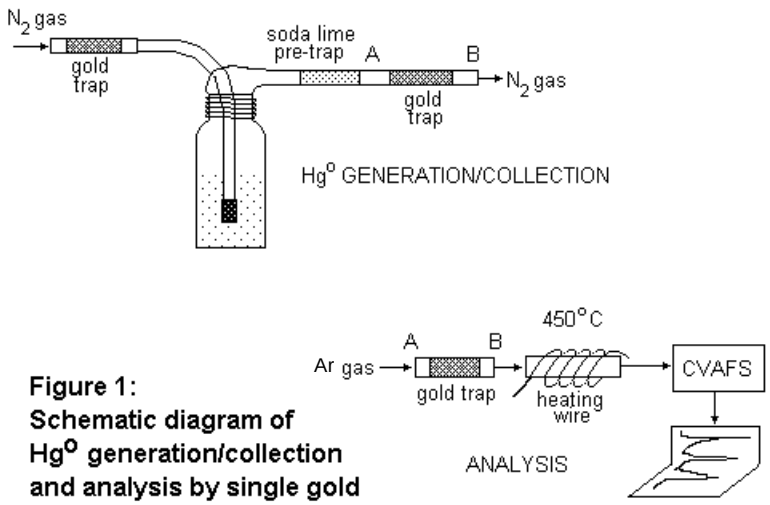

Figure 1: Schematic diagram of Hg^0 generation/collection and analysis by single gold amalgamation/CVAFS

● **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. BRL uses pretraps consisting of 6-12 mesh soda lime chunks and which are purged for 15 minutes with nitrogen.

● **Section 6.5** discusses the use of a dual-trap Hg(0) pre-concentration system. In contrast, BRL uses a single trap pre-concentration system. This allows for shorter analysis times without masking of potential problems on the sample collection trap.

● BRL uses direct data acquisition with the BRL "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, BRL uses a 1.0 ng Hg/mL standard, along with the 10 ng Hg/mL standard, for calibration.

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- **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. After the determination of the concentration of the native sample, appropriate spike volumes will be added to an aliquot of the native sample in the Teflon split bottle prior to pre-reduction.

- **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, BRL subtracts the average peak area measurement of the first four split bottle blanks 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.

- **Section 9.4.7** specifies that 5% of any lot of bottles should be tested. BRL has found that there is little variation within any given lot of FLPE bottles, glass bottles, or glass vials. Therefore, BRL 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. BRL 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. BRL 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. BRL uses a calibration curve with points of 25 pg, 100 pg, 500 pg, 2500 pg, and 10000 pg. These points are produced by adding 0.025 mL and 0.100 mL of a 1.0 ng Hg/mL standard (50 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). BRL 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. BRL purges all samples for 15 minutes at 400 mL/min. High-level standard recoveries and subsequent bubbler blanks indicate that 15 minutes is an adequate purge time to volatilize and collect mercury.

- BRL 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.

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