Sample Splitting. Preservation, Storage, and Disposal
Edward S. Babcock & Sons
Standard Operating Procedure
Effective Date____09/08/06_____

1.0 The following procedures are in place to safeguard samples from deterioration, contamination, loss, or damage.

2.0 All samples must be thoroughly mixed prior to removing subsamples. Please be aware of client id and special instructions in the comments. Once a sample is preserved or split wrong it cannot be fixed so be careful**!**

3.0 All sample preservation and screening is noted on the Work Order Printout.

3.1 Samples that are received preserved are checked for proper pH. If more preservative is required a ++ is written next to the preserved bottle listed on the Work Order Printout.

3.2 Samples that are received unpreserved may require a separate bottle split out for analyses listed below. This will be indicated by a →. Make sure to include the entire Lab Number that subsample was split from including the bottle letter.

3.3 If the entire unpreserved sample bottle is preserved for an analysis, this is indicated the preservative code only.

3.4 If the sample is not logged in correctly for the type of bottle received, Login is informed and a note is made on the Work Order Printout (e.g. "rec UNP" for received unpreserved).

3.5 Samples must be brought to a method specified pH however do not add excessive volumes of preservative as this might dilute the sample. Use no more than 20 ml of preservative per quart or liter of sample. If the sample is still not at the proper pH, note on the Work Order Printout and sample bottle; **NOT>12** or **NOT<2.**

3.6 Sample compositing will be requested in the notes and needs to be done prior to any analysis of the sample. Place a **checkmark** ✓ next to the note on the Work Order Printout. Label the bottle COMP with date and initials and apply the printed label if it is provided. Enter this information into the computer in "Update Status" under comments. It is very important that this information be entered into Element as soon as it has been done or directly after the splitting process. Go to "Update Status" under comments and enter "COMPOSITED or COMP mmddyyinitials" in front of the "needs to be composited prior to analysis" note.

3.7 Sample filtering will be requested in the notes and needs to be done prior to any analysis of the sample. Place a **checkmark** ✓ next to the note on the Work Order Printout and then follow the appropriate procedure listed below. Use the splitter code F on the bottle and Work Order Printout to indicate what has been filtered and preserved. It is very important that this information be entered into Element as soon as it has been done or directly

after the splitting process. Go to "Update Status" under comments and enter "FILTEREDmmddyyinitials" in front of the "needs to be filtered" note.

3.7.1 For metals filtering: Acid rinse filtration apparatus with 1:1 HNO_3. Filter sample through a 0.45 micron filter prior to preservation. Use a portion of the sample to rinse the filter flask, discard this portion and collect the required volume of filtrate. A filtered blank must be included with the samples. Label blank with date/initials along the side of the bottle and list the sample #(s) associated with it. Apply the printed label to the samples if it is provided. Preserve for metals as per section 6.0 placing a NF on the sample bottle and Work Order Printout.

3.7.2 For phosphate or nitrogen filtering: Acid rinse filtration apparatus with 1:1 HCl. Filter sample through a 0.45 micron filter prior to preservation. Use a portion of the sample to rinse the filter flask, discard this portion and collect the required volume of filtrate. A filtered blank must be included with the samples. Label blank with date/initials along the side of the bottle and list the sample #(s) associated with it. Apply the printed label to the samples if it is provided. Preserve as specified in section 8.0 placing a SF on the sample bottle and Work Order Printout.

3.7.3 For all other analysis: Filter sample through a 0.45 micron filter. Make sure to filter enough sample for all requested analysis. Label the bottle as filtered with date and initials and apply the printed label if it is provided. If filtered sample needs to be split out for any other preserved analysis, make sure to include a preserved filter blank that is labeled with date/initials and sample #(s) associated with it.

3.8 Odd notes in comments: Place a **checkmark** ✓next to the note on the Work Order Printout once the directions have been carried out.

3.9 All interference screening and preservative addition is noted on the Work Order Printout by a code explained below and in Table 1.

3.10 The splitter signs and dates each Work Order Printout after the statement "Preservation confirmed by_____".

4.0 **Cyanides (CN)**

4.1 Preserve samples with approximately 1 - 2 mL of 35% sodium hydroxide per 500 mL of sample to pH ≥ 12 at the time of collection or as soon as possible at the laboratory. Place an **OH** on the Work Order Printout and sample lid. If a sample is received preserved section 4.4 cannot be performed. If possible, screen for chlorine (section 4.3) prior to preservation.

4.1.1. 35% NaOH = 175g NaOH into 500 mL of D.I. Stored at room temperature for up to one year.

4.2 The samples should be stored in a refrigerator or in an ice chest filled with water and ice to maintain temperature at 4 °C. The maximum holding time is 14 days.

4.3 Oxidizing agents such as chlorine decompose most of the cyanides. Test drinking water and final effluent samples by filling a snap cap one quarter full with sample and adding a drop of Orthotolidine Solution. A yellow-orange color indicates the need for treatment. Add sodium thiosulfate a few drops at a time, (no more than 16mL per liter of sample), until a retesting of the sample produces no color change. Place an **TS** on the sample cap and Work Order Printout whenever sodium thiosulfate is used. Document amount added on the Work Order Printout. Place an **X** on the Work Order Printout if no chlorine was detected.

4.3.1. Orthotolidine Solution: Dissolve 1.35g orthotolidine dihydrochloride into 500 mL D.I. water. Prepare separately a solution of 150 mL HCl and 350 mL D.I. water (always add acid to water). Combine the two solutions. Stored at room temperature for up to one year.

4.3.2. Sodium Thiosulfate solution (BOD titrant): Dissolve 6.205 g $Na_2S_2O_3 \cdot 5H_2O$ in 1 liter Nanopure water. Add 4 pellets (~ 0.4 g) of NaOH. This solution is made fresh every month and stored at room temperature.

4.4 Sulfides adversely affect the colorimetric procedure. Prior to NaOH preservation, test liquid samples for sulfide by placing a drop of sample on lead acetate paper previously moistened with acetate buffer. If paper turns brown, sulfide is present. Add lead acetate (or lead carbonate) to the neutralized sample until the sulfide is gone as indicated by retesting. Filter the sample before raising the pH for stabilization. Place a **Pb** on the sample cap and on Work Order Printout if treatment was necessary. Place a **NS** on the Work Order Printout if no sulfide was detected. Make a lead carbonate blank for the analyst to use as a calibration check. In a 500mL volumetric flask, add 200mL D.I. and 5g of NaOH, dissolve. Add lead carbonate to the volumetric flask replicating the exact amount that was added to the sample (per 500mL) and bring up to 490-495mL leaving space for the Cyanide analyst to add spiking solution.

4.4.1. Lead acetate paper = Cut up available filter paper into strips. Soak strips in a saturated solution of lead acetate and D.I.

4.4.2. Saturated lead acetate solution: Add lead acetate salt to D.I. water until solution is saturated and salt will no longer dissolve.

4.4.3. Acetate buffer -pH 4 (SM 4500-Cl C 3e: 243g anhydrous $NaC_2H_3O_2 \cdot 3H_2O$ into 400 mL D.I. water. Add 480 mL acetic acid and bring up to a liter with D.I.

4.4.4. Above solutions are stored at room temperature for up to one year.

4.5 Aldehydes convert cyanide to cyanohydrin that does not respond to this method. Stabilize all liquid samples with NaOH and add 1ml 35% ethylenediamine solution per 500ml of sample. Place an **E** on the sample and on the Work Order Printout. EDA is not necessary for drinking water samples.

4.5.1. 35% Solution – Dilute 35ml pharmaceutical grade anhydrous $NH_2CH_2CH_2NH_2$ to 100ml D.I. Store at room temperature, make fresh monthly.

5.0 **Phenol**

5.1 Samples must be collected in a glass container. Preserve samples with approximately 2 mL of 1:1 H_2SO_4 per liter to pH < 2 and refrigerated to 4^o C ± 2 °C. Place an **S (PHE)** on the Work Order Printout and sample lid. The holding time for Phenols is 28 days. If possible, screen for chlorine prior to preservation.

5.2 Oxidizing agents such as chlorine will oxidize phenols. Test each drinking water and final effluent sample by filling a snap cap one quarter full with sample and adding a drop of Orthotolidine Solution. A yellow-orange color indicates the need for treatment. Add sodium thiosulfate a few drops at a time, (no more than 16mL per liter of sample), until a retesting of the sample produces no color change. Place an **TS** on the sample cap and Work Order Printout whenever sodium thiosulfate is used. Document amount added on the Work Order Printout. Place an **X** on the Work Order Printout if no chlorine was detected.

5.3 Orthotolidine Solution - See section 4.3.1 above.

5.4 Sodium Thiosulfate Solution – See section 4.3.2 above.

6.0 **Metals**

6.1 To preserve for trace metals, acidify the sample with 1:1 nitric acid to a pH of less than 2 units. Place a **N** on the Work Order Printout and sample.

6.2 If the sample is reactive upon addition of acid, the splitter lets the reaction finish and then rechecks the pH. If the pH is not <2 place a **NOT<2** on the lid of the sample next to the **N** and on the Work Order Printout. Note on sample and Work Order that sample is reactive.

6.3 If metals require filtration, see section 3.7.1 for instructions..

7.0 **Chemical Oxygen Demand COD**

7.1 Collect the samples in glass bottles if possible. Use of plastic containers is permissible if it is known that no organic contaminants are present in the containers.

7.2 Samples should be analyzed as soon as possible or preserved with approximately 3 to 4 drops 1:1 sulfuric acid per snap cap of sample to a pH < 2 and maintained at 4 ^oC until analysis. Analysis must take place within 28 days. Samples are kept in plastic bottles or snap caps in the refrigerator on the sulfuric preserved cart. Place a **COD** on the Work Order Printout and a **S** on the sample.

8.0 **Kjeldahl Nitrogen (TKN)/ Ammonium (NH4)/ Total Phosphate (TP)**

8.1 Preserve samples with approximately 2ml of 1:1 sulfuric acid per quart of sample to pH <2. After a homogeneous subsample has been separated into a quart plastic bottle, add H_2SO_4. Mix the bottle, test the pH by pouring a portion of the

preserved sample over a pH strip. Place a **S** on the Work Order Printout and sample. If the sample is not <2 add more H_2SO_4 and repeat the above steps until the sample is <2. See section 3.5.

9.0 **Oil and Grease (O&G)**

9.1 Oil and grease samples must be sampled in glass. If they are not sampled in glass, a note is placed in "analyte comments". Preserve samples with approximately 1ml of 1:1 sulfuric acid per 500 mL jar of sample to pH<2. Dip strip into jar to check pH. Place an **S (O/G)** on the Work Order Printout and sample.

10.0 **Total Petroleum Hydrocarbons (TPH)**

10.1 Total Petroleum Hydrocarbons samples must be sampled in glass. Preserve samples with approximately 1-2 ml of 1:1 sulfuric acid per 1L jar of sample to pH<2. Place an **S (TPH)** on the Work Order Printout and sample.

11.0 **Oxyhalides (chlorate, chlorite, bromate)**

11.1 Add 0.15 mL (3 drops) of 35% EDA solution per quart bottle or 2 drops per ½ pint. Place an **E** on the Work Order Printout and sample.

11.1.1. Note – A sample for bromide only does not require preservation however it may be analyzed on the preserved sample.

11.1.2. 35% Solution EDA– Dilute 35ml pharmaceutical grade anhydrous $NH_2CH_2CH_2NH_2$ to 100ml D.I. Store at room temperature, make fresh monthly.

12.0 **Total Organic Carbon (TOC)**

12.1 Preserve samples with approximately 0.1 ml of 1:1 sulfuric acid per 43 mL amber vial to pH<2. Place a **S** on the Work Order Printout and sample

13.0 **Organic Analyses** – Chlorine is an interferant for almost all organic analytes. Dechlorination is handled in the following manner:

13.1 Methods: 504/8011, 524/624/8260, 531, and 547:
It is more appropriate for Organic analyses to be sampled prior to chlorination therefore that vast majority of our samples are not chlorinated. If it is unclear whether a sample will be taken from a chlorinated source, the client will be contacted

for clarification. If it is known that a sample will be from a chlorinated source, the appropriate sample container will be provided containing the method specified dechlorination salt (sodium thiosulfate or sodium sulfite).

13.2 Methods: 507/8141, 508/608/8081, 515/8151, 525/625/8270, 548:
As a precaution, method specified dechlorination salt is added prior to extraction to drinking water and final effluent samples.

14.0 **Separate Carts**

14.1 The following tests have their own carts. When a sample is received requesting only one of these analytes, the sample bottle is placed on this special cart.

14.1.1. Color, Odor, and Turbidity – A **COT** is recorded on the bottle cap and Work Order printout.
14.1.2. Nitrate – A **NO3** is recorded on the bottle cap and Work Order printout.

15.0 **Unpreserved Line-Up**

15.1 The following samples are placed on the unpreserved cart:

15.1.1. Unpreserved bottles (**UNPRES**)
15.1.2. Dissolved sulfide bottles (**DH2S**) - should be zero headspace
15.1.3. Total sulfide bottles (**ZNAC**)

16.0 **Sample Storage**

16.1 Metal sample bottles are stored on the Bakers carts in the metals laboratory.
16.2 The inorganic unpreserved carts are on the east wall of the walk-in refrigerator.
16.3 Along the west wall of the walk-in refrigerator are the following carts from left to right: hydrocarbons, other organics, haz mat, oil and grease, NO_3, cyanide and phenol, sulfuric preserved, and COT only carts.
16.4 Organics:
16.4.1. refrigerator # 1 – wastewater VOA, wastewater TOX, TOC, soils, solids, sludges
16.4.2. refrigerator # 6 – other samples (possibly contaminated) that cannot be in any other refrigerator.
16.4.3. refrigerator # 7 – drinking water VOA's, 531.1, 547, and TOX.
16.5 Soils (agricultural) – are placed in the rolling and sieve area next to the soils drying oven
16.6 Bacti: analyzed immediately or stored in the walk-in on the Bacti cart
16.7 Extracts:

16.7.1. TCLP and CAM extracts: stored in the walk-in, on the standards cart.

16.7.2. Water extracts: usually made and used within 24 hours, however if the analysis has a longer holding time, the extract is stored in the walk-in refrigerator.

16.7.3. Soil-ag extracts: BEX and WEX if not analyzed immediately are placed in the walk-in refrigerator for a maximum of 48 hours. AEX and SEX stored on the metals lab bench prior to analysis. Extracts are not stored after analysis.

16.7.4. Organic extracts: prior to analysis - may be stored in the extraction refrigerator R3 or the refrigerator in the main organics laboratory R4 or R5. After analysis, extracts are either dumped out, kept on lab bench or in one of the above refrigerators depending on the nature of the analytes.

16.7.5. Digests:

16.7.5.2. Metals: stored on multilevel cart near metals samples

16.7.5.3. Kjeldahl: prior to analysis – may be stored under the Kjeldahl counter prior to bringing up to volume for a couple days. Extracts are not stored after analysis

17.0 **Sample Disposal**

17.1 Inorganic Aqueous Samples:

17.1.1. Unpreserved – Stored for 5 –6 weeks and then dumped into an empty barrel. The pH is checked, adjusted if necessary and then the contents are pumped down the sink.

17.1.2. Preserved – See ESB SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes

17.2 Soil, Solid, Sludge, and Nonaqueous Liquid Samples:

17.2.1. Nonhazardous - Samples are kept for 3 months and then placed in the dumpster.

17.3 Hazardous –

17.3.1. Supervisors or chemists reviewing sample results will note when a sample exceeds hazardous limits by placing a red sticker on the sample with the hazardous constituent's name. The sample is recorded in a notebook.

17.3.2. The notebook includes: the sample number, date and initials of the person recording the sample, the hazardous constituent name and concentration.

17.3.3. After 3 months, the sample is placed downstairs until it is lab packed for disposal by a professional waste hauler.

18.0 Table 1. Splitter Documentation Codes

CODE	SPLITTER'S KEY
✓	Special instructions have been followed.
UNP	Unpreserved
S	Pres w/sulfuric acid
N	Pres w/nitric acid
OH	Pres w/sodium hydroxide
NOT<2	pH could not be adjusted low enough (matrix)
NOT>12	pH could not be adjusted high enough (matrix)
F	Filtered
COMP	Composited
E	Pres w/EDA
NS	Negative for sulfide
Pb	Positive for sulfide, lead carbonate added
X	Negative for chlorine
TS	Positive for chlorine, sodium thiosulfate added
REC	Received
-->	Split into another bottle or vial
++	Extra added to already pres bottle or vial
COT	Bottle for Color,Odor,Turbiditiy only
COD or C	Cod vial
NO3	Bottle for nitrate only
(CN)	Cyanide bottle
(O/G)	Oil and grease bottle
(TPH)	Total Petroleum Hyderocarbon bottle
(PHE)	Phenol bottle

COLIFORMS BY
MULTIPLE TUBE FERMENTATION
SM 9221 B,C,E
E.S. BABCOCK STANDARD OPERATING PROCEDURE
Effective Date_____

1.0 Scope and Application

1.1 The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. This group is defined as all aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 h at 35°C.

1.2 Results of the examination of replicate tubes are reported in terms of Most Probable Number (MPN) per 100 mL of sample. This number, based on certain probability formulas, is an estimate of the mean density of coliforms in the sample. Coliform density, together with other information obtained by engineering or sanitary surveys, provides the best assessment of water treatment effectiveness and the sanitary quality of untreated water.

1.3 This method is used for drinking water, surface water, source water, wastewater samples, and solids.

1.4 Reporting limit: 2MPN/100mL

1.5 Range: 2 - 1600MPN/100mL

2.0 Summary of Method

2.1 Sample is placed at various dilutions into tubes containing Lauryl sulfate broth and incubated for up to 48 hours, at $35^{o}C \pm 0.5^{o}$. Each broth tube which shows CO_2 formation inside the Durham tube is transferred to a brilliant green bile tube. These tubes are incubated at 35°C for a total of 48 hours. If gas formation is present in their Durham tubes the sample is considered positive for total coliform bacteria. The positive broth tubes are also transferred to E.C. media (wastewaters and source waters), or E.C. + MUG (drinking waters). The tubes are incubated at $44.5°C \pm 0.2\ ^{o}C$ for 24 ± 2 hours in the fecal water bath. If CO_2 formation is observed in the EC tube after the incubation period, the sample is considered positive for fecal coliform bacteria. If CO_2 formation is observed in the EC + MUG tube and the tube fluoresces in UV light, the sample is considered positive for *E. coli*.

3.0 Definitions: See SOP Q15 – SOP Definitions

4.0 Interferences

4.1 If the sample is not adequately shaken before portions are removed or if clumping of bacterial cells occurs, the MPN value will be an underestimate of the actual bacterial density.
4.2 Turbidity: See sections 11.2 and 11.2.
4.3 An arbitrary 48 hour limit for observation doubtless excludes occasional members of the coliform group that grow very slowly.
4.4 Even when five fermentation tubes are used, the precision of the results obtained is not of a high order. Consequently, exercise great caution when interpreting the sanitary significance of coliform results obtained from the use of a few tubes with each sample dilution, especially when the number of samples from a given point is limited.

5.0 Safety

5.1 When dealing with bacteriological testing it is important to ensure that all working surfaces are clean. Gloves are recommended to protect the analyst from exposure to bacteria.

6.0 Equipment and Supplies

6.1 Media tubes with Durham tube inserts (see ESB SOP B01 section 6.0 for washing instructions.)
6.2 Metal racks
6.3 Glass Pipets: 10mL
  6.3.1 Pipets are placed in disinfectant and then washed thoroughly with D.I. water and then sterilized in a drying oven overnight.
6.4 Transfer loops - sterilized in a drying oven overnight
6.5 Labels
6.6 365 nm Fluorescent light
6.7 Water bath at 44.5°C ± 0.2°
6.8 Incubators at 35°C ± 0.5°

7.0 Reagents and Standards

7.1 Lauryl Sulfate Broth, Brilliant Green Bile, EC Media, and EC + MUG.
  7.1.1 Weekly Preparation:
    7.1.1.1 Directions on media labels are followed for proper rehydration.
      7.1.1.1.1 LSB: 356g media for every 5 liters of Nanopure.
      7.1.1.1.2 BGB: 120g media for every 3 liters of Nanopure
      7.1.1.1.3 EC: 166.5g media for every 4.5 liters of Nanopure
      7.1.1.1.4 EC + MUG: 111g media for every 3 liters of Nanopure

7.1.1.2 Lauryl Sulfate Broth is rehydrated at double strength with Nanopure water as indicated in Standard Methods when used with 10 mL aliquots of sample. 10 milliliters of the rehydrated medium is added to each test tube along with an inverted Durham tube which sits inside the medium tube. A loosely fitting lid is then added to each test tube before it is autoclaved.

7.1.1.3 Prepared media is autoclaved for 12-15 minutes at 250°F (121°C) and 15 lb/in^2 pressure.

7.1.1.4 All sterilized media is stored for later use in a cool cabinet out of direct sunlight, with loose fitting caps, for up to two weeks. A previous study showed that evaporation of media does not exceed 1 mL until 3 months have elapsed. Each rack of media is identified and dated and rotated to ensure the oldest media is used first. Tubes stored longer than two weeks must have either a foam plug or screw cap

7.1.2 The pH of media is recorded after autoclaving. One tube from each batch of media made is autoclaved in a beaker along with the prepared media. The tube is allowed to cool to room temperature before the pH is taken. If pH is outside the following acceptance criteria new media is prepared.

7.1.2.1 Lauryl Sulfate Broth: 6.8 ± 0.2

7.1.2.2 Brilliant Green Bile: 7.2 ± 0.2

7.1.2.3 EC Media and EC + MUG: 6.9 ± 0.2

7.1.3 Each batch of media prepared is tested with a positive and negative control.

7.1.3.1 When a batch of media is prepared, four tubes are designated as QC tubes.

7.1.3.2 After being autoclaved, one tube is inoculated with *Escherichia coli*, one tube with *Enterobacter aerogenes*, one tube with *Pseudomonas aeruginosa*, and the last tube is designated as a blank.

7.1.3.3 These tubes are incubated for 48 hours at 35.0 °C ± 0.5 °C. Their results are recorded in the Bacteriology QC log book.

7.2 Eosin methylene blue agar – 18.8g media for every 500 mLs of Nanopure. Final pH must be 7.1 ± 0.2. See ESB SOP B01 sections 5.2 and 5.3 for agar preparation instructions.

7.3 Dilution water bottles – See ESB SOP B01 section 5.0

7.4 Reagent water - Nanopure

7.5 Our laboratory uses reference cultures of organisms obtained from ATCC, (American Type Culture Collection). The reference cultures are stored at 4^{o}C until manufacturers due date. Reference cultures are rehydrated in lauryl sulfate broth at 35°C ± 0.5°C for 15 minutes or until dissolved. A loop full of the suspension is streaked onto a Standard Methods agar slant and kept at 4°C for up to a month.

	LSB	BGB	EC	EC+MUG	EMB
Enterobacter aerogenes	+	+	-	-	NA
Pseudomonas aeruginosa	-	-	-	-	NA
Escherichia coli	+	+	+	+	+
Enterococcus faecalis	NA	NA	NA	NA	-

8.0 Sample Collection, Preservation and Storage

8.1 Samples are collected in sterile 120 mL containers containing $Na_2S_2O_3$ for chlorine removal.

8.2 Samples must be kept cold, <10^{o}C from sampling until analysis. If a sample arrives <30 ^{o}C and on ice it is assumed that the cooling process has begun. If a sample arrives >30 ^{o}C (even if it is on ice) or >10 ^{o}C without ice, the client is advised that the sample temperature may affect test results.

8.3 Samples must be analyzed within 30 hours for drinking water and within 6 hours for wastewater.

9.0 Inoculation of Drinking Water Samples:

9.1 10 milliliters of sample is transferred to each of 10 tubes containing 10 mL of double strength sterile broth.

9.2 All broth tubes are placed in incubators. The broth tubes are allowed to incubate for 24 ± 2 hours, examined, and then incubated for an additional 24 ± 3 hours, if needed at 35^{o}C ± 0.5^{o}.

9.3 Note: Heterotrophic plate counts are also set up at this time. (see SOP B07).

10.0 Inoculation of non-drinking waters such as effluents, stormwaters, source waters and other surface waters and solids.

10.1 Liquids and semi-solids:

10.1.1 Generally a multiple dilution or serial dilution is used on non-drinking water which may contain high amounts of bacteria. A series of dilutions are needed in order to calculate the most probable number of bacteria per 100 mL (MPN/100mL).

10.1.1.1 Five 10 mL portions of sample are inoculated into the first 5 broth tubes.

10.1.1.2 Ten mL of sample is then transferred into 90 mL of sterile dilution water. This dilution represents 1 mL of sample per 10 mL aliquot of solution. Five 10 mL portions of this first dilution are inoculated into the following five broth tubes.

10.1.1.3 10 mL of the first dilution are transferred into 90 mL of sterile dilution water. This second dilution represents 0.1 mL of sample per 10 mL aliquot of solution. Five 10 mL portions of this second dilution are inoculated into the third set of five broth tubes.

10.1.2 If the sample is cloudy or contains a small amount of dirt, a third dilution may be considered. If the sample is very dirty, additional dilutions will be needed. Raw liquid influents are generally diluted to $1x10^{-3}$ mL at the beginning of the series and ending at $1x10^{-6}$mL. Semi-solid raw influents usually are started at $1x10^{-5}$ mL and end at $1x10^{-8}$ mL. 990 mL and 999 mL dilution bottles may be used for these higher dilutions.

10.2 Solids:

10.2.1 If a sample is solid, generally 10 grams is used to ensure a representable sample. The sample should be broken up with a sterile transfer loop and then added to 100 mL of sterile *dilution water*. *The sample mixture should be allowed to soak for 10* minutes and shaken repeatedly to ensure a homogenous mixture.

10.2.2 Depending on the type of sample, different serial dilutions may be considered. Dry soils are generally inoculated at 1 mL to 0.01 mL. Dried sludges are started at 1 mL and diluted to 0.001 mL.

10.2.3 The MPN for a solid sample is calculated using the following equation: [(MPN from Chart) X A/B] X any dilution factor.

Where:
A = number of grams customer wants the MPN calculation based on (usually one gram).
B = Number of grams added to 100 mL of sterile dilution water (usually 10 grams).

11.0 Daily reading and recording of incubated samples.(EC+MUG)

11.1 Presumptive Phase: The tubes are incubated at 35°C ± 0.5°C. 24 ± 2 hours after the sample has been added into the tubes of sterile broth, the tubes are removed from the incubator and examined. A negative result will show no CO_2 formation inside the Durham tube. The negative broth tubes are re-incubated for another 24 ± 3 hours. After the total 48 ± 3 hours of incubation shows no CO_2 formation in the Durham tubes, the test is completed and the sample is considered negative for coliform bacteria. The broth tubes which do contain CO_2 bubbles are considered presumptively positive for coliform bacteria and proceed to the confirmed phase. Any drinking water sample producing turbid cultures (heavy growth) which inhibit an accurate gas production reading, is automatically transferred to confirming media. All positives on confirming media are reported as such, all negatives are reported as invalid and a resample is requested. In either case, a note indicating the presence of turbidity is placed on the bottom of the lab sheet.

11.2 Confirmed Phase: Each broth tube which shows CO_2 formation inside the Durham tube is transferred to a brilliant green bile tube. Insert a transfer loop into the positive tube, stir, and then place loop into the brilliant green bile tube. These tubes are incubated at 35°C ± 0.5°C for a total of 48 ± 3 hours. If gas formation is present in their Durham tubes when examined after 24 ± 2 or 48 ± 3 hours, the sample is considered positive for total coliform bacteria. The positive broth tubes are also transferred to E.C. media (wastewaters), or E.C. + MUG (drinking waters). The tubes of media also contain Durham tubes. After the sample has been transferred to the E.C. tubes, the tubes are incubated at 44.5°C ± 0.2°C for only 24 ± 2 hours in the fecal water bath. Tubes are placed in the water bath within 30 minutes of inoculation. If CO_2 formation is observed in the EC tube after the incubation period, the sample is considered positive for fecal coliform bacteria. EC tubes that are so turbid that the Durham tube is difficult to see are considered positive if there is foam on the top and the Durham tube sounds hollow. If CO_2 formation is observed in the EC + MUG tube and the tube fluoresces in UV light, the sample is considered positive for *E. coli*. Any tube that is positive for fecal coliform bacteria or *E. coli* must also be positive for total coliform bacteria.

12.0 Quality Control:

Note: See also ESB SOP B01 for general QC requirements

12.1 Positive and Negative controls:

12.1.1 To check for the possible occurrence of false positives due to fecal water bath malfunction during a fecal coliform test, one EC fermentation tube is inoculated with *Enterobacter aerogenes*. Another tube is inoculated with *Pseudomonas aeruginosa*. They are incubated for 24 + 2 hours. If the fecal water bath has maintained a constant temperature of 44.5°C ± 0.2°C, the *E. aerogenes* and *P. aeruginosa* will no longer be viable and will not produce gas.

12.1.2 As an additional fecal water bath QC check, another EC fermentation tube is inoculated with *Escherichia coli* and is incubated for 24 ± 2 hours. If the water bath temperature has been properly maintained, the *E. coli* will produce gas.

12.1.3 A fourth tube of EC media is incubated. It is a blank to ensure sterility.

12.2 Duplicates:

12.2.1 On a monthly basis each analyst shall make parallel analyses of at least one positive sample.

12.2.2 Sample duplicates are performed at a frequency of 5% or once per batch (day) whichever is more.

12.2.3 A precision criterion is generated from historical data as described in Standard Methods 9020:VI. Results $\geq$ 10 times the reporting limit are evaluated against the precision criterion. Attach the qualifier N-Brp to duplicates that do not meet acceptance criteria. If duplicates are extremely erratic, report the higher result with the qualifier N-Bru and notify the client

12.3 Completed Phase: The completed test is run on a quarterly basis.

12.3.1 A completed test is used to establish definitely the presence of coliform bacteria and to provide quality control data. A positive green bile tube containing CO_2 is streaked on an eosin methylene blue agar dish. The streaking method must ensure presence of some discrete colonies after a 24 ± 2 hour incubation period at 35°C ± 0.5°C. The colonies which grow on the E.M.B. agar dish fall in two categories: typical and atypical. The typical colonies are nucleated, with or without a metallic sheen. The atypical colonies are unnucleated, opaque, and mucoid. From the plate, pick one or more typical, well isolated colonies and transfer to a tube of lauryl sulfate broth. Incubate this tube at 35°C ± 0.5°C for 24 ± 2 hours. If no gas production is observed, reincubate for an additional 24 ± 3 hours. Formation of gas within this lauryl sulfate broth tube within the 48 ± 3 hour incubation period demonstrates the presence of a member of the coliform group.

12.3.2 Completed test results are recorded.

12.4 Media Checks:

12.4.1 A blank, known negative and a known positive culture is analyzed with each set of samples. Tubes of Lauryl sulfate broth are inoculated each with *E. coli* and *P. aeruginosa*. These are incubated along with the lauryl sulfate broth samples to ensure reproducibility of results. This is also done with green bile and incubated along with the green bile tubes to ensure reproducibility.

13.0 Calculation of Most Probable Number (MPN):

13.1 The most probable number estimates the number of specific bacteria in water and wastewater by the use of probability tables. The MPN is based on the number of tubes that have confirmed for or are present for bacteria (i.e. total coliform, fecal coliform, etc.). The MPN can also be determined by a combination of confirmed tubes when using multiple dilutions. When more than three dilutions were analyzed, use the highest dilution that gives confirmed results in all five test tubes along with the next two dilutions. Both tables are listed in Standard Methods, 18th edition and is based on the amount of bacteria per 100 mL of sample. If the combination of positive tubes cannot be found on the MPN charts, the MPN can be calculated by the following:

$$\frac{\text{Number of Positive Tubes} \times 100}{\sqrt{\left(\text{mL of sample in negative tubes}\right) \times \left(\text{mL of sample in all tubes}\right)}}$$

This formula was found in Standard Methods, 18th edition.

14.0 Method Performance

14.1 Refer to in-house quality control performance records.

15.0 Corrective Action For Out of Control Or Unacceptable Data:

15.1 If any of the above QC samples do not meet acceptance criteria, the analyst must take measures to correct the problem.

- 15.1.1 The analyst examines the results of other controls performed that day.
- 15.1.2 The analyst examines sample results either for historical trends or for none detects.
- 15.1.3 If the above investigation indicates that the batch is still valid and the out of control QC appears to be an anomaly, results are reported.
- 15.1.4 If it is unclear whether sample results can be verified, any affected samples must either be resampled or results must be reported with a note qualifying the data.
- 15.1.5 Out of control QC is noted on the QC data page along with the corrective action taken. A QC follow up form is filled out in the computer and added behind the QC data page.

16.0 Pollution Prevention and Waste Management

16.1 All positive samples and QC are autoclaved prior to disposal.
16.2 See also SOP S07 – Pollution Prevention

References:
Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WPCF, 18th edition. 9221 B,C,E.

Note: All *italicized items* except bacteria names are an indication of a variation from the method.

Approved by _____ **Date** _____

TABLE 9221.III. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN TEN 10-mL PORTIONS ARE USED

No. of Tubes Giving Positive Reaction Out of 10 of 10 mL Each	MPN Index/ 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0	< 1.1	0	3.0
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12.0	4.3	27.1
8	16.1	5.9	36.8
9	23.0	8.1	59.5
10	>23.0	13.5	Infinite

TABLE 9221.IV. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 mL, 1.0 mL, 0.1 mL)

Combination of Positives	MPN Index/ 100 mL	95% Confidence Limits		Combination of Positives	MPN Index/ 100 mL	95% Confidence Limits	
		Lower	Upper			Lower	Upper
				4-2-0	22	9.0	56
0-0-0	< 2	—	—	4-2-1	26	12	65
0-0-1	2	1.0	10	4-3-0	27	12	67
0-1-0	2	1.0	10	4-3-1	33	15	77
0-2-0	4	1.0	13	4-4-0	34	16	80
				5-0-0	23	9.0	86
1-0-0	2	1.0	11	5-0-1	30	10	110
1-0-1	4	1.0	15	5-0-2	40	20	140
1-1-0	4	1.0	15	5-1-0	30	10	120
1-1-1	6	2.0	18	5-1-1	50	20	150
1-2-0	6	2.0	18	5-1-2	60	30	180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
				5-5-0	240	100	940
4-0-0	13	5.0	38	5-5-1	300	100	1300
4-0-1	17	7.0	45	5-5-2	500	200	2000
4-1-0	17	7.0	46	5-5-3	900	300	2900
4-1-1	21	9.0	55	5-5-4	1600	600	5300
4-1-2	26	12	63	5-5-5	≥1600	—	—

BIOCHEMICAL OXYGEN DEMAND - 5 DAY TEST
Edward S. Babcock & Sons
STANDARD OPERATING PROCEDURE
(**Standard Methods 5210 B**)
Date Effective:____02/01/06_____

1. **Scope and Application:** This method is used to determine the relative oxygen requirements of wastewaters. This method may also be used for other matrices if both client and regulator agree to this variation.

2. **Reporting limits:** The reporting limit for this method is 5.0 mg/L.

3. **Working Range:** Reporting limit = 5 mg/L. The upper end of the working range is dependent upon the dilutions used.

4. **Summary of Method:** The method consists of placing a sample in a full airtight bottle and incubating the bottle under specified conditions for a specific time. Dissolved oxygen is measured initially and after incubation. The BOD is computed from the difference between the initial and final dissolved oxygen.

5. **Environmental Relevence:** The biochemical oxygen demand determination has its widest application in measuring waste loadings to treatment plants and in evaluating BOD-removal efficiency of such treatment systems.

6. **Sample Collection, Preservation, and Holding Times:**

   6.1. The sample for BOD is collected with a minimum of headspace and refrigerated at 4^{o}C prior to analysis.
   6.2. The maximum holding time prior to BOD incubation is 48 hours (CFR part 136). Since even a short time can significantly change the BOD results, the sample is set up for incubation as soon as possible after the sample has been taken.

7. **Interferences:**

   7.1. Adjusting the temperature of the dilution water to 20°C prior to use is important for reproducible results.

7.2. Any chemical present in the sample that is detrimental to the seed bacteria would cause a negative interference (i.e. chlorine, acid, or alkaline).
7.3. See SOP I24 for interferences affecting the dissolved oxygen measurement.

8. Safety Issues: Some of the reagents used in the analysis are corrosive. Proper care must be taken to prevent contact. The use of gloves and safety glasses are essential when performing this test.

See SOP S01 – Concentrated Acids and Bases
SOP S03 – Spill Control Policy

9. Instrumentation/Equipment:

9.1. Incubator with temperature setting at 20 ± 1°C (excluding all light to prevent the possibility of photosynthetic production of dissolved oxygen).
9.2. 300 ml capacity bottles with ground glass stoppers.
9.3. Plastic cup covers.
9.4. Dissolved Oxygen Probe. (see I24)
9.5. General laboratory glassware: pipets, graduated cylinders,

Note: All glassware is cleaned immediately after use by thorough rinsing with three portions of D.I. water. If glassware still appears dirty, further steps are taken, such as 1:1 acid rinse. Glassware is always finished with a final D.I. rinse.
Clean BOD bottles with 1:1 HCL and three rinses of D.I. water. Inspect bottles for residue. *If a more vigorous cleaning is required, a stronger acid and heat may be used. Always ensure that all acid is thoroughly removed from the bottle prior to use.*

10. Reagents and Standards:

Reagents are stored at room temperature for up to six months. Discard if there is any sign of biological growth. Standard Glucose-Glutamic Acid is made fresh daily and stored in the incubator.

10.1. Phosphate buffer solution: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$ and 1.7 g NH_4Cl in 500 ml Nanopure water and dilute to 1 liter. PH should be 7.2.

10.2. Magnesium Sulfate solution: Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in Nanopure water and dilute to 1 liter.

10.3. Calcium Chloride solution: Dissolve 27.5 g $CaCl_2$ or 36.5 g $CaCl_2 \cdot H_2O$ in Nanopure water and dilute to 1 liter.

10.4. Ferric Chloride solution: Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in Nanopure water and dilute to 1 liter.

10.5. Standard Glucose (or Dextrose)-Glutamic Acid solution: Dissolve 0.15 g $C_6H_{12}O_6$ and 0.15 g $HO_2CCH_2{\cdot}CH_2{\cdot}CH(NH_2)CO_2H$ in Nanopure water and dilute to 500 ml. Prepare fresh prior to use. Plant 3 mL of solution into the BOD 300 mL bottle. The working standard solution is made at double strength, therefore 3 mL spiked into a BOD bottle represents a 2% dilution.

10.6. Sulfuric Acid Solution: Add 5 drops concentrated sulfuric acid to 100 mL of Nanopure Water.

10.7. 0.25N NaOH Solution: Add 1 gram of NaOH to 100 mL of Nanopure Water.

10.8. Sodium Sulfite Titrant: Dissolve 0.7895g of Na_2SO_3 into 500 mL of Nanopure water. Prepare fresh daily.

10.9. Orthotolidine Solution: Dissolve 1.35g orthotolidine dihydrochloride into 500 mL Nanopure water. Prepare separately a solution of 150 mL HCl and 350 mL Nanopure water (always add acid to water). Combine the two solutions.

10.10. Nitrification Inhibitor -contains 2-chloro-6-(trichloro methyl) pyridine (TCMP).

10.11. Reagent water: Nanopure or D.I. Water

11. Procedure:

11.1. Sample Pretreatment

11.1.1. Test to determine if residual chlorine is present in the sample.

11.1.1.1. Pre-screen for chlorine with ortho-tolidine solution. Add one or two drops of ortho-tolidine solution to 5 ml of sample. Yellow color = chlorine present.

11.1.1.2. Place 200 ml of sample into a flask. Add 0.5 mL Sulfuric Acid, a few crystals of Potassium Iodide and a few drops of starch solution (see Dissolved Oxygen SOP I24). Titrate using Sodium sulfite solution until blue color disappears. Determine the amount of Thiosulfate titrant needed to destroy the residual Chlorine in the amount of sample used for dilution.

11.1.1.3. Calculation -- Amount of Sodium sulfite solution from 11.1.1.2 x Amount of sample needed for BOD divided by 100 = Amount of Sodium sulfite needed to destroy the residual Chlorine in the BOD sample. Add this amount to fresh sample.

11.1.2. Adjust pH to between 6.5 and 7.5. Check the pH with pH paper or meter. Adjust acidic samples with 0.25 N NaOH (1g/100ml DI) and basic samples with Sulfuric Acid (5 drops to 100ml DI). Do not dilute by more than 0.5% (about 10 drops/100 ml). Use a higher concentration acid or base, if more than 0.5% is needed to neutralize the sample or adjust the pH of the diluted sample.

11.2. Sample Incubation and Analysis

11.2.1. Place 19 liters of Nanopure water into blue jug. Add 1 ml each of Phosphate buffer, Magnesium Sulfate, Calcium Chloride, and Ferric

Chloride solutions per liter of water. Aerate water in refrigerator until temperature reads 19 - 21°C. (Temperature of water will affect D.O. Regulate the temperature with a thermometer.) Fill up a bottle for unseeded blank, then seed Nanopure water in the blue jug with *10 mL* plant influent received in lab no longer than 24 to 48 hours, preferably one to be run the same day, if possible, use as a duplicate. Check with lab director. When planting seed, pipet sample out of unshaken bottle.

11.2.2. Arrange bottles so two - three dilutions of each sample can be made. If the determination of D.O. is by probe, then $D.O_1$ and $D.O_2$ are made from the same bottle, so only one bottle is needed for each dilution. Set up labeled bottles for blank, standards and Winkler standards.

11.2.3. Determine dilution amount by past experience or odor of sample. Check with lab director. Clean samples require a 40% dilution. *If the dilution is greater than 0.1 ml in 300 ml than a serial dilution is needed.*

11.2.4. Using wide tip pipette or graduated cylinder deliver desired amount of sample to each bottle by predetermined dilutions. Sample has been stored at 4°C and may or *may not be 20°C upon dilution.*

11.2.5. The dilution of the lab control is set to result in 200 ppm. This is achieved by adding 3 ml of the Standard Glucose-Glutamic Acid solution into the 300 ml BOD bottle.

11.2.6. Fill each bottle with prepared water, preventing any air bubbles in the bottles. Form a water seal by allowing water to fill flare top to the lip of the bottle to ensure no air will be trapped under stoppers after $D.O_1$ reading.

11.2.7. Determine the initial dissolved Oxygen (see Dissolved Oxygen procedure SOP I24) of samples using DO_1. bottles.

11.2.7.1. If the DO_1 is considerably lower (1 mg/L) than the other DO_1's and blanks then redo that sample with a lesser volume.

11.2.7.2. If the DO_1 is considerably higher (more than 9 mg/L) reduce DO to saturation by bringing another portion of sample to about 20 degrees Centigrade in a partially filled BOD bottle while agitating by vigorous shaking or by aerating.

11.2.8. Before placing ground glass stoppers into bottles, add more water to top of bottle if necessary. Cover each bottle ($D.O_2$) that is to be placed in the incubator with plastic cup caps. Put the covered bottles in the incubator.

11.2.9. After incubating $D.O_2$. samples for 5 days, determine the final Dissolved Oxygen (see Dissolved Oxygen procedure) on the $D.O_2$. bottles.

11.3. Other BOD Products

11.3.1. BOD-dissolved - dissolved BOD: Filter sample though 0.45 micron filter prior to analysis. Do the same to a blank and duplicate sample. Filter 120 mL of Nanopure water and set up a 40% dilution for the method blank.

11.3.2. BOD-Carb – cabonaceous BOD: Add 0.16g Nitrification Inhibitor (containing 2-chloro-6-(trichloro methyl) pyridine -TCMP) to BOD bottle containing sample. Do the same to a blank, LCS, and duplicate sample.

11.4. Other matrices such as solids and sludges: Discuss with a supervisor. Perform a duplicate on a solid or sludge sample.

12. Quality Control:

Note: See also ESB SOP Q01 for general QC requirements

12.1. Blanks are analyzed with every 20 samples or one per batch, whichever is more to check for possible interference from seed and reagents. Blanks are reported per matrix type. The target range is <0.5 mg/L DO. An average blank value from the batch is subtracted out of the sample results. Analysts should double check DO readings and notify a supervisor if blank readings exceed the target range.

12.2. Duplicate analyses are performed at a frequency of one in 20 samples, per matrix type or one per batch, whichever is more.
The relative percent difference is calculated for the duplicate samples run as follows:

$$RPD = \frac{ABS(Run\ 1 - Run\ 2)}{(Run\ 1 + Run\ 2) / 2} \times 100$$

Duplicates must be within a maximum RPD of 20%.

12.3. A laboratory control standard is analyzed every 20 samples or once per batch, whichever is more and reported for every matrix type. The laboratory control is compared to acceptance ranges generated from in-house historical data. The method target range for historical Limits is 85-115% based on a series of Laboratory studies which gave the following results: "For the 300 mg/L mixed primary standard, the average 5-d BOD was 198 mg/L with a standard deviation of 30.5 mg/L). Lab control standards outside the acceptance range cause the analysis to be put into question. Troubleshooting must be performed to attempt to identify the problem. The Winkler LCS may be used to accept a sample batch. The Supervisor, QA Manager or Laboratory Technical Director is consulted to determine if data is still reportable. If data is reported in a batch without an acceptable LCS, all client reports must be flagged with NLbod. See ESB SOP Q06 for more details.

12.4. Probe Blank: An unseeded blank is analyzed with each batch to monitor probe performance. Target range should read <0.4 mg/L.

12.5. An MDL study is completed whenever major equipment or procedural changes are made. A standard Glucose-Glutamic Acid solution is prepared as described in section 10.5. The dissolved oxygen depletion of seven replicates is tabulated. See QA Manual for calculation. Results must be below the reporting limit.

12.6. Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration of 4 mg/L for BOD and cBOD. Plant 3 mL of Standard Glucose-Glutamic Acid solution from section

10.5 into a BOD bottle. This is calculated as a 100% solution. Acceptance ranges are 80-120% with a maximum %RSD of 10.

12.7. Demonstration of Continuing Proficiency: On an annual basis, each analyst must turn in valid LCS data from four consecutive batches or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed twice a year.

13. Calculations:

13.1. For seeded samples:

$$\text{BOD mg/L} = \frac{(D.O_1. - D.O_2) - (B_1 - B_2)\, f}{P}$$

or

$$\text{BOD mg/L} = \frac{(D.O._1 - D.O._2) - (B_1 - B_2)\, f}{W} \text{ X } 100$$

Where:

$D.O_1.$ = D.O. of diluted sample immediately after preparation.
$D.O_2.$ = D.O. of diluted sample after a 5 day incubation at 20°C.
$B_1.$ = D.O. of seeded blank immediately after preparation.
$B_2.$ = D.O. of seeded blank after a 5 day incubation at 20°C.
P = Decimal volumetric fraction of sample used, or W = % of sample in BOD bottle.
F = Ratio of seed in diluted sample to seed in seed control = (% seed in diluted sample)/(% seed in seed control)

Note: Since several blanks are prepared during a BOD run, the average blank depletion is subtracted from each sample depletion. An exception is made if one blank bottle depletes by more than 0.5 from the others. The analyst must then evaluate the blank result based on the depletion of the associated LCS. If the blank appears to be an anomaly then it is not used in the average. If the blank indicates contamination in the dilution water, that blank depletion is subtracted from all associated samples and QC.

Note: For in house calculations, f is considered to be equivalent to 1. Any variation in f due to sample dilutions is negligible after result is rounded to two significant figures.

14. Reporting:

14.1. The following criteria is followed when choosing a reportable result:

14.1.1. Residual DO of at least 1mg/L.
14.1.2. DO depletion of at least 2mg/L.

14.2. *If more than one dilution meets this criteria, the optimal result is chosen.*

14.2.1. Choose the answer with the least dilution (i.e. the answer which used more of the original sample)
14.2.2. If more than one valid answer is available and they differ greatly, refer to historical data, COD or TDS result to pick the result that will fit best.

14.3. If the above criteria are not met, the supervisor is consulted to determine the most appropriate course of action.

14.3.1. If residual DO is < 1mg/L then too much sample was used. Qualify the sample as follows:
14.3.1.1. A "greater than" result will be reported using the _BOD qualifier. The greater than value is calculated as the highest value that the sample could have been had its smallest dilution depleted to 1.0mg/L Use also qualifier N-Nae, result not available due to lab error.

14.3.2. If DO depletion < 2mg/L. then insufficient sample was used. Qualify the sample as follows:
14.3.2.1. Data will be reported using _BOD qualifier with an adjusted reporting limit based on sample size. This qualifier allows the result to be displayed even though it is less than the elevated reporting limit. Add N-BOD to explain results did not meet method calculation criteria. The reported result is an estimated value only.
14.3.2.2. Exceptions to this rule include samples with a dilution of 5% or greater; in those cases, no qualifications are necessary and the client will see ND at an RL of 40mg/L or less.

14.3.3. Each of the above scenarios requires that a BOD cancellation form be filled out, with estimated results and client information.
14.3.3.1. Write, "Do not recalc" in the top left-hand corner. Go into "Update work pricing" in Element, and for each invalid result, find the login number and change the BOD price to $0.
14.3.3.2. Print a copy of the bench sheet
14.3.3.3. Make two copies of every cancellation form – one for Gail and the other for peer review. Give the original to the project manager associated with that client.

14.4. Turn in for peer review:
- Original, completed data page
- Copy of bench sheet
- Copies of BOD cancellation forms (if applicable)
- Completed BOD peer review sheet

14.5. The reporting units for this analysis are mg/L.

14.6. The raw result entered into LIMS is the sample depletion minus the method blank depletion (see Note sec 13).

14.7. The number entered in the DIL column in LIMS is the value:

percentage planted

14.8. Customers can refer to this SOP section 6 for temperature and duration of storage.

15. Definitions: See SOP Q15 – SOP Definitions
Seed Control = Method Blank

16. Corrective Action For Out of Control Or Unacceptable Data:
See SOP Q06 – Corrective Action

17. Pollution Prevention and Waste Management:

17.1. See SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes

17.2. SOP S07 – Pollution Prevention

18. References:

Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WPCF, 18th edition. 5210B
EPA 405.1, EPA Methods for the Chemical Analysis of Water and Wastes.

Note: All *italicized items* are an indication of a variation from the method.

Approved by: Susann K. Thomas Date: 01/06/06

Draft
Standard Operating Procedure
Edward S. Babcock & Sons
METHOD #: SM 5220D
Effective Date: _____

TITLE: Chemical Oxygen Demand (Colorimetric)

ANALYTE: COD Chemical Oxygen Demand

INSTRUMENTATION: Spectrophotometer-Genesys 10uv

1.0 Scope and Application

1.1. This method covers the determination of COD in surface waters, domestic and industrial wastes.

1.2. This method may also be used for solid samples if both client and regulator agree to this variation.

1.3. The applicable range of the colorimetric method is 10-500 mg/L. The reporting limit is 10mg/L.

2.0 Summary of Method

2.1 Sample, blanks and standards in sealed tubes are heated in an oven block digester in the presence of dichromate at 150^{o}C. After two hours, the tubes are removed from the oven or digester, cooled and measured spectrophotometrically at 620 nm.

3.0 Sample Handling and Preservation

3.1 Collect the samples in glass bottles if possible. Use of plastic containers is permissible if it is known that no organic contaminants are present in the containers.

3.2 Samples should be analyzed as soon as possible or preserved with sulfuric acid to a pH < 2 and maintained at 4^{o}C until analysis. Analysis performed on unpreserved samples are qualified N_UNP. Analysis must take place within 28 days. Samples are kept in plastic bottles or snap caps in the refrigerator on the sulfuric preserved cart.

4.0 Interferences

4.1 Chlorides—Chlorides are quantitatively oxidized by dichromate and represent a positive interference. Mercuric sulfate is added to the digestion tubes in the Hach reagent to complex the chlorides. This is effective for chloride levels of 2000mg/L or less. High concentrations of chloride will precipitate and become turbid when added to the COD solution. Samples with higher amounts of chloride are *diluted* until the chloride level is no longer an interferent.

4.2 Discard vials that are scratched or blemished.

5.0 Safety

5.1 Safety goggles and gloves are recommended during this procedure since the reagent vials contain sulfuric acid which will heat up when the sample is added as well as other hazardous chemicals such as dichromate and mercury.

5.2 See SOP S01 – Concentrated Acids and Bases
SOP S03 – Spill Control Policy

6.0 Apparatus

6.1 Biospec Tissue Tearer

6.2 Drying oven or block digestor, 150^{o}C.

6.3 HACH Digestion vials with digestion solution for COD 0-1500ppm.

6.4 Spectrophotometer – Genesys 10uv

6.5 Standard laboratory glassware: volumetric flasks, beakers, graduated cylinders, pipettes.

Note: All glassware is cleaned immediately prior to and after use by thorough rinsing with three portions of D.I. water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

7.0 Reagents and Standards

7.1 Stock potassium acid phthalate (potassium biphthalate or potassium Hydrogen Phthalate)

Draft

7.1.1 LCS/MS Intermediate Standard-Dissolve 0.425g in Nanopure water and dilute to 1 liter. 1 mL = 250 mg/L COD, 0.2ml = 50mg/L COD. Store LCS solution in the refrigerator in an amber bottle for up to 3 months.

7.1.1.1 Midlevel LCS 250 ppm: 1 mL of above intermediate and 1 mL Nanopure into COD vial, made fresh each run

7.1.1.2 High Level LCS 500ppm: 2 mLs of above intermediate into COD vial, made fresh each run. (High CCV)

7.1.1.3 Working MS 125 ppm: 0.5 mL of above intermediate, 0.5 mL of Nanopure, and 1 mL sample into COD vial, made fresh each run.

7.1.2 Calibration Intermediate Standard: Follow above procedure using a second source of potassium acid phthalate. Store STD Intermediate in the refrigerator in amber bottles for up to 3 months.

7.1.2.1 Dilute Intermediate Standard further to create a seven point curve.

7.1.2.2 Single point calibrator 250 ppm: 1 mL of 500ppm non LCS intermediate (made fresh when recalibration is necessary) and 1 mL Nanopure into COD vial.

7.2 Digestion Solution : purchased premade reagent from HACH Vials are stored at room temperature until manufacturer's specified holding time.

7.3 Reagent water: Nanopure

8.0 Colorimetric Procedure

8.1 Uncap and number 25 HACH vials (MB),2,3,4,5,etc.

8.2 Turn on COD reactor to infinity to warm up.

8.3 Add 2mls of Nanopure to MB vial, 1ml of the LCS/MS standard to LCS vial(#2), 0.5ml of the LCS/MS standard to the MS and MSD vials, and 2mls of the LCS/MS standard to the high level LCS vial.

8.4 Add 2mls of aqueous sample to corresponding numbered vial. Add 1ml of sample chosen for spike (if 2mls used) to the MS and MSD vials. For solid samples weigh out approximately 0.25g of solid sample to the corresponding numbered vial, as well as the MS and MSD vials. A 1ml autopipetter with the end of the tip clipped off is used for QC samples and samples where 1ml or less is used. Use a 5ml autopipetter with the end of the tip clipped off for all other samples. Should the analyst feel that the subsample obtained in not a homogenous representative of the original sample due to suspended material that either settles out quickly, is too large, or does not mix well, the TOC Tissue Tearer is used prior to taking a subsample. A digested reagent blank used to zero the spectrophotometer must be run for each new lot of Hach tubes. *This may be kept as long as it is stable*. Store in a dark place. A

method blank that is digested and read must be analyzed once a week and for each new lot of Hach tubes.

8.5 If a sample is dirty or turns a greenish color when added to the vial less sample must be used. If sample turns turbid or precipitates see section 4.1. Choose dilutions according to sample color, clearness, and odor. Mark the amount of sample used on the COD prep sheet. Add Nanopure water to bring up QC samples and any diluted samples up to 2mls. Verify that the liquid level is the same throughout the batch.

8.6 Cap tightly and shake to mix layers. A thorough mixing is necessary before heating to prevent local heating of sulfuric acid layer on bottom of tube that may cause an explosive reaction.

8.7 Process standards and blanks exactly as the samples.

8.8 Place in oven or block digester at 150-C for two hours. Set timer for 120 minutes. Leave tubes in reactor for approximately 20 minutes after timer goes off.

8.9 Invert cooled samples several times. Return vials to rack until cool to the touch, approximately 30 minutes, before measuring absorbance. Dislodge solids that adhere to container by gently tapping. If sample is turbid, let it settle longer, use a centrifuge, or reanalyze with less sample volume.

8.10 Measure color intensity on Genesys 10uv by setting the spec to read at *620 nm*. On the spec select "Set nm" and enter 620. This wavelength was determined to be optimal for our laboratory conditions.

8.11 Check the digested reagent blank by placing a vial containing 5mls of Nanopure into the spec. Set transmittance to 100% by selecting "Change Mode" until %T appears and then select "Measure Blank." Then read the transmittance of the reagent blank. Record this value on the data page. If the transmittance has changed more than 2% or a new lot of vials is started, a new reagent blank must be digested and used to zero the spec. (per manufacturers instructions – Hach COD Reaction Model 45600 pg51). Generally the method blank for the week will serve as the reagent blank for the following week unless transmittance varies more than 2% or a new lot of vials is started.

8.12 Select "Change Mode" until absorbance appears. Select "Measure Blank" and read the samples.

8.13 Wipe method blank vial#1 off with a damp tissue and dry off making sure vial is clean and free of fingerprints. Place vial in the spec with the label facing forward. Read the absorbance and remove the blank. Read LCS and samples without changing anything.

9.0 Product Variation

9.1 Dissolved COD results are obtained by filtering the sample thru a 0.45 micron filter and then proceeding with the analysis in step 8.4.

10.0 Calibration and Calculation

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10.1 Five calibration standards are required by the method. In general calibration standards are prepared at seven concentration levels including zero. A new curve is prepared on a *yearly* basis covering the referenced concentration range.

10.2 An acceptable curve yields an $r^2 = 0.99$ or better.

10.3 A regression equation is generated from the curve.

10.4 Results are calculated using the regression equation. See most current curve for proper equation.

10.5 Any sample that reads an absorbance above the high standard must be redone at a higher dilution. Also any sample that is cloudy or turbid or may cause any colorimetric interference must be diluted.

10.6 Immediately after calibration a 250mg/L lab control serves as the ICV. It must be within 95% - 105% of the expected value or the calibration curve is redone.

10.7 See below for CCV information.

11.0 Quality Control

Note: See also ESB SOP Q01 for general QC requirements

11.1 The 250mg/L lab control is used as a CCV. It is analyzed every twenty samples per matrix type or one per analysis batch whichever is greater. If recovery is not within 95% - 105% of the expected value either the analysis is repeated or it is recalculated based on a single point calibrator.

11.2 A MS/MSD is analyzed every twenty samples per matrix type or one per analysis batch whichever is greater. The acceptance range is 80% - 120% of the expected value and 75%-120% for solids/sludges. The relative percent difference between the spikes must be no greater than 20%.

11.3 The 500mg/L lab control is used as the ending CCV. It is analyzed at the end of the batch. If recovery is not within 95% - 105% of the expected value a new one is digested and reanalyzed. If the redigested CCV fails, a 250mg/L calibrator is digested from a non LCS source. It is used as a single point calibrator to recalculate the rest of the batch as follows:

$$\text{Samp. Conc.} = \frac{(\text{Samp. Abs.})(\text{Conc. Single Pt. Cal.})}{\text{Single Pt. Cal. Abs}}$$

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11.4 The method blank is run once a week. It must be below the reporting limit. Sample results at or above the reporting limit must be accompanied with a qualifier if the method blank exceeds a concentration greater than 1/10 the measured raw concentration of the sample. Blank results below the reporting limit are considered to be ND and will not require a note.

11.5 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at the reporting limit or 2.5-5 times the estimated MDL and a minimum of seven replicates is analyzed. See QA Manual for calculation. Results must be $\leq$ 2.2 times the reporting limit.

11.5.1 LOD Verification: On an annual basis, an aliquot of water is spiked at a level of 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL.

11.6 Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration 5-50 times the MDL. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 10.

11.7 Demonstration of Continuing Proficiency: On an annual basis, analysts must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies performed twice a year.

12.0 Definitions: See SOP Q15 – SOP Definitions

13.0 Corrective Action For Out of Control Or Unacceptable Data:

13.1 See SOP Q06 – Corrective Action

14.0 Pollution Prevention and Waste Management:

14.1 See SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S07 – Pollution Prevention

14.2 Waste Disposal: Each sample vial's contents must be poured into a COD only waste receptacle and the empty vials must be discarded only into the GLASS ONLY waste receptacle. The COD only waste receptacle is handled by a Waste Disposal company.

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15.0 Method Performance

Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QA Office.

References

EPA 410.4 Methods for the Chemical Analysis of Waters and Wastes.
Standard Methods For the Examination of Water and Wastewater APHA, AWWA, WPCF 18th Edition 5220 D.

Note: All *italicized items* are an indication of a variation from the method.

Approved by: _____ Date: __ __

ELECTRICAL CONDUCTIVITY
Edward S. Babcock & Sons
STANDARD OPERATING PROCEDURE
(Standard Methods 2510 B)
Date Effective: 10/30/06

1.0 Scope and Application: Conductivity is the measurement of an aqueous solution's ability to conduct electricity. This measurement depends on the presence of ions--their total concentration, valence, and relative concentrations--and the temperature of the solution. Conductivity is an indication of salinity of the solution. This method is applicable to waters and wastewaters. This method may also be used for solid samples if both client and regulator agree to this variation.

2.0 Reporting Limit: 1.0 µmho/cm

3.0 Precision and Bias: The RPD of this test is < 10 and the relative error <10% in a conductivity range of 147 to 303 µmhos/cm (Standard Methods study). Our laboratory's generated data is expected to achieve similar results. Refer to Initial Demonstration of Capability Studies, and quality control charts maintained in the QA Office.

4.0 Working Range: 1.0-200,000 µmhos/cm.

5.0 Summary of Method: The resistance to electrical current across a gap in the conductivity cell is measured. Conductivity is the reciprocal of the resistance.

6.0 Sample Collection, Preservation, and Holding Times:

6.1 The sample may be collected and stored in either plastic or glass.

6.2 The sample is refrigerated as soon as possible after collection.

6.3 The regulatory holding time for this test is 28 days. We analyze most samples within 24 hours according to EPA 120.1.

7.0 Interferences:

7.1 The specific conductance cell can become coated with oil and other materials. It is essential that the cell be thoroughly rinsed.

7.2 Platinum electrodes can degrade and cause erratic results. The electrode should be inspected periodically. If the coating appears to be thin or if it is flaking off, the electrode should be re-platinized or a new electrode should be purchased.

8.0 Safety Issues: General laboratory safety procedures are sufficient for this test (See Chemical Hygiene Plan).

9.0 Instrumentation/Equipment:

9.1 Conductivity meter capable of measuring conductivity with an error < 1% or 1 µmho/cm (whichever is greater).
YSI 3100 Conductivity, Temperature Instrument.

9.2 Platinum electrode. YSI Model 3252

9.3 Thermometer.

9.4 Water bath with tube rack and tubes.

9.4.1 Tubes are cleaned immediately after use by thorough rinsing with three portions of D.I. water and stored upside down to dry.

Note: If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

10.0 Standards:

10.1 0.01M KCl Calibration Standard: 0.7456 g KCl pre-dried in 105^{o} oven, dissolved in 1 liter of Nanopure water. The adjustment factor is acquired from this reading. This solution is stored at room temperature for up to one year.

10.2 0.01M KCl Lab Control: 0.7456 g KCl pre-dried in 105^{o} oven, from a separate source than the standard, dissolved in 1 liter of Nanopure water. This

solution is stored at room temperature for up to one year. This solution is used full strength and diluted 1:1 with Nanopure water to yield a calibration check at two levels.

10.3 Reagent water: Nanopure

11.0 Procedure:

11.1 Waters:

11.1.1 Make sure electrode is hooked up securely.

11.1.2 The instrument should be on.

11.1.3 Pour samples and standards into clean dry test tubes. Place the tubes in a rack. Bring all samples and standards to 24-26^{o}C using a water bath. Warm tap water of approximately 27^{o}C is added to the rack and cold samples are allowed to reach room temperature. Place thermometer in water bath to measure and monitor the temperature.

11.1.4 Rinse the electrode off with the D.I. wash bottle and dry by shaking and blotting the end.

11.1.5 Calibration: Place the electrode in the Calibration standard solution that has been poured into the test tube (about ¼ full), ensuring that the probe does not rest on the bottom of the test tube. Immerse electrode several times in and out of solution ensuring that no bubbles are trapped under the electrode and a stable reading is acquired. *The probe is not placed in successive portions of standard solution as specified in the method, however if unusual variations are observed, standard solution is re-poured and reanalyzed.*

11.1.6 Read the conductance and make sure the reading falls within 90-110%. The temperature is also displayed at the bottom of the screen.

11.1.7 Take the electrode out of the standard, rinse it off with D.I. wash bottle,

dry off and place in lab control. It must read 90-110%.

11.1.8 Rinse it off with D.I. wash bottle, dry off and place in a tube of D.I. water. An EC reading of the D.I. water is read as an internal monitoring of the deionization process.

11.1.9 Place the clean dry electrode into the first sample. Immerse electrode several times in and out of sample ensuring that no bubbles are trapped under the electrode and a stable reading is acquired. *The probe is not placed in successive portions of sample as specified in the method, however if unusual variations are observed, samples are re-poured and reanalyzed.*

Note: If the conductivity is elevated the result will be displayed in mmhos/cm. Record your raw result, multiply by 1000, and record your final result in µmho/cm. This must be noted on your data sheet.

11.1.10 Take the electrode out of the each sample, rinse it off with D.I. wash bottle, dry off and place in the next sample.

11.1.11 If the conductivity is very low and if there is enough sample, re-rinse the electrode and sample tube with the sample, re-pour the sample, and take another reading.

11.1.12 It is best to store cells so that the electrodes are immersed in D.I water. Change the water frequently to prevent any growth that may cause electrode fouling. The electrodes in cells stored in water will require less frequent replatinization than those that dry out between periods of use. Any cell that has been stored dry should be soaked in distilled water for 24 hours before use to assure complete wetting of the electrodes.

11.2 HMS/Solid:

11.2.1 Add deionized water in a 1:10 ratio with the soil (i.e. 5g of sample to 50mL

water); or any ratio that is convenient. Document the ratio used on the data page.

11.2.2 Allow sample to leach for 1 hour with frequent stirring.

11.2.3 Follow the procedure in section 11.1.

12.0 Quality Control:

Note: See also ESB SOP Q01 for general QC requirements

12.1 Duplicate analysis will be run with every analytical batch, at a minimum frequency of 1 for every 10 samples per matrix type.

12.1.1 The Relative Percent Difference (RPD) of each duplicate analysis will be calculated and compared to control limits.

The RPD is calculated as follows:

$$RPD = \frac{ABS\ (Run\ 1 - Run\ 2)}{(Run\ 1 + Run\ 2)/2} \times 100$$

A maximum of 20 is allowed.

12.2 The reading of the KCL standard is used in the following calculation (section 13.1) to establish the calculation factor.

12.3 The second source of KCl is used as a lab control and calibration check. It is analyzed at two different concentrations, at the beginning of the analysis, every 20 samples per matrix type, and at the end of the run.

12.3.1 If the percent recovery does not fall within the control limits 90%-110%, the analysis is considered to be out of control. The problem must be corrected before the analysis can proceed.

12.4 Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration of

1413µmhos/cm. The average percent recovery must be 80-120% with a maximum %RSD of 10.

12.5 Demonstration of Continuing Proficiency: On an annual basis, analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed twice a year.

13.0 Calculations:

13.1 Calculate the answer as follows:

$$\text{Sample Reading (µmho's)} \times \frac{1413}{\text{Standard Reading (µmho's)}}$$

14.0 Reporting:

14.1 Conductivity is reported as µmhos/cm. Since all EC readings are taken at 25°C, *temperature is not reported with the result.*

14.2 The reporting limit is 1.0 µmho/cm.

14.3 Report the result as 2 significant figures for all answers under 1000. Above 1000, 3 significant figures are reported.
If EC is > 5000 µmhos/cm please note this in Sample Comments in Element/Update Status.

15.0 Definitions: See SOP Q15 – SOP Definitions

16.0 Corrective Action For Out of Control or Unacceptable Data:

See SOP Q06 – Corrective Action

17.0 Pollution Prevention and Waste Management:
See SOP S07 – Pollution Prevention

References/Method Source:

EPA 120.1 Methods for Chemical Analysis of Water and Wastes.

Standard Methods for the Examination of Water and Wastewater: AWWA/APHA/WPCF, 18th edition. 2510B

Note: All *italicized items* are an indication of a variation from the method.

Approved by: Susann K. Thomas Date:10/30/06

Standard Operating Procedure
Edward S. Babcock & Sons
EPA 300.0
Effective Date____02/07/07_____

TITLE: The Determination of Inorganic Anions in Water by Ion Chromatography

ANALYTE:	CAS #	MCL (mg/L)
Chloride Cl	7782-50-5	250/500/600*
Nitrate NO_3	14797-55-8	45
Sulfate SO_4	14808-79-8	250/500/600*

Non NELAP Analytes:

Fluoride F	7782-41-4	2.0
Nitrite NO_2		3.29
Phosphate PO_4		
Para-Chlorobenzene Sulfonic Acid PCBSA		98-66-8

*Secondary MCL (nonhealth limit) recommended/upper/short term

1.0 Scope and Application

1.1. This method covers the determination of the following inorganic anions.

		RL, mg/L
1.1.1.	Fluoride	0.1
1.1.2.	Chloride	1
1.1.3.	Nitrate-N / Nitrate	0.2 / 1
1.1.4.	Nitrite-N	0.1
1.1.5.	Phosphate-P	0.05
1.1.6.	Sulfate	0.5
1.1.7.	PCBSA	10

1.2. The matrices applicable to each method are shown below:
Drinking water, surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, solids (after extraction 11.11).

1.3. The working range for these analytes is as follows:

1.3.1.	Fluoride	0.1-5 mg/L
1.3.2.	Chloride	1-250 mg/L
1.3.3.	Nitrate	1-100 mg/L
1.3.4.	Nitrite-N	0.1-5.0 mg/L
1.3.5.	Phosphate-P	0.05 -5.0 mg/L
1.3.6.	Sulfate	0.5-250 mg/L
1.3.7.	Perchlorate	0.004-0.25 mg/L
1.3.8.	PCBSA	1-100 mg/L

1.4. This method is recommended for use only by or under the supervision of analysts experienced in the use of ion chromatography and in the interpretation of the resulting ion chromatogram. Each analyst must demonstrate the ability to generate acceptable results with this method, using the procedure described in Section 10.2.

1.5. When this method is used to analyze unfamiliar samples for any of the above anions, anion identification should be supported by the use of fortified sample matrix covering the anions of interest if the identity of an analyte is in question.

1.6. Environmental Relevance:

1.6.1. Nitrate is regulated as a health concern because it has been identified as a cause of methemoglobinemia. In freshwater or estuarine systems nitrate can reach high levels that can potentially cause the death of fish. Over 30 ppm of nitrate can inhibit growth and impair the immune system of fish.

1.6.2. Chloride increases the electrical conductivity of water and increases its corrosivity. In metal pipes, chloride reacts with metal ions to form soluble salts thus increasing levels of metals in drinking water.

1.6.3. Sulfate may have a laxative effect that can lead to dehydration. With time, the symptoms will disappear as people adapt to the sulfate in the water. Sulfate can also cause scale buildup in water pipes and corrode plumbing.

1.7 Nitrate Notification: If results are 40mg/L or above, the analyst will notify and hand one of the four Project Managers their results noted on their bench sheets. The Project Manager will then notify the client within 24 hours, via email or phone call until the client is reached. Contact information depends on client requests. Client specifications for contact are noted on their Chain of Custody forms or on Element LIMS.

2.0 Summary of Method

2.1 An 25 µL aliquot of sample is injected into an eluent stream and passed through a series of ion exchangers. The system is comprised of a guard column, separator column, and suppressor device. These separate the ions based on their affinity for a low capacity, strongly basic ion exchanger. They are then directed onto a strongly acidic cation exchanger where they are converted to their highly conductive acidic forms. The conductivity of these acid forms is measured. Identification is based on retention time. Quantitation is based on peak height or peak area.

2.2 In order to use this method for solids an extraction procedure must be performed (See Sec 11.11).

3.0 Definitions (see SOP Q15 for definitions)

4.0 Interferences

4.1 Interferences can be caused by substances with retention times that are similar to and overlap those of the anion of interest. Large amounts of an anion can interfere with the peak resolution of an adjacent anion. Sample dilution and/or spiking can be used to solve most interference problems.

4.2 The water dip or negative peak that elutes near and can interfere with the fluoride peak can usually be eliminated by the addition of the equivalent of 1 mL of concentrated eluent (7.3 100X) to 100 mL of each standard and sample.

4.3 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baseline in ion chromatograms.

4.4 Samples that contain particles larger than 0.45 microns and reagent solutions that contain particles larger than 0.20 microns require filtration to prevent damage to instrument columns and flow systems. Caution: filtration may remove perchlorate.

4.5 Any anion that is not retained by the column or only slightly retained will elute in the area of fluoride and interfere. Known coelution is caused by carbonate and other small organic anions. At concentrations of fluoride above 1.5 mg/L this interference may not be significant, however, it is the responsibility of the user to generate precision and accuracy information in each sample matrix.

4.6 The acetate anion elutes early during the chromatographic run. The retention times of the anions also seem to differ when large amounts of acetate are present. Therefore, this method is not recommended for leachates of solid samples when acetic acid is used for pH adjustment.

4.7 The quantitation of unretained peaks should be avoided, such as low molecular weight organic acids (formate, acetate, propionate, etc.) which are conductive and coelute with or near fluoride and would bias the fluoride quantitation in some drinking and most waste waters.

5.0 Safety

5.1 Normal, accepted laboratory safety practices should be followed during reagent preparation and instrument operation. No known carcinogenic materials are used in this method.

5.2 See SOP S01 – Concentrated Acids and Bases
SOP S02 – Compressed Gas Cylinder Handling
SOP S03 – Spill Control Policy

6.0 Apparatus and Materials

6.1 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2 Ion chromatograph - Analytical system complete with ion chromatograph and all required accessories including syringes, analytical columns, compressed gasses and detectors.

6.2.1 IC: Dionex DX –120 1 and 2
Pump Rate: 2.0 mL/min.
Eluent: as specified in 7.3.1
Sample Loop: 25 uL

6.2.2 Data Handling: Pentium Processor with Peak-Net software on Windows NT platform.

6.2.3 Printer: HP Laser Jet 2100

6.2.4 Autosampler: Alcott Micromeritics 728, 708

6.2.5 Anion guard column: AG4A 4mm Dionex Guard Column. A protector of the separator column. If omitted from the system the retention limes will be shorter. Usually packed with a substrate which is the same in the separator column.

6.2.6 Anion separator column: AS-4A 4mm Dionex Column

6.2.7 Anion suppressor column: Anion self-regenerating ASRS-11.

6.2.8 Detector – Conductivity cell

6.3 Standard laboratory glassware: volumetric flasks, beakers, graduated cylinders, pipets.

Note: All glassware is cleaned immediately prior to and after use by thorough rinsing with three portions of D.I. water.

7.0 Reagents and Consumable Materials

7.1 Sample bottles: Glass or polyethylene, sufficient volume to allow replicate analyses of anions of interest.

7.2 Reagent water: Nanopure, free of the anions of interest. Water should contain particles no larger than 0.20 microns with a conductance of <0.1uS/cm.

7.3 Eluent solution: Dissolve 0.571 g sodium bicarbonate ($NaHCO^3$) and 0.763 g of sodium carbonate (Na^2CO^3) in 1 liter of nanopure water (7.2) and dilute to 4 liters. Store at room temperature for up to 6 months.

7.4 Stock standard solutions 1000 mg/L and 10,000 mg/L solutions: Stock standard solutions are purchased as certified solutions. Store standards at 4°C until manufacturer specified holding date. Once opened, the expiration date is 1 year from date opened as long as this does not exceed manufacturer's original expiration date.

7.5 Working standard solutions: See section 9.5 for concentrations and recipes. Working standards are stored at room temperature. Calibration standards are stored for up to *3 months.* LCS solutions are prepared weekly.

8.0 Sample Collection, Preservation and Storage

8.1 Samples are collected in scrupulously clean glass or polyethylene bottles.

8.2 Sample preservation and holding times for the anions that can be determined by this method are as follows:

Analyte	Preservation	Holding Time
Chloride	None required	28 days
Fluoride	None required	28 days
Nitrate-N/Nitrite-N		
Unchlorinated	Cool to 4°C	48 hours
chlorinated	Cool to 4°C	14 days
combined	conc. H_2SO_4 pH<2*	28 days
o-Phosphate-P	Cool to 4°C	48 hours
Sulfate	Cool to 4°C	28 days
PCBSA	Cool to 4°C	28 days

8.3 The method of preservation and the holding time for samples analyzed by this method are determined by the anions of interest. In a given sample, the anion that requires the most preservation treatment and the shortest holding time will determine the preservation treatment. It is recommended that all samples be cooled to 4-C and held no longer than 28 days. *If a preserved sample is used it must be neutralized prior to injection.

9.0 Calibration and Standardization

9.1 Calibrators at three levels are required for each analyte of interest. Generally calibration standards are prepared at eight
concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with reagent water as specified in section. A quadratic curve fit is used. The curve is forced through the zero point. 9.5.4.

9.2 An acceptable curve has an $r^2 \geq 0.99$. *A method blank is analyzed after the calibration to verify the zero point since method software will not allow a blank to be quantified as part of the calibration.*

9.3 Using 25 uL injections of each calibration standard, tabulate peak height or area responses against the concentration. The results are used to prepare a calibration curve for each analyte. During this procedure, retention times must be recorded.

9.4 The calibration curve is verified on each working day, prior to sample analysis after every 10 samples, at the end of the run. The IPC, an Instrument Performance Check, is essentially equivalent to the ICV/CCV required in other methods. If the response for any analyte varies from the expected values by more than 10%, the test must be repeated, using fresh calibration standards. If the results are still out of range, a new calibration curve must be prepared for that analyte and results that are not bracketed by working QC must be qualified or reanalyzed. *If the retention time for any analyte varies outside the curtain window, reset the retention time to the current CCV value and reprocess the run.*

9.5 Standard Concentrations and Recipes:

9.5.1 ICV/IPC Standard: (Initial Calibration Verification/Instrument Performance Check)

Analyte	**Conc.**	**Acceptance Range %**
Cl	50ppm	90-110
NO_3	50ppm	90-110
SO_4	50ppm	90-110

9.5.2 CCV/IPC Standards: For recipes see Calibration Standard #5 and #8.

Analyte	**Conc.**		**Acceptance Range %**
	Mid	High	

Cl	50	250ppm	90-110
NO_3	50	100ppm	90-110
SO_4	50	250ppm	90-110

9.5.3 Low-level Check Standards:

9.5.3.1 Low Level CCV (Standard #3): A low level CCV, Chloride, Nitrate, and Sulfate all at 20ppm is analyzed at the end of each analytical run. If the Low-Level CCV varies by more than 15%, samples having results less than 35ppm are reanalyzed.

9.5.3.2 RL Check (Standard #1): A standard at the reporting limit is analyzed. This is used to validate samples reported as ND and to evaluate instrument sensitivity should calibration checks show a low bias. The standard should have a signal greater than the method blank.

9.5.4 Calibration Standards (Cl, NO_3, SO_4): The eight standards below are made from a 1000ppm stock solution into a volumetric flask or a graduated cylinder and brought up to volume with Nanopure water.

Std #1		mL into 1L
Cl	1 ppm	1.0
NO_3	1 ppm	1.0
SO_4	0.5ppm	0.5

Std #2		mL into 50mL
Cl	10ppm	0.5
NO_3	10ppm	0.5
SO_4	10ppm	0.5

Std #3		mL into 50mL
Cl	20ppm	1.0
NO_3	20ppm	1.0
SO_4	20ppm	1.0

Std #4		mL into 50mL
Cl	35ppm	1.75
NO_3	35ppm	1.75
SO_4	35ppm	1.75

Std #5		mL into 200mL
Cl	50ppm	10
NO_3	50ppm	10
SO_4	50ppm	10

Std #6		mL into 50mL
Cl	75ppm	3.75
NO_3	60ppm	3.0
SO_4	100ppm	5.0

Std #7		mL into 50mL
Cl	100ppm	5.0
NO_3	75ppm	3.75
SO_4	150ppm	7.5

Std #8		mL into 200mL
Cl	250ppm	50
NO_3	100ppm	20
SO_4	250ppm	50

9.5.5 PCBSA Calibration Standards

9.5.5.1 Stock Standard Salt: 4-Chlorobenzenesulfonic acid purchased from a certified vendor. Store at room temperature for up to 10 years from date received.

9.5.5.2 Intermediate Standard 100ppm: Weigh 0.1g of 4-Chlorobenzenesulfonic acid into 1L of Nanopure.

9.5.5.3 Working Calibration Standards: Dilute Intermediate Standard solution to make the following concentrations:

Std #1		mL into 50ml
PCBSA	1ppm	0.5

Std #2		mL into 40mL
PCBSA	5ppm	2

Std #3		mL into 50mL
PCBSA	10ppm	5

Std #4		mL into 40mL
PCBSA	50ppm	20

Std #5		g into 1L
PCBSA	100ppm	0.1

*same as the Int. Std.

9.5.6 Lab Controls: The LCS used is from a noncalibration source.

9.5.6.1 The standards below for Cl, NO_3, and SO_4 are made from 1000ppm stock solution into a graduated cylinder and brought up to volume of 100ml with nanopure.

		mL of 1000ppm stock
Cl	50ppm	5
NO_3	50ppm	5
SO_4	50ppm	5

9.5.6.2 PCBSA LCS Standard 25ppm;

9.5.6.2.1 Stock Standard Salt: 4-Chlorobenzenesulfonic acid purchased from a certified vendor that is from a noncalibration source.

9.5.6.2.2 Intermediate Standard 100ppm: Weigh 0.1g of Chlorobenzenesulfonic acid into 1L of nanopure.

9.5.6.2.3 Working Standard: Dilute 10ml of the intermediate standard into 40ml of nanopure.

9.5.7 Matrix Spikes (Cl, NO_3, SO_4) : Add the below aliquots into a 5mL sample. Apply a dilution factor of 1.035 to the matrix spike result.

			Aliquot
Cl	High	50 ppm	25 uL of 10,000ppm stock
NO_3	High	20 ppm	100 uL of 1000ppm stock
SO_4	High	100 ppm	50 uL of 10,000ppm stock

10.0 Quality Control

Note: See also ESB SOP Q01 for general QC requirements

10.1 Our laboratory has a formal quality control program. The minimum requirements of this program are stipulated below.

10.1.1 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made the analyst is required to repeat the procedure in Section 10.2.

10.2 Initial Demonstration of Proficiency: Before performing any analyses, the analyst demonstrates the ability to generate acceptable accuracy and precision with this method.

10.2.1 Each analyst will analyze four replicates of a standard at the NELAP level of 1-4x RL. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 10.

10.3 Demonstration of Continuing Proficiency: On an annual basis, analyst must turn in valid LCS data from four consecutive LCS's or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed twice a year or more. Quality control check samples are analyzed *twice a year* with the performance evaluation sample studies required to maintain state certification. *Study limits are used to evaluate results.*

10.4 Calibration Blank/Method Blank :

10.4.1 An aliquot of Nanopure water is analyzed at the beginning of each run, every ten samples, and at the end of the run for the calibration

blank. One of these blanks is reported for every 20 samples to satisfy the method blank requirement.

10.4.2 Results must be below but not more negative than the *reporting limit*. Sample results at or above the reporting limit must be accompanied with a qualifier if the method blank exceeds a concentration greater than 1/10 the measured concentration of the sample. Blank results below the reporting limit are considered to be ND and will not require a note.

10.5 Lab Control:

10.5.1 In order to verify that standards have been prepared correctly a LCS is performed using a standard of known concentration from an independent source. This laboratory control sample containing each analyte of concern is analyzed with each batch of samples processed. If more than 20 samples are run in a batch analyze one LCS for every 20 samples and report per matrix type.

10.5.2 Evaluate the accuracy by comparing to laboratory acceptance criteria. Results may be generated historically but must be as tight as 90-110%. The acceptance criteria for PCBSA is 80-120%.

10.5.3 If acceptable data cannot be obtained, locate the problem and correct it. If during the course of a run a LCS is out of range, it is rerun on the spot if possible. If this is not possible the analyst may reevaluate the data based on peak height rather than peak area. If this does not solve the problem a fresh calibration is performed and all samples are rerun starting from the last acceptable LCS.

10.6 Matrix Spike and Matrix Spike Duplicate:

10.6.1 Matrix spikes are performed at a frequency of 10% by analysis of a MS every 10 samples per matrix type. MSD is done per 20 samples.

10.6.2 Historical limits are used for percent recovery requirements with a maximum RPD of 20%. If the concentration of fortification is less than 25% of the background concentration of the sample, the matrix recovery should not be calculated and a qualifier (QM4x) is attached.

10.7 Replicates are analyzed for solid samples. The maximum allowable RPD is 40%.

10.8 When doubt exists over the identification of a peak in the chromatogram, confirmatory techniques such as sample dilution and fortification must be used.

10.9 Linear Calibration Range: *This method uses an eight point calibration curve so that if any portion of the range is shown to be nonlinear, that portion is clearly defined. Results are not accepted over the high standard value.*

10.10 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at the reporting limit or 2-3 times the estimated MDL. A minimum of seven replicates is analyzed. See QA Manual for calculation. Results must be below the reporting limit. The MDL of the least sensitive instrument is entered into LIMS.

10.10.1 LOD Verification: On a yearly basis, an aliquot of water is spiked at the level of 2-3 times the MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the MDL for each column.

11.0 Procedure

11.1 Set-up:

11.1.1 Prepare Eluant. Turn He valve to 5psi. Check that the He line is connected to the eluant bottle. Set pump rate as per table 1.

11.1.2 On peaknet program – click on run icon. Under file click on load method – anion 300.

11.1.3 Wait for conductivity and pressure to stabilize.

11.2 Standardization and Calibration:

11.2.1 Using a clean syringe, fill one vial with the Method Blank by filtering Nanopure through a 0.2 µm disc filter.

11.2.1.1 Place vial in position #1 of autosampler.

11.2.1.2 Press <**START**> enter.

11.2.1.3 Init V <**1**> enter.

11.2.1.4 Rinse <**0**> enter.

11.2.1.5 Last V <**1**> enter.

11.2.2 Using a clean syringe, fill one vial with an initial calibration verification standard.

11.2.2.1 Place vial in position #2 of autosampler.

11.2.2.2 Press <**START**> enter.

11.2.2.3 Init V <**1**> enter.

11.2.2.4 Rinse <**0**> enter.

11.2.2.5 Last V <**2**> enter.

11.2.3 The initial calibration verification standard should read within the established control limits. If it does not, reinject it, if it still does not work, recalibrate.

11.2.3.1 Load calibration standards on the autosampler
11.2.3.2 Inject eight calibration standards.
11.2.4 Check an initial calibration verification standard again.

11.3 Analysis:
11.3.1 Fill vials with sample. Shake sample and filter through a 0.2 µm disc filter.
11.3.2 Start the autosampler on vial 1 through 64.
11.3.2.1 Press **<START>** enter.
11.3.2.2 Init V **<1>** enter.
11.3.2.3 Rinse **<0>** enter.
11.3.2.4 Last V **< # of last vial>** enter.
11.3.3 Make sure the peaknet software is calculating appropriately by observing peak heights and retention times.

11.4 Shutdown
11.4.1 Under Run – load stop method.
11.4.2 Turn pressure valve to 0 psi.

11.5 Check system calibration daily and, if required, recalibrate as described in Section 9.

11.6 The injection loop is flushed thoroughly, using each new sample. The same size loop is used for standards and samples. Record the resulting peak size in area or peak height units.

11.7 The width of the retention time window used to make identifications is based on measurements of actual retention time variations over the course of a day. Three times the standard deviation of a retention time is used to calculate the window size unless experience shows that the window requires adjustment. The experience of the analyst weighs heavily in the interpretation of chromatograms. The current window is $\pm$ 0.5 minutes for chloride and nitrate and $\pm$ 0.75 minutes for sulfate.

11.8 If a sample analyte concentration exceeds the calibration range, the sample is diluted to fall within the range. Samples with results near but above the reporting limit are reanalyzed if proceeded by a sample of elevated concentration (250 mg/L for Cl and SO_4, 100 mg/L for NO_3) to eliminate possible carry over contamination.

11.9 If the resulting chromatogram fails to produce adequate resolution, or if identification of specific anions is questionable, fortify the sample with an appropriate amount of standard and reanalyze.

11.10 Wherever possible evaluate coeluding peaks by redrawing the baseline rather than sample dilution and document on your raw data.

11.11 The following extraction should be used for solid materials. Add an amount of reagent water equal to ten times the weight of solid material taken as a sample. This mixture is agitated for sixty minutes by shaking intermittently. Filter the resulting slurry before injecting using a *0.20* micron membrane type filter. This can be the type that attaches directly to the end of the syringe. *A sample is duplicated for every batch prepared*.

12.0 Calculation

12.1 Prepare separate calibration curves for each anion of interest by plotting peak size in area, or peak height units of standards against concentration values. The system will then compute sample concentration by comparing sample peak response with the standard curve.

12.2 Report results in mg/L.

12.3 Report:
NO_2- as N
NO_3- as N or as NO_3 if desired by the client
$H(PO_4)_2$- as P

12.4 Dilutions:
Raw results are displayed on the chromatograms. Dilutions are noted on the schedule and entered into LIMS by editing the primary prep volume.

12.5 Calculations associated with this method:

12.5.1 Total Anions (TA)
mequiv. of OH + CO3 + HCO3 + SO4 + Cl + NO3 = TA

12.5.2 Electrochemical Balance (ECB)
Total Cations (TC) – Total Anions (TA)

12.5.3 Total Dissolved Solids by Summation (TDSSUM)
mg/L of 0.6(Total Alkalinity) + Na + K + Ca + Mg + SO4 + Cl + NO3 + F + SiO3 = TDSSUM

13.0 Transferring Data

13.1 Update the Schedule/Run Log. (PeakNet Main Menu\Schedule\Schedule#) Analyst will usually make notes on the printed schedule the night before for

such things as dilution changes, sample bottle letters, and referenced samples for spikes. These things are not generally typed in initially because they are not always known for sure when they start loading the ICs.

13.2 Reference Batch QC on Bench Sheet. (Element\Laboratory\Bench Sheet\Batch#) Source sample(s) for MS/MSD(s) are referenced. Dilutions are entered by editing the Initial column with the appropriate amounts for a Final amount of 50mL (for solids – any dilution on top of the normal 1:10 extraction is entered in the Diln column of the Data Entry/Review screen). Spike Amounts and Spike IDs are entered.

13.3 Create a Data Entry Table. (Element\Laboratory\Data Entry/Review\Batch#) Select the analytes associated with the batch, click Create, click Export and select an Elmnt User File to save the batch in (ex. IC-WATERTEMP), click Save and then click Cancel back in Data Entry/Review.

13.4 Process/Transfer Batch. (PeakNet Main Menu\Batch\Processing\Input) Select the Input tab. Click Select to acquire a complete schedule of samples to send or click Build to select individual data files (if Build is selected, the file will need to be saved as a new file name – do not save as an existing file name). Also in the Input tab under Process Methods click the circle next to From Schedule. Once you have selected or created a schedule click the Export tab and there click Browse to select a file name (ex. WATERS) in which to save the data to send to Data Tool and click Save. Also in the Export tab select a report type by clicking the circle next to Full report type. When finished click OK at the bottom of the Processing window. Then click the Start icon (looks like an arrow circling clockwise) or under the Processing menu select Start. This sends the data to Data Tool.

13.5 Merge Files in Element DataTool and make edits. (Element\Laboratory\Data Tool Main) In Data Tool under Element Data Entry Table click Browse. Select the Element User File that was created above (ex. IC-WATERTEMP) and click Open. Under Instrument Data Files click Browse. Click Clear to remove previous work. From the bottom right box double click the file exported from PeakNet Batch (ex. WATER), the samples will appear in the top left box. Highlight the desired samples, click Include, and then click Done. In Data Tool Main click Merge Files.

13.6 Make Necessary edits. In Data Transfer select the Instrument Data tab. Do a print screen of samples and paste on to a Word page and print each page (these pages are useful for documenting any needed edits or problems with QC). Upon reviewing chromatograms and noting any edits or problems, make any sample name or result edits to the appropriate lines in the Instrument Data table. Click Refresh. Select the Merged Upload tab. Remove the lines of samples that should not be entered (ex. need dilution, associated with bad CCV, etc.). When all edits have been made and lines removed click Save. Save as the User File from above (ex. IC-WATERTEMP).

13.7 Enter, Save, and QC review. (Element\Laboratory\Data Entry/Review) In Data Entry/Review click Open and double click the saved User File (ex. IC-WATERTEMP). Click Save. Click Query for each analyte to see if there are any red lines (QC problems, holding time issues, etc.). Make any necessary edits. Add appropriate qualifiers where needed.

14.0 Corrective Action For Out of Control Or Unacceptable Data:

See SOP Q06 – Corrective Action

15.0 Pollution Prevention and Waste Management:

15.1 Instrument Waste is dumped in the sink and neutralized.

15.2 SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S07 – Pollution Prevention

16.0 Method Performance

See Method 300 Tables 3-9.
Our intralaboratory generated data is expected to achieve similar results. Refer to Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QA Office.

References:

EPA SW846 method 9056

EPA Methods for the Determination of Inorganic Substances in Environmental Samples, (August 1993) Method 300.0 revision 2.1

California Department of Health Services IC Rev 0

Note: All *italicized items* are an indication of a variation from the method.

Approved by Susann K. Thomas Date 01/29/07

Standard Operating Procedure
Edward S. Babcock & Sons
Date Effective: 08/23/06

METHOD #: Standard Methods 4500-NO_2 B

TITLE: Nitrogen, Nitrite (Spectrophotometric)

1.0 Scope and Application

1.1 This method is applicable to the determination of nitrite in drinking, surface and saline waters, domestic and industrial wastes. This method may also be used for water extractions of solid materials if both client and regulator agree to this variation.

1.2 The method is applicable in the range from 0.01 to 1.0 mg NO2-N/L as proven by a yearly curve. The reporting limit is 0.10 mg NO2-N/L.

1.3 Environmental Relevance:

1.3.1 Contamination is caused by runoff from fertilizer use, leaching from septic tanks, sewage, and erosion of natural deposits. Nitrites are very soluable and do not bind to soils. They are likely to remain in water until consumed by plants or other organisms.

1.3.2 Nitrite-N has a MCL of 1mg/L and is the etiologic agent methemoglobinemia.

2.0 Summary of Method

2.1 The diazonium compound formed by diazotation of sulfanilamide by nitrite in water under acid conditions is coupled with N-(1-naphthyl)ethylenediamine dihydrochloride to produce a reddish-purple color which is read in a spectrophotometer at 543 nm.

3.0 Sample Handling and Preservation

3.1 Samples should be analyzed as soon as possible. They may be stored for 24 to 48 hours at 4^{o}C. Never use acid preserved samples.

4.0 Interferences

4.1 There are very few known interferences at concentrations less than 1,000 times that of the nitrite; however, the presence of strong oxidants or reductants in the samples will readily affect the nitrite concentrations.

4.2 High alkalinity (>600 mg/L) will give low results due to a shift in pH. The ideal pH for nitrite analysis is 5-9. After our initial sample dilution of ten with D.I., it is unlikely that any sample will need pH adjustment. Upon review of data, should the supervisor observe an alkalinity of >6000 or a pH outside the range, the sample pH will be adjusted to 6 with 1:3 HCl and the nitrite reanalyzed.
4.3 NCl_3 imparts a false red color when color reagent is added.
4.4 The following ions cause precipitation: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^{+}, $PtCl_6^{2-}$, and VO_3^{2-}.
4.5 Cupric ion may cause low results by catalyzing decomposition of the diazonium salt.
4.6 Chlorine converts NO_2 and may be the reason for low results.
4.7 Suspended material is removed by filtration. Color is removed by filtration or by subtracting a background absorbance reading.

5.0 Apparatus

5.1 Filtration apparatus or 0.45 micron disk filters
5.2 Spectrophotometer equipped with 1 cm or larger cells for use at 543nm.
5.3 Standard laboratory glassware: volumetric flasks, 100 mL beakers, graduated cylinders, pipets.

Note: All glassware is cleaned immediately after use by thorough rinsing with three portions of D.I. water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

6.0 Reagents and Standards

6.1 Reagent water: D.I. water free of nitrite and nitrate is to be used in preparation of all reagents and standards and for sample dilutions.
6.2 Color Reagent:
- 6.2.1 To 800 mL of D.I. add 100 mL 85% phosphoric acid and 10 grams of sulfanilamide
- 6.2.2 Mix until sulfanilamide is dissolved completely.
- 6.2.3 Add 1 gram N-(1 naphthyl)-ethylenediamine dihydrochloride, mix to dissolve
- 6.2.4 Dilute to 1L with D.I. water.
- 6.2.5 Solution is stable for about a month when stored in a dark bottle in the refrigerator.

6.3 Stock Standard: Solution is stored in the walk-in refrigerator until manufacturer specified holding dates. Once opened, the expiration date is 1 year from date opened as long as it does not exceed mmmanufacturuer's original expiration date. Stock standards can be replaced sooner if comparison with QC samples indicate a problem.

6.3.1 Stock #1: *Purchase 304 ppm certified nitrite-nitrogen standard solution.*
6.3.2 Stock #2: *Purchase 1000 ppm certified nitrite-nitrogen standard solution.*

6.4 Working Standard: These solutions are prepared daily in D.I. water.
6.4.1 Midpoint Check (0.12mg/L):
6.4.1.1 Dilute 1 ml of stock standard #1 to 500 ml in a volumetric flask.
6.4.1.2 Use 10 mL in the beaker and dilute to 50 mL.
6.4.2 Lab Control and Matrix Spike (0.1 mg/L):
6.4.2.1 Dilute .25 ml of stock standard #2 to 500 ml in a volumetric flask.
6.4.2.2 Use 10 mL in the beaker and dilute to 50 mL.
6.4.3 Calibration Standards:
6.4.3.1 When a new curve is necessary, stock standard #1 is diluted to the necessary concentrations.

7.0 Procedure

7.1 Sample Set Up
7.1.1 Use 3 beakers for the blank, midpoint check and lab control.
7.1.1.1 Add 50 ml of D.I. water to the first beaker for the reagent blank *used to tare the instrument.*
7.1.1.2 Add 40 ml of D.I. water and 10 ml of midpoint working standard to the last beaker in the batch.
7.1.1.2 Add 40 ml of D.I. water and 10 ml of lab control working standard to the second beaker.
7.1.2 Use 3 beakers for the spiked sample.
7.1.2.1 In one beaker, add 5 ml of sample (filter all samples if not clear) and 45 ml of D.I. water.
7.1.2.2 In the other two beakers (Spike and Duplicate Spike) add 5 ml of sample, 10 ml of lab control working standard solution, and 35 ml of D.I. water.

7.1.3 Set up the rest of the samples in beakers by adding 5 ml of sample and 45 ml of D.I. water.

7.1.3.1 If the sample is not clear, filter it. If filtrate is discolored or turbid, take a background reading. Zero the spec to a blank prior to addition of color reagent. Read the sample prior to addition of color reagent. This absorbance is subtracted from the reading acquired in section 7.5. If results are still out of range, a serial dilution may be necessary.

7.1.4 For solid samples: Add an amount of reagent water equal to ten times the weight of solid material taken as a sample. This mixture is agitated for sixty minutes by shaking intermittently. Filter the resulting slurry through a 0.45 micron membrane type filter. Place 1-5 ml of extract and 45 ml of D.I. water into sample beaker. Document the ratio used on the data page.

7.2 Add 2 ml of the color reagent to each sample. Swirl.

7.3 Let sit for at least 10 minutes for color development.

7.4 Zero the spectrophotometer to the reagent blank.

7.5 Read samples on UV/Vis Spectrometer at 543 nm. Record the answer in absorbance units.

8.0 Calibration and Calculation

8.1 Three calibration standards are required by the method. In general calibration standards are prepared at six concentrations, including a calibration blank. A calibration curve is analyzed on a yearly basis covering the referenced concentration range.

8.2 An acceptable curve yields an $r^2 = 0.99$ or better.

8.3 A regression equation is generated from the curve.

8.4 Results are calculated from the regression equation.

8.5 Enter the raw concentration into the LIMS. Ensure that the proper initial volume is entered on the bench sheet.

8.6 Immediately following calibration a 0.1 mg/L ICV is analyzed using the LCS source to verify the curve. Results must be within 90-110% of the expected value or a new curve may be necessary.

8.7 Continuing Calibration Verification Checks: The calibration is verified on a daily basis by analysis of the Lab Control, at the beginning of the day's batch and the Midpoint Check at the end of the day's batch. See acceptance criteria below.

8.8 Results greater than the highest calibrator must not be reported. Dilute result into the calibration range.

9.0 Quality Control:

Note: See also ESB SOP Q01 for general QC requirements

9.1 A Laboratory Control Sample is analyzed with each batch of 20 or fewer samples per matrix type. Compare the Laboratory Control results to the acceptance ranges. If the results are not within the acceptance ranges of 90% - 110%, the analysis is considered to be out of control and the problem must be corrected before the analysis proceeds.

9.2 Matrix Spikes (MS and MSD) are analyzed with each batch of 20 or fewer samples per matrix type. Calculate the percent recoveries of the spikes by the following equation:

(Spiked sample - sample) /1 X 100

If the spike recovery does not fall within acceptance ranges of 80%-120% max RPD of 20% for aqueous samples and historically generated limits for solid samples, the spike must be re-analyzed. If the second spike still does not fall within acceptance ranges, the client's results are flagged.

9.3 The Midpoint Check is analyzed with each batch of 20 or fewer samples. It must fall within the same criteria as the lab control or the validity of the curve is in question. If the midpoint check is remade and it is still out of range, a new curve may have to be drawn.

9.4 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at the reporting limit or 2.5-5 times the

estimated MDL and a minimum of seven consecutive replicates is analyzed. See QA Manual for calculation. Results must be below the reporting limit.

9.5 LOD Verification: On a yearly basis, a QC sample is spiked at a level of not more than 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than the method blank response to verify the Level of Detection or MDL.

9.6 Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration 5-50 times the MDL. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 10.

9.7 Demonstration of Continuing Proficiency: On an annual basis, each analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed twice a year.

10.0 Reporting:

10.1 Results are reported as mg/L Nitrite-N.

10.2 The reporting limit is 0.1 mg/L.

10.3 The results are reported to 2 significant figures.

11.0 Definitions: See SOP Q15 – SOP Definitions

12.0 Safety:

See SOP S01 – Concentrated Acids and Bases
SOP S03 – Spill Control Policy

13.0 Corrective Action For Out of Control Or Unacceptable Data:

See SOP Q06 – Corrective Action

14.0 Pollution Prevention and Waste Management:

See SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S06 – Disposal of Chlorinated Solvents
SOP S07 – Pollution Prevention

15.0 Method Performance:

Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QC Office.

16.0 References/Method Source:

EPA 354.1 Methods for Chemical Analysis of Water and Wastes.

Standard Methods for the Examination of Water and Wastewater: APHA/AWWA/WEF, 18th edition. 4500-NO_2 B

Note: All *italicized items* are an indication of a variation from the method.

Approved by Susan K. Thomas Date 08/23/06

DISSOLVED OXYGEN
STANDARD OPERATING PROCEDURE
Edward S. Babcock & Sons
AZIDE MODIFICATION/D.O. Probe
(Standard Methods 4500-O C and G)

Date Effective: 02/01/06

1. Scope and Applications

1.1. This method determines the dissolved oxygen levels in water and wastewater. The analysis for DO is a common test for water pollution and for use in waste treatment process control.

2. Reporting Limits

2.1. The reporting limit for this method is 0.1 mg/L.

3. Working Range

3.1. The working range starts at 0.1 mg/L and continues to the saturation point (about 8.9 mg/L at STP).

4. Summary of Method

4.1. The azide modification is one example of the iodometric test which is a commonly used titrimetric procedure. It is based on the addition of divalent manganese solution which, in the presence of a strong alkali forms manganese hydroxide. DO rapidly oxidizes an equivalent amount of manganese hydroxide to a higher valency state. In the presence of acid, an equivalent amount of iodide in the solution is liberated as iodine. The iodine is titrated with sodium thiosulfate.

4.2. The oxygen membrane electrode measures the "diffusion current" caused by oxygen traveling across a semipermeable membrane.

5. Sample Collection, Preservation, and Holding Times

5.1. The sample is collected in a 300 mL glass bottle. Analyze as quickly as possible if using probe method. For Winkler method, the sample may be set in the field and analyzed within 8 hours. See sections 11.1.1 through 11.1.7 for procedure.

6. Interferences

6.1. The azide modification: Oxidizing or reducing materials and iron levels of over 1 mg/L can cause interferences.

6.2. Membrane electrode: Membrane electrodes are very sensitive to changes in temperature. Prolonged use of the electrode containing other gasses that will permeate the membrane (such as hydrogen sulfide) will decrease cell sensitivity.

6.3. Helpful Hints: Do not remove the lids from the DO bottles prior to analysis. Keep the lids on the bottles at all times when not being used.

7. Safety Issues

Safety Issues: Prepared sample is corrosive. Wear gloves and safety goggles when performing this analysis.

7.1. See SOP S01 – Concentrated Acids and Bases
SOP S03 – Spill Control Policy

8. Instrumentation/Equipment

8.1. 4-25mL burets -- one each for manganese sulfate solution, alkali iodide azide solution, sulfuric acid solution, and sodium thiosulfate titrant -- for use in Winkler titration (azide modification).

8.2. 300 ml DO bottles.

8.3. Dissolved Oxygen meter: YSI Model 5000. (Allow 30 minutes to warm up or leave instrument on.)

8.4. Dissolved Oxygen probe: YSI 5010. (Store probe in BOD bottle filled with about 1 inch of D. water.)

9. Reagents and Standards

9.1. Manganous Sulfate solution: Dissolve 400 g $MnSO_4 \cdot 2H_2O$ or 364 g $MnSO_4 \cdot H_2O$ in 1 liter of Nanopure water. Store at room temperature for up to six months.

9.2. Alkali-Iodide-Azide reagent: Dissolve 500 g NaOH in Nanopure water. Allow to cool. Add 150g KI and dissolve. Dilute to 1 liter. Dissolve 10.0 g NaN3 into 40 mL of Nanopure water. Add to 40 mL to 1L mix. Store at room temperature for up to six months.

9.3. Starch solution: Dissolve 20 g soluble starch and 2 g $C_7H_6O_3$

9.4. Salicylic Acid) in 1 liter of heated Nanopure water or purchase 1% starch indicator.

9.5. Concentrated Sulfuric Acid.

9.6. Standard Sodium Thiosulfate Titrant(Hypo): Dissolve 6.205 g $Na_2S_2O_3 \cdot 5H_2O$ in 1 liter Nanopure water. Add 4 pellets (~ 0.4 g) of NaOH. This titrant is standardized against a Potassium bi-iodate standard and is made fresh every month.

9.7. KI crystals.

9.8. Standard Potassium bi-iodate solution(0.0021M): Dissolve 812.4 mg $KH(IO_3)_2$ in distilled water and dilute to 1000 ml. This standard is stored at room temperature and replaced every 6 months.

Standardization: Dissolve approximately 2 g KI in an Erlenmeyer flask with 100 to 150 ml distilled water. Add 1 ml 6 N Sulfuric acid or a few drops of concentrated Sulfuric acid and 20 ml of the standard bi-iodate solution. Dilute to 200 ml and titrate the liberated iodine with thiosulfate titrant, adding starch towards the end of the titration, when a pale straw color is reached. When the solutions are of equal strength, 20 ml of Sodium thiosulfate should be required to titrate the 20 ml of standard bi-iodate solution. If not, adjust the Sodium thiosulfate to match the standard bi-iodate.

10. **Procedure**:

10.1. Winkler Titration.

10.1.1. Start with a 300 ml BOD bottle filled with sample to the top with no air bubbles.

10.1.2. Using a buret, add 1 ml Manganese Sulfate solution and 1 ml Alkali-Iodide-Azide solution to BOD bottle.

10.1.3. Place the stopper carefully in the bottle to exclude any air bubbles.

10.1.4. Mix each bottle by inverting a few times.

10.1.5. When flocculant has settled to approximately half the bottle volume, mix and allow it to settle once more.

10.1.6. Add 1 ml of Sulfuric Acid.

10.1.7. Mix by inverting several times until flocculant is completely dissolved.

10.1.8. Measure a volume corresponding to 201 ml original sample after correction for sample loss by displacement with reagents and pour into beaker.

10.1.9. Add a few drops of starch solution to form a blue color. Drain the tip of the titration buret and titrate with 0.025 M $Na_2S_2O_3$ solution until the disappearance of the blue color. Disregard subsequent recolorations.

10.2. D.O. Probe Calibration

10.2.1. Air Calibration Check

10.2.1.1. Dry membrane and place in BOD bottle containing 50mL Nanopure.
10.2.1.2. Press calibration mode.
10.2.1.3. Wait approximately 15 minutes for probe to stabilize.
10.2.1.4. If necessary, adjust saturation percentage to 96%.

Note: For probe calibration, operation, and maintenance, see YSI Model 5000 Operations Manual, section 6.1, page 21 and section 7.1, page 26. See also YSI 5905/5010 BOD Probe Instruction Manual for further details. If the membrane is replaced refill the KCl solution and allow probe to sit for a half hour.

10.2.2. Winkler Calibration Check

10.2.2.1. Blank
10.2.2.1.1. Set up a BOD bottle filled with bubbled unseeded water.
10.2.2.1.2. (Probe Blank) Set meter in Main mode and take a DO_1 reading.
10.2.2.1.3. (Winkler Blank) Perform Winkler titration on the same bottle as per above procedure (11.1.1 through 11.1.9).
10.2.2.1.4. (Probe Blank) Set up another bottle to incubate. Perform a DO_2 reading by probe and then by Winkler.

10.2.2.2. Standard
10.2.2.2.1. (Winkler Standard) Set up a standard check by adding 3 ml of the Standard Glucose-Glutamic Acid solution (BOD SOP I05 section 10.5) into the 300 ml BOD bottle.
10.2.2.2.2. DO_1: Take a probe reading.
10.2.2.2.3. DO_2 : Probe reading and Winkler reading

10.2.2.3. If the probe result varies more than 0.4 from the Winkler titration, try the titration again. If result still varies, perform proper maintenance and repeat probe reading.

10.2.2.4. When evaluating results, consider several factors to determine if probe is performing correctly: membrane appearance, Winkler standard, Winkler blank, LCS and MB results.

10.2.3. Samples are read by taking the LED readout when a stable reading has been reached. It is important that no air bubbles are trapped in between the neck of the bottle and the probe. Make sure to rinse probe between sample readings.

10.2.4. After reading the sample, the probe should be carefully removed from the bottle with a gentle twisting motion and rinsed to remove any excess sample. The probe is ready for the next sample.

10.2.5. Clean BOD bottles with 1:1 HCL and three rinses of D.I. water. Inspect bottles for residue. *If a more vigorous cleaning is required, a stronger acid and heat may be used. Always ensure that all acid is thoroughly removed from the bottle prior to use.*

11. Calculations

11.1. The number of milliliters used for the titration is the Dissolved Oxygen content of the sample in mg/L for a 200 ml sample.

11.2. The probe is calibrated daily so the reading is the Dissolved Oxygen Content of the sample in mg/L.

12. Reporting

12.1. Reporting Units = mg/L

12.2. Reporting Limits = 0.1 mg/L.

13. Definitions: See SOP Q15 – SOP Definitions

14. Pollution Prevention and Waste Management:

14.1.1. See SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S07 – Pollution Prevention

15. Quality Control:

Note: See also ESB SOP Q01 for general QC requirements

15.1. Duplicates are analyzed if provided. Results must be within $\pm$ 0.2 mg/L.

15.2. Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by Winkler and probe analysis on four replicates of buffered reagent water. Acceptance criteria is a maximum %RSD of 10.

15.3. See SOP I05 for further BOD quality control and corrective action.

16. References/Method Source

Note: All *italicized items* are an indication of a variation from the method

16.1. Standard Methods, AWWA/APHA/WEF, 18th edition method 4500-O C and G.

16.2. Methods of Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Method 360.1 and 360.2

Approved by Susann K. Thomas Date 01/06/06

pH
Edward S. Babcock & Sons
STANDARD OPERATING PROCEDURE
(**EPA 9040B, 9045C**)
(SM 4500-H^+ B)
Date Effective: 10/30/06

1.0 Scope and Applications:

1.1 This method is applicable for all aqueous solutions as a measure of the hydrogen ion activity of the sample. This is important for all phases of water supply and wastewater treatment, since almost all treatment processes are pH controlled.

1.2 Natural waters usually have pH values in the range of 4-9 units and most are slightly basic due to the interaction with alkali and alkaline earth metals.

1.3 Solid samples may be analyzed as described later in section 9.13.

2.0 Working Range: 1 to 14 units.

3.0 Summary of Method: This method determines the hydrogen ion activity by potentiometric measurement using a glass electrode. The system is calibrated by comparison to buffers of known pH.

4.0 Sample Collection, Preservation, and Holding Times:

4.1 Samples for pH analysis may be collected in either plastic or glass and should be analyzed as soon as possible after collection. Analysis on site is preferable to analysis in the lab.

5.0 Interferences:

5.1 Changes in the properties of the electrodes during analysis and chemical effects caused by equilibrium changes. There must be correction for drift caused by temperature variations.

5.2 Coatings of oily material or particulate matter can impair electrode response. These coatings can usually be removed by gentle wiping or detergent washing, followed by D.I. water rinsing. An additional treatment with hydrochloric acid (1 + 9) may be necessary to remove any remaining film.

5.3 Helpful Hints: Allow sufficient time for temperature of sample to equilibrate in water bath.

6.0 Safety Issues (specific to the method): General laboratory safety precautions are sufficient for this test.

7.0 Instrumentation/Equipment:

7.1 Orion Model 720 pH/ISE Meter

7.2 Electrode: An electrode with special low sodium error glass is recommended - Orion Ross combination probe 8156
7.2.1 VWR# 34107-377

7.3 Water bath and tubes

7.3.1 Tubes are cleaned immediately after use by thorough rinsing with three portions of D.I. water and stored upside down to dry.
Note: If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

7.4 Thermometer

8.0 Standards and Reagents:

8.1 pH buffers: 4,7, and 10 purchased premixed from a certified vendor. Buffers are stored at room temperature. Manufacturer expiration dates are observed.

8.2 Reagent water: D.I. water.

9.0 Procedure:

9.1 The pH electrode should be hooked up to the specific ion meter (on the back of the black box).

9.2 Set the specific ion meter dial to pH mode.

9.3 Rinse the electrode with the D.I. wash bottle and dry the electrode.

9.4 Pour all the samples and standards in test tubes (fill to ¼ of the volume) and place in the water bath at 25°C.

9.5 Calibration: Calibrate the specific ion meter on Standard buffers of *pH 7 and 10* as specified by the manufacturer. Check the calibration by reading the Standard pH 4 buffer. *The probe is not placed in successive portions of buffer solution as specified in the method, however if unusual variations are observed, buffer solutions are repoured and reanalyzed.*

9.5.1 Press down on calibrate one. Place the clean electrode in Standard buffer pH 7. Wait until the green ready light appears making sure to allow sufficient time for settling. Set the reading to 7. Press <ENTER>.

9.5.2 Take the electrode out of the standard, rinse and dry the electrode, and then place it in Standard buffer pH 10. Wait until the ready light appears on calibrate 2. Set the reading to 10, press <ENTER>.

9.5.3 Take the electrode out, rinse it with the D.I. wash bottle, dry, and place it in Standard buffer pH 4 (which is also the LCS). The green light should now be on sample, and read 4.0 $\pm$ 2.5%. Wait for the ready light.

9.6 The instrument is now calibrated and the electrode may be rinsed, dried, and placed in the first sample. Pull electrode in and out of tube with a slight bouncing action to ensure that no air is trapped under the electrode. *The probe is not placed in successive portions of sample and samples are not stirred during analysis as specified in the method. Samples are thoroughly mixed prior to subsampling. In the event that unusual variations are observed, samples are repoured and reanalyzed.*

9.7 If sample reading is less than 5 or greater than 9 and this value is not confirmed by historical data or sample id, place probe in buffer that most closely matches the sample value to standardize electrode. Then pour a fresh aliquot to verify result. Finish by placing electrode into buffer 7 and allowing electrode to return to neutral before proceeding.

9.8 Rinse and dry the electrode between each sample.

9.9 Record the answers in the EC/pH log book to the nearest 0.1 units.

9.10 The first two runs in each set of samples should be duplicates or two runs of the same sample. If the two duplicate readings are not within 0.1 of each other, repour the sample and take another reading. If they are still out of range, reread the LCS, the meter may need to be recalibrated.

9.11 When the analysis is complete, rinse the test tubes 3 times with D.I. water and place test tubes in a box so that the water can drain out.

9.12 Leave the pH electrode in storage solution specified by the probe manufacturer: 200mL pH 7 buffer with 1g KCl. This solution has an expiration date of 6 months from date made. Be sure to cover the filling hole with its protective cap when not in use.

9.13 Solid/Hazardous waste samples:

9.13.1 Weigh 10g of sample.
9.13.2 Add 10ml of D.I. water to sample.
9.13.3 If most of the water is absorbed by the sample, more water may be added. Additional dilutions are allowed if working with hygroscopic soils or other problematic matrices. Document the ratio used on the data page.
9.13.4 Stir periodically for 30 minutes.
9.13.5 Let settle for one hour.
9.13.6 Immerse probe into supernate for reading.
9.13.7 If sample is a liquid but non-aqueous, a 1:1 dilution must be used.

10.0 Quality Control:

Note: See also ESB SOP Q01 for general QC requirements

10.1 Duplicates are analyzed at least once with every analytical batch at a minimum of once for every 10 samples per matrix type. A maximum difference of ± 0.1 pH units is allowed between sample duplicates.

10.2 Calibration Checks: After calibration the pH 4.0 standard is read for an ICV. The results are compared to acceptance ranges ± 0.1 pH units (97.5 – 102.5%). If the results do not fall within acceptance ranges a new calibration is necessary. The pH 7.0 standard is checked at the end of the analysis as a continuing calibration check. Results must be within 90%-110% for the batch to be acceptable.

10.3 The pH 4 standard is also used as the Lab Control. It is analyzed at least once with very analytical batch and at a minimum of once for every 20 samples per matrix type. The results are compared to acceptance ranges ± 0.1 pH units (97.5 – 102.5%). If the results do not fall within acceptance ranges, the analysis is considered to be out of control. No further samples are analyzed until the out of control situation is corrected.

10.4 Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a pH of 4. Acceptance criteria 80-120% maximum %RSD of 20.

10.5 Demonstration of Continuing Proficiency: On an annual basis, analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed *twice* a year.

11.0 Calculations/Reporting:

11.1 Report meter reading in pH units. Since all pH readings are taken at 25^{o}C, temperature is not reported with the result.

11.2 Report 2 significant figures for all readings below 10, 3 significant figures for readings above.

11.3 Even if the electrode with special low sodium error glass is used, pH readings between 12.0 to 12.5 may require a correction factor from the attached nomograph. See Supervisor to determine if correction is necessary.

11.3.1 First prescreen the sample to determine if the sodium content is negligible.

11.3.2 A quick conductivity reading will give the analyst a value above the actual sodium content of the sample. Although the conductivity reading is not equal to the sodium concentration, we can use the reading as a limit of how high the actual sodium concentration is in the sample.

11.3.3 Apply the Ec reading in the following fashion on the nomograph:

Ec reading	Na in Molarity
2300	0.1
11,500	0.5
23,000	1.0
46,000	2.0

115,000 5.0

11.3.4 If the "pH correction to be added" is negligible using the above screening steps then the original pH is reported.

11.3.5 If however, the "pH correction to be added" does change the original pH reading, an actual sodium content must be determined by the ICP. Once this value is obtained, convert the mg/L result to molarity and find the actual "pH correction to be added" value from the nomograph.

11.3.5.1 Conversion to molarity:

$$\frac{\text{Na in mg/L}}{23{,}000} = \text{Molarity of Na}$$

11.4 For sample readings below 1 report <1 in the text field.

11.5 For sample readings above 14 report >14 in the text field.

12.0 Definitions: See SOP Q15 – SOP Definitions

13.0 Corrective Action For Out of Control or Unacceptable Data:

13.1 See SOP Q06 – Corrective Action

14.0 Pollution Prevention and Waste Management:

14.1 See SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes

14.2 See SOP S07 – Pollution Prevention

References/Method Source:

Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WPCF, 18th edition. 4500-H^+ B

EPA **9040B, 9045C** Methods for the Chemical Analysis of Waters and Wastes.

Note: All *italicized items* are an indication of a variation from the method.

Approved by: Susann K. Thomas Date: 10/30/06

ORTHO-PHOSPHATE PHOSPHORUS
Edward S. Babcock & Sons
STANDARD OPERATING PROCEDURE
ASCORBIC ACID METHOD
(SM 4500-P E)
Effective Date: 082806

1.0 Scope and Application

This method covers the determination of ortho phosphate phosphorus in drinking, surface and saline waters, domestic and industrial wastes.

1.1 Health Effects of Phosphorus

1.1.1 Too much phosphate can cause health problems, such as kidney damage and osteoporosis. While burning, white phosphorus may cause damage to the liver, the heart, or the kidneys.

1.2 Environmental Effects of Phosphorus

1.2.1 In water, white phosphorus will accumulate in the bodies of aquatic organisms. In soil, phosphorus will remain for several days, but in deep soils and the bottom of rivers and lakes phosphorus will remain for a thousand years or so. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff.

1.2.2 The increasing phosphorus concentrations in surface waters raises the growth of phosphate-dependent organisms, such as algae and duckweed. These organisms use great amounts of oxygen and prevent sunlight from entering the water. This is known as eutrophication.

2.0 Working Range: 0.05 – 1.0mg/L

3.0 Summary

Ammonium molybdate and antimony potassium tartrate reacts in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration. Only orthophosphate forms a blue color in this test.

4.0 Sample Preservation

4.1 Samples must be unpreserved and stored in the refrigerator.

4.2 Analysis must be done within 48 hours.

5.0 Interferences

5.1 Copper, iron, or silicate at concentrations many times greater than their reported concentration in seawater causes no interference. However, high iron concentrations can cause precipitation of and subsequent loss of phosphorus.

5.2 Arsenate is determined similarly to phosphorus reacting with molybdate reagent to produce a blue color and should be considered when present in concentrations higher than phosphorus. Concentrations as low as 0.1mg As/L can interfere.

5.3 Hexavalent chromium and nitrite interfere to give results about 3% lower at concentrations of 1mg/L and 10-15% lower at 10mg/L.

6.0 Safety

General laboratory safety procedures are sufficient for this analysis. Recommended safety equipment includes gloves and safety glasses.

7.0 Equipment

7.1 Standard laboratory glassware: volumetric flasks, beakers, graduated cylinders, pipettes.

7.2 Syringe and 0.45 micron filter disk: Test each new lot of filters by comparing an unfiltered and filtered LCS and a Blank. Zero the spectrophotometer with an unfiltered Blank and document the result for the filtered Blank.

7.3 Spectrophotometer: Spectronic Genesys 10uv and Spectronic 20D+ set at 880nm.

8.0 Glassware Preparation

8.1 All glassware is acid washed in 1:1 HCl immediately after use, followed by thorough rinsing with three portions of D.I. water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

9.0 Phosphate Reagents

9.1 Reagent water: Nanopure

9.2 P-Reagent - Let all reagents reach room temperature before mixing. Mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 hours at room temperature. *If refrigerated, the reagent can be kept and used for 3 days assuming all QC samples meet the proper acceptance criteria*. Place reagent immediately back in the refrigerator after use to preserve its integrity. When fresh the reagent should be light yellow. If

the reagent turns gold, it is too old. Store reagent in glass.

9.2.1 Prepare two beakers as follows:

9.2.1.1 Beaker #1 0.53 g Ascorbic acid and 30mL nanopure water. Make fresh before each batch analysis.

9.2.1.2 Beaker #2 50mL 5N H_2SO_4, 5mL Potassium Antimonyl Tartrate solution, and 15mL Ammonium Molybdate solution.

9.2.2 Add the contents of beaker #1 to beaker #2. This will make 100mL P Reagent.

9.3 5N Sulfuric Acid

9.3.1 Start with 430mL of nanopure water and slowly, with stirring, add 70mL of concentrated H_2SO_4. Let cool and dilute to 500mL. Store in a glass bottle at room temperature for up to one year.

9.4 Ammonium Molybdate solution

9.4.1 Dissolve 20 g of Ammonium Molybdate 4-hydrate $[(NH_4)_6Mo_7O_{24}.4H_2O]$ in 500mL nanopure water. Store in a glass bottle at room temperature for up to three months.

9.5 Potassium Antimonyl Tartrate Hemihydrate

9.5.1 Dissolve 1.3715g $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$ in 400mL nanopure water. Dilute to 500mL. Store in a glass bottle at room temperature for up to six months.

9.6 Calibration Standards for Curve:

9.6.1 Stock: KH_2PO_4 previously dried in a 105^oC oven and stored in a desiccator.

9.6.2 Intermediate 1000ppm: Weigh up 4.394g KH_2PO_4 into 1L of Nanopure. Keep at room temperature. This standard may be kept for up to 12 months or replaced sooner if comparison with QC samples indicate a problem.

9.6.3 Working Standards:

9.6.3.1 Pipette 1mL of 1000ppm PO4-P Intermediate standard into 1L of Nanopure water.

9.6.3.2 Analyze various amounts to create a six- point curve. Solutions are made fresh each time.

9.7 Laboratory Control 0.5ppm:

9.6.4 Stock: Non-calibration source of KH_2PO_4 previously dried in a 105^{o}C oven and stored in a desiccator.

9.6.5 LCS Intermediate 500ppm: Dilute 2.197g KH_2PO_4 into 1L of Nanopure. This standard may be kept for up to 12 months or replaced sooner if comparison with QC samples indicate a problem.

9.6.6 Working LCS 0.5ppm: Spike 10uL of LCS Intermediate into 10mL of Nanopure water. Filter and analyze as specified in sections 12.4 - 12.6.

9.6.7 Calibration Check 0.25ppm: Spike 5uL of LCS Intermediate into 10mL of Nanopure water. Analyze as specified in sections 12.4 - 12.6.

10.0 Quality Control

Note: See also ESB SOP Q01 for general QC requirements

10.1 A LCS is analyzed once per batch, or 1 for every 20 samples, whichever is greater and reported per matrix type. The result must be within acceptance ranges of 90-110% or the analysis is considered to be out of control. The problem must be corrected before the analysis can continue.

10.2 Matrix Spike and Matrix Spike Duplicate 0.5ppm:

10.2.1 Spike 10uL of LCS Intermediate into 10mL of sample. Analyze as specified in sections 12.4 - 12.6.

10.2.2 An MS/MSD is analyzed one per batch, or for every 20 samples per matrix type, whichever is greater.

10.2.3 If the matrix spike and matrix spike duplicate is not within the control limits of 80-120%, maximum RPD of 20%, the spikes should be re-analyzed. If the results are the same, the sample used as the spike must be qualified.

10.3 Method Blank

10.3.1 One method blank per batch of 20 samples is analyzed using 10mL of Nanopure water. The blank must be below the reporting limit, but not more negative than the RL for batch acceptance. Sample results at or above the reporting limit must be accompanied with a qualifier if the method blank exceeds a concentration greater than 1/10 the measured concentration of the sample. Blank results below the reporting limit are considered to be ND and will not require a note.

10.3.2 If the instrument is zeroed to a blank *a method blank is not required*.

10.4 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at the reporting limit or 2.5-5 times the estimated MDL and a minimum of seven replicates is analyzed. See QA Manual for calculation. Results must be below the reporting limit.

10.4.1 LOD Verification: On a yearly basis, a vial of reagent water is spiked at a level of at least 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL.

10.5 Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of

Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration 5-50 times the MDL. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 10.

10.6 Demonstration of Continuing Proficiency: On an annual basis, analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed twice a year.

11.0 Calibration:

11.1 A six-point curve plus the zero point, required by the method, is analyzed on a yearly basis covering the referenced concentration range.

11.2 An acceptable curve yields an $r^2 = 0.99$ or better.

11.3 A regression equation is generated from the curve.

11.4 Results are calculated from the regression equation.

11.5 Immediately following calibration a 0.5mg/L ICV is analyzed using the LCS source to verify the curve. Results must be within 90-110% of the expected value or a new curve may be necessary.

11.6 Continuing Calibration Verification Checks: The calibration is verified on a daily basis by analysis of the 0.25 mg/L Calibration Check analyzed at the end of the day’s batch. Recovery must be within 85-115% of expected value.

12.0 Procedure for Ortho-Phosphorus

12.1 Pour up to 10mL of sample into beaker. Adjust the pH of the sample prior to measurement *if it is suspected* to be <6.5 or >8.5. The sample must be clear. Filter through 0.45micron disk filter if

necessary. If the filtrate is still turbid or discolored read the background.

12.1.1 Zero the spec to a blank *prior to addition of reagents.* Read the sample *prior to addition of reagents.* This absorbance is subtracted from the reading acquired in section 12.5.

12.2 If using less than 10mL of sample, bring up the volume to 10ml with nanopure water. Record the volume of sample used.

12.3 Spike 10uL of LCS Intermediate into the lab control, matrix spike, and matrix spike duplicate.

12.4 Add 1.6mL of "P-Reagent" to all samples.

12.5 Swirl samples then let them stand at least 10 minutes but not more than 30 minutes.

12.6 Read the absorbance of the samples in the spectrophotometer at 880 nm.

12.7 If the sample is a solid:

12.7.1 Add deionized water in a 1:10 ratio with the soil. (I.e. 5g of sample to 50mL water.) or any ratio that is convenient.

12.7.2 Allow leaching for 1 hour with frequent stirring.

12.7.3 Follow the procedure in section 12.1 – 12.5.

13.0 Reading Samples

13.1 Before reading samples on the spectrophotometer, zero instrument on the following blank.

13.1.1 Blank: 10mL of nanopure water to which 1.6mL of "P-Reagent" have been added.

13.2 Read samples one at a time, rinsing, blotting and wiping the cuvette in between each one.

14.0 Calculations

14.1 Compare each sample absorbance to a current graph.

14.2 Multiply concentration by Dilution Factor if 10mL were not used.

14.3 Read to 2 significant figures.

14.4 The reporting limit is 0.05 mg/L.

14.5 All results are reported as ortho-phosphate phosphorus. If the sample is filtered, no distinction is made for “dissolved” versus “total” since 40 CFR, part 136 Table II instructs all ortho-phosphates to be filtered immediately.

15.0 Definitions: See SOP Q15 – SOP Definitions

16.0 Corrective Action For Out of Control Or Unacceptable Data:

16.1 See SOP Q06 – Corrective Action

17.0 Pollution Prevention and Waste Management:

17.1 See SOP S05 – Neutralization Procedure for Acid and Alkaline Waste

17.2 SOP S07 – Pollution Prevention

17.3 Wastes are dumped in the “High Acid Concentration Waste” barrel.

18.0 Method Performance

18.1 Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QC Office.

References

Standard Methods for the Examination of Water and Wastewater, AWWA/APHA/WEF, 18th Edition 4500-P E

Note: All *italicized items* are an indication of a variation from the method.

Approved by: Susan K. Thomas
Date:08/23/06

RESIDUE, TOTAL FILTERABLE (Total Dissolve Solids)
Edward S. Babcock & Sons
STANDARD OPERATING PROCEDURE
(Standard Methods, 2540C)
Effective Date: 09/22/06

1.0 Scope and Application

1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
1.2 This method may also be used for solid samples if both client and regulator agree to this variation. See section for details.
1.3 Environmental Relevance –
1.3.1 TDS are both inorganic and organic in origin. Inorganic sources are minerals such as calcium, magnesium, and bicarbonate from rocks. Organic TDS can come from leaves, industrial waste, and sewage. TDS in a water sample comes from natural sources such as the bedrock and decaying plant material and introduced sources such as waste, urban runoff, and chemicals left over from water treatment processes. A constant level of TDS is essential to the health of aquatic organisms as the density of the TDS determines water flow in and out of cells. Elevated TDS levels are not generally health hazards for humans, but have an effect on the smell and taste of drinking water.
1.3.2 The EPA classified TDS as a Secondary Drinking Water Standard and has set a limit of TDS less than 500 mg/L for drinking water. High concentrations of TDS due to carbonates can lead to scale formation and a bitter taste to the water. If a water source tastes salty and an increase in corrosivity is observed, it may have a high TDS concentration due to chlorides. These effects are not necessarily dangerous but are unpleasant. Scale formation is a nuisance as it can be damaging to water heaters and plumbing.

2.0 Working Range

10 mg/L to 2000 mg/L

3.0 Summary of Method

A well-mixed aliquot of the sample is filtered through a standard glass fiber filter. The filtrate is evaporated and dried at 180^{o}C.

4.0 Definitions

See SOP Q15 – SOP Definitions

5.0 Sample Handling

Sample may be stored at 4°C for up to 7 days as specified in 40 CFR part 136, table II.

6.0 Interferences

6.1. Highly mineralized waters containing significant concentrations of calcium, magnesium, chloride and/or sulfate may be hydroscopic and will require prolonged drying, desiccation and rapid weighing.

6.2. Samples containing high concentrations of bicarbonate will require careful and possibly prolonged drying at 180-C to insure that all the bicarbonate is converted to carbonate.

6.3. Too much residue in the evaporating dish will crust over and entrap water that will not be driven off during drying. Total residue in the dish should be limited to about 200 mg.

7.0 Safety

General laboratory safety procedures apply. Care should be taken when reaching over the hot water bath, handling dishes or reaching into the ovens.

8.0 Apparatus

8.1. 500 ml side-arm flask.

8.2. Filtration apparatus: Membrane filter funnel with a Gelman type 47mm/90mm (A/E glass fiber) filter disk.

8.3. Drying oven, for operation at 180°C ± 2°C.

8.4. Vacuum aspirator.

8.5. Desiccator with fresh desiccant

8.6. Balance # 78 with a sensitivity of 0.1 mg. Calibrated on a daily basis with 5g and 100g class "S" weights on a daily basis. Calibration must be within ±

5mg. If values are not within these limits, recalibrate the balance.

8.7. 12 Unit steam bath. On a quarterly basis drain the water bath and scrap out the residue. Try to turn off the bath heat at night so that cool water can circulate

8.8. Standard laboratory glassware: volumetric flasks, beakers, graduated cylinders, pipets.

Note: All glassware is cleaned immediately prior to and after use by thorough rinsing with three portions of D.I. water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

9.0 Standards

0.01M KCl Lab Control: 0.7456 g KCl predried in 105^{o} oven, dissolved in 1 liter of Nanopure water. This solution is stored at room temperature for up to 6 months.

10.0 Procedure

10.1. Prepare dishes by heating at 180°C for a minimum of 1 hour.

10.2. Take hot dishes out of 180°C oven. Cool 15-20 minutes on a counter.

10.3. Cool dishes to room temperature in a desiccator at least half an hour. Weigh dishes on balance for tare weight and record. Place dishes on water bath.

10.4. Place a small amount of sample into a tube for electrical conductivity screening. Samples that are high in salts (TDS) will have an elevated conductivity. Filter the appropriate aliquot of sample to give a result of less than 2000 mg/L prior to dilution factors.

Estimated Ec	mLs of sample to analyze
001 – 200	200
200 – 2000	100
2001 – 4000	50
4001 – 8000	25
8001 – 20,000	10
>20,000	See supervisor

10.5.Measure proper aliquot of *well shook* sample, usually 100 ml into a 100 ml graduated cylinder and filter.

10.6. Some samples may be difficult to filter. In order to maximize sample size, try one of the following. If possible do not use less than 10 ml of sample.

10.6.1. A bigger filtration apparatus may be necessary for samples that are not high in salts but contain a great deal of suspended material. It is recommended that the filter time not exceed 10 minutes.

10.6.2. Change filter papers as often as necessary to filter sample ensuring that each filter is rinsed thoroughly.

10.7. After the sample has filtered through, make sure that the filter is completely dry prior to turning off the pump. Drips should be at least 5 seconds apart and the filter must be visually dry. Rinse into the filtrate, the filter paper and cylinder with D.I. water. Pour filtrate into the evaporating dish. Rinse flask with a small amount of D.I. water. Add the rinse to the evaporating dish with the filtrate.

10.8. After each sample rinse all apparatus three times with deionized water.

10.9. Place samples on water bath until they are dry. After samples are dry, put in 180°C oven for 1.5 hours which is sufficient time to bring the sample to constant weight *as proven by an annual constant weight study.* Wipe down the outside of each dish prior to placing them in the oven.

10.10. Cool dishes in a desiccator at least half an hour, until room temperature. Weigh dishes. Record weight.

10.11. Wash evaporating dishes with a green scrubbie,1:1 HNO_3 if necessary, and plenty of D.I. Put dishes back in 180°C oven for 1 hour. Cool and place in desiccator for the next set.

10.12. Solid samples may be analyzed by first preparing a 1:10 water extract. Add an amount of reagent water equal to ten times the weight of solid material taken as a sample. This mixture is agitated for sixty minutes by shaking intermittently. Filter the resulting slurry through a 0.45 micron membrane type filter. Add filtrate to TDR dish. Report result in the extract, as mg/L, attaching qualifier N-WXr.

11.0 Calculation and Reporting

$$(A-B) \times 1{,}000{,}000$$

$$\frac{\text{(A - B) x 1000}}{\text{\# ml of sample used}}$$

Where A = Weight in grams of dish with residue, and
B = Tare weight in grams of dish.

11.1. Alternatively, you may subtract the actual numbers in the weight readings (without any decimal points) and multiply the difference by the factor of 100/(ml of sample used).

11.2. Results are entered into LIMS by taking the

$$\frac{\text{Difference (from data page) X Dilution Factor (based on a 200mL default volume)}}{2}$$

The dilution factor is placed in the DIL column to account for initial volume differences. The initial and final volumes are set at 200 and 100 mL and are not adjusted by the analyst.

11.3 Reporting Units: mg/L

11.4 Reporting Limit: 10 mg/L for 200ml aliquot or 20 mg/L for 100ml aliquot

12.0 Quality Control:

Note: See also ESB SOP Q01 for general QC requirements

12.1 Duplicates are analyzed daily or every ten samples per matrix type whichever is more. The Relative Percent Difference is calculated and compared to the acceptance range. Sample acceptability however is based on a maximum RPD of *20%*. If the RPD does not fall within this acceptance range, the sample is re-analyzed. If the RPD still does not fall within the acceptance range, a note is placed on the client's results.

12.2 Laboratory Control: An aliquot of the KCL standard is analyzed daily or one every twenty samples whichever is more and reported per matrix type. The result from this analysis is compared to the control limit (90 - 110% recovery). If the result fall outside the control limit, the analysis is considered to be out of control and all the other results in the analytical run are questionable. Troubleshooting is performed to

attempt to determine the cause. If the cause is not determined, or the cause is something that could effect the other samples in the batch, the analyses will be re-run.

12.3 A method blank is analyzed daily or one every twenty samples whichever is more and reported per matrix type. 200 mL of D.I. water is filtered exactly like a sample. It must read less than 10 mg/L and greater than –10 mg/L for batch acceptance. Reportable sample results must be accompanied with a qualifier if the method blank exceeds a concentration greater than 1/10 of the measured raw concentration of the sample. However, blank results below the reporting limit are considered to be ND and will not require a note.

12.4 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at 2.5-5 times the estimated MDL and a minimum of seven replicates is analyzed. See QA Manual for calculation. Results must be below the reporting limit.

12.5 Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration 5-50 times the MDL. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 10.

12.6 Demonstration of Continuing Proficiency: On an annual basis, each analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed twice a year.

12.7 LOD Verification: On a yearly basis, a QC sample is spiked at a level of 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL.

12.8 Constant Weight Study: On an annual basis a constant weight study is performed. 200mg of calcium sulfate is placed in three TDR dishes by filtering a 100mL aliquot of a 2000mg/L solution. This hydroscopic salt is intended to represent the

most challenging sample. Dishes are placed in the oven for one hour, cooled and weighed. Dishes are placed back in the oven for 15 minutes, cooled and then weighed. This process is repeated until the weights are constant. Constant weight is defined as, "weight change less than 4% of the previous weight or a change less than 0.5mg, whichever is less". The constant weight determined by this study will be used as the drying time for the procedure. Empty dishes are also tested to determine the necessary dish preparation time.

14.0 Corrective Action For Out of Control or Unacceptable Data:

See SOP Q06 – Corrective Action

15.0 Pollution Prevention and Waste Management:

See SOP S07 – Pollution Prevention

16.0 Method Performance

Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QC Office.

17.0 References

17.1. Standard Methods for the Examination of Water and Wastewater, AWWA/APHA/WEF, 18th edition Method 2540C.

17.2. Methods of Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Method 160.3

Note: All *italicized items* are an indication of a variation from the method.

Approved by: Susann K. Thomas Date: 07/25/06

RESIDUE, TOTAL SUSPENDED
Edward S. Babcock & Sons
STANDARD OPERATING PROCEDURE
(SM 2540 D)
Effective Date: 081106

1.0 Scope and Application:

1.1 This method is applicable to all aqueous samples.

2.0 Working Range:

2.1 The working range is 5mg/L (the reporting limit) to 2000mg/L.

3.0 Method Summary:

3.1 A measured volume of sample is filtered through a pretared filter. The residue that remains on this filter after drying in a 105 degree Celsius oven is considered the suspended solid portion of the sample.

4.0 Sample Collection, Preservation and Holding Time:

4.1 The sample must be unpreserved. It must be stored at 4 degrees Celsius until analysis. Analysis must take place within 7 days of sampling per CFR part 136, Table II.

5.0 Interferences:

5.1 Non-representative particulates such as leaves, rocks, and sticks may be excluded.
5.2 To avoid water entrapment, limit the sample size to that yielding no more than 200mg residue on the filter. (This would be a final result of 2000mg/L since we are analyzing 100 mLs of sample.)
5.3 For samples high in dissolved solids thoroughly wash the filter with D.I. water after the sample has passed through the filter.
5.4 Prolonged filtration times resulting from filter clogging may produce high results owing to

increased colloidal materials captured on the clogged filter.

6.0 Apparatus and Standards

6.1 Side-arm flask of sufficient capacity for sample size selected.

6.2 Filtration apparatus: Membrane filter funnel with a Gelman type A/E glass fiber filter disk with a suitable diameter for the funnel.

6.3 Drying oven, for operation at 105°C ± 2°C.

6.4 Vacuum aspirator.

6.5 Desiccator – Check on the desiccant to make sure that it is always blue. Replace desiccant when it turns purple.

6.6 Balance #58 with a sensitivity of 0.1 mg. Calibrated with 0.1 g, 5g, and 100 g class "S" weights on a daily basis. Calibration must be within 5mg (for 5g & 100g) and 0.5mg (for 0.1g). If values are not within these limits, recalibrate the balance.

6.7 *Filter garages* to hold glass fiber filters.

6.8 Standard laboratory glassware: volumetric flasks, graduated cylinders, pipets.

Note: All glassware is cleaned immediately prior to and after use by thorough rinsing with three portions of D.I. water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

6.9 Stock Standards:
6.9.1 Cellulose – stored at room temperature for up to 10 years.

6.10 Lab Control: 500mg of cellulose is weighed into a liter of D.I. water. This solution is kept at room temperature for up to a 6 months. Use a 100mL aliquot.

6.11 Reagent water: D.I. water.

7.0 Procedure

7.1 Prepare glass fiber filters by rinsing three times with D.I. water and heating at 105°C for a minimum of 1 hour. *A constant weight study is performed yearly to establish the minimum time required to bring the filter to a constant weight.*

7.2 Take hot filters out of 105°C oven.

7.3 Cool filters to room temperature then place in a desiccator. Use forceps when handling filters. Place filter on balance and record tare weight. Place the filter onto the filtering apparatus. Wet filter with a small amount of D.I. to seat it.

7.4 Mix sample well by *shaking* sample bottle. Measure an appropriate volume of sample in a graduated cylinder normally 100 mL. Use a 200 mL sample volume for method blank and samples expected to contain very minute amounts of suspended material. Filter through apparatus collecting suspended residue on filter. Rinse cylinder and filter 2 to 3 times with a small amount of D.I. water. Apply suction three *until no visible free liquid is present.*

7.4.1 For samples with a lot of suspended matter, a smaller volume of sample (10 – 50 mL) may be used. **Avoid using less than 10mL.** If less than 10mLs must be used, an auto pipette can be used to obtain a representative sample by carefully cutting the tip. Verify the accuracy of the altered tip by using the scale. Make sure the tip is rinsed well.

7.5 Place samples in 105°C oven for 1.5 hours which is longer than the *time proven to be sufficient to bring the sample to a constant weight.*

7.6 Cool filters in an area free of moisture or contamination for 10-15 min. Weigh filters or place in a desiccator until ready to weigh. Record the final weight.

8.0 Calculation:

$$\frac{(A-B) \times 1{,}000{,}000}{\#\ \text{mL of sample used}}$$

Where A = Weight in grams of filter with residue, and

B = Tare weight in grams of filter.

8.1 Alternatively, you may subtract the actual numbers in the weight readings (without any decimal points) and multiply the difference by the factor of 100/(mL of sample used).

8.2 The reporting limit for this procedure (based on a 200 mL aliquot) is 5 mg/L.

8.3 Report all results to three significant figures.

8.4 The difference is entered as the initial raw result.

8.5 The dilution factor based on a 200mL sample aliquot, is entered into the Dil column in LIMS.

9.0 Quality Control:

Note: See also ESB SOP Q01 for general QC requirements

9.1 Duplicates are run with every batch or one every ten samples per matrix type whichever is more frequent. The Relative Percent Difference is calculated. If the RPD does not fall within the acceptance limit maximum of *25%*, the sample is re-analyzed. If the RPD still does not fall within the acceptance range, a note is placed on the client's results.

9.2 A method blank is analyzed with every batch of samples, at a minimum of once for every 20 samples and reported per matrix type. Results must be less than 5 mg/L and greater than –5 mg/L for batch acceptance. Sample results at or above the reporting limit must be accompanied by a qualifier if the method blank exceeds a concentration greater than 1/10 of the measured raw concentration of the sample. Blank results below the RL are considered to be ND and will not require a note.

9.3 A lab control is analyzed with every batch at a minimum of once for every 20 samples and reported

per matrix type. Results must be between 90-110% recovery for batch acceptance. If the result falls outside the control limits, the analysis is considered to be out of control and all other results in the analytical run are questionable. Troubleshooting is performed to attempt to determine the cause. If the cause is not determined, or the cause is something that could affect the other samples in the batch, the analyses will be re-run.

9.4 An MDL study is completed whenever major equipment or procedural changes are made. Past studies were performed at 50 mg/L. Standards should be spiked at 2.5-5 times the estimated MDL. A minimum of six to seven replicates is analyzed. See QA Manual for calculation. Results must be below the reporting limit.

9.5 LOD Verification: On a yearly basis, a QC sample is spiked at a level of 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL.

9.6 Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four consecutive replicates of a QC sample made at a concentration of 5-50 times the MDL. Past studies were performed at 25 or 50mg/L. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 10.

9.7 Demonstration of Continuing Proficiency: On an annual basis, each analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed twice a year.

9.8 Constant Weight Study: On an annual basis a constant weight study is performed. 200mg of cellulose is placed on three TSS filters by filtering a 100ml aliquot of a 2000mg/L solution. This solution is intended to represent the most challenging sample. Filters are placed in the oven for an hour, cooled and weighed. This process is

repeated until the weights are constant. Constant weight is defined as, "weight change less than 0.5mg or 4% of the previous weight, whichever is less." The constant weight determined by this study will be used as the drying time for the procedure. Empty filters are also tested to determine the necessary filter preparation time.

10.0 Corrective Action For Out of Control Or Unacceptable Data:
See SOP Q06 – Corrective Action

11.0 Pollution Prevention and Waste Management:
SOP S07 – Pollution Prevention

12.0 Definitions: See SOP Q15 – SOP Definitions

13.0 Safety

13.1 General laboratory safety procedures are sufficient for this analysis. Recommended safety equipment includes gloves and safety glasses.

14.0 Method Performance:
Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QC Office.

15.0 References

Standard Methods For the Examination of Water and Wastewater 18th Edition APHA/AWWA/WEF 2540D.

Methods for the Chemical Analysis of Waters and Wastes EPA 160.2.

Note: All *italicized items* are an indication of a variation from the method.

Approved by: Susan K. Thomas Date: 08/10/06

Standard Operating Procedure
Edward S. Babcock & Sons
Effective Date: 10/06/06

METHOD #: Standard Methods 2130 B

TITLE: Turbidity (Nephelometric)

1.0 Scope and Application

This method is applicable to drinking, surface, and saline waters in the range of turbidity from 0.2 to 4000 nephelometric turbidity units (NTU).

1.1 Health Effects of Turbidity

1.1.1 High turbidity levels are often associated with high levels of disease-causing microorganisms such as viruses, parasites, and some bacteria. These organisms can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.

NOTE 1: NTU's are considered comparable to the previously reported Formazin Turbidity Units (FTU) and Jackson Turbidity Units (JTU).

2.0 Summary of Method

2.1 The method is based upon a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, the higher the turbidity. Readings, in NTU's, are made in a nephelometer designed according to specifications outlined in Apparatus. A standard suspension of Formazin, prepared under closely defined conditions, is used to calibrate the instrument.

2.2 Formazin polymer is used as the turbidity reference suspension for water because it is more reproducible than other types of standards previously used for turbidity standards.

2.3 A commercially available polymer standard is also approved for use for the National Interim Primary Drinking Water Regulations. This standard is identified as AMCO-AEPA-1 available from Amco Standard International, Inc.

3.0 Sample Handling and Preservation

3.1 Samples may be stored in either plastic or glass.

3.2 Preservation consists of refrigeration or icing to 4-C, to minimize microbiological decomposition of solids.

3.3 Analysis must be performed within 48 hours per 40CFR section 136, Table II. Samples must be stored at $4^{o}C$.

4.0 Interferences

4.1 The presence of floating debris and coarse sediments which settle out rapidly will give low readings. Finely divided air bubbles will affect the results in a positive manner.

4.2 The presence of true color, that is the color of water which is due to dissolved substances which absorb light, will cause turbidities to be low, although this effect is generally not significant with finished waters.

4.3 Dirty glassware or sample vibration will cause a positive interference.

5.0 Apparatus

5.1 The 2100N Hach turbidimeter consists of a nephelometer with light source for illuminating the sample a photo-electric detector with a readout device to indicate the intensity of light scattered at right angles to the path of the incident light. The turbidimeter is designed so that little stray light reaches the detector in the absence of turbidity should be free from significant drift after a short warm-up period.

5.2 The sensitivity of the instrument permits detection of a turbidity difference of 0.05 unit or less in waters having turbidities less than 1 unit. (The minimum detection level reported is 0.05 NTU.) The instrument is able to measure from 0.05 to 4000 units turbidity. Several ranges are available to obtain both adequate coverage and sufficient sensitivity for low turbidities.

5.3 The sample tubes are made of clear, colorless glass. They must be kept scrupulously clean, both inside and out, and discarded when they become scratched or etched. They must not be handled at all where the light beam from the instrument strikes them, so they are provided with sufficient extra length so that they may be handled at the top.

5.4 Differences in physical design of turbidimeters will cause differences in measured values for turbidity even though the same suspension is used for calibration. To minimize such differences, the following design criteria are observed:

5.4.1 Light source: Tungsten lamp operated at a color temperature between 2200-3000-K.

5.4.2 Distance traversed by incident light and scattered light within the sample tube: Total not to exceed 10 cm.

5.4.3 Detector: Centered at 90- to the incident light path and not to exceed +/- 30 C- from 90 C. The Detector, and filter system if used, shall have a spectral peak response between 400 and 600 nm.

5.5 Standard laboratory glassware: volumetric flasks, beakers, graduated cylinders, pipets.

Note: All glassware is cleaned immediately prior to and after use by thorough rinsing with three portions of NANOPURE water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final NANOPURE rinse.

6.0 Reagents and Standards

6.1 Turbidity-free water: Nanopure water is used. It has a background turbidity reading of approximately 0.05 NTU.

6.2 Stock formazin turbidity suspension: 4000 NTU Formazine Solution purchased for supplier, stored at room temperature until Manufacturer specified expiration date. Stock standards can be replaced sooner if comparison with QC samples indicate a problem. Once opened, the expiration date is 1 year from date opened as long as this does not exceed manufacturer's original expiration date. Two sources are purchased, one for calibration standard preparation and the second source for calibration verification (LCS) preparation.

6.3 Standard formazin turbidity suspension prepared:

6.3.1 LCS : Prepare daily as specified below for from noncalibration stock source

6.3.2 Calibration Standards: Prepare each time a calibration is necessary as specified below for instrument calibration

For: Standard Concentration, NTU	Pipette mls From Stock	*Dilute To: (Volume in mls)with NANOPURE
4000	fill cell	0
1000	25	100
800	20	100
400	10	100
200	5	100
80	2	100
40	1	100
20	1	200
8	2	1000
4	1	1000
0.85*	0.2	1000

*0.05 NTU is added from the dilution water.

7.0 Procedure

7.1 Turbidimeter calibration: The manufacturer's operating instructions are followed which specify calibration every 90 days for USEPA reporting. However, should the Electronic P.C. Board, the Photo Detectors, or the Light Source be replaced or if very carefully prepared Formazine Suspensions indicate a need for recalibration, this will be done more often.

7.2 Calibration

7.2.1 Always mix the contents of each cell by inverting several times before placing in the Optical Well for reading.

7.2.2 Keep the outside surface of the cell clean and dry. Apply a drop of silicone oil to the outside and wipe with a cloth or tissue. Finish with a chem wipe.

7.2.3 When placing any standards in the well, always use the light shield to cover the well in order to keep out ambient light.

7.3 Carry out the following steps:

7.3.1 First place a cell of NANOPURE water in the cell holder.

7.3.2 Press the CAL key.

7.3.3 Press ENTER.

7.3.4 The instrument will advance to the next standard, display the expected value, and the S1 light.

7.3.5 Place the 20 NTU standard in the cell holder.

7.3.6 Press ENTER.

7.3.7 Once the instrument displays the next standard value and S# light, place that standard in the cell holder, press ENTER and so on until all the standards have been read.
7.3.8 The standard values are: 20, 200, 1000, and 4000.
7.3.9 After the last standard is processed, press the CAL key.

7.4 Analysis:
7.4.1 Clean and rinse the cell with Nanopure, wiping all excess water from the sides with tissue. Apply a drop of silicone oil to the outside and wipe with a cloth or tissue. Finish with a chem wipe. Never handle the cell where the light strikes.
7.4.2 Check the calibration by reading the 0.8 Lab Control. If it is not within range repour or remake it. If it is still out, examine the instrument for problems. The calibration may have to be repeated. Sample results may not be taken until a Lab Control falls within the acceptance ranges.
7.4.3 Shake the sample and pour it into the cell. Wipe the cell with lens paper to make sure there are not smudges, then put the cell into the turbidity meter with the line on the cell pointing foreword. If the sample reading is higher than the 0.80 range, the calibration must be checked using a lab control (from the previous table) that encompasses the sample's range. Record the lowest stabilized reading before the readings begin to climb back up or before sample particles have settled.
7.4.4 *Do not dilute the sample!(*Standard Methods, 20th Edition, Method 2130B 4a and Hach Model 2100N Turbidity Meter Manual 2.3.8.) Readings above 4000 are reported as >4000 NTU, using the qualifier _T.

8.0 Calculation and Reporting Requirements:

8.1 Results are reported in NTU's.

8.2 Report results to *2* significant figures.

8.3 Reporting Limit (RL) = 0.2 NTU

9.0 QA/QC Requirements:

Note: See also ESB SOP Q01 for general QC requirements

9.1 The 0.85 NTU LCS serves as an Initial Calibration check analyzed at the beginning of the analysis. Another LCS, made at a different concentration is analyzed at the end of the run to verify calibration. An LCS is analyzed with every batch or one per twenty samples whichever is greater. If the lab control does not read within acceptance limits 90-110%, re-make and read again. If the lab control still does not read correctly, re-calibrate the instrument.

9.2 Analyze a Nanopure blank with every batch or one per twenty samples whichever is greater. Results must be below but not more negative than the reporting limit. Results should be approximately 0.05 NTU or the 0.85 LCS true value may need to be adjusted. Sample results at or above the reporting limit must be accompanied with a qualifier if the method blank exceeds a concentration greater than 1/10 the measured raw concentration of the sample. Blank results below the reporting limit are considered to be ND and will not require a note. If more than one sample tube is used, check each one with a method blank.

9.3 Duplicates are analyzed with each batch or every 10 samples per matrix type which ever is more. Results must have a RPD $\pm$ 20%.

9.4 Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration of 0.4-0.9 NTU. The average percent recovery of the four replicates must be 80-120% with a maximum %RSD of 10.

9.5 Demonstration of Continuing Proficiency: On an annual basis, analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed twice a year.

9.6 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at a concentration 2.5-5 times the estimated MDL and a minimum of seven replicates analyzed. See QA manual for calculation. Results must be below the reporting limit.

9.6.1 LOD Verification: On an annual basis, an aliquot of water is spiked at a level of 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL.

10.0 Definitions: See SOP Q15 – SOP Definitions

11.0 Safety: General laboratory safety procedures are adequate for this analysis.

12.0 Corrective Action For Out of Control Or Unacceptable Data:
See SOP Q06 – Corrective Action

13.0 Pollution Prevention and Waste Management:
See SOP S07 – Pollution Prevention

14.0 Method Performance
Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QC Office.

References

1. EPA Method 180.1 (1993) Methods for the Chemical Analysis of Waters and Wastes.

2. Standard Methods for the Examination of Water and Wastewater, APHA/AWWA/WEF, 18th Edition, Method 2130B.

Note: All *italicized items* are an indication of a variation from the method

Approved by Susan K. Thomas Date 10/02/06

TOTAL-PHOSPHORUS
Edward S. Babcock & Sons
STANDARD OPERATING PROCEDURE
ASCORBIC ACID METHOD
(SM 4500-P B, 4 E)
Date Effective: 082806

1.0 Scope and Application

These methods cover the determination of specified forms of phosphorus in drinking, surface and saline waters, domestic and industrial wastes.

1.1 Health Effects of Phosphorus

1.1.1 Too much phosphate can cause health problems, such as kidney damage and osteoporosis. While burning, white phosphorus may cause damage to the liver, the heart, or the kidneys.

1.2 Environmental Effects of Phosphorus

1.2.1 In water, white phosphorus will accumulate in the bodies of aquatic organisms. In soil, phosphorus will remain for several days, but in deep soils and the bottom of rivers and lakes, phosphorus will remain for a thousand years or so. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff.

1.2.2 The increasing phosphorus concentrations in surface water raises the growth of phosphate-dependent organisms, such as algae and duckweed. These organisms use great amounts of oxygen and prevent sunlight from entering the water. This is known as eutrophication.

2.0 Working Range: 0.05 – 1.0mg/L

3.0 Summary

Ammonium molybdate and antimony potassium tartrate reacts in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration. Only orthophosphate forms a blue color in this test. Polyphosphates and organic phosphorus compounds may be converted to the orthophosphate form by nitric-sulfuric digestion.

4.0 Sample Preservation

The samples should be preserved by adding 0.5ml/L of 1:1 H_2SO_4 as specified in CFR part 136 Table II. Check that the pH is less than 2. Samples may be stored in the refrigerator for up to 28 days.

5.0 Interferences

5.1 Copper, iron, or silicate at concentrations many times greater than their reported concentration in seawater causes no interference. However, high iron concentrations can cause precipitation of and subsequent loss of phosphorus.

5.2 Arsenate is determined similarly to phosphorus reacting with molybdate reagent to produce a blue color and should be considered when present in concentrations higher than phosphorus. Concentrations as low as 0.1mg As/L can interfere.

5.3 Hexavalent chromium and nitrite interfere to give results about 3% lower at concentrations of 1mg/L and 10-15% lower at 10mg/L.

6.0 Safety

Safety glasses and gloves must be worn when dealing with acid digests.

See SOP S01 – Concentrated Acids and Bases
SOP S03 – Spill Control Policy

7.0 Equipment

7.1 Hot plate
7.2 100ml beakers
7.3 Watch glass
7.4 Spectrophotometer: Spectronic Genesys 10uv and Spectronic 20D+ set at 880nm.

8.0 Glassware Preparation

8.1 Glassware is stored separately for Total Phosphorus and Orthro-Phosphate phosphorus use only.

8.2 All glassware is acid washed in 1:1 HCl immediately after use, followed by thorough rinsing with three portions of D.I. water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

9.0 Phosphate Reagents and Standards

9.1 Reagent water: Nanopure

9.2 P-Reagent

9.2.1 Ascorbic Acid solution: Dissolve 0.53g Ascorbic acid in 30ml Nanopure water. Make fresh before each batch analysis.

9.2.2 5N Sulfuric Acid: Slowly with stirring, add 70ml concentrated H_2SO_4 to 430ml nanopure water. Let cool and dilute to 500ml. Reagent is stored in a glass bottle at room temperature for up to one year.

9.2.3 Ammonium Molybdate solution: Dissolve 20 g of Ammonium Molybdate 4-hydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] in 500ml Nanopure water. Reagent is stored in a glass bottle at room temperature for up to three months.

9.2.4 Potassium Antimonyl Tartrate: Dissolve 1.3715 g $K(SbO)C_4H_4O_6\cdot 1/2H_2O$ in 400ml Nanopure water. Dilute to 500ml Nanopure water. Reagent is stored in a glass bottle at room temperature for up to six months.

9.2.5 Mix in a beaker 50ml 5N Sulfuric Acid, 5ml Potassium Antimonyl Tartrate solution, and 15ml of the Ammonium Molybdate solution. To this, add Ascorbic Acid solution. This makes 100ml P Reagent.

9.2.6 Let all reagents reach room temperature before mixing. Mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 hours at room temperature. *If refrigerated, the reagent can be kept and used for 3 days assuming all QC samples meet the proper acceptance criteria.*

9.3 Phenolphthalein indicator: Dissolve 1 g of Phenolphthalein in 100ml of reagent alcohol: ethyl or isopropyl. Add 100ml nanopure water. Indicator is stored in a glass bottle at room temperature for up to six months.

9.4 6 N Sodium Hydroxide: Dissolve 240 g NaOH into 900ml nanopure water. Stir on magnetic stir plate under fume hood. (Caution: sample will become hot, fumes are noxious.) Let cool. Dilute to 1 liter.

Reagent is stored in a Nalgene bottle at room temperature for up to one year.

9.5 Sulfuric Acid Solution (1:4): Gradually, add 100ml concentrate sulfuric acid to 300ml nanopure water, cool. Warning, solution will get HOT. Reagent is stored in a glass bottle at room temperature for up to one year.

9.6 Instra analyzed Nitric Acid (conc.).

Note: The curve standards, Laboratory control samples, and blanks are all made with Nanopure water.

9.7 Laboratory Control(0.5ppm):

9.7.1 Stock: 100ppm solution containing organic phosphate, purchased from a certified vendor. Solution is stored at room temperature until manufacturer specified expiration date. Once opened the expiration date is 1 year from date opened.

9.7.2 Working LCS (0.5ppm): Pipet 50uL of 100ppm Stock standard into 10ml Nanopure water. Digest 10ml of Working standard to yield a 0.5ppm LCS.

9.7.3 Calibration Check (0.25ppm): Pipet 25uL of 100ppm Stock standard into 10ml Nanopure water and digest.

9.7.4 MS/MSD (0.5ppm): Pipette 50uL 100ppm Stock Standard into 10ml of a sample or a dilution of sample with the final volume of 10ml and digest.

9.8 Calibration Standards for Curve:

9.8.1 Stock: KH_2PO_4 previously dried in a 105^{o}C oven and stored in a desiccator.

9.8.2 Intermediate 1000ppm: Weigh up 4.394g KH_2PO_4 into 1L of Nanopure. Keep at room temperature. This standard may be kept for up to 12 months or replaced sooner if comparison with QC samples indicate a problem.

9.8.3 Working Standards:

9.8.3.1 Pipette 1ml of 1000ppm PO4-P Intermediate standard into 1 L of nanopure water.

9.8.3.2 Once a year or when calibration checks indicate a need, digest various amounts of above solution to create a six-point curve. Solutions are made fresh each time.

10.0 Procedure for Total Phosphorus:

10.1 Digestion

10.1.1 Acid-rinse all glassware. Use 100ml beakers for digestion.

10.1.2 Transfer 10ml of well-mixed sample (preserved with Sulfuric acid) into each beaker.

10.1.3 Spike 50ul of 100ppm Stock standard into 10ml nanopure water for the lab control, matrix spike, and matrix spike duplicate.

10.1.4 Add *1ml* of Nitric Acid (conc.) to each beaker.

10.1.5 Add *1ml of* Sulfuric Acid Mixture to each beaker.

10.1.6 Digest on edge of hot plate (setting 4-6) to Sulfuric Acid (white) fumes. Allow water and Nitric acid to steam off. Once there appears to be a thin coating of H2SO4 fumes in the beaker, cover with a watch glass, move beaker to the center and continue to digest with rolling fumes, about 2 minutes. If yellow fumes are present after watch glass is put on the beaker, remove the watch glass until yellow fumes are

gone and cover again. The beaker will fill with fumes and then the fumes will decrease. Drag the bottom of the watch glass along the rim of the beaker if moisture is accumulating. Do not allow the beaker to go dry. The entire digest takes about 10-15 minutes depending on volume and organic content of the sample.

10.1.7 Let cool. Rinse watch glass into the beaker and rinse down sides of beaker with water from the nanopure squirt bottle.

10.1.8 Add 2 drops of phenolphthalein to each beaker.

10.1.9 Neutralize with 6N Sodium Hydroxide by adding one drop at a time using a plastic dropper until it turns pink.

10.1.10 Transfer sample to 10ml-graduated cylinder. Dilute to 10ml with Nanopure water. Transfer the sample back to the beaker. Check for turbidity at this time. Redigest sample with less volume if turbid.

10.2 Colorimetric Reading

10.2.1 Add 1.6ml P-reagent, swirl, and then allow to stand for minimum of 10 minutes, maximum of 30 minutes.

10.2.2 Set Spectrophotometer wavelength to 880 nm.

10.2.3 Set zero with digested blank.

10.2.4 Read ABS of standard and record.

10.2.5 Record absorbance of samples. If result is over
1 mg/L, dilute and re-digest a new aliquot of
sample.

11.0 Calibration:

11.1 A six-point curve plus the zero point, required by the method, is analyzed on a yearly basis covering the referenced concentration range.

11.2 An acceptable curve yields an $r^2 = 0.99$ or better.

11.3 A regression equation is generated from the curve.

11.4 Results are calculated from the regression equation.

11.5 Immediately following calibration a 0.5 mg/L ICV is analyzed using the LCS source to verify the curve. Results must be within 90-110% of the expected value or a new curve may be necessary.

11.6 Continuing Calibration Verification Checks: The calibration is verified on a daily basis by analysis of the 0.25 mg/L Calibration Check analyzed at the end of the day's batch. Recovery of these checks must be within 85-115% of expected value.

12.0 Quality Control

Note: See also ESB SOP Q01 for general QC requirements

12.1 Laboratory Control (Conc 0.5ppm):

12.1.1 A LCS is analyzed once per batch, or for every 20 samples, whichever is greater and reported per matrix type.

12.1.2 The results must be within the acceptance range of *80-120%* or the analysis is considered to be out of control. The

problem must be corrected before the analysis can continue.

12.2 Matrix Spike and Matrix Spike Duplicate (Conc 0.5ppm):

12.2.1 A MS/MSD is analyzed once per batch, or 1 for every 20 samples per matrix type, whichever is greater.

12.2.2 If the matrix spike and matrix spike duplicate are not within the control limits of 80-120% with a maximum RPD of 20%, the spikes should be re-analyzed.

12.3 A Reagent Blank is digested with each batch and used to zero the instrument.

12.4 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at the reporting limit or 2.5-5 times the estimated MDL and a minimum of seven replicates is analyzed. See QA Manual for calculation. Results must be less than the reporting limit.

12.4.1 LOD Verification: On a yearly basis, a vial of reagent water is spiked at a level of at least 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL.

12.5 Demonstration of Capability: Prior to initial analysis of samples or when a significant change is made to the method, a Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration 5-50 times the MDL. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 10.

12.6 Demonstration of Continuing Proficiency: On an annual basis, analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or

equal to 20%. Performance Evaluation Studies are performed twice a year.

13.0 Calculations

13.1 For Total Phosphorus

13.1.1 Convert Absorbance to concentration by plugging spectrophotometer reading into regression equation. Do not report results higher than the highest calibrator.

13.1.2 Enter initial digestion volume used for samples on the Bench Sheet.

13.1.3 Enter raw result into the LIMS.

13.1.4 If the digest requires further dilution this value is entered in the DIL column of LIMS.

13.1.5 Results are reported in mg/L to 2 significant figures.

13.1.6 Results below 0.05mg/L are reported as ND.

13.2 Other Forms of Phosphorus

13.2.1 Total Dissolved Phosphorus (TPD) – Sample is filtered thru a 0.45micron filter prior to analysis. *To test the filters include a blank with the analysis that has also been filtered.*

13.2.2 Total Insoluble Phosphorus (TPI) – Total Phosphorus minus Total Dissolved Phosphorus.

13.2.3 Organic Phosphorus (ORGP) = Total Phosphorus minus phosphorus generated from digest SM 4500-P B 2 below.

13.2.4 Acid Hydrolyzable Phosphorus (PAHYDRO) - see Standard Methods 4500-P B 2. Phosphorus resulting from this digest minus ortho phosphate = acid hydrolysable phosphorus.

13.2.5 Total Phosphate (PO4) = Total Phosphorus x3.

14.0 Definitions: See SOP Q15 – SOP Definitions

15.0 Corrective Action For Out of Control Or Unacceptable Data:

15.1 See SOP Q06 – Corrective Action

16.0 Pollution Prevention and Waste Management:

16.1 See SOP S05 – Neutralization Procedure for Acid and Alkaline Waste

16.2 SOP S06 – Disposal of Chlorinated Solvents

16.3 SOP S07 – Pollution Prevention

16.4 Wastes are dumped in the "High Acid Concentration Waste" barrel.

17.0 Method Performance

17.1 Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QC Office.

References

Standard Methods for the Examination of Water and Wastewater, AWWA/APHA/WEF, 18th Edition 4500-P B, E

Methods of Chemical Analysis of Water and Wastes, EPA Method 365.2

Note: All *italicized items* are an indication of a variation from the method.

Approved by: Susan K. Thomas _ Date: 08/23/06

AUTOMATED KJELDAHL NITROGEN
Edward S. Babcock & Sons
STANDARD OPERATING PROCEDURE
EPA Method 351.2
Effective Date: **__09/22/06_____**

1.0 Scope and Application:

1.1 This method covers the determination of total Kjeldahl nitrogen in drinking and surface waters, domestic and industrial wastes. This method may also be used for solid samples if both client and regulator agree to this variation.

1.2 Analytically organic nitrogen and ammonia are determined together as Kjeldahl nitrogen. Organic nitrogen includes such natural materials as proteins and peptides, nucleic acids and urea, and numerous synthetic organic materials. Typical organic nitrogen concentrations vary from a few hundred ug/L in some lakes to more than 20 mg/L in raw sewage. This procedure converts nitrogen components of biological origin, but may not convert all the nitrogenous compounds of some industrial wastes.

2.0 Working Range:

0.1mg/L to 3.0mg/L. This range is for photometric measurements made at 660nm in a 10 mm tubular flow cell. Higher concentrations can be determined by sample dilution.

3.0 Summary:

The sample is heated in a block digester in the presence of sulfuric acid, potassium sulfate and mercuric sulfate. The residue is cooled and diluted with Nanopure water. The ammonium ion is determined using an automated colorimetric analyzer.

4.0 Collection, Preservation, and Holding Time:

4.1 Samples are preserved to pH<2 with sulfuric acid. They are stored at 4 degrees Celsius.

4.2 Samples must be analyzed within 28 days.

5.0 Interferences:

5.1 The acid and salt content of the digestion reagent is intended to produce a digestion temperature of about 360 to 370 degrees Celsius. If the sample contains a very large quantity of salt or inorganic solids that dissolve during digestion, the temperature may rise causing pyrolytic loss of nitrogen. To prevent this from occurring, the analyst may use less sample.

5.2 If large amounts of organic matter are present an increase in digestion temperature may also occur. To prevent this from occurring, the analyst may use less sample volume.

5.3 Be cautious of turbidity that may result in the SEAL tubes from the boiling stones if the digestate is not properly decanted. Once the digested KJN samples have been poured into the SEAL tubes, discard the remaining digestate to prevent such problems.

5.4 The color reaction chemistry for TKN analysis is sensitive to changes in acid concentration in the diluted digests. The acid concentration may vary from the nominal value for various reasons. Typically-observed symptoms are significant change in the calibration absorbance range or excessive upward or downward curvature of the calibration line.

6.0 Safety:

All digests must be performed under the hood. Take care when removing digests from the Block digester. Use gloves and safety goggles. Sulfuric acid and Sodium Nitroprusside have the potential to be highly toxic or hazardous.

See SOP S01 – Concentrated Acids and Bases
SOP S03 – Spill Control Policy

7.0 Apparatus:

7.1 Standard laboratory glassware: volumetric flasks, beakers-tall 200mL capacity, graduated cylinders, pipettes.

Note: Prior to use, glassware is acid rinsed with 1:1 HCl and three rinses of D.I. All glassware is cleaned immediately after use by thorough rinsing with three portions of D.I. water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

7.2 Block Digester: SEAL BD-46

7.3 SEAL – Discrete Automated Colorimetry

7.4 Vortex

7.5 Hengar Granules

8.0 Standards and Reagents:

8.1 Reagent water: Prepare all intermediate standards and reagents (including dilutions) in ammonium free (D.I.) water.

8.2 Laboratory Control Sample (LCS):

8.2.1 Stock LCS: 100 mg/L Stock TKN Standard purchased from ERA. Solution is stored at room temperature. Manufacturer expiration dates are observed. Once a stock standard is opened, the expiration date is one year as long as this date does not exceed the original manufacturer's date. Stock standards can be replaced sooner if comparison with QC samples indicate a problem.

8.2.2 LCS: Digest 0.2mL Stock LCS. Dilute to 20mL with Nanopure water after digestion for a final concentration of 1mg/L.

8.2.3 Acceptability: If the LCS doesn't fall within the acceptance range of 80% to 120% recovery, the analysis is stopped until the cause is determined and the LCS is in control.

8.3 Matrix Spikes and Matrix spike duplicates:

8.3.1 Spiking solution: Pipette 0.2mL of stock LCS on top of sample prior to digestion for MS and MSD. The spike concentration is 1 mg/L ammonia nitrogen in solution.

8.4 Calibration Standard:

8.4.1 Stock: Ammonium chloride (NH_4Cl)

8.4.2 Intermediate Standard (1000ppm):

8.4.2.1 Dehydrate Ammonium chloride (NH_4Cl) in a 105°C oven. Allow to cool in a dessicator.

8.4.2.2 Weigh out 3.819 g Ammonium Chloride. Dilute to 1 liter with D.I. water in a volumetric flask.

8.4.2.3 Pour the solution into a 1 liter amber bottle. Keep out of sunlight. Solution is stored at room temperature for up to one year. Standards can be replaced sooner if comparison with QC samples indicate a problem.

8.4.3 Intermediate Standard A (50ppm): Dilute 1.0mL of above intermediate standard (8.4.2) up to 20mL with digested digestion reagent. This solution is prepared fresh.

8.4.4 Working Calibration Standards: Fresh solutions are made each time a calibration is performed. *A 3ppm standard is diluted by adding making 600 uL of 50ppm Intermediate standard up to 10 mL with digested digestion reagent*. The other *standards are diluted from the 3ppm standard by the instrument with digested digestion reagent.* Working Calibration standards concentrations are: 3.0, 2.0, 0.99, 0.51, 0.24 and 0.09 mg/L.

8.5 ICV and CCV: A second stock source of NH_4Cl is purchased. An intermediate standard is prepared as specified in section 8.4.2. Standards are prepared as follows:

8.5.1 Standard A (50ppm): Digest 1.0mL of above intermediate standard and dilute to 20mL with Nano water. This solution is stored at 4°C for up to one month.

8.5.2 Working Standards: Solutions are stored at 4°C for up to 2 weeks.

8.5.2.1 CCV - 2.0 mg/L standard: 400uL of 50ppm Intermediate into 10mL of digested digestion reagent.

8.5.2.2 ICV - 1.0 mg/L standard: 200 uL of 50ppm Intermediate into 10mL of digested digestion reagent.

8.5.2.3 RL Check - 0.1 mg/L standard: 0.5 mL of 2ppm CCV into 10mL of digested digestion reagent.

8.6 Stock sodium hydroxide (10% w/v)

8.6.1 Sodium hydroxide (NaOH) - 50 g

8.6.2 Dilute to 500 mL with DI water.

8.6.3 Dissolve 50 g sodium hydroxide pellets in 400 mL ammonia-free deionized water. Caution, the flask will become warm! Cool and dilute to 500 mL.

8.6.4 Store at room temperature for up to six months.

8.7 Mercury (II) sulfate solution

8.7.1 Red mercury (II) oxide (HgO) - 8 g

8.7.2 20% (v/v) sulfuric acid (see below) - 50 mL

8.7.3 Dilute to 100 mL with DI water.

8.7.4 Prepare 20% (v/v) sulfuric acid by carefully adding 20 mL concentrated sulfuric acid to about 70 mL deionized water. Caution, the container will become hot! Swirl, cool to room temperature and dilute to 100 mL.

8.7.5 Dissolve 8 g red mercury (II) oxide (HgO) in 50 mL 20% (v/v) sulfuric acid and dilute to 100 mL with deionized water. Filter this reagent.

8.7.6 Store at 4°C for up to three months.

8.8 Digestion reagent

8.8.1 Potassium sulfate (K2SO4) - 133 g

8.8.2 Concentrated sulfuric acid (H2SO4) - 200 mL

8.8.3 Mercury (II) sulfate solution (above) - 25 mL

8.8.4 Dilute to 1 L with DI water.

8.8.5 Add 133 g potassium sulfate (K2SO4) to about 700 mL of deionized water and carefully add 200 mL concentrated sulfuric acid (H2SO4). Caution, the container will become hot! Add 25 mL filtered mercuric sulfate solution and dilute to 1 Liter.

8.8.6 Store at room temperature for up to six months.

8.9 Alkaline EDTA

8.9.1 Ethylenediaminetetraacetic acid, disodium salt dihydrate - 2.5 g

8.9.2 Sodium hydroxide (NaOH) - 10.0 g

8.9.3 Dilute to 500 mL with DI water.

8.9.4 To a 500 mL volumetric flask, add 2.5 g disodium EDTA dehydrate (ethylenediaminetetraacetic acid, disodium salt dihydrate) and 10.0 g sodium hydroxide pellets (NaOH). Stir to dissolve. Dilute to the mark with ammonia-free deionized water.

8.9.5 Store at room temperature for up to six months.

8.10 Stock sodium potassium tartrate solution (10% w/v)
- 8.10.1 Sodium potassium tartrate - 50 g
- 8.10.2 Dilute to 500 mL with DI water.
- 8.10.3 To prepare this reagent, dissolve 50 g sodium potassium tartrate in 400 mL ammonia-free deionized water and dilute to about 500 mL. Add 3 to 5 pellets of sodium hydroxide. Boil the solution, with stirring, for at least 1 hour to drive off ammonia contamination. Add make-up water if needed. Cover the flask and cool to room temperature. Add acid dropwise (sulfuric or hydrochloric, about 5 normal) to reach a pH of 7.5 ± 0.4. Dilute to 500 mL.
- 8.10.4 Store in the refrigerator up to six months.

8.11 Stock buffer solution
- 8.11.1 Sodium phosphate, dibasic anhydrous (Na2HPO4) -- 67 g
- 8.11.2 Sodium hydroxide - 10 g
- 8.11.3 Dilute to 500 mL with DI water.
- 8.11.4 Dissolve 67 g sodium phosphate, dibasic anhydrous (Na2HPO4) or 127 g sodium phosphate, dibasic heptahydrate (Na2HPO4•7H2O) in about 350 mL ammonia-free water. Add 10.0 g sodium hydroxide and stir to dissolve. Dilute to 500 mL.
- 8.11.5 Store at room temperature for up to six months.

8.12 Working buffer solution
- 8.12.1 10% Sodium potassium tartrate solution (above) – 240 mL
- 8.12.2 Stock buffer solution (above) - 100 mL
- 8.12.3 10% Sodium hydroxide stock solution (above) – 160 mL
- 8.12.4 Dilute to 500 mL with DI water.
- 8.12.5 Combine reagents in the stated order; add 100 mL stock buffer solution to 240‡ or 250 mL 10% sodium potassium tartrate solution and mix. Add 10% sodium hydroxide stock solutionl. Dilute to 500 mL and invert to mix.
- 8.12.6 Store at room temperature for up to six months.

8.13 Alkaline sodium salicylate stock solution
- 8.13.1 Sodium salicylate, anhydrous - 75 g
- 8.13.2 Sodium hydroxide - 10 g
- 8.13.3 Dilute to 500 mL with DI water.
- 8.13.4 Add 10 g sodium hydroxide pellets to about 400 mL ammonia-free water and stir to dissolve. Add 75 g sodium salicylate and stir to dissolve. Dilute to 500 mL.
- 8.13.5 Store this solution in an opaque bottle. Discard if the solution darkens significantly.

8.14 Sodium hypochlorite reagent (6.15% NaOCl (w/v)) Important: The bleach should not contain any additives other than NaOCl.
- 8.14.1 Refrigerate this reagent. Replace this solution every 4 months.
- 8.14.2 Dilute 10 mL, respectively, of purchased bleach to 50 mL with D.I.
- 8.14.3 Prepare fresh daily.

8.15 Stock sodium nitroprusside solution (30 g/L)
- 8.15.1 Sodium nitroprusside - 3 g
- 8.15.2 Dilute to 100 mL with DI water.
- 8.15.3 Dissolve 3 g sodium nitroprusside in 100 mL ammonia-free water.
- 8.15.4 Store this solution in an opaque bottle in the refrigerator. Discard at 6 months or if a blue-green tint is seen.

8.16 Working salicylate/nitroprusside solution
- 8.16.1 Stock sodium nitroprusside solution (above) 0.25 mL
- 8.16.2 Stock sodium salicylate solution (above) 40 mL
- 8.16.3 Add 0.25 mL stock sodium nitroprusside solution and 40 mL stock sodium salicylate solution. Install the pierced cap and mix gently yet thoroughly over a sink.
- 8.16.4 Replace this reagent wedge monthly.

Note: The above reagent recipes are specified by the instrument manufacturer (SEAL AQ2 Method No: EPA-125-A Rev 3).

9.0 Procedure:

9.1 Digestion:

- 9.1.1 Add 3-4 Hengar Granules (use once only)
- 9.1.2 Pipet 5mL of digestion reagent into each tube.
- 9.1.3 Pipet 20mL of aqueous sample into each block digestion tube or use a dilution as needed. The method blank will contain digestion reagent only.
- 9.1.4 Make sure tubes are dry on the outside surfaces. Place digestion tubes into the block.
- 9.1.5 Flip the stand by switch to On. The yellow light will appear. It will take about 20-30 minutes to reach 180^{o}C. When the red light goes on indicating it has reached temperature, press the green Start button. The green light will go on. Samples will heat for about one hour evaporating the water.
- 9.1.6 When the buzzer sounds, press the green start button again and add the cold fingers.
- 9.1.7 After 1½ hours at 380^{o}, the buzzer will sound and the block will cool down to 180^{o}C.
- 9.1.8 Remove fingers and digestion tubes.
- 9.1.9 After a few minutes, while samples are still warm, add 19mL of Nanopure to each sample and spin with vortex to mix.
- 9.1.10 Allow at least 2 hours for the turbidity to settle.
- 9.1.11 Pour into cuvettes, cool, and analyze on the SEAL.
- 9.1.12 See pages 17 – 20 in BD-46 User Manual for more details of Block Digestion procedure.

9.2 Solid Samples:

- 9.2.1 Weigh up 0.1g of solid sample into digestion vessel.
- 9.2.2 LCS 1ppm: 800 uL of 100ppm LCS Stock (section 8.2.1)
  MS 0.5ppm: 2mL of 100ppm LCS Stock (The digest is usually diluted 5 times prior to analysis.)

9.2.3 Add 20mL of digestion reagent.
9.2.4 Continue digest starting in section 9.1.4.
9.2.5 After digestion, while samples are still warm, bring up to 80mL with Nanopure and spin with vortex to mix.
9.2.6 Pour into cuvettes, cool, and analyze on the SEAL.

9.3 Leaf Tissue (N):

9.3.1 Wash leaves with D.I. water.
9.3.2 Dry in feed oven overnight.
9.3.3 Grind leaves into a powder.
9.3.4 Digest sample according to section 9.2.
9.3.5 Report result as %N.

9.4 Analysis:

9.4.1 See SEAL SOP I41 for general operating instructions.
9.4.2 Load standard, reagents and samples.
9.4.3 Choose method: TKN.
9.4.4 Type in sample table.
9.4.5 After system has stabilized and the heater temperature has reached at least 60^{o} C, calibration may begin.

9.5 Calibration

9.5.1 Calibrate the instrument every run or whenever the calibration check indicates a need. A 3 point curve including zero is required by method. Normally the laboratory performs a 5-point curve, plus a blank. An acceptable curve yields an r = 0.995 or better.
9.5.2 The Linear Calibration Range is proven *with each calibration* since 5 points are used and the curve is linear. Results are not reported over the highest calibrator, see 11.3.
9.5.3 Calibration Check Standards: Prepare standards at 1.0ppm and 2.0ppm (section 8.5) to check the calibration. Analyze prior to sample analysis every 10 samples and at the end of the run to prove that the existing calibration is still valid. Results must be within 90 – 110% recovery.
9.5.4 A calibration Blank is analyzed after calibration, every 10 samples and at the end. Results must be less than the reporting limit.
9.5.5 A standard at the reporting limit is analyzed at the end of the run. This is used to evaluate instrument sensitivity should calibration checks show a low bias and to validate samples reported as none detected. This standard should have a signal greater than the method blank.

10.0 Quality Control:

Note: See also ESB SOP Q01 for general QC requirements

10.1 Matrix Spikes -- Matrix Spikes and Matrix Spike Duplicates will be run with every analytical batch and at a minimum of once for every 20 samples per matrix type. This satisfies the 10% frequency requirement of the method. They will be compared to acceptance ranges *generated from historical data*. See LIMS for most current limits. If they do not fall within the acceptance range, the sample must be re-analyzed. If they still do not fall into the acceptance range, the data is flagged.

10.2 Laboratory Control -- A standard from a different source than the calibration standards shall be processed as a laboratory control with each analytical batch. One LCS will be run with every analytical batch and at a minimum of once for every 20 samples, per matrix type. This control will be compared to acceptance ranges of *80-120% recovery*. If the control does not fall within acceptance ranges, the analysis is considered to be out of control. No further samples shall be analyzed until the out of control condition is corrected.

10.3 Method Blank – A sample consisting of 5mL of digestion reagent is digested and analyzed as the method blank. One Method Blank will be run with every analytical batch and at a minimum of once for every 20 samples, per matrix type. It must read below but not more negative than the RL for batch acceptance. Samples results reported must be accompanied with a note if the method blank exceeds a concentration greater than 1/10 of the measured raw concentration of the sample, however blank results below the RL are considered to be ND and will not require a note.

Note: The blank value noted in the NBLK qualifier is the raw blank result. If the sample being qualified was diluted, in addition to NBLK, the analyst must also attach the qualifier NBLKd which includes the sample raw result.

10.4 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at the reporting limit or 2-3 times the estimated IDL and a minimum of seven replicates is analyzed. See QA Manual for calculation. Results must be below the reporting limit.

10.4.1 LOD Verification: On a yearly basis, a QC sample is spiked at a level of not more than 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL

10.5 Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QCS sample made at a concentration 5-50 times the MDL (or as of August 2006: 1-4 times the “Level of Quantitation” {RL} per 2003 NELAP Standards). The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 20.

10.6 Demonstration of Continuing Proficiency: On an annual basis, analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%.

10.7 Performance Evaluation Studies are performed *twice* a year. A QCS sample acquired from an independent source such as ERA is analyzed. *Acceptance criteria supplied by the manufacturer is used to access performance.*

11.0 Calculations:

11.1 The instrument compares peak *areas* of samples, rather than peak height, against the calibration curve. Raw results are transferred into LIMS.
- 11.1.1 Digestion volumes are entered in LIMS as the initial and final volumes.
- 11.1.2 Dilutions of the digest are entered into the DIL column of LIMS.

11.2 If diluted samples read below 0.1 mg/L, re-analyze using more sample and diluting to a final volume of 20mL. Ideally diluted samples should fall in the middle or upper part of the calibration curve.

11.3 Do not report results higher than the highest calibrator. Digestate may be diluted in half with digested digestion reagent. If further sample dilution is necessary, re-digest a smaller aliquot.

11.4 Watch for possible carry over. Studies have shown that carry over may occur over a concentration of mg/L. Rerun samples at or above the reporting limit that following a sample at this concentration. Be mindful of carry over that may happen in the digestion block.

11.5 If a sample produces a negative peak that is more negative than the reporting limit, redigest sample at full strength and reanalyze. If the result is still negative, the sample is spiked at the reporting limit to demonstrate that matrix interference is not masking actual reportable Kjeldahl nitrogen in the sample. Recovery of the 0.1 mg/L spike should be at 50-150%. If recovery is <50%, dilute sample, re-spike and rerun. If problem persists, re-digest a smaller aliquot of sample.

11.6 Organic Nitrogen (mg/L) = Kjeldahl nitrogen (mg/L) – Ammonia nitrogen (mg/L)

11.7 Total Nitrogen (mg/L) = Kjeldahl nitrogen (mg/L) + Nitrate nitrogen (mg/L) + Nitrite nitrogen (mg/L).

11.8 Report all results to two significant figures.

12.0 Definitions: See SOP Q15 – SOP Definitions

13.0 Pollution Prevention and Waste Management:

13.1Waste Disposal

13.1.1 Instrument waste is placed in the "KjN waste" barrel.

13.1.2 Sample waste after digestion is placed in the "High Acid Concentration Waste" barrel.

13.2 See also SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S06 – Disposal of Chlorinated Solvents
SOP S07 – Pollution Prevention

14.0 Corrective Action For Out of Control Or Unacceptable Data:

See SOP Q06 – Corrective Action

15.0 Method Performance

Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts.

References:

Standard Methods for the Examination of Water and Wastewater APHA, AWWA, WPCF 18th Edition. 4500-O_{org}

Standard Methods for the Examination of Water and Wastewater APHA, AWWA, WPCF 20th Edition. 4500-O_{org}

SEAL AQ2 Method No: EPA-125-A Rev 3

EPA Method 351.2 (1993) Methods for the Chemical Analysis of Waters and Wastes.

Note: All *italicized items* are an indication of a variation from the method.

Approved by: Susann K. Thomas Date: 09/18/06

Standard Operating Procedure
Edward S. Babcock & Sons
SM 4500-NH_3 H
Nitrogen, Ammonia (Colorimetric, Automated Phenate)
Effective Date: 09/22/06

1.0 Scope and Application

1.1 This method covers the determination of ammonia in drinking, surface, and saline waters, domestic and industrial wastes in the range of 0.1 to 2.0 mg/L NH3 as N. This range is for photometric measurements made at 660nm in a 10 mm tubular cell. Higher concentrations can be determined by sample dilution. This method may also be used for solid samples if both client and regulator agree to this variation.

1.2 Environmental Relevance: Ammonia is present naturally in surface and wastewaters. Its concentration is generally low in groundwaters because it adsorbs to soil particles and clays and is not leached readily from soils. It is produced largely by deamination of organic nitrogen containing compounds and by hydrolysis of urea. At some treatment plants ammonia is added to react with chlorine to form combined chlorine residual. In the chlorination of wastewater effluents containing ammonia, virtually no free residual chlorine is obtained until ammonia has been oxidized. The chlorine reacts with ammonia to form mono and dichloramines.

2.0 Summary of Method

2.1 Samples are distilled (if under NPDES program) or filtered and analyzed on an automated colorimetric system. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.

3.0 Sample Handling and Preservation

3.1 Preservation by addition of conc. H_2SO_4 to a pH < 2 and refrigeration at 4-C.

3.2 Samples must be analyzed within 28 days.

4.0 Interferences

4.1 Calcium and magnesium ions, may be present in concentrations sufficient to cause precipitation problems during analysis. A 7.5% EDTA solution is used to prevent the precipitation of calcium and magnesium ions from river water and industrial waste. For sea water a sodium potassium tartrate solution may be used. (See SM 4500-NH_3 H, 3e. for recipe.)

4.2 Sample turbidity and color may interfere with this method. Turbidity must be removed by filtration prior to analysis. Sample color that absorbs in the photometric range used will also interfere. If necessary, sample is diluted.

4.3 Urea and cyanates will hydrolyze on distillation at pH of 9.5.

4.4 Dechlorination is not necessary since the colorimeteric analysis will detect chloramines formation.

4.4 Marked variation in acidity and alkalinity are eliminated by sample preservation with H_2SO_4. The pH is then checked to ensure that it is <2. Due to the reducing nature of this environment, residual chlorine is not expected to be a problem. *The sample is neutralized prior to analysis by the addition of the first reagent that is a NaOH buffer.*

4.4 Distillation is required for all samples under the NPDES permit program. All other samples are filtered and may be distilled if the sample presents special matrix problems.

5.0 Apparatus

5.1 Test tube rack from SEAL.

5.2 13 x 100mm disposable culture tubes.

5.3 SEAL Analyzer

5.4 Whatman 2 and Whatman 4 (11.0cm) filter paper or Gelmin 0.45 micron disk filters.

5.5 100ml beakers.

5.6 1 ml, 2 ml, 5 ml, 10 ml and 50 ml pipets.

5.7 25 ml, 50 ml, and 100 ml graduated cylinders.

5.8 Distillation apparatus

5.9 VWR boiling chips 26397-409

5.10 Antifoaming Agent JT Baker B531-05

Note: Rinse all glassware prior to use with three portions of D.I. water. Immediately after use thoroughly rinse with three portions of D.I. water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 HCl, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

6.0 Reagents

6.1 Reagent Water: D.I. water

6.2 Diluant or preserved water: Add 1ml of Sulfuric acid to 1 L of D.I.. This solution is stored for up to 6 months at room temperature.

6.3 Sodium phenate: To a 100 ml beaker, add 2g of sodium hydroxide and about 10ml DI. Swirl to dissolve and cool to room temperature. In the fume hood, add 5g of crystalline phenol and swirl to dissolve. Transfer to a 50ml graduated cylinder and fill to the mark with DI water. Store in the refrigerator for three weeks or until the reagent becomes dark brown.

6.4 Sodium hypochlorite solution: bleach solution containing 5.25% NaOCl (such as "Clorox").

6.5 Buffer: Disodium ethylenediamine-tetraacetate (EDTA) (7.5%): Dissolve 18.75g of EDTA (disodium salt) and 0.5g of NaOH in 250ml of D.I. water. This solution is stored for up to 6 months at room temperature.

6.6 Sodium nitroferricyanide (0.2%): Dissolve 0.5g of sodium nitroferriocyanide in 250ml of D.I. water. This solution is stored for up to 6 months at room temperature.

6.7 1 N NaOH: Dissolve 40g of NaOH into 1L of D.I. water. This solution is stored for up to 6 months at room temperature.

6.8 0.04N Sulfuric Acid: Add 1.12mL of concentrated sulfuric acid into 1L of D.I. water. This solution is stored for up to 6 months at room temperature.

6.9 Borate Buffer:

6.9.1 0.1 N NaOH: Dissolve 4g of NaOH into 1L of D.I. water
6.9.2 Mix 0.95g $Na_2B_4O_7 \cdot 10H_2O$ into 100mL of Nnaopure water.
6.9.3 Add 8.8 mL of 0.1N NaOH and 50 mL of borate solution into a 100 mL flask and bring up to volume with D.I. water.
6.9.4 This solution is stored for up to 6 months at room temperature.

Note: The above reagent recipes are specified by the instrument manufacturer (SEAL Analytical AQ2 Method No: EPA 103-A Rev 4 © 6/01/05) and by Standard Methods 20th Edition 4500-NH_3.

7.0 Standards and Quality Control Requirements:

Note: See also ESB SOP Q01 for general QC requirements

7.1 Lab Control Sample (LCS):

7.1.1 Stock Solution: EM 1000 mg/L NH_3 Standard - stored at room temperature using manufacturer specified holding times. Once a stock standard is opened, the expiration date is one year as long as this date does not exceed the original manufacturer's date and percent recovery is acceptable.

7.1.2 LCS: Dilute 50 uL of stock standard (7.1.1) to 50 ml of diluant (6.2). The concentration is 1 mg/L NH_3 or 0.78 mg/L NH_3-N. This solution is stored at 4°C for up to 2 weeks.

7.1.3 A LCS is analyzed for every 20 samples per matrix type or one per analysis batch whichever is greater. LCSs are distilled unless the sample batch contains only undistilled samples.

7.1.4 If the LCS analysis does not fall within the acceptance range of 90-110%, the analysis is stopped until the cause is determined and the LCS is within the acceptance range.

7.2 Matrix Spike (MS) / Matrix Spike Duplicate (MSD)

7.2.1 Spike solution: Spike 50uL of stock standard (7.1.1) into 50 ml of sample. The spike concentration is 1 mg/L NH_3 or 0.78 mg/L NH_3-N.

7.2.2 A MS/MSD is analyzed for every twenty samples per matrix type or one per analysis batch whichever is greater. Spikes are distilled unless the sample batch contains only undistilled samples.

7.2.3 Acceptability: Acceptance ranges are 80-120% recovery with a maximum RPD of 20%.

7.3 Method Blank

7.3.1 Use diluant from section 6.2

7.3.2 A MB is analyzed for every twenty samples per matrix type or one per analysis batch whichever is greater and reported per matrix type. Blanks are distilled unless the sample batch contains only undistilled samples.

7.3.3 Acceptability: MB must read below but not more negative than the RL of 0.1mg/L for batch acceptance. Samples results reported must be accompanied with a note if the method blank exceeds a concentration greater than 1/10 of the measured raw concentration

of the sample however, blank results below the RL are considered to be ND and will not require a note.

Note: The blank value noted in the NBLK qualifier is the raw blank result. If the sample being qualified was diluted, in addition to NBLK, the analyst must also attach the qualifier NBLKd which includes the sample raw result..

Note: Since the intensity of the color used to quantify the concentration is pH dependent, the acid concentration of the diluant and the standard ammonia solutions should approximate that of the samples.

7.4 Calibration Standard:

7.4.1 Stock: Ammonium chloride (NH_4Cl)
7.4.2 Intermediate Standard 1000ppm:
7.4.2.1 Dehydrate Ammonium Chloride (NH_4Cl) in a 105°C oven.
7.4.2.2 Allow salt to cool in a dessicator. Weigh out 3.819 g NH4Cl.
7.4.2.3 Dilute to 1 liter with D.I. water in a volumetric flask containing 1 mL sulfuric acid.
7.4.2.4 Pour the solution into a 1 liter amber bottle. Keep at room temperature, out of sunlight. This standard may be kept for up to 1 year or replaced sooner if comparison with QC samples indicate a problem.
7.4.3 Intermediate standard (50ppm): Dilute 2.5 ml of 1000ppm stock standard into 50 ml of diluant. This solution is stored at 4^oC up to 28 days.
7.4.4 Dilute to make the following calibration standards:
7.4.4.1 2.0 mg/L standard: 2 mL of 50ppm Intermediate into 50 mL of diluant.
7.4.4.2 The instrument dilutes the 2ppm standard to the following concentrations: 0.02, 0.05, 0.1, 0.4, 0.8, and 1.6ppm.
7.4.4.3 Calibration Blank: diluant.

Note: These solutions are stored at 4^oC for up to two weeks.

7.5 ICV and CCV: A second stock source of NH_4Cl is purchased. An intermediate standard is prepared as specified in section 7.4.2 and section 7.4.3. Working standards are stored at 4^oC for up to two weeks. Solutions are prepared as follows:

7.5.1 1.5 mg/L standard: 1.5 mL of 50ppm Intermediate into 50 mL of diluant.
7.5.2 1.0 mg/L standard: 1 mL of 50ppm Intermediate into 50 mL of diluant.

7.6 A calibration blank is analyzed at the beginning of the run, every 10 samples and at the end of the run. Results must be less than but not more negative than the RL.

7.7 A standard at the reporting limit is analyzed at the end of the run. This is used to validate samples reported as none detected and to evaluate instrument

sensitivity should calibration checks show a low bias. This standard should have a signal greater then the method blank.

7.8 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at the reporting limit or 2.5-10 times the estimated MDL and a minimum of seven replicates is analyzed. See QA Manual for calculation. Results must be below the reporting limit.

7.8.1 On an annual basis perform the LOD verification check. Spike a QC sample of reagent water at a level of no more than 2-3 times analyte MDL. Analyte response must be greater than method blank response.

7.9 Demonstration of Capability: Prior to initial analysis of samples or when a significant change is made to the method, a Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration 5-50 times the MDL. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 10.

7.10 Demonstration of Continuing Proficiency: On an annual basis, analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed *twice* a year.

8.0 Procedure:

8.1 Sample Preparation

8.1.1 Distillation is required for all samples under the NPDES permit program. First check the distillation comparison study records. If a sample from the same site has been distilled in the past, and the RPD between the distilled and undistilled results is less than or equal to 20, then the sample does not need to be distilled. Raw results $\leq$1.0 mg/L will not generate useful RPD statistics. Instead these results must be within 0.1 mg/L of each other to eliminate the distillation step in the future. If a sample site is not in the study then it must be distilled and added to the study.

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8.1.1.1 If the apparatus sits idle for an extended period of time or if samples have left a residue clean the apparatus as follows:

8.1.1.1.1 Rinse all glassware with D.I.

8.1.1.1.2 Make clean out solution by adding 25 mL of borate buffer to 500 mL of D.I. Adjust pH to 9.5 with 6N NaOH solution. Fine tune with 1N NaOH.

8.1.1.1.3 Fill distillation flasks to the line with clean out solution. Add boiling chips.
8.1.1.1.4 Distill over at least 30 mL
8.1.1.1.5 Re-rinse all glassware.
8.1.1.2 Then add 50 mL of sample, LCS, MS, MSD and D.I. method blank to individual distillation flasks.
8.1.1.3 Add 2.5 mL of borate buffer to each flask.
8.1.1.4 Adjust to pH 9.5 with 1N or 6N NaOH.
8.1.1.5 Add several porous VWR boiling chips.
8.1.1.6 Distill at 190 ^{O}C into an Erlenmeyer flask containing 5 mL of 0.04N sulfuric acid. Ensure that the tip of the delivery tube is placed below the sulfuric acid level.
8.1.1.7 Collect 50ml of distillate.
8.1.1.8 Move the delivery tube out of the distillate; add extra water to the distillation flask to prevent dryness until the unit is turned off.
8.1.1.9 Turn off unit when the last sample is finished.
8.1.1.10 Pour distillate into SEAL tubes.

8.1.2 Undistilled samples
8.1.2.1 Filter all samples through 0.45 micron microdisk filters directly into SEAL test tubes unless a dilution is made. If a dilution is required, dilute sample, bring up to 50 mL, and filter prior to pouring into SEAL tube. If less than 5 ml of sample is used, dilute with diluant otherwise D.I. water may be used.
8.1.2.2 Use the following volumes based on sample matrix:
8.1.2.2.1 Industrial or Influent Wastewater – 2-5mL.
8.1.2.2.2 Effluent Wastewater – 25-50 mL.
8.1.2.2.3 Well water - 50 ml.
8.1.2.2.4 Solids – Make a 1:10 water extract, extract and swirl periodically for one hour. Filter prior to analysis.

8.2 Calibration:

8.2.1 The instrument is calibrated every run, when a major instrumentation change is made, or when the calibration check fails.
8.2.2 The analyst first analyzes a 1.0 mg/L standard. If the initial calibration check (ICV) is valid, within 90 – 110% of the expected value, a new calibration is not necessary.
8.2.3. If the ICV is out, calibrate the instrument. Although a 3-point curve is required, a 5-point curve plus blank is usually prepared. An acceptable curve yields an r = 0.995 or better.
8.2.4. Calibration Check Standard: Prepare a 1.0ppm standard to check the calibration. Analyze prior to sample analysis, every 10 sample,

and at the end of the run to prove that the existing calibration is still valid. Recovery must be 85 – 115%.

8.2.5. Calibration Check Blank: Analyze a calibration blank prior to sample analysis, every 10 sample, and at the end of the run to prove that the existing calibration is still valid. Results must be less but not more negative than the reporting limit.

8.2.6. A standard at the reporting limit is analyzed at the end of the run. This is used to evaluate instrument sensitivity should calibration checks show a low bias. This standard should have a signal greater than the method blank

8.3 Analyze samples on the SEAL.

8.4 Allow instrument to warm up

8.5 Load standard, reagents and samples.

8.6 See SEAL SOP I41 for general operating instructions.

8.7 Choose method and begin analysis.

8.8 When an acceptable calibration has been performed, submit the tray of samples.

8.9 If diluted samples read below 0.1 mg/L, re-analyze using more sample and diluting to a final volume of 50 ml. Ideally diluted samples should not fall in the lower quarter of the calibration curve.

8.10 If any sample reads above 2.0 mg/L, re-analyze using less sample.

8.11 Watch for possible carry over. Studies have shown that carry over may occur over a concentration of 50mg/L. Rerun samples at or above the reporting limit following a sample at this concentration. Be mindful of carry over that may happen in the distillation apparatus.

8.12 If a sample produces a negative peak that is more negative then the reporting limit, the sample pH is checked, adjusted to pH 2 if needed and reanalyzed.

9.0 Calculations

9.1 Compute concentration of samples by comparing sample peak areas rather than peak height with the standard curve.

9.2 Enter raw results into the LIMS. Preparation aliquots are entered as the initial and final volumes. Dilution factors performed after preparation are entered in the DIL column in LIMS.

9.3 The reporting limit is 0.1mg/L.

9.4 Report 2 significant figures.

9.5 Inorganic Nitrogen = $NH_3N + NO_3N + NO_2N$

10.0 Definitions: See SOP Q15 – SOP Definitions

11.0 Safety: The toxicity or carcinogenicity of chemicals used in this method has not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. A reference file of material data handling sheets is made available to all personnel involved in the chemical analysis.

See SOP S01 – Concentrated Acids and Bases
SOP S03 – Spill Control Policy

12.0 Corrective Action For Out of Control or Unacceptable Data:
See SOP Q06 – Corrective Action

13.0 Pollution Prevention and Waste Management:

13.1Waste Disposal
- 13.1.1 Instrument waste is placed in the "KjN waste" barrel.
- 13.1.2 Sample waste after distillation is placed in the "High Acid Concentration Waste" barrel.
- 13.1.3 Unused distillate or filtrate is placed in the "Low Acid Concentration Waste" barrel.

13.2 See also SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S06 – Disposal of Chlorinated Solvents
SOP S07 – Pollution Prevention

14.0 Method Performance
Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QC Office.

15.0 References

Standard Methods for the Examination of Water and Wastewater APHA, AWWA, WPCF 18th Edition 4500-NH_3 H

SEAL Analytical AQ2 Method No: EPA 103-A Rev 4 © 6/01/05

EPA Method 350.1. Methods for the Chemical Analysis of Waters and Wastes.

Note: All *italicized items* are an indication of a variation from the method.

Approved by: Susann K. Thomas Date: 09/18/06

METHOD #: 200.7
Determination Of Metals And Trace Elements In Water And Wastes By Inductively Coupled Plasma-Atomic Emission Spectrometry
Standard Operating Procedure of Edward S. Babcock & Sons
Effective Date: 09/22/06

1.0 SCOPE AND APPLICATION

Inductively coupled plasma-atomic emission spectrometry (ICP-AES) is used to determine metals and some nonmetals in solution. This method is a consolidation of existing methods for water, and wastewater. Our laboratory does not use this method for the determination of solid samples. This method is applicable to the following analytes:

Table 1

ANALYTE:	CAS #	MCL (ppb)
Aluminum	7429-90-5	1000
Antimony	7440-36-0	6
Arsenic	7440-38-2	50
Barium	7440-39-3	1000
Beryl1ium	7440-41-7	4
Boron*	7440-42-8	--
Cadmium	7440-43-9	5
Calcium	7440-70-2	--
Chromium	7440-47-3	50
Cobalt	7440-48-4	--
Copper	7440-50-8	1000
Iron	7439-89-6	300
Lead	7439-92-1	15
(Lithium)	7439-93-2	--
Magnesium	7439-95-4	--
Manganese	7439-96-5	50
(Mercury)	7439-97-6	2
Molybdenum	7439-98-7	--
Nickel	7440 02-0	100
Potassium	7440-09-7	--
Selenium	7782-49-2	50
Silica (a)	7631-86-9	--
Silver	7440-22-4	100
Sodium	7440-23-5	--
(Strontium)	7440-24-6	--
Thallium	7440-28-0	2
Tin	7440-31-5	--
(Titanium)	7440-32-6	--
Vanadium	7440-62-2	--
Zinc	7440-66-6	5000

Note: Analytes in parenthesis are not certified by NELAP.
* ELAP Certified

1.1 INSTRUMENTATION: Perkin Elmer Optima 5300DV

1.2 For reference where this method is approved for use in compliance monitoring programs [e.g., Clean Water Act (NPDES) or Safe Drinking Water Act (SDWA)] consult both the appropriate sections of the Code of Federal Regulation (40 CFR Part 136 Table 1B for NPDES, and Part 141 141.23 for drinking water), and the latest Federal Register announcements.

1.3 ICP-AES can be used to determine dissolved analytes in aqueous samples after suitable filtration and acid preservation. To reduce potential interferences, dissolved solids should be < 2000 mg/L (Sect. 4.2).

1.4 Samples may be analyzed directly by pneumatic nebulization without acid digestion if the sample has been properly preserved with acid and has turbidity of < 1 NTU at the time of analysis. This total recoverable determination procedure is referred to as "direct analysis". This method is not sensitive enough to determine some drinking water analytes without preconcentration. The preferred method for those analytes is ICP-MS (SOP M12).

1.5 For the determination of total recoverable analytes in aqueous samples a digestion is required prior to analysis when the elements are not in solution (e.g., aqueous samples that may contain particulate and suspended solids). See SOP M02 for digestion procedures.

1.6 When determining boron and silica in aqueous samples, only plastic, PTFE or quartz labware *should be* used from time of sample collection to completion of analysis. Borosilicate glass is avoided to prevent contamination of these analytes.

1.7 Detection limits and linear ranges for the elements will vary with the wavelength selected, the spectrometer, and the matrices. Table 2 provides estimated instrument detection limits for the listed wavelengths. However, actual method detection limits and linear working ranges will be dependent on the sample matrix, instrumentation, and selected operating conditions.

1.8 Data-quality objectives are understood by the analyst prior to analysis. ESB has on file documentation of required initial demonstration performance data.

1.9 Working range of analytes: Reporting limit to 90% of Linear Dynamic Range Maximum.

2.0 SUMMARY OF METHOD

2.1 An aliquot of a well mixed, homogeneous aqueous sample is accurately weighed or measured for sample processing. See SOP M02 for information on digestion methods. For the determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is < 1 NTU, the sample is made ready for analysis by the appropriate addition of nitric acid and hydrochloric acid.

2.2 The analysis described in this method involves multielemental determinations by ICP-AES. The instrument measures characteristic atomic-line emission

spectra by optical spectrometry. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element specific emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the line spectra are monitored at specific wavelengths by a photosensitive device. Photocurrents from the photosensitive device are processed and controlled by a computer system. A background correction technique compensates for variable background contribution to the determination of the analytes. Background is measured adjacent to the analyte wavelength during analysis. Interferences are more completely discussed in section 4.0.

2.3 The plasma converts metals in the sample from complex molecules to their atomic form and provides energy to excite them. Each metal will absorb energy at a specific wavelength. Eventually the metal will return to its ground state by emitting the energy it absorbed. The ICP uses this emission of energy to quantify each metal. Each metal emits light at a different wavelength. This is called polychromatic. These wavelengths are separated by a monochromater and detected by a photomultiplier tube.

3.0 DEFINITIONS

3.1 See SOP Q15 – SOP Definitions

4.0 INTERFERENCES

4.1 Spectral interferences are caused by background emission from continuous or recombination phenomena, stray light from the line emission of high concentration elements, overlap of a spectral line from another element, or unresolved overlap of molecular band spectra.

4.1.1 Background emission and stray light are usually compensated for by subtracting the background emission determined by measurement(s) adjacent to the analyte wavelength peak. Spectral scans of samples or single element solutions in the analyte regions indicate not only when alternate wavelengths are desirable because of severe spectral interference, but also show whether the most appropriate estimate of the background emission is provided by an interpolation from measurements on both sides of the wavelength peak or by the measured emission on one side or the other. The location(s) selected for the measurement of background intensity are determined by the complexity of the spectrum adjacent to the wavelength peak. The selected location(s)used for routine measurement are free of off-line spectral interference (interelement or molecular) or adequately corrected to reflect the same change in background intensity as occurs at the wavelength peak.

4.1.2 Spectral overlaps may be avoided by using an alternate wavelength or can be compensated for by equations that correct for interelement contributions, which involves measuring the

interfering elements. On-line spectral interferences observed for the recommended wavelengths are given in Table 3. When operative and uncorrected, these interferences will produce false-positive determinations and be reported as analyte concentrations. The interferences listed are only those that occur between method analytes.

4.1.3 When determining trace analytes, the analyst is aware of potential spectral interferences and makes correction where necessary by calculating the interference factor and utilizing a computerized correction routine or utilizing another wavelength.

4.1.4 The interference effects are evaluated for each individual instrument whether configured as a sequential or simultaneous instrument. Interferences are specific to the instrument and operating conditions. The analyst is required to determine and document for each wavelength the effect from the known interferences given in Table 2 and to check for the presence of interferents in samples and utilizing a computerized correction routine. To determine the appropriate location for off-line background correction, the analyst scans the area on either side adjacent to the wavelength and records the apparent emission intensity from all other method analytes. This spectral information is documented and kept on file. The location selected for background correction is free of off-line interelement spectral interference. If a wavelength other than the recommended wavelength is used, the analyst determines and documents both the on-line and off-line spectral interference effect from all method analytes and provide for their automatic correction on all analyses. Tests to determine the spectral interference are done using analyte concentrations that will adequately describe the interference.

4.2 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they are reduced by using a peristaltic pump and an internal standard. Additionally the analyst may utilize such means as diluting the sample if the recovery on the internal standard is low. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. This problem is controlled by diluting the sample when necessary. Also, it has been reported that better control of the argon flow rates, especially for the nebulizer, improves instrument stability and precision; this is accomplished with the use of mass flow controllers.

4.3 Chemical interferences include molecular-compound formation, ionization effects, and solute-vaporization effects. Normally, these effects are not significant with the ICP-AES technique. If observed, they can be minimized by careful selection of operating conditions (such as incident power and

observation height), by buffering of the sample, by matrix matching, and by standard-addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.4 Memory interferences are controlled by the use of a rinse blank between analyses. The instrument automatically increases the rinse time following a high sample. If a memory interference is still suspected, the sample is re-analyzed after the system is free of contamination.

4.4.1 Necessary rinse times for a particular element are estimated. This is achieved by aspirating a standard containing elements at a concentration near the top of the linear dynamic range. The aspiration time for this sample should be the same as a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to *less than one half the reporting limit* is applied. If one half the reporting limit is less than the MDL, signals must be less than the MDL.

4.4.2 When there is suspected additional memory interference between samples, the rinse cycle is repeated and the sample is reanalyzed.

5.0 SAFETY

The toxicity or carcinogenicity of chemicals used in this method has not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. A reference file of material data handling sheets is made available to all personnel involved in the chemical analysis.

See SOP S01 – Concentrated Acids and Bases
SOP S02 – Compressed Gas Cylinder Handling
SOP S03 – Spill Control Policy

General laboratory safety procedures are sufficient for this analysis.
Recommended safety equipment includes gloves and safety glasses

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled plasma emission spectrometer: Perkin Elmer Optima 5300DV
Computer-controlled emission spectrometer with background- correction capability.

6.1.1 Radio-frequency generator compliant with FCC regulations per PE manual.

6.1.2 Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders.

6.1.3 A variable speed peristaltic pump is required to deliver both standard and sample solutions to the nebulizer.

6.1.4 (optional) Mass flow controllers to regulate the argon flow rates, especially the aerosol transport gas, are highly recommended. Their use will provide more exacting control of reproducible plasma conditions.

6.2 Autopipetters

6.3 Labware - A clean laboratory work area designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by contributing contaminants through surface desorption or leaching, depleting element concentrations through adsorption processes. All reusable labware (glass, quartz, polyethylene, PTFE, FEP, etc.) is sufficiently cleaned for the task objectives. Plastic volumetric flasks are rinsed with 1:1 nitirc and Nanopure water. See preparation SOP for washing instructions of digestion glassware.

6.3.1 Glassware - Volumetric flasks, graduated cylinders, funnels and centrifuge tubes (metal-free plastic).

6.3.2 Assorted calibrated autopipettes.

6.3.3 Microwave cylinders with Teflon sleeves

6.3.4 15 dram snap cap vials.

6.3.5 Plastic autosampler tubes.

6.3.6 One-piece stem FEP wash bottle with screw closure, 125-mL capacity.

6.4 Plastic sample bottles: New bottles are used to store samples. Each new lot of bottles is tested for trace contaminants by analysis of an acid blank placed in one sample bottle from that lot. Results must be below the MDL.

7.0 REAGENTS AND STANDARDS

7.1 Reagents may contain elemental impurities that might affect analytical data. Only high-purity reagents that conform to the American Chemical Society specifications 13 should be used whenever possible. If the purity of a reagent is in question, analyze for contamination. All acids used for this method must be purchased from the manufacturer instra analyzed (spectro grade).

7.2 Hydrochloric acid, concentrated (sp.gr. 1.19) - HCl.

7.3 Nitric acid, concentrated (sp.gr. 1.41) - HNO3.

7.4 Reagent water. All references to water in this method refer to ASTM Type I grade water. Our lab uses Nanopure

7.5 Standard Stock Solutions - Stock standards are purchased from a reputable supplier either separately or as mixed solutions. Two sources are acquired to verify instrument performance. Solutions are stored at room temperature. Manufacturer specified holding times are observed.

7.6 Working Standard Solutions – Solutions are made from above stock solutions in 5% HCl and 10 % HNO_3. Solutions are stored at room temperature for up

to six months. Solutions will be replaced sooner if comparison with QC samples indicate a problem. See standard log for recipes.

Note: *If the other QC standards indicate a problem with a mixed standard, each stock solution should be analyzed separately to determine the problem.* There can be slight high bias of Fe, Al, or other metals in mixed standards since stock sources of some metals contain trace amounts of other metals. If this bias causes QC samples to be out of range, each stock standard will be tested for the bias and this bias will be added to the true value of the metal in the standards

7.7 Blanks - Four types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure, the laboratory fortified blank is used to assess routine laboratory performance and a rinse blank is used to flush the instrument uptake system and nebulizer between standards, check solutions, and samples to reduce memory interferences.

7.7.1 The calibration blank for aqueous samples and extracts is prepared by acidifying reagent water to the same concentrations of the acids as used for the standards. The calibration blank is stored in a contaminant free plastic bottle.

7.7.2 The laboratory reagent blank (LRB) (method blank (MB)) must contain all the reagents in the same volumes as used in the processing of the samples. The LRB is carried through the same entire preparation scheme as the samples including sample digestion, when applicable.

7.7.3 The laboratory fortified blank (LFB) [Laboratory Control Sample (LCS) or Blank Spike (BS)] is prepared by fortifying an aliquot of the laboratory reagent blank with all analytes to a suitable concentration (approximately 100 times their respective MDL). 200 uL of an Intermediate standard is spiked into 5 mL of Nanopure water. See batch standard log for individual metal concentrations. The LFB must be carried through the same entire preparation scheme as the samples including sample digestion, when applicable.

7.7.4 The rinse blank is prepared by acidifying reagent water to 5% nitric acid and 2.5% hydrochloric acid.

7.8 Instrument Performance Check (IPC/CCV) Solution - The IPC is a mixed standard obtained from a reputable vendor. . This standard is the same source as the calibration stock standards. The IPC solution is stored in a plastic bottle and analyzed as needed to meet data quality needs (Section 9.5.5). See standard log for recipe.

7.9 Quality Control Sample (QCS) – The QCS is a noncalibration source mixed standard containing all certified metals. This standard is used to initially prove method capability (section 9.2.3), demonstrate continuing acceptable instrument performance semiannually via the Performance Testing program

(section 9.4) and to verify calibration standards after new standard preparation (section 9.5.6). See standard log for recipe.

7.10 Initial Calibration Verification (ICV) – The ICV is a mixed standard obtained from a reputable vendor. This is a noncalibration source standard. The ICV solution is stored in a plastic bottle and analyzed as needed to meet data quality needs. See standard log for recipe.

7.11 Spectral Interference Check (SIC) Solution and Blank - When interelement corrections are applied, SIC solutions are needed containing concentrations of the interfering elements at levels that will provide an adequate test of the correction factors. See standard log for preparation information.

7.12 Intensity Check (Plasma Solution) - The plasma solution is used for determining the optimum viewing height of the plasma above the work coil prior to using the method. The plasma solution (tuning solution) utilized on the Optima is 10 mg/L Manganese per manufacturers instruction PE CH 8. The instrument is subjected prior to analysis to the autotune procedure provided with the software.

7.13 Internal Standard: A Lanthanum solution is added to all standards and samples. See standard log for recipe.

7.14A high check is performed with each run to prove linearity. Results must be within 90-110% recovery to accept sample with elevated results.

8.0 SAMPLE COLLECTION, PRESERVATION. AND STORAGE

8.1 Prior to the collection of an aqueous sample, consideration should be given to the type of data required, (i.e., dissolved or total recoverable), so that appropriate preservation and pretreatment steps can be taken. If properly acid preserved, the sample can be held up to 6 months before analysis.

8.2 For the determination of the dissolved elements, the sample must be filtered through a 0.45-um pore diameter membrane filter at the time of collection or as soon thereafter as practically possible. (Glass or plastic filtering apparatus are recommended to avoid possible contamination. If the filtered blank shows a problem with the glass apparatus, only plastic apparatus should be used when the determinations of boron and silica are critical. Use a portion of the filtered sample to rinse the filter flask, discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) nitric acid immediately following filtration to pH < 2.

8.3 For the determination of total recoverable elements in aqueous samples, samples are not filtered, but acidified with (1+1) nitric acid to pH < 2 (normally, 3 mL of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples). Preservation may be done at the time of collection, however, to avoid the hazards of strong acids in the field, transport restrictions, and possible contamination it is recommended that the samples be returned to the laboratory within two weeks of collection and acid preserved upon receipt in the laboratory. The pH of all preserved samples is *verified upon receipt by the laboratory*, and if necessary, additional acid is

added to bring the pH to <2. If the sample is reactive upon addition of acid, the splitter lets the reaction finish and then rechecks the pH.

8.4 A field blank is prepared and analyzed if required by the data user. Use the same container and acid as used in sample collection.

8.5 Samples containing turbidity < 1 NTU will be determined by "direct analysis". Screen acidified samples by testing an aliquot in a turbidimeter meter. Results are recorded in the logbook. Samples containing turbidity >1 NTU must be digested prior to analysis.

8.6 If analysis begins <16 hours after sample preservation and pH verification a qualifier will accompany the data.

9.0 QUALITY CONTROL

Note: See also ESB SOP Q01 for general QC requirements

9.1 Edward S. Babcock & Sons operates a formal quality control (QC) program. The requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks (LCS's) and other laboratory solutions as a continuing check on performance. The laboratory maintains performance records that define the quality of the data thus generated.

9.2 Demonstration of Method Capability.

9.2.1 The Demonstration of Method Capability is used to characterize instrument performance (analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to analyses of this method conducted by this laboratory.

9.2.2 Linear dynamic range (LDR) –The upper limit of the linear dynamic range must be established. The LDR should be determined by analyzing succeedingly higher standard concentrations of the analyte until the observed value is no more than 10% below the stated concentration of the standard. Analyte concentrations above 90% of the determined upper range are diluted. For those analyses that are known interferences, and are present at above the linear range, the analyst ensures that the interelement correction has been applied correctly. New dynamic ranges should be determined annually or whenever there is a significant change in instrument response.

9.2.3 Quality control sample (QCS) - When beginning the use of this method, verify calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. The determined mean concentrations from 3 analyses of the QCS must be within +/- 5% of the stated values. If the calibration standard cannot be verified, performance of the determinative step of the method is unacceptable. The source of the problem must be

identified and corrected before either proceeding on with the initial determination of method detection limits.

9.2.4 Method detection limit (MDL) – MDL's are established whenever there is a change in instrumentation, or a major modification to the analysis. MDL's must be established for all wavelengths utilized, using reagent water (blank) fortified at a concentration of approximately 2-5 times the estimated detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

MDL = (t) x (S)

where:
t = students' t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].
S = standard deviation of the replicate analyses.
See the attached LIMS information for the current MDL study results.

9.3 Analyst Demonstration of Capability

9.3.1 Initial Demonstration: Prior to initial analysis of samples or when a significant change is made to the method, a Demonstration of Capability Study is performed by each analyst. This is accomplished by analysis of four replicates of a QC sample made at a concentration of 1-4 times the RL. Concentrations of cation metals may be higher. Past studies have been performed at 50-2000ppb. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 20.

9.3.2 Demonstration of Continuing Proficiency: On an annual basis, each analyst must turn in valid LCS data from four consecutive batches or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%.

9.4 Performance Evaluation Studies are performed *twice* a year. This is accomplished by obtaining a QCS sample from an outside source and evaluating it's performance based on study acceptance criteria.

9.5 Assessing Laboratory Performance (mandatory)

9.5.1 Laboratory reagent blank (LRB) or Method Blank (MB)- The laboratory must analyze at least one LRB with each batch of 20 or

fewer samples of the same matrix. LRB data are used to assess contamination from the laboratory environment. For batch acceptance, LRB values must be < ½ the reporting limit. If one half the reporting limit is less than the MDL, LRB values must be < the MDL for those analytes. If the LRB is unacceptable, fresh aliquots of the samples must be prepared and analyzed again for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained. Sample results at or above the reporting limit must be accompanied with a qualifier if the method blank exceeds a concentration greater than 1/10 the measured raw concentration of the sample. Blank results below the ½ the reporting limit (or below the MDL) are considered to be ND and will not require a note.

9.5.2 Laboratory fortified blank (LFB) or Blank Spike (BS)- The laboratory analyzes at least one LFB with each batch of samples per matrix type. This check is made from a noncalibration source. Calculate accuracy as percent recovery using the following equation:

$$R = \frac{LFB - LRB}{s} \times 100$$

where:
R = percent recovery.
LFB = laboratory fortified blank.
LRB = laboratory reagent blank.
s = concentration equivalent of analyte added to fortify the LBR solution.

If the recovery of any analyte falls outside the required control limits of that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.5.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 85-115%. When sufficient internal performance data become available (usually a minimum of twenty to thirty analyses), optional control limits can be developed from the mean percent recovery (x) and the standard deviation (S) of the mean percent recovery. These data can be used to establish the upper and lower control limits as follows:

UPPER CONTROL LIMIT = x + 3S
LOWER CONTROL LIMIT = x - 3S

The optional control limits must be equal to or better than the required control limits of 85-115%.The standard deviation (S) data are used to establish an on-going precision statement for the level of concentrations included in the LFB.

9.5.4 Calibration Blank - A calibration blank is analyzed immediately following daily calibration, after every tenth sample and at the end of the sample run. *Analytes must be < ½ the reporting limit*. If one half the reporting limit is less than the MDL, calibration blank values must be < *the MDL* for those analytes.

9.5.5 Instrument performance check/ Continuing calibration verification (IPC/CCV) solution - The IPC(CCV) is a solution of metals from the same source as the calibration. The laboratory analyzes the IPC solution following daily calibration, after every tenth sample and at the end of the sample run. Analysis of the IPC solution immediately following calibration must verify that the instrument is within +/- 5% of calibration with a relative standard deviation < 3% from *3* replicate integrations. Subsequent analyses of the IPC solution must be within +/- 10% of calibration. If the calibration cannot be verified within the specified limits, reanalyze either or both the IPC solution and the calibration blank. If the second analysis of the IPC solution or the calibration blank confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined, corrected and/or the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data. Alternately data may be reprocessed using the most recent CCB as the calibrator. Reprocess all samples back thru the last working calibration check. An earlier ICV and CCB reprocessed under the new calibration must work to accept the calibration. All data must be bracketed by working QC. The decision to accept any data when a IPC is out must be clearly documented and made by the QA Officer.

9.5.6 Quality Control Sample (QCS) - The QCS is a solution of metals from a noncalibration source. After the preparation of new calibration standards, analyze three QCS standards. Tabulate the mean concentration of the three QCS analyses. It must be within 5% of the true value to verify the new standards.

9.5.7 Initial Calibration Verification (ICV) - The ICV is a solution of metals from a noncalibration source. The ICV is analyzed immediately following initial calibration. Apply the following acceptance criteria:

9.5.7.1 The ICV is used to verify initial calibration – acceptance criteria 10% recovery

9.5.7.2 If data must be reprocessed under a new calibration due to instrument drift later in the run, a later CCV may be used as the calibrator The ICV must be processed under the new calibration to verify it - acceptance criteria: 10% recover.

9.5.8 Spectral interference check (SIC) solution - The laboratory verifies the interelement spectral interference correction routine by

analyzing a SIC solution and blank with each run that contains analytes of interest needing correction. It is analyzed at the beginning, after every 20 samples, and at the end of the run. Target analytes in the SIC solution must be within ± 20% of expected value and less than ± the RL in the SIC blank to verify the correction routine. (If SIC blank is high, see section 9.5.1 for qualifying criteria.) All interelement spectral correction factors are updated if the SIC solution and SIC blank do not consistently meet acceptance criteria. To update correction factors separate standards of each metal are analyzed at a level of 100ppm (except Fe -300ppm and Al -200ppm). Target metals are examined for any false response caused by the elevated metal.

9.6 Assessing Analyte Recovery and Data Quality

9.6.1 Laboratory Fortified Matrix (LFM) or Matrix Spike (MS) - Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess the effect.

9.6.2 The laboratory adds a known amount of each analyte for every 20 routine samples per matrix type per batch. When combined with the MSD (see section 9.6.5, this constitutes a frequency of 10%). In each case the LFM aliquot is a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. The added analyte concentration is the same as that used in the laboratory fortified blank. See section 7.7.3 for recipe. Samples are chosen at random.

9.6.3 Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range of 70-130%. Recovery calculations are not required if the concentration added is less than *25%* of the sample background concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{C(s) - C}{s} \times 100$$

where:

R = percent recovery.
C(s) = fortified sample concentration.
C = sample background concentration.
s = concentration equivalent of analyte added to fortify the sample.

9.6.4 If the recovery of any analyte falls outside the designated LFM recovery range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.3), the recovery problem

encountered with the fortified sample is judged to be matrix related, not system related. The data user should be informed that the result for that analyte in the unfortified sample is suspect due to either the heterogeneous nature of the sample or matrix effects.

9.6.5 Laboratory Fortified Matrix Duplicates (LFMD) or Matrix Spike Duplicates (MSD) are analyzed every 20 samples per matrix type per sample batch. Relative Percent Difference must be $\leq$20%.

9.6.6 Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably. Reference materials containing high concentrations of analytes can provide additional information on the performance of the spectral interference correction routine.

9.7 Internal standards are used with all analyses. Directions for using internal standard(s) are given in Section 11.2.4.

9.8 A Detection Limit Check is analyzed with every calibration at the detection limit concentration. 50 – 150% of the expected value is considered acceptable.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Specific wavelengths are listed in Table 2. Other wavelengths may be substituted if they can provide the needed sensitivity and are corrected for spectral interference. Instrument Operating Conditions: The analyst follows the instructions provided by the instrument manufacturer unless other conditions provide similar or better performance for a task.

10.1.1 Prior to using this method optimize the plasma operating conditions using the 10 mg/L manganese solution and the autotune program provided with the software.

10.1.2 Calibrate the instrument per manufacturers instructions using a single point calibration.

10.2 Before using the procedure (Section 11.0) to analyze samples, there must be data available documenting initial demonstration of performance. The required data and procedure is described in Section 9.2. This data must be generated using the same instrument operating conditions and calibration routine to be used for sample analysis. These documented data must be kept on file and be available for review by the data user.

10.3 After completing the initial demonstration of performance, but before analyzing samples, the laboratory must establish and initially verify an interelement spectral interference correction routine to be used during sample analysis. A general description concerning spectral interference and the analytical requirements for background correction and for correction of interelement spectral interference in particular is given in Section 4.1. To determine the appropriate location for background correction and to establish the interelement interference correction routine, repeated spectral scan about the analyte wavelength and repeated analyses of the single element solutions

may be required. Criteria for determining an interelement spectral interference is an apparent positive or negative concentration on the analyte at the *MDL level*. Once established, the entire routine must be initially and periodically verified by successful analysis of the SIC blank and check solution with each run, or whenever there is a change in instrument operating conditions.

11.0 PROCEDURE

11.1 Aqueous Sample Preparation - Dissolved Analytes

11.1.1 Samples are received, from the splitter, filtered through a 0.45 um filter and preserved. The sample is then processed like the samples in paragraph 11.2. The final report indicates that the results are for dissolved analytes.

11.2 Preparation for "direct analysis" of total recoverable analytes in samples containing turbidity < 1 NTU – Place in autosampler tube:

11.2.1 5 mL of sample

11.2.2 0.5 mL conc HNO_3

11.2.3 *250 µL HCl*

11.2.4 50µL of 2000ppm La Internal Standard.

11.3 Total Recoverable Analytes – See SOP M02 for sample preparation.

11.4 Sample Analysis

11.4.1 When a problem is suspected, inspect the sample introduction system including the nebulizer, torch, injector tube and uptake tubing for salt deposits, dirt and debris that would restrict solution flow and affect instrument performance. Clean the system when needed or on a daily basis.

11.4.2 Configure the instrument system per manufacturer's instructions.

11.4.3 The instrument must be allowed to become thermally stable before calibration and analyses. This usually requires at least 30 to 60 minutes of operation. After instrument warm-up, complete any required optical profiling or alignment particular to the instrument.

11.4.4 For initial and daily operation calibrate the instrument according to the instrument manufacturer's recommended procedures, using mixed calibration standard solution and the calibration blank. A peristaltic pump is used to introduce all solutions to the nebulizer. To allow equilibrium to be reached in the plasma, aspirate all solutions for 30 sec after reaching the plasma before beginning integration of the background corrected signal to accumulate data. When possible, use the average value of replicate integration periods of the signal to be correlated to the analyte concentration. Flush the system for a total of 60 seconds with the rinse blank and then the next sample. The calibration line should consist of a minimum of a calibration blank and a high standard. Replicates of the blank and highest standard provide an optimal distribution of calibration standards to minimize the confidence band for a

straight-line calibration in a response region with uniform variance.

11.4.5 After completion of the initial requirements of this method, samples should be analyzed in the same operational manner used in the calibration routine with the rinse blank also being used between all sample solutions, LFBs, LFMs, and check solutions.

11.4.6 During the analysis of samples, the laboratory must comply with the required quality control described in Sections 9.5 and 9.6. Only for the determination of dissolved analytes or the "direct analysis" of drinking water with turbidity of < 1 NTU is the sample digestion step of the LRB, LFB, and LFM not required.

11.4.7 Any elements with a concentration above the verified linear range must be diluted. Also, for the interelement spectral interference correction routines to remain valid during sample analysis, the interferant concentration must not exceed its verified linear range. In these circumstances analyte detection limits are raised and determination by another approved test procedure that is either more sensitive and/or interference free is recommended.

11.4.8 Report data as directed in Section 12.

11.5 Our laboratory uses the internal standard technique (as an alternate to the method of standard addition) by adding one or more elements (not in the samples and verified not to cause an uncorrected interelement spectral interference) at the same concentration (which is sufficient for optimum precision) to the prepared samples (blanks and standards) that are affected the same as the analytes by the sample matrix. The ratio of analyte signal to the internal standard signal is used for calibration and quantitation. This technique is very useful in overcoming matrix interferences especially in high solids matrices. The analyst monitors internal standard response for large drops that might indicate improper spiking of La or sample matrix interference.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Sample data should be reported in units of ug/L for aqueous samples.

12.2 For dissolved aqueous analytes report the data generated directly from the instrument with allowance for sample dilution. Do not report analyte concentrations below the MDL.

12.3 For total recoverable aqueous analytes, most results are reported directly from the instrument readout (since normal digestion dilutions are accounted for in the software). Any additional dilutions prior to analysis are accounted for by entering the factors into the software. Apply correction factors for any additional dilution of the prepared sample solution to report samples within the verified linear range.

12.4 All data is sent to the LIMS. If an aliquot other than the default volume is used during preparation, this variation is documented in the LIMS initial volume. If a dilution is made at the instrument, the change in internal

standard response will automatically adjust the result prior to data transfer. To correctly report the MDL and RL, the transferred result is divided by the dilution factor and the dilution factor is placed in the LIMS dilution column.

12.5 The result labeled in the run as "Si" is actually a silica (SiO_2) result and will be entered as such. Silicon standards used to calibrate for silica are entered as silica to account for this conversion.

12.6 Total Hardness may be calculated from the calcium and magnesium results as follows (SM 2340 B):

$$\frac{\text{Ca mg/L}}{20} + \frac{\text{Mg mg/L}}{12} \times 50 = \text{Total Hardness in mg eqiv. } CaCO_3/L$$

12.7 Exporting and Entering Data: ICP-OPTIMA 5300DV

Element:
File
Utilities
Data manager
Choose result name
Report
Use existing design
Browse
La, open
Preview , l> print
Xclose
Cancel
Export
Use existing design
Browse
Data tool, open
Next next next Finish
Export data Xclose Xclose Xclose

Element:
Laboratory:
Data entry/review
Choose bench sheet
Select analytes
Create
Export double click
Yes
Xclose
Laboratory:
Data tool clear
Browse (left)

export, double click
Browse (right)
Clear
Select data (PE Winlab-final), double click
Auto select
Scan for typos
Done
Merge files
Change analyst
Save
export, double click
Yes
Xclose, Xclose

Laboratory:
Data entry/review
Open
export, double click
Save, ok
Select analytes
Max page
Query
Edit
Lock
Change analyst initials, fill cells

12.7.1. Delete calculations from QC and the source sample if not requested by the client i.e.: total hardness, total cations, sodium percentage,caco3, sar
Filtered blanks need to be qualified: Qbfilt
Add qualifier to source sample if the analyte is not requested: ?QC
Check for perfect zero - 0 could be saturated sample

12.7.2. Qualify:

12.7.2.1. Blank**:** A blank that does not meet the criteria must be qualified QBLK. A blank does not turn red if the result is too negative. The result may not be greater than +/- ½ the RL.

12.7.2.2. BS**:** A BS that is out of range may be qualified QLMS if the MS or MSD meet the BS criteria. If a BS is out of range and can not be qualified the analytes in question must be re-analyzed unless the results are ND and the BS is high. Qualify analytes NLOhi

12.7.2.3. MS, MSD:

12.7.2.3.1. If both spikes are red check prep dilution and source sample prep dilution,correct on bench sheet if necessary.

12.7.2.3.2. Check if source sample is greater than four times the BS value. If it is Qualify the MS, MSD: QM-4X

12.7.2.3.3. If only one spike is out and the RPD is less than twenty and if there are ten or less analytes you can make the red spike the MSD and use the qualifier: QMSDr

12.7.2.3.4. QFnt: If the source sample does not require the reporting of the analyte.

12.7.2.3.5. QOcal if result is over LDR.

12.7.2.4. Verify dilutions on prep sheet and in lims

12.7.2.5. Samples, diluted, that result in an answer of ND or J flag**:** "N_RLm"

12.7.2.6. Right click Sort by analyte order

12.7.2.6.1. Check ND results for any negatives greater than RL.

12.7.2.6.2. If a blank has been qualified, check for any analytes less than 10 times the blank result. This result may be passable if it is ND, if it is not it must be RE'd and Qualified SUS; or qualified NBLK. The result may be reported if it is 10x greater than the blank result. The re's should be addressed now in Project Management, update status. Edit comment , create bench sheets if necessary.

12.7.2.6.3. Check for results over the LDR, these need to be RE'd , entered as 9999 and qualified ?SUS NOcal

12.7.2.7. Sort by Sample ID.

12.7.2.7.1. Calculate any sodium percentage or SAR's

12.7.2.7.2. Highlight page, right click, custom status, update to peer review.

12.7.2.7.3. Save, Ok, Done

12.7.3. Fill out peer review sheet

13.0 METHOD PERFORMANCE

13.1 See EPA Method 200.7 for data validation information.

14.0POLLUTION PREVENTION and WASTE MANAGEMENT

See SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S06 – Disposal of Chlorinated Solvents
SOP S07 – Pollution Prevention

15.0 REFERENCE

15.1 U.S. Environmental Protection Agency. Inductively Coupled Plasma-Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes-Method 200.7, Supplement 1, Rev 4.4, May 1994

Note: All *italicized items* are an indication of a variation from the method.

Approved by Susann K. Thomas Date 09/18/06

Table 2
Estimated Detection Limit (ppb)

Metal	Wavelength	P.E. SM DL	SM Est. DL	EPA 200.7 Est. DL	EPA 6010 Est. IDL	ESB RL 2/25/03	ESB MDL 2/25/03
Ag	328.07	7	7	7.0	4.7	10	4
Al	396.15	28				100	31
As	188.98					100	42
B	208.89	12				100	10
Ba	233.53	4				20	2.2
Be	313.11	0.7	0.3	0.3	0.18	5	1.7
Ca	317.93	10	10		6.7	1000	50
Cd	228.8	2.7				5	3.6
Co	228.62	7	7	7.0	4.7	10	2.5
Cr	267.72	7.1	7	0.24		10	1.8
Cu	327.39	9.7				10	6.5
Fe	238.2	4.6				50	9.1
K	766.49		100	700		1000	130
Li	670.78		4	3.7	2.8	100	100
Mg	285.21	1.6	30			1000	40
Mn	257.61	1.4	2	1.4	0.93	10	3
Mo	202.03	7.9	8		5.3	10	1.8
Na	589.59	69	30			1000	50
Ni	231.6	15	15	15	10	10	2.4
Pb	220.35	42	40	42	28	20	15
Sb	206.84	32	30	32	21	100	34
Se	203.99	115				100	46
Tot Si	251.61	26		26		500	33
Sn	189.93			25		100	9.6
Sr	407.77	0.4	0.5		0.28	100	100
Ti	334.94	3.8		3.8	5	10	1.2
Tl	190.8		40	40	27	100	48
V	310.23	6.4				10	2.5
Zn	206.2	5.9				10	1.2

Run MDL at 2-5 times estimated MDL

Table 3

		Interfering Metals																
Analyte	Wave-length	Al	Ba	Be	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Mo	Ni	Ti	Tl	V	Zn
Ag	328.07								x				x				x	
Al	396.15				x	x	x			x		x	x			x	x	
As	188.98		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
B	249.72						x						x					
Ba	233.53												x				x	
Be	313.11							x										
Cd	228.8						x											
Ca	317.93																	
Cd	228.80																	
Co	228.62		x					x		x				x	x			
Cr	267.72									x		x	x				x	
Cu	327.39						x						x		x		x	
Fe	238.2						x				x		x					
K	766.49																	
Mg	285.21																	
Mn	257.61										x							
Mo	202.03											x						x
Na	589.59																	
Ni	231.6		x				x									x		
Pb	220.35	x		x	x		x		x	x		x	x		x			x
Sb	206.84						x	x					x	x			x	
Se	196.03	x			x		x	x		x		x	x				x	x
Tot Si	251.61			x						x			x	x	x			
Sn	189.93				x					x					x		x	
Sr	407.77				x													
Ti	334.94							x										
Tl	190.8											x	x					
V	310.23	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
Zn	206.2				x			x		x	x		x					

x - This metal interferes with this analyte. Spectral correction must be applied.

E. S. BABCOCK STANDARD OPERATING PROCEDURE FOR
METHOD #: EPA 200.8
Effective Date: __09/26/06_____

TITLE: Determination Of Trace Elements In Waters And Wastes By Inductively Coupled Plasma - Mass Spectrometry

1. SCOPE AND APPLICATION

1.1. This method provides procedures for determination of dissolved elements in ground waters, surface waters and drinking water. It may also be used for determination of total recoverable element concentrations in these waters as well as wastewater. This method is applicable to the following elements:

ANALYTE:	CAS #	MCL(ppb)	ANALYTE:	CAS #	MCL
Aluminum (Al)	7429-90-5	1000	Mercury (Hg)	7439-97-6	2
Antimony (Sb)	7440-36-0	6	Molybdenum (Mo)	7439-98-7	---
Arsenic (As)	7440-38-2	50	Nickel (Ni)	7440-02-0	100
Barium (Ba)	7440-39-3	1000	Selenium (Se)	7782-49-2	50
Beryllium (Be)	7440-41-7	4	Silver (Ag)	7440-22-4	100
Cadmium (Cd)	7440-43-9	5	Thallium (Tl)	7440-28-0	2
Chromium (Cr)	7440-47-3	50	(Thorium (Th))	7440-29-1	--
Cobalt (Co)	7440-48-4	----	(Tin (Sn))	7440-31-5	--
Copper (Cu)	7440-50-8	1000	Uranium (U)	7440-61-1	--
Lead (Pb)	7439-92-1	15	Vanadium (V)	7440-62-2	--
Manganese (Mn)	7439-96-5	50	Zinc (Zn)	7440-66-6	5000

Analytes in parenthesis are not NELAP certified.

INSTRUMENTATION: ICP/MS

Reporting limits for these elements are listed in Element attachment. Method detection limits (MDLs) and linear working ranges determined by the lab will be dependent on the sample matrix, instrumentation and selected operating conditions. Working range: waters RL-1000ppb, wastewater RL-2900ppb.

1.2. For reference where this method is approved for use in compliance monitoring programs [e.g., Clean Water Act (NPDES) or Safe Drinking Water Act (SDWA)] consult both the appropriate sections of the Code of Federal Regulation (40 CFR Part 136 Table 1B for NPDES, and Part 141 Section 141.23 for drinking water), and the latest Federal Register announcements.

1.3. Dissolved elements are determined after suitable filtration through 0.45 micron filter and acid preservation. In order to reduce potential interferences, dissolved solids should not exceed 0.2% (w/v) (Sect. 4.1.4).

1.4. Samples may be analyzed directly by pneumatic nebulization without acid digestion if the samples have been properly preserved with acid and have turbidity of < 1 NTU at the time of analysis. This total recoverable determination procedure is referred to as "direct analysis".

1.5. For the determination of total recoverable analytes in aqueous samples a digestion/extraction is required prior to analysis when the elements are not in solution (e.g., aqueous samples that may contain particulate and suspended solids). Aqueous samples containing suspended or particulate material >/= 1% (w/v) are diluted and digested by EPA 3015.

1.6. The total recoverable sample digestion procedure given in this method is not suitable for the determination of volatile organo-mercury compounds. However, for "direct analysis" of drinking water (turbidity < 1 NTU), the combined concentration of inorganic and organo-mercury in solution can be determined by "direct analysis" pneumatic nebulization provided gold is added to both samples and standards alike to eliminate memory interference effects. Gold is added to the internal standard spiking solution to address this concern.

1.7. Silver is only slightly soluble in the presence of chloride unless there is sufficient chloride concentration to form the soluble chloride complex. Therefore, low recoveries of silver may occur in samples, fortified sample matrices and even fortified blanks if determined as a dissolved analyte or by "direct analysis" where the sample has not been processed using the total recoverable mixed acid digestion. HCl acid is added to all silver *AAR* and digests to address this issue.

1.8. The total recoverable sample digestion procedure given in this method will solubilize and hold in solution only minimal concentrations of barium in the presence of free sulfate. For the analysis of barium in samples having varying and unknown concentrations of sulfate, analysis is completed as soon as possible after sample preparation.

1.9. This method should be used by analysts experienced in the use of inductively coupled plasma mass spectrometry (ICP-MS), the interpretation of spectral and

matrix interferences and procedures for their correction. ESB personnel are to be trained by the Metal Section Leader or the Laboratory Director.

1.10. Our laboratory has on file the required initial demonstration performance data described in Section 9.2 that was generated prior to using the method for analysis.

2. SUMMARY OF METHOD

2.1. An aliquot of a well mixed, homogeneous aqueous sample is accurately measured for sample processing. For total recoverable analysis of an aqueous sample containing undissolved material, analytes are first solubilized by microwave digestion with nitric and hydrochloric acid. After cooling, the sample is decanted or filtered prior to analysis. For the determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is < 1 NTU, the sample is made ready for analysis by the appropriate addition of nitric and hydrochloric acid, and then diluted to predetermined volume and mixed before analysis.

2.2. The method describes the multi-element determination of trace elements by ICP-MS(1-3). Sample material in solution is introduced by pneumatic nebulization into a radio frequency plasma where energy transfer processes cause desolvation, atomization and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a quadrupole mass spectrometer having a minimum resolution capability of 1 amu peak width at 5% peak height. Our laboratory uses the Perkin-Elmer Elan 6000 and 9000 for this analysis. The ions transmitted through the quadrupole are detected by a electron multiplier or Faraday detector and the ion information processed by a data handling system. Interferences relating to the technique (Sect. 4) are recognized and corrected. Such corrections must include compensation for isobaric elemental interferences and interferences from polyatomic ions derived from the plasma gas, reagents or sample matrix. Instrumental drift as well as suppressions or enhancements of instrument response caused by the sample matrix are corrected by the use of internal standards.

2.3. Environmental Relevance – Metals pose a variety of health risks and are monitored for various reasons. Some are monitored simply for as aesthetic nuisance while others may cause serious damage to the liver, kidney, circulatory or nervous systems. Metals occur in the environment both naturally and from various manufacturing processes.

3. DEFINITIONS

3.1. See SOP Q15 – SOP Definitions

4. INTERFERENCES

4.1. Several interference sources may cause inaccuracies in the determination of trace elements by ICP-MS. These are:

4.1.1. Isobaric elemental interferences - Are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer in use. All elements determined by this method have, at a minimum, one isotope free of isobaric elemental interference. Of the analytical isotopes recommended for use with this method (Table 4), only molybdenum-98 (ruthenium) and selenium-82 (krypton) have isobaric elemental interferences. If alternative analytical isotopes having higher natural abundance are selected in order to achieve greater sensitivity, an isobaric interference may occur. All data obtained under such conditions must be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest. A record of this correction process should be included with the report of the data. It should be noted that such corrections will only be as accurate as the accuracy of the isotope ratio used in the elemental equation for data calculations. Relevant isotope ratios should be established prior to the application of any corrections.

4.1.2. Abundance sensitivity - Is a property defining the degree to which the wings of a mass peak contribute to adjacent masses. The abundance sensitivity is affected by ion energy and quadrupole operating pressure. Wing overlap interferences may result when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the spectrometer resolution adjusted to minimize them.

4.1.3. Isobaric polyatomic ion interferences - Are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer in use. These ions are commonly formed in the plasma or interface system from support gases or sample components. Most of the common interferences have been identified, and these are listed in Table 2 together with the method elements affected. Such interferences must be recognized, and when they cannot be avoided by the selection of alternative analytical isotopes, appropriate corrections must be made to the data. Equations for the correction of data should be established at the time of the analytical run sequence as the polyatomic ion interferences will be highly dependent on the sample matrix and chosen instrument conditions. In particular, the common 82Kr interference that affects the determination of both arsenic and selenium, can be greatly reduced with the use of high purity krypton free argon.

4.1.4. Physical interferences - Are associated with the physical processes, which govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma-mass

spectrometer interface. These interferences may result in differences between instrument responses for the sample and the calibration standards. Physical interferences may occur in the transfer of solution to the nebulizer (e.g., viscosity effects), at the point of aerosol formation and transport to the plasma (e.g., surface tension), or during excitation and ionization processes within the plasma itself. High levels of dissolved solids in the sample may contribute deposits of material on the extraction and/or skimmer cones reducing the effective diameter of the orifices and therefore ion transmission. Dissolved solids levels not exceeding 0.2% (w/v) have been recommended to reduce such effects. Brine samples are routinely diluted. Internal standardization may be effectively used to compensate for many physical interference effects. Internal standards ideally should have similar analytical behavior to the elements being determined.

4.1.5. Memory interferences - Result when isotopes of elements in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the sampler and skimmer cones, and from the buildup of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples (Sect. 7.6.3). The possibility of memory interferences is recognized within an analytical run and suitable rinse times are used to reduce them. The rinse times necessary for a particular element are estimated and monitored *by the analysis of a blank following the 500ppb linearity check at the end of every run.*. Memory interferences may also be assessed within an analytical run by using a minimum of three replicate integrations for data acquisition. If the integrated signal values drop consecutively, the analyst is alerted to the possibility of a memory effect, and examines the analyte concentration in the previous sample to identify if this was high. If memory interference is suspected, the sample is reanalyzed after a long rinse period.

4.1.6. Environmental interferences – see section 6.3.

5. SAFETY

5.1. The toxicity or carcinogenicity of reagents used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonable achievable. A reference file of material data handling sheets is available to all personnel involved in the chemical analysis. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper mixing when working with these reagents.

5.2. The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

5.3. Analytical plasma sources emit radio frequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection from UV emissions.

5.4. It is the responsibility of the user of this method to comply with relevant disposal and waste regulations. For guidance see Sections 15.0.

6. EQUIPMENT AND SUPPLIES

6.1. Inductively coupled plasma - mass spectrometer:

6.1.1. Instrument capable of scanning the mass range 5-250 amu with a minimum resolution capability of 1 amu peak width at 5% peak height. Instrument may be fitted with a conventional or extended dynamic range detection system. Our laboratory uses the ELAN 6000 and 9000 DRCe with the extended dynamic range detection system.

NOTE: If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise changes in instrument response or damage to the multiplier may result.

6.1.2. Radio-frequency generator compliant with FCC regulations.

6.1.3. Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders.

6.1.4. A variable-speed peristaltic pump is required for solution delivery to the nebulizer. Our laboratory uses a Gilson system.

6.1.5. A mass-flow controller on the nebulizer gas supply is required. A water-cooled spray chamber is used for reducing some types of interferences (e.g., from polyatomic oxide species).

6.1.6. Samples having high concentrations of elements beyond the linear range of the instrument and with isotopes falling within scanning windows should be diluted prior to analysis.

6.2. Labware - For the determination of trace levels of elements, contamination and loss are of prime consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust, etc. A clean laboratory work area designated for trace element sample handling is used. Sample containers can introduce positive and negative errors in the determination of trace elements by contributing contaminants through surface desorption or leaching, depleting element concentrations through adsorption processes. All reusable labware is sufficiently cleaned for the task objectives. Labware is washed thoroughly with acetone, laboratory-grade detergent, and D.I. and then soaked at least half an hour, usually overnight in 1:1 nitric acid, followed by rinsing with D.I.

6.2.1. Plastic ware - 50 mL and 10-mL centrifuge tubes (metal free plastic).

6.2.2. Assorted calibrated air displacement pipetters with metal-free tips.

7. REAGENTS AND STANDARDS

Note: All standards and reagents are stored at room temperature.

7.1. Reagents may contain elemental impurities that might affect the integrity of analytical data. Owing to the high sensitivity of ICP-MS, high-purity reagents are used whenever possible. All acids used for this method are of ultra high-purity grade. Nitric acid is preferred for ICP-MS in order to minimize polyatomic ion interferences. Several polyatomic ion interferences result when hydrochloric acid is used (Table 2), however, it should be noted that hydrochloric acid is recommended to maintain stability in solutions containing antimony and silver. When hydrochloric acid is used, corrections for the chloride polyatomic ion interferences must be applied to all data.

7.1.1. Nitric acid, concentrated (sp.gr. 1.41) stored at room temperature for up to 10 years.

7.1.2. Nitric acid (1+1) for rinsing – Add, for example 100-mL conc. nitric acid to 100 mL of reagent grade water in a beaker. Prepare fresh daily.

7.1.3. Hydrochloric acid, concentrated (sp.gr. 1.19) stored at room temperature for up to 10 years.

7.2. Reagent Water - All references to reagent grade water in this method refer to nanopure water. Suitable water is prepared by passing distilled water through a mixed bed of anion and cation exchange resins and then through the Nanopure system to 18 mega-ohm purity.

7.3. Standard Stock Solutions - High purity stock solutions (10000, 1000, 100, 20, or 10 ppm) are purchased from reputable commercial sources. Most metals are

purchased as a mixture, however Hg and Sn are purchased separately and should be checked for the presence of impurities prior to mixing with the other elements. Solutions are stored at room temperature. Manufacturer specified holding times are observed.

7.4. Preparation of calibration standards - Fresh multielement calibration standards are prepared every 48 hours or daily for Hg and Ag. Mixed calibration standard solutions are prepared by diluting the stock-standard solutions to levels in the linear range for the instrument in a solution consisting of 2 percent (v/v) HNO3 and 1 percent (v/v) HCl in reagent water. The calibration standard is 10 ppb for all metals of interest except Hg, which is at 0.5ppb. Two linearity check standards are also analyzed, one at 100 ppb with Hg at 2ppb and the second at 500ppb, containing no Hg. Refer to the standard preparation log book for details of the preparation dilutions.

7.5. Internal Standards Stock Solution: Scandium, indium, terbium, and germanium stock standards solutions are purchased at a concentration of 1000 µg/ml from a reputable commercial vender. See standard prep log for dilution details. All standards, blanks, and samples contain each internal standard at 50 and/or 16.7µg/L.

NOTE: If mercury is to be determined by the "direct analysis" procedure, add an aliquot of the gold stock standard (1000 mg/L in 5% HCl) to the internal standard solution sufficient to provide a concentration of *500* ug/L in final the dilution of all blanks, calibration standards, and samples.

7.6. Blanks - Three types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure and to assess spectral background and the rinse blank is used to flush the instrument between samples in order to reduce memory interferences.

7.6.1. Calibration blank - Consists of 2% (v/v) nitric acid and 1% HCl in reagent grade water with internal standards.

7.6.2. Laboratory reagent blank (LRB) or Method Blank (MB) - Consists of 2% (v/v) nitric acid and 1% HCl in reagent grade water with internal standards. The LRB is carried through the entire sample digestion and preparation scheme as the samples including digestion, when applicable.

7.6.3. Rinse blank - Consists of 2% (v/v) nitric acid and 200µg/L Au in reagent grade water.

7.7. Tuning Solution (Daily Performance Check) - This solution is used for instrument tuning and mass calibration prior to analysis. The solution is

purchased from Perkin Elmer. It contains Ba, Cd, Ce, Cu, Pb, Mg, Rh, and U at 10 ng/mL ± 0.5% in 1% HNO_3.

7.8. The quality control sample (QCS) is the initial calibration verification solution (ICV), which is prepared in the same acid matrix as the calibration standards. This solution is an independent standard near the midpoint of the linear range at a concentration other than that used for instrument calibration (50ppb, 1ppb Hg). An independent standard is defined as a standard composed of the analytes from a source different from those used in the standards for instrument calibration. The QCS (ICV) is not processed. This solution is made every 48 hours or daily for Hg and Ag.. Refer to the standard preparation logbook for details of the preparation dilutions.

7.9. Laboratory Fortified Blank (LFB) or (LCS or BS) and Laboratory Fortified Matrix (LFB) or (MS) - To an aliquot of LRB, add aliquots from noncalibration multielement stock standards. The fortified concentration range for drinking waters is 25 µg/L except for mercury, which is 2.5 ug/L for wastewater *200* ug/L, Hg 4 ug/L. The LFB and MS/MSD are carried through the entire sample preparation scheme as the samples including sample digestion, when applicable. Add internal standards to this solution after preparation has been completed. This solution is made fresh daily. Refer to the standard preparation logbook for details of the preparation dilutions.

7.10. Autolens Solution: See standard log for preparation.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. Prior to sample collection of an aqueous sample, consideration is given to the type of data required (i.e., dissolved or total recoverable), so that appropriate preservation and pretreatment steps can be taken. The pH of all aqueous samples is tested *after sample receipt* to ensure the sample has been properly preserved. If properly acid preserved, the sample can be held up to 6 months before analysis for all metals except mercury which can be held for 28 days.

8.2. For the determination of dissolved elements, the sample is filtered through a 0.45-um pore diameter membrane filter at the time of collection or as soon thereafter as practically possible. Use a portion of the sample to rinse the acid rinsed filter flask, discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) nitric acid immediately following filtration to pH < 2. Filter a blank and attach QBfil.

8.3. For the determination of total recoverable elements in aqueous samples, samples are not filtered, but acidified with (1+1) nitric acid to pH < 2. Preservation may be done at the time of collection, however, to avoid the hazards of strong acids in the field, transport restrictions, and possible contamination, most samples are returned to the laboratory within two weeks of collection and

acid preserved upon receipt in the laboratory. *Following acidification, the sample is mixed, and the pH is verified.* If for some reason, such as high alkalinity the sample pH is verified to be > 2, more acid is added until verified to pH < 2. If after the addition of up to 2 mL of acid per 100 mL of sample, the pH is still >2 this is noted in the prep log and (N-pH) is added in LIMS.. When feasible, samples are held after preservation for 16 hours prior to withdrawing an aliquot for processing or "direct analysis". *If samples are processed prior to16 hours, a notation is made in the prep log and a qualifier (N-M) is added in LIMS.*

8.4. For aqueous samples, a field blank should be prepared and analyzed if required by the data user. Use the same container and acid as used in sample collection.

8.5. Samples containing turbidity < 1 NTU will be determined by "direct analysis". Screen acidified samples by testing an aliquot in a turbidimeter meter. Results are recorded in the logbook. Samples containing turbidity >1 NTU must be digested prior to analysis.

9. QUALITY CONTROL

Note: See also ESB SOP Q01 for general QC requirements

9.1. Our laboratory operates a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and calibration solutions as a continuing check on performance. The laboratory maintains performance records that define the quality of the data thus generated.

9.2. Initial demonstration of Laboratory Performance (mandatory)

9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to analyses conducted by this method.

9.2.2. Linear calibration ranges -. The range at which, at the high end, the analyte response is not less than 10% below what is expected. Our laboratory proves that we are operating within this range by analysis of standards that bracket the concentrations of any analytes reported. Analytes that are higher than our highest standard are diluted. A 500 µg/L and a 100 µg/L (2 µg/L Hg) is used to check upper concentration ranges.

9.2.3. Quality control sample (QCS or ICV) - When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. To verify the calibration standards the mean concentration from 3 replicates of the QCS1, prepared to a

concentration value of 50 ug/L for all metals except Hg which is at 1 ug/L. Must be within +/- 10% of the stated QCS value. The QCS is used for determining acceptable on-going instrument performance, the QCS is prepared as stated in section 7.8 and analysis of the QCS must be within +/- 10% of the stated value or within the acceptance limits listed in Table 8, whichever is the greater. (If the QCS is not within the required limits, an immediate second analysis of the QCS is recommended to confirm unacceptable performance.) If the calibration standards and/or acceptable instrument performance cannot be verified, the source of the problem is identified and corrected before either proceeding on with the initial determination of method detection limits or continuing with on-going analyses.

9.2.4. Method detection limit (MDL) – MDL's are established whenever there is a change in instrumentation, or a major modification to the analysis. The MDLs must be sufficient to detect analytes at the required levels according to compliance monitoring regulation (Sect. 1.2). Method detection limits (MDL) are established for all analytes, using reagent water (blank) fortified at various concentrations (for example, 0.1 ppb, 0.5 ppb, 0.625 ppb & 2.5 ppb for drinking waters and 0.5, 2 & 10 ppb for wastewater). The level that performs the best and most closely represents a concentration approaching 2-5 times the estimated detection limit (EDL) is chosen. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

MDL = (t) x (S)

where:
(t) = Student's t value for a 99% confidence level and a standard) deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].
(S) = standard deviation of the replicate analyses.

NOTE: If additional confirmation is desired, reanalyze the seven replicate aliquots on two more nonconsecutive days and again calculate the MDL values for each day. An average of the three MDL values for each analyte may provide for a more appropriate MDL estimate. If the relative standard deviation (RSD) from the analyses of the seven aliquots is < 10%, the concentration used to determine the analyte MDL may have been inappropriately high for the determination. If so, this could result in the calculation of an unrealistically low MDL. Concurrently, determination of MDL in reagent water represents a best case situation and does not reflect possible matrix effects of real world samples. However, successful analyses

of LFMs (Sect. 9.4) can give confidence to the MDL value determined in reagent water.

9.2.4.1 LOD Verification: On a yearly basis, a QC sample is spiked at a level of not more than 1-4 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL for each instrument.

9.2.5. Analyst Demonstration of Capability: Prior to initial analysis of samples or when a significant change is made to the method, a Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration 1-4 times the RL* The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 10.

9.2.5.1. *Studies in the past have been performed at 100 ppb per the QCS level mentioned in 200.8 or at 20 ppb.

9.2.6. Analyst Demonstration of Continuing Proficiency: On an annual basis, each analyst must turn in valid LCS data from four consecutive LCS's or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative Standard deviation between the four replicates must be less that or equal to 20%. Performance Evaluation Studies are performed twice a year.

9.3. Assessing Laboratory Performance (mandatory)

9.3.1. Laboratory reagent blank (LRB) - The laboratory analyzes at least one LRB (Sect. 7.6.2) with each batch of 20 or fewer of samples of the same matrix. LRB data are used to assess contamination from the laboratory environment and to characterize spectral background from the reagents used in sample processing. For batch acceptance, *LRB values must be less, but not more negative than ½ the reporting limit for all metals.* If the LRB is not acceptable, fresh aliquots of the samples must be prepared and analyzed again for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained. Samples results reported must be accompanied with a note if the method blank exceeds a concentration greater than 1/10 of the measured concentration of the sample or is greater than 1/10 of the specified regulatory limit if known. However, blank results below ½ the RL are considered to be ND and will not require a note.

9.3.2. Laboratory fortified blank (LFB) - The laboratory analyzes at least one LFB (Sect. 7.9) with each batch of samples per matrix type or 1/20 whichever is more. Calculate accuracy as percent recovery using the following equation:

$$P = \frac{LFB}{s} \times 100$$

where:
R = percent recovery.
LFB = laboratory fortified blank.
s = concentration equivalent of analyte added to fortify the LRB solution.

9.3.3. If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem is identified and resolved before continuing analyses.

9.3.4. The laboratory uses LFB analyses data to assess laboratory performance against the required control limits of 85-115% (Sect.9.3.2). When sufficient internal performance data becomes available (usually a minimum of twenty to thirty analyses), optional control limits can be developed from the mean percent recovery (x) and the standard deviation (S) of the mean percent recovery. These data can be used to establish the upper and lower control limits as follows:

UPPER CONTROL LIMIT = x + 3S
LOWER CONTROL LIMIT = x - 3S

9.3.5. The optional control limits must be equal to or better than the required control limits of 85-115%.. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. Historical recovery is examined on a yearly basis and is available in the LIMS.

9.3.6. Instrument performance (CCB/CCV) - For all determinations the laboratory checks instrument performance and verifies that the instrument is properly calibrated on a continuing basis. To verify calibration run the calibration blank and calibration standards immediately following each calibration routine, after every ten analyses and at the end of the sample run. The results of the analyses of the standards will indicate whether the calibration remains valid. All metals in the calibration blank must be less but not more negative than ½ the reporting limit. The analysis of all analytes within the standard solutions must be within ± 10% of calibration. If the calibration cannot be verified within the specified limits, the instrument must be recalibrated. (The instrument responses from the last working calibration check may be used for recalibration purposes, however, it must be verified before continuing sample analysis by an acceptable reprocessed CCV and CCB.) If the continuing calibration check is not confirmed within ± 15%, the previous ten samples must be reanalyzed after recalibration. Therefore ± 10% is considered the "recalibration limit" and ± 15% is considered the "re-

analysis limit". If the sample matrix is responsible for the calibration drift, it is recommended that the previous ten samples be reanalyzed in groups of five between calibration checks to prevent a similar drift situation from occurring.

9.4. Assessing Analyte Recovery and Data Quality

9.4.1. Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess the effect.

9.4.2. The laboratory adds a known amount of analyte to a minimum of every 20 routine samples per matrix type per batch. When combined with the MSD (see section 9.4.5, this constitutes a frequency of 10%). In each case the LFM aliquot is a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration is the same as that used in the laboratory fortified blank (Sect. 7.9). Over time, samples from all routine sample sources are fortified.

9.4.3. Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range of 70-130%. Recovery calculations are not required if the concentration of the analyte added is less than 30% of the sample background concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{C(s) - C}{s} \times 100$$

where:
R = percent recovery.
C(s) = fortified sample concentration.
C = sample background concentration.
s = concentration equivalent of analyte added to fortify the sample.

9.4.4. If recovery of any analyte falls outside the designated range and laboratory performance for that analyte is shown to be in control (Sect. 9.3), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The data user should be informed that the result for that analyte in the unfortified sample is suspect due to either the heterogeneous nature of the sample or an uncorrected matrix effect.

9.4.5. Laboratory Fortified Matrix Duplicates (LFMD) or Matrix Spike Duplicates (MSD) are analyzed every 20 samples per matrix type per sample batch. RPD must be $\leq$ 20%.

9.4.6. Internal standards responses - The analyst is expected to monitor the responses from the internal standards throughout the sample set being

analyzed. Ratios of the internal standards responses against each other should also be monitored routinely. This information may be used to detect potential problems caused by mass dependent drift, errors incurred in adding the internal standards or increases in the concentrations of individual internal standards caused by background contributions from the sample. The absolute response of any one internal standard must not deviate more than 60-125% of the original response in the calibration *standard*. If deviations greater than these are observed, flush the instrument with the rinse blank and monitor the responses in the calibration blank.

9.4.6.1. If the responses of the internal standards in the calibration blank are within the limit,

9.4.6.1.1. And the internal standard in the sample is bias low, take a fresh aliquot of the sample, dilute by a factor of two to five, add the internal standards and reanalyze. This procedure is repeated until the internal-standard intensities fall within the prescribed window.

9.4.6.1.2. And the internal standard in the sample was bias high it may be naturally occurring in the sample. Remove the IS and regroup the metals that are affected with the next closest internal standard or if there is not another internal standard dilute as indicated above.

9.4.6.2. If after flushing, the response of the internal standards in the calibration blank are out of limits, terminate the analysis and determine the cause of the drift. Possible causes of drift may be a partially blocked sampling cone or a change in the tuning condition of the instrument.

Note: For additional information concerning corrective action for out-of-control or unacceptable data see SOP Q06 – Corrective Action.

10. CALIBRATION AND STANDARDIZATION

10.1. Operating conditions - The analyst is advised to follow the recommended operating conditions provided in the ELAN 6000 and 9000 manual. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions satisfy the analytical requirements and to maintain quality control data verifying instrument performance and analytical results. Instrument operating conditions, which were used to generate precision and recovery data for this method are included in Table 6.

10.2. Precalibration routine:

10.2.1. Initiate proper operating configuration of instrument and data system. Allow a period of not less than 30 min for the instrument to warm up. During this process, conduct resolution checks using the tuning solution. If this solution is out perform, consult ELAN 6000 or 9000 manual.

10.2.2. Instrument stability is demonstrated every day by running the tuning solution (Sect. 7.7) a minimum of five times with resulting relative standard deviations of absolute signals for all analytes of less than 5%. Insert the TUNE report with the daily batch reports. The tune solution must pass the following criteria:

	ELAN 6000	ELAN 9000DRCe
Background:	<30 cps @ Mass 220	<2 cps @ Mass 220
Rh sensitivity:	>150,000 cps	In >300,000 cps
Mg sensitivity:	>20,000 cps	Mg >50,000 cps
Pb sensitivity:	>100.000 cps	U >200,000 cps
CeO:	≤0.03 %	≤0.03 %
Ba++:	≤0.03 %	≤0.03 %

If the oxides are high reduce the nebulizer flow. If the tune solution still does not pass, refer to the ELAN manual for guidance.

10.3. Internal Standardization - Internal standardization is used in all analyses to correct for instrument drift and physical interferences. A list of acceptable internal standards is provided in Table 3. For full mass range scans, four internal standards are used; scandium, indium, germanium, and terbium. Internal standards are present in all samples, standards and blanks at identical levels. This is achieved by directly adding an aliquot of the internal standards to the CAL standard, blank or sample solution (Method A, Sect. 10.3). The concentration of the internal standard is sufficiently high that good precision is obtained in the measurement of the isotope used for data correction and to minimize the possibility of correction errors if the internal standard is naturally present in the sample. A concentration of 50 and/or 16.7μg/L of each internal standard is used. Internal standards are added to blanks, samples and standards in like manner, so that dilution effects resulting from the addition may be disregarded. See internal standard log for preparation detail.

10.4. Calibration - Prior to initial calibration, set up proper instrument software routines for quantitative analysis. The instrument is calibrated using the internal standard routine described above. The instrument is calibrated for the analytes to be determined using the calibration blank (Sect. 7.6.1) and a calibration standard prepared at 10 μg/L for all metals except mercury which is at 0.5μg/L. A minimum of three replicate integrations are used for data acquisition. The average of the integrations is used for instrument calibration and data reporting. Analyze a 1-ppb (0.25ppb Hg) standard at the reporting limit. Results should be 50-150%. Analyze a 100ppb and if needed a 250ppb or 500ppb standard to verify linearity. Results should be +/- 10% of the expected value.

10.5. The rinse blank is used to flush the system between solution changes for blanks, standards and samples. Allow sufficient rinse time to remove traces of the previous sample (Sect. 4.1.5). Solutions should be aspirated for 30 sec prior to the acquisition of data to allow equilibrium to be established.

11. PROCEDURE

Note: Due to occasional client requests for lower RL's, the analyst may vary prep amounts.

11.1. Aqueous Sample Preparation - Dissolved Analytes (Refer to Sample Prep SOP--M02A)

11.2. Aqueous Sample Preparation - Total Recoverable Analytes (Refer to Sample Prep SOP--M02A)

11.3. Samples with turbidity < 1 NTU are prepared as follows:

Add the following to a sample tube:	water	liquid
internal standard	50 µL	50 µL
sample	5 mL	2 mL
4% HNO_3/2% HCl acid water	5 mL	5 mL
Nanopure	0 mL	3 mL

11.4. Sample Analysis

11.4.1. For new or unusual matrices, *samples are often prediluted*.

11.4.2. Initiate instrument operating configuration. Tune and optimize the instrument for the analytes of interest. Follow ELAN 6000 or 9000 software instructions.

11.4.3. Establish instrument software run procedures for quantitative analysis. For all sample analyses, a minimum of three replicate integrations is required for data acquisition. Use the average of the integrations for data reporting.

11.4.4. All masses that might affect data quality are monitored during the analytical run. As a minimum, those masses prescribed in Table 4 are monitored in the same scan as is used for the collection of the data. This information is used to correct the data for identified interference.

11.4.5. During the analysis of samples, the laboratory must comply with the required quality control described in Sections 9.3 and 9.4. Only for the determination of dissolved analytes or the "direct analysis" of drinking water with turbidity of < 1 NTU is the sample digestion step of the LRB, LFB, and LFM not required.

11.4.6. The rinse blank is used to flush the system between samples. Allow sufficient time to remove traces of the previous sample *(35 seconds for wastewaters and ground waters, 25 seconds for drinking waters)*. Samples are aspirated for a minimum of 30 sec prior to the collection of data.

11.4.7. Samples having concentrations higher than the high check standard are diluted into range and reanalyzed. The sample is first analyzed for the trace elements in the sample, protecting the detector from the high concentration elements, if necessary, by the selection of appropriate scanning windows. The sample is then diluted for the determination of the remaining elements.

11.4.8. Analytical Summary (Refer to ELAN 6000 or 9000 manual)

11.4.8.1. Fill Autosampler tray with blanks, standards and samples as desired. Under the "sample" portion of the method program, fill out the sample table to match the Autosampler positions.

11.4.8.2. Analyze the calibration blank.

11.4.8.3. Analyze the calibration standard. 10 µg/L (0.5µg/L Hg)

11.4.8.4. Analyze the QCS (50µg/L, Hg 1µg/L). Make certain QC is acceptable (Table 8 and sec. 9.2.3)

11.4.8.5. Analyze CCV. 10 µg/L (0.5 µg/L Hg) Make certain QC is acceptable (±10%)

11.4.8.6. Analyze CCB. Make certain QC is acceptable (no analytes found at > ½ RL).

11.4.8.7. Analyze a 1-ppb (0.25-ppb Hg) RL std. Make certain QC is acceptable (50-150%).

11.4.8.8. Analyze 100 µg/L (2µg/L Hg) linearity check standard. Linearity is dependent of 90-110% recovery.

11.4.8.9. Analyze the LRB. Make certain QC is acceptable. *LRB values must not exceed ½ the reporting limit for all metals of concern.*

11.4.8.10. Analyze the LFB (DW: 25µg/L, Hg 2.5µg/L WW 200µg/L, Hg 4µg/L). Make certain QC is acceptable (±15%).

11.4.8.11. Analyze samples and MS/MSD samples.

11.4.8.12. Analyze CCV, CCB, QCS, and CCB2 after every 10 samples and at end of run. CCB criteria are the same as in section 11.4.8.9. CCV criteria are ± 10% for re-calibration flag and ±15% for re-analysis flag. (See sec. 9.3.6) QCS and CCB2 are evaluated only when resloping is necessary.

11.4.8.13. At the end of the run, analyze 500 µg/L linearity check standard followed by a CCB to verify sufficient rinse time. Linearity is dependent of 90-110% recovery

11.4.8.14. Follow instructions in the ELAN 6000 or 9000 Software Manual for printing reports.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Elemental equations recommended for sample data calculations are listed in Table 5. Sample data is reported in units of ug/L for aqueous samples. Do not report element concentrations below the determined MDL.

12.2. *Two significant figures* are used for reporting element concentrations.

12.3. For aqueous samples prepared by total recoverable procedure (Sect.11.2), the preparation dilution factor is entered into Element as the initial/final volume. If additional dilutions were made to any samples at the instrument, the appropriate factor is applied to calculate sample concentrations by entering a value into the dilution column in Element.

12.4. Data values are corrected for instrument drift or sample matrix induced interferences by the application of internal standardization. Corrections for characterized spectral interferences are applied to the data. Chloride interference corrections are made on all samples, regardless of the addition of hydrochloric acid, as the chloride ion is a common constituent of environmental samples.

12.5. The QC data obtained during the analyses provide an indication of the quality of the sample data and should be provided with the sample results at the time of LIMS entry.

13. METHOD PERFORMANCE: See "Initial Demonstration of Proficiency" report prepared for this method.

14. CORRECTIVE ACTION FOR OUT OF CONTROL / UNACCEPTABLE DATA

See SOP Q06 – Corrective Action

15. POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1. Waste Disposal

15.1.1. Instrument waste is placed in the Low Concentration Acid Waste Drum.
15.1.2. Digests are placed in the High Concentration Acid Waste Drum.
15.1.3. Preserved samples are placed Low Concentration Acid Waste Drum.
15.1.4. Expired standards are placed in the High Concentration Acid Waste Drum.
15.1.5. Expired Hg standards are placed in the COD Waste Drum

15.2. See SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S07 – Pollution Prevention

REFERENCES

EPA Method 200.8, Supplement I, May 1994

Note: All *italicized items* are an indication of a variation from the method.

TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 2: COMMON MOLECULAR ION INTERFERENCES IN ICP-MS

BACKGROUND MOLECULAR IONS

Molecular Ion	Mass	Element Interference(a)
NH+	15	
OH+	17	
OH2+	18	
C2+	24	
CN+	26	
CO+	28	
N2+	28	
N2H+	29	
NO+	30	
NOH+	31	
O2+	32	
02H+	33	
36ArH+	37	
38ArH+	39	
40ArH+	41	
CO2+	44	
CO2H+	45	Sc
ArC+, ArO+	52	Cr
ArN+	54	Cr
ArNH+	55	Mn
ArO+	56	
ArOH+	57	
40Ar36Ar+	76	Se
40Ar38Ar+	78	Se
40Ar2+	80	Se

MATRIX MOLECULAR IONS

BROMIDE(12)

Molecular Ion	Mass	Element Interference
81BrH+	82	Se
79BrO+	95	Mo
81BrO+	97	Mo
81BrOH+	98	Mo
Ar81Br+	121	Sb

CHLORIDE

Molecular Ion	Mass	Element Interference
35ClO+	51	V
35ClOH+	52	Cr
37ClO+	53	Cr
37ClOH+	54	Cr
Ar35Cl+	75	As
Ar37Cl+	77	Se

SULFATE

Molecular Ion	Mass	Element Interference
32SO+	48	
32SOH+	49	
34SO+	50	V, Cr
34SOH+	51	V
SO2+, S2+	64	Zn
Ar32S+	72	
Ar34S+	74	

PHOSPHATE

Molecular Ion	Mass	Element Interference
PO+	47	
POH+	48	
PO2+	63	Cu
ArP+	71	

GROUP I, II METALS

Molecular Ion	Mass	Element Interference
ArNa+	63	Cu
ArK+	79	
ArCa+	80	

MATRIX OXIDES

Molecular Ion	Mass	Element Interference
TiO	62-66	Ni, Cu, Zn
ZrO	106-112	Ag,Cd
MoO	108-116	Cd

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method elements or internal standards affected by the molecular ions.

(*) Oxide interferences will normally be very small and will only impact the method elements when present at relatively high concentrations. Some samples of matrix oxides are listed of which the analyst should be aware. It is recommended that Ti and Zr isotopes are monitored in solid waste samples, which are likely to contain high levels of these elements. Mo is monitored as a method analyte.

TABLE 3: INTERNAL STANDARDS AND LIMITATIONS OF USE

Internal Standard	Mass	Possible Limitation
6Lithium	6	a
Scandium	45	polyatomic ion interference
Germanium	72	
Yttrium	89	a, b
Rhodium	103	
Indium	115	isobaric interference by Sn
Terbium	159	
Holmium	165	
Lutetium	175	
Bismuth	209	a

May be present in environmental samples.
In some instruments Yttrium may form measurable amounts of YO+ (105 amu) and YOH+ (106 amu). If this is the case, care should be taken in the use of the cadmium elemental correction equation.

Internal standards recommended for use with this method are shown in bold face. Preparation procedures for these are included in Section 7.5.

TABLE 4: RECOMMENDED ANALYTICAL ISOTOPES AND ADDITIONAL MASSES WHICH MUST BE MONITORED

Isotope	Element of Interest
27(*)	Aluminum
121, 123(*)	Antimony
75(*)	Arsenic
135, 137(*)	Barium
9(*)	Beryllium
106, 108, 111,(*) 114	Cadmium
52,(*) 53	Chromium
59(*)	Cobalt
63,(*)65	Copper
206,(*) 207,(*) 208(*)	Lead
55(*)	Manganese
95, 97,(*)98	Molybdenum
60,(*) 62	Nickel
77, 82(*)	Selenium
107,(*) 109	Silver
203, 205(*)	Thallium
232(*)	Thorium
238(*)	Uranium
51(*)	Vanadium
66,(*) 67, 68	Zinc
Krypton	
Ruthenium	
Palladium	
Tin	

NOTE: Isotopes recommended for analytical determination have an (*) next to them.

TABLE 5: EPA 200.8 RECOMMENDED ELEMENTAL EQUATIONS FOR DATA CALCULATIONS

Element	Elemental Equation	Note
Al	(1.000)(27C)	
Sb	(1.000)(123C)	
As	(1.000)(75C)-(3.127)[(77C)-(0.815)(82C)]	(1)
Ba	(1.000)(137C)	
Be	(1.000)(9C)	
Cd	(1.000)(111C)-(1.073)[(108C)-(0.712)(106C)]	(2)
Cr	(1.000)(52C)	(3)
Co	(1.000)(59C)	
Cu	(1.000)(63C)	
Pb	(1.000)(206C)+(1.000)(207C)+(1.000)(208C)	(4)
Mn	(1.000)(55C)	
Mo	(1.000)(98C)-(0.146)(99C)	(5)
Ni	(1.000)(60C)	
Se	(1.000)(82C)	(6)
Ag	(1.000)(107C)	
Tl	(1.000)(205C)	
Th	(1.000)(232C)	
U	(1.000)(238C)	
V	(1.000)(51C)-(3.127)[(53C)-(0.113)(52C)]	(7)
Zn	(1.000)(66C)	
Bi	(1.000)(209C)	
In	(1.000)(115C)-(0.016)(118C)	(8)
Sc	(1.000)(45C)	
Tb	(1.000)(159C)	
Y	(1.000)(89C)	

C - calibration blank subtracted counts at specified mass.

(1) - correction for chloride interference with adjustment for 77Se. ArCl 75/77 ratio may be determined from the reagent blank. Isobaric mass 82 must be from Se only and not BrH+.

(2) - correction for MoO interference. Isobaric mass 106 must be from Cd only not Zro+. An additional isobaric elemental correction should be made if palladium is present.

(3) - in 0.4% v/v HCl, the background from ClOH will normally be small. However the contribution may be estimated from the reagent blank. Isobaric mass must be from Cr only not ArC+.

(4) - allowance for isotopic variability of lead isotopes.

(5) - isobaric elemental correction for ruthenium.

(6) - some argon supplies contain krypton as an impurity. Selenium is corrected for 82Kr by background subtraction.

(7) - correction for chloride interference with adjustment for 53Cr. Cl0 51/53 ratio may be determined from the reagent blank. Isobaric mass 52 must be from Cr only no ArC+.

(8) - isobaric elemental correction for tin.

TABLE 8: ACCEPTANCE LIMITS FOR QC CHECK SAMPLE

METHOD PERFORMANCE (ug/L)(1)

QC Check Sample ELEMENT	Average Conc.	Standard Deviation(2) Recovery	Acceptance Limits(3) (S(r))	ug/L
Aluminum	100	100.4	5.49	84-117
Antimony	100	99.9	2.40	93-107
Arsenic	100	101.6	3.66	91-113
Barium	100	99.7	2.64	92-108
Beryllium	100	105.9	4.13	88-112(4)
Cadmium	100	100.8	2.32	94-108
Chromium	100	102.3	3.91	91-114
Cobalt	100	97.7	2.66	90-106
Copper	100	100.3	2.11	94-107
Lead	100	104.0	3.42	94-114
Manganese	100	98.3	2.71	90-106
Molybdenum	100	101.0	2.21	94-108
Nickel	100	100.1	2.10	94-106
Selenium	100	103.5	5.67	86-121
Silver	100	101.1	3.29	91-111(5)
Thallium	100	98.5	2.79	90-107
Thorium	100	101.4	2.60	94-109
Uranium	100	102.6	2.82	94-111
Vanadium	100	100.3	3.26	90-110
Zinc	100	105.1	4.57	91-119

Method performance characteristics calculated using regression equations from collaborative study, reference 11.
Single-analyst standard deviation, S(r).
Acceptance limits calculated as average recovery +/-3 standard deviations.
Acceptance limits centered at 100% recovery.
Statistics estimated from summary statistics at 48 and 64 ug/L.

Approved by Susann K. Thomas Date 09/25/06

Edward S. Babcock & Sons Standard Operating Procedure
SM 5310B
Date Effective:___09/25/06_____

TITLE: Organic Carbon, Total, (Nonpurgeable), and Dissolved (Combustion)

1.0 Scope and Application

1.1 This method includes the measurement of organic carbon in drinking, surface and saline waters, domestic and industrial wastes. Since the sample is purged to remove inorganic carbon the result is essentially nonpurgeable organic carbon*. See Definitions and Interferences for other exclusions.

1.2 The method is applicable to measurement of organic carbon for aqueous sample from 0.3 for waters and 0.7 for liquids to 20mg/L.

1.3 This method may also be used for solid samples if both client and regulator agree to this variation. Range 0.2% to 3.2% Carbon.

2.0 Summary of Method

2.1 Organic carbon in a sample is converted to carbon dioxide (CO2) by catalytic combustion. The CO2 formed can be measured directly by an infrared detector. The amount of CO2 is directly proportional to the concentration of carbonaceous material in the sample.

3.0 Definitions See also SOP Q15

3.1 The carbonaceous analyzer measures all of the carbon in a sample. Because of various properties of carbon-containing compounds in liquid samples, preliminary treatment of the sample prior to analysis dictates the definition of the carbon as it is measured. Forms of carbon that can be measured by the method are:

3.1.1 soluble, nonvolatile organic carbon; for instance, natural sugars.

3.1.2 soluble, volatile* organic carbon; for instance, mercaptans.

3.1.3 insoluble, partially volatile carbon; for instance, oils.

3.1.4 insoluble, particulate carbonaceous materials, for instance; cellulose fibers.

3.1.5 soluble or insoluble carbonaceous materials adsorbed or entrapped on insoluble inorganic suspended matter; for instance, oily matter adsorbed on silt particles.

Note: *IC interference can be eliminated by acidifying samples to pH 2 or less to convert IC species to CO_2. Subsequently, purging the sample with a purified gas removes the CO_2 by volatilization. Sample purging also removes POC so that the organic carbon measurement made after eliminating IC interferences is actually a NPOC determination: determine VOC to measure true TOC. In many surface and ground waters the VOC contribution to TOC is negligible. Therefore, in practice, the NPOC determination is substituted for TOC.

3.2 Method Definitions

3.2.1 Inorganic Carbon (IC): carbonate, bicarbonate, dissolced carbon dioxide

3.2.2 Total Organic Carbon (TOC): all carbon atoms covalently bonded in organic molecules

3.2.3 Dissolved Organic Carbon (DOC): the fraction of TOC that passes through a 0.45micron filter

3.2.4 Particulate Organic Carbon (POC) or nondissolved organic carbon: the fraction of TOC that remains on a 0.45micron filter

3.2.5 Volatile Organic Carbon (VOC) or purgeable organic carbon: the fraction of TOC removed by purging

3.2.6 Nonpurgeable Organic Carbon (NPOC): the fraction of TOC not removed by purging.

4.0 Environmental Relevance

The final usefulness of the carbon measurement is in assessing the potential oxygen demanding load of organic material on a receiving stream. This statement applies whether the carbon measurement is made on a sewage plant effluent, industrial waste, or on water taken directly from the stream. In this light, carbonate and bicarbonate carbon are not a part of the oxygen demand in the stream and therefore is removed by purging prior to analysis. The manner of preliminary treatment of the sample and instrument settings defines the types of carbon that are measured. Instrument manufacturer's instructions are followed.

5.0 Sample Handling and Preservation

5.1 Sampling and storage of samples in glass amber bottles is preferable. Sampling and storage in plastic bottles such as conventional polyethylene containers is permissible if it is established that the containers do not contribute contaminating organics to the samples. Generally sample are received in new VOA vials.

5.2 Because of the possibility of oxidation or bacterial decomposition of some components of aqueous samples, the lapse of time between collection of samples and start of analysis should be kept to a minimum. Also, samples should be kept cool (4-C) and protected from sunlight and atmospheric oxygen.

5.3 The sample may be kept for 7 days unpreserved or up to 28 days if acidified (pH </= 2) with H2SO4. If dissolved organic carbon is requested, filter sample through a 0.45 micron filter prior to preservation.

6.0 Interferences

6.1 Carbonate and bicarbonate carbon represent an interference under the terms of this test. They are removed by purging the sample with oxygen prior to analysis.

6.2 This procedure is applicable only to homogeneous samples which can be injected into the apparatus reproducibly by means of a microliter type

syringe or pipette. The openings of the syringe or pipette limit the maximum size of particles that may be included in the sample. A homogenizer is used to reduce the size of large particles and mix the sample evenly.

6.3 Any contact with organic may contaminate the sample. Avoid plastic containers and rubber tubing.

6.4 DOC can be lost on the filter or contaminated from the filter. When analyzing for DOC, filter a method blank and LCS.

7.0 Apparatus

7.1 Apparatus for blending or homogenizing samples: Biospec Tissue Tearer.

7.2 Apparatus for total and dissolved organic carbon: Shimadzu TOC Vsch.

7.3 Standard laboratory glassware: volumetric flasks, beakers, graduated cylinders, pipets, and autosampler vials.

7.4 Glass syringe, 0.45 micron disk filters

7.5 Apparatus for solid samples: TOCSSM 5000A

Note: See instrument manual for maintenance requirements.

Note: All glassware is cleaned immediately prior to use with Nanopure water, followed by rinsing with TOC Reagent Water (section 8.1). After use glassware is cleaned by thorough rinsing with three portions of D.I. water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

8.0 Reagents and Standards

8.1 TOC Reagent Water (Blank solution): 1L Nanopure w/ 1ml HCl. Water is stored at room temperature up to one year.

8.2 Inorganic Carbon Purge Blank; Carbonate solution - Weigh 1.25g of sodium carbonate and transfer into the 1000 mL volumetric flask containing 100 mL of Nanopure water. Dissolve and bring up to 1L with Nanopure water. Blank is stored at room temperature up to six months.

8.3 Calibration Standards

8.3.1 Calibration Stock Standard: Potassium Acid Phthalate purchased from a certified vendor and kept for 10 years.

8.3.2 Calibration Intermediate: Add 425mg Potassium Acid Phthalate (dried ae 105^{o}C for 4 hours) to 100ml of TOC reagent grade water. This will be equivalent to 2000mg/L of organic carbon. This solution is stored at room temperature for up to a year.

8.3.3 Calibration Working Standards: One 7-point calibration curve is prepared. The concentrations for the water curve are as follows: 0mg/L, 0.3mg/L, 2mg/L, 4mg/L, 7mg/L, 10mg/l, and 20mg/L. The concentrations for the liquid curve are as follows: 0mg/L, 0.7mg/L, 2mg/L, 4mg/L, 7mg/L, 10mg/L, and 20mg/L. These are prepared by making a 20ppm standard, (100ml of 2000ppm calibration standard to 100ml TOC reagent water in a volumetric

flask). The instrument provides automated dilution for the other concentrations. The 0.3mg/L standard is made by diluting 2mL of a 3mg/L standard up to 20mL with TOC reagent water. The Make standards before each run and place them in 40ml VOA vials. Place standards in the autosampler rack in positions according to the prep sheet. The 4mg/L and 20mg/L standards are also the continuing calibration check sources. These are prepared by adding the 2000ppm intermediate standard into 20ml TOC reagent water. in the following amounts: 40ul and 200ul.

8.4 LCS/ICV Standards

8.4.1 LCS Stock: Second source of Potassium Acid Phthalate purchased from a certified vendor and kept for 10 years

8.4.2 LCS Intermediate: Add 425mg of dried Potassium Acid Phthalate to 100ml of TOC reagent water. This will be equivalent to 2000mg/L of organic carbon. This solution is stored at room temperature for up to a year.

8.4.3 LCS Working 4mg/L: Add 40ul of the LCS Intermediate to 20ml of TOC reagent water. Make fresh daily. Place in a 40ml VOA vial.

8.4.4 ICV Working 20mg/L: Add 200ul of the LCS Intermediate to 20ml of TOC reagent water. Make fresh daily.

8.5 Solid Standards

8.5.1 Glucose (40% carbon at 99.5% purity = 39.79% carbon) purchased from a certified vendor. This salt is stored at room temperature for up to 10 years.

8.5.1.1 A calibration curve is made by analyzing 2.5mg, 10mg, 20mg, 30mg, and 40mg quantities for calibration points of 0.2%, 0.8%, 1.6%, 2.4%, and 3.2% carbon.

8.5.1.2 Calibration checks: Checks are performed every twenty samples and at the end of the run alternating between a level of 0.8%(10 mg) and 2.4%(30mg). Results must be within 80-120% of the expected value

8.5.2 Second source Glucose (40% carbon at 99.5% purity = 39.79% carbon) purchased from a certified vendor. This salt is stored at room temperature for up to 10 years.

8.5.2.1 A LCS/ICV is made by analyzing 20mg of glucose for a true value of 1.6% carbon based on a sample aliquot of 0.5g. Results must be within 70-130% of the expected value.

8.5.2.2 A MS/MSD is made by adding 20mg of glucose to 0.5g of sample for a spike concentration of 1.6% carbon. Results must be within 27-119% (based on historical data) of the expected value with a maximum RPD of 25%.

8.5.3 Method Blank: A method blank is analyzed with each batch by weighing 0.5g of baked sand. Results must be less than the reporting limit.

9.0 Procedure:

9.1 Instrument set up

9.1.1 Make sure the instrument is set to PC Control (switch found inside in the upper right of the instrument.

9.1.2 Turn on the instrument while holding the enter key on the front of the instrument. This is a reset on "stand-alone" mode. (usually the instrument stays on)

9.1.3 If you have just turned on the instrument, you will need to wait for the furnace to heat up and equilibrate to 680 ^{o}C. Select [Background Monitor] under the Instrument Menu. This will show the temperatures and baseline position. Exit this menu when the instrument has equilibrated.

9.1.4 Make sure the hydrator found in the lower right of the instrument is filled with nanopure water, NOT the acidified reagent water.

9.1.5 Check the IC reagent water bottle located on the left of the instrument, labeled H3P04.

9.1.6 Start the TOC Control program on the PC. Connect the instrument by selecting [Connect] under the Instrument Menu.

9.1.7 Perform a Residue Removal under Maintenance Menu. Ensure that there are bubbles in the IC reaction chamber.

9.1.8 Load sample table. Under Insert select [autogenerate] and enter "0-20 liquid" or "0-20 water" in the Method cell. Enter the appropriate vial numbers.

9.1.9 Press the [Start] button on the computer's TOC Control Panel once the samples have been properly placed on autosampler.

9.1.10 To view the current results as they are being analyzed, select [Sample Window] under the View Menu.

9.1.11 Enter the Lab Number in the Sample Number cell.

9.1.12 Every 15 samples (or less) you will need to check the 4ppm or 20ppm TOC Standard.

9.1.13 Every set of 20 or less will need a method blank, LCS, spike and spike duplicate. The spike and spike duplicate is simply 40ul of the LCS intermediate standard in 20ml of sample. This will make a 4ppm spike. It is best that you do a spike on a sample that you know is less than 10ppm.

9.1.14 Place 40ml vials on the autosampler rack. Use graduated cylinders if vials are not provided or if a dilution is needed to measure 20 mL of aqueous sample into 40mL vials. If sample contains solid material that is larger than autopipet tip or autosampler needle then use the Tissue Tearer, see section 6.2. Make sure to put the cover on the rack.

9.1.15 Press [Start], then [Stand by], verify vial numbers, press ok.

9.1.16 Uncheck acid addition box before starting.
9.1.17 After the run is finished and the data has been reviewed, select [Print] under the file menu.

9.2 Solid sample preparation
9.2.1 Dry approximately 2g of solid or sludge sample in the 105^{o}C oven overnight.

Note: For solid samples note weight before and after oven as well as pan weight to determine total solid result.

9.2.2 Crush and sieve dried sample using mortar, pestle and 60 mesh sieve.
9.2.3 Place 0.5g sample into a ceramic boat.
9.2.4 Add 10 drops of HCl and 10 drops of Nanopure to sample to eliminate inorganic carbon.
9.2.5 Dry on the hot plate.
9.2.6 Dry in 180^{o}C oven for about 2 hours to eliminate HCl.
9.2.7 Cool sample.
9.2.8 Place boat directly into instrument for analysis.
9.2.9 Reporting:
9.2.9.1 Sludges are reported on a dry weight basis.
9.2.9.2 Solid are reported on an as received basis. Results must be calculated back to the original sample:
%TOC on dry sample x % solids/100 = %TOC on AAR sample

9.3 Procedure for Neutralizing Acid Samples
9.3.1 500 µl of sample is added to a 50 ml blue tube and brought up to 30 mL with nanopure. Then with 6N NaOH the pH is adjusted to between 2 and 3. Finally the sample is diluted up to 50 mL with nanopure for a total dilution of 100.
9.3.2 MB and LCS are also prepared in a blue tube. 500 µl of 2% H_2SO_4 is added to 30 mL of nanopure. The pH is adjusted with 6N NaOH if needed to between 2 and 3. Finally, the sample is brought up to 50 mL with nanopure. 100µl of Intermediate.Standard is added to the LCS tube to make a 4 ppm LCS.
9.3.3 A MS and MSD are analyzed with this method.

9.4 Calibration:
9.4.1 Calibration is performed when CCVs are out of acceptance criteria.
9.4.2 Although a 3 point curve plus zero is required, a 7 point curve is prepared by plotting instrument response against standard concentration. The curve also includes zero.
9.4.3 To calibrate the instrument, make sure that the temperature of the furnace has equilibrated.
9.4.4 Load the file and place the blank, standards, and ICV in the appropriate positions.
9.4.5 Press ‘start’.

9.4.6 An acceptable curve yields an $r^2 = 0.99$ or better.

9.4.7 Calibration Checks:

9.4.7.1 ICV: Following calibration the 20ppm ICV made from the LCS source is analyzed. Results must be within 90-110% of the true value.

9.4.7.2 CCV: Every 15 samples and at the end of the run, a 4ppm or 20ppm calibration check is analyzed. Results must be within 85-115% of the true value. Standards are made from the calibration source intermediate.

9.4.7.3 CCB: Every 15 samples and at the end of the run, a calibration blank is analyzed to monitor drift. Results should be less than the reporting limit.

9.4.7.4 A standard at the reporting limit is analyzed at the end of the run. This is used to validate none detected results. This standard should have a signal greater than the method blank and ND results

9.4.8 See section 8.5.1 for solid calibration information.

9.5 Reporting:

9.5.1 Examine each sample report to make sure that the software took the proper average. Enter raw data and dilutions into the LIMS.

9.5.2 Do not report results higher than the highest calibrator.

9.5.3 Watch for possible carry over by examining multiple injections. If subsequent readings decline in concentration, the initial result may have been biased by the previous sample. Do not include this biased result in the sample average.

9.5.4 Aqueous results are reported in mg/L. Solid results are reported as %. Sludge results are reported as % dry weight.

9.5.5 For DOC analyses, the filtered date and time will be entered as the preparation date and time.

10.0 Quality Control

Note: See also ESB SOP Q01 for general QC requirements

10.1 LCS: One LCS is analyzed every 20 samples per matrix type or once per batch whichever is greater. If samples are homogenized, the LCS must be homogenized. If samples are filtered, a LCS must be filtered. LCS readings must be inside historical acceptance limits.

10.2 MB: A method blank is analyzed every 20 samples per matrix type or once per batch whichever is greater. The method blank must be less but not more negative than 0.7ppm for liquid samples and 0.3ppm for water samples. If samples are homogenized, the blank must be homogenized. If samples are filtered, a blank must be filtered. Sample results at or above the reporting limit must be accompanied with a qualifier if the method blank exceeds a concentration greater than 1/10 the measured raw concentration of the sample. Sample results below the reporting limit are considered to be ND

and will not require a note however method blank results must not be more negative than the reporting limit.

10.3 MS/MSD: A matrix spike and matrix spike duplicate is analyzed every 20 samples per matrix type or once per batch whichever is greater. Take 20 mls of the sample and add 40ul of the 2000mg/L intermediate std into a TOC vial. Upon analysis, the result must fit within an 80% to 120% window(true value=4.0mg/L), and have a RPD no greater than 10%.

10.4 See section 8.5.2 for solid QC requirements.

10.5 Inorganic Carbon Purge Blank: A blank made from sodium carbonate is acidified and purged exactly as a sample would be processed to ensure that all inorganic carbon is being adequately removed from the samples. The blank must be below the reporting limit or steps should be taken to determine the presence if inorganic carbon.

10.6 The instrument is set to report an average of several injections. Repeat injections until consecutive peaks are obtained that are reproducible to within a maximum coefficient of variation (CV) of 10% or a standard deviation (SD) of the area counts less than 200.

10.7 See section 8.5.2 for solid QC information.

10.8 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at the reporting limit or 2.5-5 times the estimated MDL and a minimum of seven replicates is analyzed. See QA Manual for calculation. Results must be below the reporting limit.

10.8.1 LOD Verification: On a yearly basis, a QC sample is spiked at a level of not more than 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL.

10.9 Demonstration of Capability: Prior to initial analysis of samples or when a significant change is made to the method, a Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration 5-50 times the MDL. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 10.

10.10 Demonstration of Continuing Proficiency: On an annual basis, analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies performed twice a year.

11.0 Safety

11.1 See SOP S01 – Concentrated Acids and Bases
SOP S02 – Compressed Gas Cylinder Handling
SOP S03 – Spill Control Policy

11.2 General laboratory safety procedures are sufficient for this analysis. Recommended safety equipment includes gloves and safety glasses.

12.0 Corrective Action For Out of Control Or Unacceptable Data:
12.1 See SOP Q06 – Corrective Action

13.0 Pollution Prevention and Waste Management:
13.1 Waste Disposal
13.1.1 Instrument waste is placed in the Low Acid Concentration Waste" barrel.

13.1.2 Sample waste is placed in the "Low Acid Concentration Waste" barrel.
13.2 See SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S06 – Disposal of Chlorinated Solvents
SOP S07 – Pollution Prevention

14.0 Method Performance
14.1 See Method 415.1 section 9 SM 5310B section 6.
14.2 Our intralaboratory generated data is expected to achieve similar results. Refer to MDL studies, Initial Demonstration of Capability Studies, and quality control charts maintained in the QC Office.

References

EPA 415.1 1974 Methods for the Chemical Analysis of Waters and Wastes.
EPA 9060 Methods for the Chemical Analysis of Waters and Wastes.
Standard Methods For the Examination of Water and Wastewater APHA, AWWA, WPCF 18th Edition 5310B.

Note: All *italicized items* are an indication of a variation from the method.

Approved by Susann K. Thomas Date 09/20/06

Standard Operating Procedure
Edward S. Babcock & Sons
Date Effective: _____9/27/04____

METHOD #: 8141B

TITLE: Determination Of Organo-Phosphorus Pesticides In Water By Gas Chromatography With A Nitrogen Phosphorus Detector and GCMS Confirmation

1.0 SCOPE and APPLICATION

1.1 This is a gas chromatographic (GC) method applicable to the determination of organo-phosphorus pesticides in groundwater samples, sludge and soil samples. The following compounds can be determined using this method:

Range: Groundwater samples: 4 µg/L - 50µg/L
Solid samples: 1 mg/kg – 3.33 mg/kg
Sludge samples: 3 mg/kg – 10 mg/kg

Table 1

Analyte	CAS #
(Aspon)	3244-90-4
Atrazine	1912-24-9
(Azinphos-ethyl)	2642-71-9
Azinphos-methyl	86-50-0
(Bolstar (Sulprofos))	35400-43-2
Carbophenothion	786-19-6
Chlorfenvinphos	470-90-6
Chlorpyrifos	2921-88-2
Chlorpyrifos methyl	5598-13-0
(Coumaphos)	56-72-4
(Crotoxyphos)	7700-17-6
Demeton-O	8065-48-3
Demeton-S	8065-48-3
Diazinon	333-41-5
(Dichlorofenthion)	97-17-6
(Dichlorvos)	62-73-7
(Dicrotophos)	141-66-2
Dimethoate	60-51-5
(Dioxathion)	78-34-2
Disulfoton	298-04-4
EPN	2104-64-5
Ethion	563-12-2
(Ethoprop)	13194-48-4
Famphur	52-85-7
(Fenitrothion)	122-14-5
(Fensuffothion)	115-90-2
(Fenthion)	55-38-9
(Fonophos)	944-22-9
(Hexamethyl phosphoramide) (HMPA)	680-31-9
(Leptophos)	21609-90-5

Malathion	121-75-5
(Merphos)	150-50-5
Mevinphos	7786-34-7
(Monocrotophos)	6923-22-4
Naled	300-76-5
Parathion, ethyl	56-38-2
Parathion, methyl	298-00-0
Phorate	298-02-2
(Phosmet)	732-11-6
Phosphamidon	13171-21-6
(Prothiofos)	
Ronnel	299-84-3
Simazine	122-34-9
(Stirophos)	22248-79-9
Sulfotepp	3689-24-5
TEPP	
(Terbufos)	13071-79-9
(Tetrachlorvinphos)	
(Tetraethyl pyrophosphate)	107-49-3
Thionazin	297-97-2
(TOCP)	
(Tokuthion)	34643-46-4
(Triazine Herbicides)	
(Trichlorfon)	52-68-6
(Trichloronate)	327-98-0
(Tri-o-cresyl phosphate)	78-30-8
(Zinophos)	

Note: Analytes in parenthesis are not certified by NELAP.

2.0 SUMMARY OF METHOD

2.1 A one liter aliquot of aqueous sample is extracted with methylene chloride by shaking in a separatory funnel. The methylene chloride extract is isolated, dried and concentrated to a volume of 5mL during a solvent exchange to methyl tert-butyl ether (MTBE). A 15g aliquot of solid or 5g aliquot of sludge sample is mixed with anhydrous sodium sulfate to form a free-flowing powder. This is solvent extracted three times with 1:1 methylene chloride and acetone using sonication. The extract is ready for analysis following solvent exchange with methyl tert-butyl ether (MTBE) and concentration. 15g of a soil sample is concentrated down to 5mL of extract. 5g of a sludge sample is concentrated down to 5mL of extract. Chromatographic conditions are described which permit the separation and measurement of the analytes in the extract by Capillary Column GC with a nitrogen-phosphorus detector (NPD) and confirmation by GCMS.

3.0 DEFINITIONS See SOP Q15

4.0 INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks.

4.1.1 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by thoroughly rinsing with *hot-water* and A*lconox followed by* three D.I. rinses. *Glassware is then rinsed with acetone.* Prior to and between use syringes are rinsed with the appropriate solvent.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems.

4.2 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. After the run is completed, the analyst will evaluate the data with this concern in mind and rerun any samples the may have been affected by carry over.

4.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that all the analytes listed in the scope and application section are not resolved from each other on any one column, i.e., one analyte of interest may be an interferant for another analyte of interest. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. Further processing of sample extracts may be necessary. Positive identifications are confirmed by GCMS.

4.4 It is important that samples and working standards be contained in the same solvent. The solvent for working standards must be the same as the final solvent used in sample preparation. If this is not the case, chromatographic comparability of standards to sample may be affected.

4.5 Analytical difficulties encountered for target analytes include:

4.5.1 Tetraethyl pyrophosphate (TEPP) is an unstable diphosphate which is readily hydrolyzed in water and is thermally labile (TEPP decomposes at 170°C). Care must be taken to minimize loss during GC analysis and during sample preparation. Identification of bad standard lots is difficult since the electron impact (EI) mass spectrum of TEPP is nearly identical to its major breakdown product, triethyl phosphate.

4.5.2 The water solubility of Dichlorvos (DDVP) is 10g/L at 20°C, and recovery is poor from aqueous solution.

4.5.3 Naled is converted to Dichlorvos (DDVP) on column by debromination. This reaction may also occur during sample workup. The extent of debromination will depend on the nature of the matrix being analyzed. The analyst must consider the potential for debromination when Naled is to be determined.

4.5.4 Trichlorfon rearranges and is dehydrochlorinated in acidic, neutral, or basic media to form Dichlorvos (DDVP) and hydrochloric acid. If this method is to be used for the determination of organophosphates in the presence of Trichlorfon, the analyst should be aware of the possibility of rearrangement to Dichlorvos to prevent misidentification.

4.5.5 Demeton (Systox) is a mixture of two compounds; O,O-diethyl O-[2-(ethylthio)ethyl]phosphorothioate (Demeton-O) and O,O-diethyl S-[2-(ethylthio)ethyl]phosphorothioate (Demeton-S). Two peaks are observed in all the chromatograms corresponding to these two isomers. It is recommended that the early eluting compound (Demeton-S) be used for quantitation.

4.5.6 Dioxathion is a single-component pesticide. However, several extra peaks are observed in the chromatograms of standards. These peaks appear to be the result of spontaneous oxygen-sulfur isomerization. Because of this, Dioxathion is not included in composite standard mixtures.

4.5.7 Merphos (tributyl phosphorotrithioite) is a single-component pesticide that is readily oxidized to its phosphorotrithioate (Merphos oxone). Chromatographic analysis of Merphos almost always results two peaks (unoxidized Merphos elutes first). As the relative amounts of oxidation of the sample and the standard is probably different, quantitation based on the sum of both peaks may be most appropriate.

4.5.8 Retention times of some analytes, particularly Monocrotophos may increase with increasing concentrations in the injector. Analysts should check for retention time shifts in highly contaminated samples.

4.5.9 Many analytes will degrade on reactive sites in the chromatographic system. Analysts must ensure that injectors and splitters are free from contamination and are silanized. Columns should be installed and maintained properly.

4.5.10 Performance of chromatographic systems will degrade with time. Column resolution, analyte breakdown and baselines may be improved by column washing. Oxidation of columns is not reversible.

4.6 NP Detector interferences: Triazine herbicides, such as Atrazine and Simazine, and other nitrogen-containing compounds may interfere.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis.

5.2 See also SOP S02 – Compressed Gas Cylinder Handling
SOP S03 – Spill Control Policy

6.0 APPARATUS and EQUIPMENT

6.1 1L amber bottles for sampling aqueous samples. Wide mouth jars of various sizes for sampling solids.

6.2 Autosampler Vials – glass with crimp cap

6.3 Standard laboratory glassware is used. See SOP O05 and O51 for extraction glassware.

6.4 GAS CHROMATOGRAPH -- Analytical system complete with temperature programmable GC suitable for use with capillary columns and all required accessories including syringes, analytical columns, gases, detector and data . All results are reported from the primary column and confirmed using the secondary column, unless analytical conditions and quality control samples indicate that the secondary column results are more accurate. See ESB SOP Q20 for details. If in any case the secondary column is used for quantification, the analyst must document reasoning for doing so.

6.4.1 Column 1 (Primary detector-NPD) --30m long x 0.25mm I.D. (Equity) SPB-5 bonded fused silica column, 0.25μm film thickness. Helium carrier gas flow is established at 1mL/min linear velocity. The injection volume is 2μL splitless pulsed injection with purge flow to split vent at 30mL/min for 0.75 minutes. Initial pulse is 45psi for 0.5min. The injector temperature must be 250°C and the detector temperature 330°C.

GC Conditions:
Initial temp: 40°C
Hold1: 0 min.
Ramp1: 25°C/min.
Temp1: 165°C
Second Hold: 5 min.
Ramp2: 5°C/min.
Temp2: 290°C
Hold2: 0 min.
Constant flow: 2mL/min.

Detector -- Nitrogen-phosphorus (NPD)
Air flow = 55mL/min H_2 flow = 3.3mL/min.
Helium make up = 3mL/min

6.4.2 Column 2 (Confirmation detector-MSD) - 30m long x 0.25mm I.D. (Equity) SPB-5 bonded fused silica column, 0.25μm film thickness. Helium carrier gas flow is established at 40mL/min linear velocity. The injection volume was 2μL. The injector temperature must be 250°C and the detector temperature was 330°C.

6.4.3 Detector – Mass Spectrometer scanning 35-450 m/e EM = 82 + Autotune Setting Threshold 250. Aux 2 = 300°C. Solvent delay = 2.0 min.

7.0 REAGENTS and CONSUMABLE MATERIALS

7.1 Solvents: Acetone, methylene chloride, methyl tert.-butyl ether (MTBE)

7.2 Reagent Water- Reagent water (Nanopure) is defined as a water that is reasonably free of contamination that would prevent the determination of any analyte of interest.

7.3 Stock Standard Solutions- Stock standard solutions are purchased as certified solutions. Solutions are stored in sealed vials, protected from light, at 4°C. *Manufacturer expiration dates are observed.* Stock standards will be replaced sooner if comparison with QC samples indicate a problem. Two sources are purchased, one for calibration standards, and the other as a Lab Control and Matrix Spike source.

7.4 Working Standard Solutions- The following solutions are stored at 4°C (Intermediate standards) and *-10°C* (Working standards) for up to *6* months. Standards will be replaced sooner if comparison with QC samples indicate a problem.

7.4.1 Internal Standard Solution- The internal standard solution is a 500ppm solution of Triphenylphosphate (TPP) purchased from Ultra Scientific. No dilution is made. Spike 8µL of the 500ppm stock Internal Standard solution into 1mL of sample extract for a final concentration of *4ppm* in the extract.

7.4.2 Surrogate Standard Solution- The surrogate standard solution is a 250ppm solution of *1,3-dimethyl-2-nitrobenzene* purchased from Ultra Scientific. *Manufacturer expiration dates are observed* and the standard should be replaced when ongoing QC indicates a problem. No dilution is made. Samples are spiked with 100µL of this 250ppm standard, which results in a concentration of *25ppb* in the sample and *5000ppb* in the extract.

7.4.3 Calibration Standards (primary source)– A five-point calibration is required for a linear (first order) model, six point calibration for a quadratic (second order) model, and seven point for a polynomial (third order) model. Six standards are purchased from Ultra Scientific.

SPM-824:

Azinphos Methyl	Diazinon	Fenthion	Phorate
Bolstar	Dichlorvos	Merphos	Ronnel
Chlorpyrifos	Disulfoton	Methyl Parathion	Stirofos
Coumaphos	Ethoprop	Mevinphos	Tokuthion
Demeton	Fensulfothion	Naled	Trichloronate

SPM-834:

Dimethoate	Malathion	Parathion	TEPP
EPN	Monocrotophos	Sulfotepp	

SPM-844:

Azinphos Ethyl	Dioxathion	Leptophos	Phosphamidon
Carbophenothion	Ethion	Phosmet	Terbuphos
Chlorfenvinphos	Famphur		

SPM-854:

Aspon	Dichlofenthion	Fenitrothion	Thionazin
Chlorpyrifos	Methyl Dicrotophos	Fonofos	Trichlorfon
Crotoxyphos			

EPA-1176A: Atrazine
NPM-107A: Simzine

7.4.3.1 Intermediate Calibration Standard: Dilute the following amounts to 10mL with MtBE in a 10mL volumetric flask.

Ultra #	Stock Concentration (ppm)	Amount (mL)	Intermediate Concentration (ppm)
SPM-824	200	2.0	40
SPM-834	200	2.0	40
SPM-844	200	2.0	40
SPM-854	200	2.0	40
EPA-1176A	1000	0.4	40
NPM-107A	1000	0.4	40

7.4.3.2 Working Calibration Standard: Seven Calibration Standards are made by diluting the following amounts of 40ppm Intermediate Standard in autosampler vials as follows:

Working Concentration (ppm)	Amount of Intermediate (µL)	Amount of MtBE (µL)
0.8	20	960
2.0	50	930
4.0	100	880
5.0	125	855
7.0	175	805
8.0	200	780
10.0	250	730

Add 20µL of 250ppm surrogate standard (*5ppm*) and 8µL of 500ppm internal standard (*4ppm*) to each calibration standard.

7.4.3.3 Reporting Limit Standard: Dilute 20µL of 40ppm Intermediate Standard into 960µL of MtBE for a final concentration of 0.8ppm. Also add 20µL of 250ppm surrogate standard and 8µL of 500ppm internal standard.

7.4.3.4 Continuing Calibration Verification Standard: Dilute 100µL of 40ppm Intermediate Standard into 880µL of MtBE for a final concentration of 4.0ppm. Also add 20µL of 250ppm surrogate standard and 8µL of 500ppm internal standard.

7.4.4 Lab Control/Matrix Spike Standard (second source)– Four standards are purchased from Protocol.

8140-A-1000:

Chlorpyrifos	Diazinon	Disulfoton	Fensulfothion
Coumaphos	Naled	Fenchlorphos	Fenthion

Demeton	Dichlorvos		
8141-A-1000:			
Azinphos Ethyl	Dioxathion	Ethyl Parathion	Malathion
Carbophenthion	EPN	Famphur	Sulfotepp
Chlorfenvinfos	Ethion	Monocrotophos	Turbofos
Demethoate			
8141-B-1000:			
Aspon	Dicrotophos	Fenitrothion	Phosphamidon
Chlorpyrifos Methyl	Fonofos	Phosmet	TEPP
Crotoxyphos	Trichlofon	Leptophos	Thionazin
Dichlorfenthion			
8141-D-1000:			
Atrazine	Simazine		

7.4.4.1 Working Spiking Standard: Dilute the following amounts to 50mL with MtBE in a 50mL volumetric flask.

Protocol #	Stock Concentration (ppm)	Amount (mL)	Working Concentration (ppm)
8140-A-1000	1000	1.0	20
8141-A-1000	1000	1.0	20
8141-B-1000	1000	1.0	20
8141-D-1000	1000	1.0	20

Spike 1mL of the 20ppm spiking solution into reagent water for aqueous samples, sodium sulfate for non-aqueous samples, or sample for MS. The final concentration is 20ppb in the sample and 4000ppb in the extract.

7.4.4.2 Initial Calibration Verification Standard: Dilute 200µL of 20ppm spiking solution into 780µL of MtBE for a final concentration of 4.0ppm. Also add 20µL of 250ppm surrogate standard and 8µL of 500ppm internal standard.

8.0 SAMPLE COLLECTION, PRESERVATION, and STORAGE

8.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed; however, the bottle must not be pre-rinsed with sample before collection.

8.2 Sample Preservation and Storage

8.2.1 If residual chlorine is present in aqueous samples, add 80mg of sodium thiosulfate per liter of sample to the sample bottle prior to collecting the sample.

8.2.2 After the sample is collected in a bottle containing preservative(s), seal the bottle and shake vigorously for 1 min.

8.2.3 The samples must be iced or refrigerated at 4°C away from light from the time of collection until extraction. Samples must be extracted within 7 days when stored under these conditions.

8.2.4 *Immediately prior to extraction, aqueous samples are adjusted to a pH of 7 by adding 50mL of Phosphate buffer.*

8.3 Extract Storage -- Extracts should be stored at 4°C away from light. A 40-day maximum extract storage time is recommended.

9.0 CALIBRATION

9.1 A 5-7 point calibration is used. Prepare standards according to recipes in section 7.4.3.

9.2 Tabulate response (peak height or area) against concentration for each compound and internal standard. Calculate the response factor (RF) for each analyte and surrogate using Equation 1.

Equation 1

$$RF = \frac{(As)(Cis)}{(Ais)(Cs)}$$

where:
As = Response for the analyte.
Ais = Response for the internal standard.
Cis = Concentration of the internal standard (µg/L).
Cs = Concentration of the analyte to be measured (µg/L).

9.3 If the RF value over the working range is constant (20% RSD or less) the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios (A_s/A_{is}) vs. C_s. A linear curve must have a correlation coefficient, $r \geq 0.99$. A nonlinear curve must have a coefficient of determination, $r^2 \geq 0.99$.

9.3.1 The above criteria applies to all analytes that appear on the LIMS reporting lists.

9.3.2 If due to the long list of compounds every analyte cannot meet one of the above criteria a calibration can be accepted if the mean of the RSD values of all analytes in the calibration is $\leq 20\%$. If the mean criteria is used instead of individual %RSD or coefficients, reportable results must be qualified for analytes outside 20% criteria.

9.4 Calibration checks are analyzed at the beginning and end of every run and every 12 hours. The Initial Calibration Verification Standard is analyzed after the calibration for

validation. The continuing calibration check is analyzed every 12 hours and at the end of the run.

9.4.1 ICV results must be within 15% of the expected value for each target analyte *or the average of all the analyte responses must be within 15% of the expected value. If average criteria is used, all reportable results for target analytes must be qualified if that analyte was outside 15% criteria. See Q06 for additional details.*

9.4.2 *At least 85%* of all target analytes in each CCV must be within 15% of the expected value. If this is not achieved, samples must be reanalyzed or qualified. See Q06 for details.

9.5 A standard at the reporting limit is analyzed at the end of the run. *This is used to evaluate instrument sensitivity should calibration checks show a low bias. This standard should have a signal greater then the method blank.*

10.0 QUALITY CONTROL

Note: See also ESB SOP Q01 for general QC requirements

10.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, determination of surrogate compound recoveries. Monitoring internal standard peak area or height in each sample and blank, analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and QC samples.

10.2 Laboratory Reagent Blanks. Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed per matrix type. If within the retention time window of any analyte of interest the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Sample results at or above the reporting limit must be accompanied with a qualifier if the method blank exceeds a concentration greater than 1/10 of the measured concentration of the sample. Blank results below the reporting limit are considered to be ND and will not require a qualifier.

10.3 Laboratory Demonstration of Capability.

10.3.1 Prior to initial analysis of samples or when a significant change is made to the method, a Demonstration of Capability Study is performed. Select a concentration 10-50 times the estimated MDL or midrange and analyze 4 aliquots. For aqueous studies spike the selected concentration in reagent water and for non-aqueous studies spike in sodium sulfate. Analyze each aliquot according to procedures beginning in Sect. 11. A cell change may be performed in lieu of an IDOC (See ESP SOP Q01).

10.3.2 For each analyte the mean recovery value of four of these samples must fall in the proper acceptance range. For aqueous samples: 70 - 130% with a RSD $\leq$ 20%. For poorly performing analytes, the criteria from Table 6 of EPA Method 8141B, Rev 2A is utilized. For analytes not found in Table 6, the range is set to equal that

of a similar poor performing analyte. For nonaqueous samples; 70 - 130% with a RSD ≤ 20%. For poorly performing analytes, the criteria from Table 7 of EPA Method 8141B, Rev 2A is utilized. For analytes not found in Table 7, the range is set to equal that of a similar poor performing analyte. Since our data is compared to performance data developed from single-laboratory data, certain analytes may be outside the limits however, the majority should be within the acceptance limits (per 8000B sec 8.4.7.)

10.4 Assessing Surrogate Recovery

10.4.1 Acceptance ranges are generated from historical data and updated periodically in Element. When surrogate recovery from a sample or method blank is out of acceptance criteria, check calculations to locate possible errors, fortifying solutions for degradation, contamination, and instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract, or re-extract the sample (if sample is available).

10.4.2 If a blank extract reanalysis/re-extract fails the recovery criterion, the problem must be identified and corrected before continuing.

10.4.3 If sample reanalysis/re-extract meets the surrogate recovery criterion, report only this data. If sample reanalysis/re-extraction continues to fail the recovery criterion, report all data for that sample with the proper qualifier.

10.5 Assessing the Internal Standard

10.5.1 When using the internal standard calibration procedure, the analyst monitors the IS response of all samples during each analysis day. The IS response for any sample chromatogram should not deviate by more than 50% from the average area calculated during calibration.

10.5.2 If >50% deviation occurs with an individual extract, optimize instrument performance and inject a second aliquot of that extract.

10.5.2.1 If the reinjected aliquot produces an acceptable internal standard response report results for that aliquot.

10.5.2.2 If a deviation of greater than 50% is obtained for the reinjected extract, extraction of the sample is repeated, provided the sample is still available. Otherwise, report results obtained from the re-injected extract, with the proper qualifier.

10.6 Assessing Laboratory Performance - Laboratory Fortified Blank

10.6.1 The laboratory analyzes one laboratory fortified blank (LFB) sample with every twenty samples per matrix type or one per sample set (all samples extracted within a 24-h period) whichever is greater. A laboratory fortified blank duplicate may also be analyzed. Acceptance ranges are generated from historical data and updated periodically in Element. If recovery of *>20%* of the analytes falls outside the control limits, that LFB is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

10.7 Assessing Analyte Recovery - Laboratory Fortified Sample Matrix

10.7.1 The laboratory adds a known concentration of analytes to a minimum of 5% of the routine samples per matrix type or one sample concentration per set, whichever is greater. A laboratory fortified sample matrix duplicate or a sample duplicate may be analyzed as well.

10.7.2 Acceptance ranges are generated from historical data and updated periodically, or a range of 70-130% for aqueous samples, and *50-150%* for solid and sludge samples is used until historical data can be generated. If recovery of *>20%* of the analytes falls outside the control limits, that LFSM is judged out of control, and a follow up must be performed on that sample. The RPD must also pass based on historical limits, or ≤40% until enough date is acquired to generate these limits.

10.8 An MDL study is completed upon initial set-up of the method and whenever major equipment or procedural changes are made. Standards are spiked at 2.5 – 5 times the estimated MDL and a minimum of seven replicates is analyzed. See QA Manual for calculation. Results must be $\leq$ 2.2 times the reporting limit.

10.9 Analyst Demonstration of Capability:

10.9.1 Initial Demonstration of Capability:

10.9.1.1 Prior to initial analysis of samples or when a significant change is made to the method, a Demonstration of Capability Study is performed. Select a concentration 10-50 times the estimated MDL or midrange and analyze 4 aliquots. For aqueous studies spike the selected concentration in reagent water and for non-aqueous studies spike in sodium sulfate. Analyze each aliquot according to procedures beginning in Sect. 11. A cell change may be performed in lieu of an IDOC (See ESP SOP Q01).

10.9.1.2 For each analyte the mean recovery value of four of these samples must fall in the proper acceptance range. For aqueous samples: 70 - 130% with a RSD $\leq$ 20%. For poorly performing analytes, the criteria from Table 6 of EPA Method 8141B, Rev 2A is utilized. For analytes not found in Table 6, the range is set to equal that of a similar poor performing analyte. For nonaqueous samples; 70 - 130% with a RSD $\leq$ 20%. For poorly performing analytes, the criteria from Table 7 of EPA Method 8141B, Rev 2A is utilized. For analytes not found in Table 7, the range is set to equal that of a similar poor performing analyte. Since our data is compared to performance data developed from single-laboratory data, certain analytes may be outside the limits however, the majority should be within the acceptance limits (per 8000B sec 8.4.7.)

10.9.2 Demonstration of Continuing Proficiency: On an annual basis, analysts must turn in valid LCS data from four consecutive LCS samples, results from a successful Performance Evaluation Study, or a valid cell change form (See ESP SOP Q01). If four consecutive LCS samples are used, percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 40%. Performance Evaluation Studies are performed *twice* a year.

11.0 PROCEDURE

11.1 Extraction: See SOP O05, O03, and O51

11.2 Gas Chromatography

11.2.1 Sect. 6.3 summarizes the recommended operating conditions for the gas chromatograph.

11.2.2 The system may be calibrated daily. The standards and extracts must be in MTBE.

11.2.3 Add 8µL of the internal standard solution to the sample extract, QC, and calibration standards, seal, and shake to distribute the internal standard.

11.2.4 Inject 2µL of the sample extract. Record the resulting peak size in area units.

11.2.5 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

11.2.6 Watch for possible carry over. Studies have shown that no carry over occurs when a blank is run after the high calibration standard (10ppm). Rerun samples at or above the reporting limit following a sample at this concentration. Be mindful of carry over that may also occur during the extraction process.

12.0 IDENTIFICATION OF ANALYTES

12.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits to the retention time of a standard compound, then identification is considered positive.

12.2 Retention time windows - The computer software allows the setting of retention time windows. This is used to make identifications, unless experience shows that the window requires adjustment. Three times the standard deviation of a retention time within a period of 72 hours can be used to calculate a suggested window size for a compound (or a percentage RT window approximating the 3_{n-1}); however, the experience of the analyst weighs heavily in the interpretation of chromatograms. If this window is too narrow, the lab uses ±30sec with MS confirmation. See Method 8000 section 7.6 for retention time study details.

12.3 Identification requires expert judgement when sample components are not resolved chromatographically. When peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak on a chromatogram, appropriate alternative techniques to help confirm peak identification, are employed. See EPA method 8141B section 3.8 for a discussion concerning analytical difficulties for specific analytes. Coeluding analytes can be identified separately by the MS. If both analytes appear to be present based on the MS evaluation, quantification is done by applying a ratio, derived from the MS response, to the sample result.

13.0 CALCULATIONS

13.1 Calculate analyte concentrations in the sample from the response for the analyte using the calibration procedure described in Sect. 9.

13.2 If the internal standard calibration procedure is used, calculate the concentration (C) in the sample using the response factor (RF) determined in Sect. 9.2 and Equation 2, or determine sample concentration from the calibration curve.

Equation 2

$$C\ (\mu g/L) = \frac{[(A(S)(I(s)]}{[A(is)(RF)Vo)]}$$

where:
A(s) = Response for the parameter to be measured.
A(is) = Response for the internal standard.
I(s) = Amount of internal standard added to each extract (μg).
Vo = Volume of water extracted (L).

13.3 LIMS Calculation

13.3.1 Aqueous samples: 1L of sample is concentrated down to 5mL.

13.3.1.1	LIMS Initial	volume: 1000 mL		units: μg/L
13.3.1.2	LIMS Final	volume: 5 mL		units: μg/L
13.3.1.3	Run Data	multiplier: 5	misc: 200	units: μg/L

Data in the run is calculated with calibration standards at the ppm level however results are displayed as ppb. This 1000 times factor plus a multiplier of 5 takes into account the prep concentration so that results in the run are displayed as final results. When data is transferred to LIMS, the misc. field converts results back to raw results so that LIMS can apply the initial and final concentration value. Since the miscellaneous field also affects RL's and MDL's, the analyst must manually adjust these limits back in LIMS by dividing the limits by 200.

13.3.2 Solid samples: 15g of sample is concentrated down to 5mL.

13.3.2.1	LIMS Initial	volume: 15g		units: mg/L
13.3.2.2	LIMS Final	volume: 5mL		units: mg/kg
13.3.2.3	Run Data	multiplier: 0.333	misc: 3	units: mg/kg

13.3.3 Sludge samples: 5g of sample is concentrated down to 5mL.

13.3.3.1	LIMS Initial	volume: 5g		units: mg/L
13.3.3.2	LIMS Final	volume: 5mL		units: mg/kg
13.3.3.3	Run Data	multiplier: 1	misc: 1	units: mg/kg

So that data in the run can display the final result the multiplier is used to account for the preparation concentration. Since LIMS will apply the concentration factor again based on the initial and final volumes, the miscellaneous field takes the multiplier back out before data is transferred to LIMS. Since the miscellaneous field also affects RL's and MDL's, the analyst must manually adjust these limits back in the LIMS by multiplying the solid limits by 0.333.

14.0 CORRECTIVE ACTION FOR OUT OF CONTROL / UNACCEPTABLE DATA

14.1 See SOP Q06 – Corrective Action

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 See SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S06 – Disposal of Chlorinated Solvents
SOP S07 – Pollution Prevention

16.0 METHOD PERFORMANCE

16.1 See Method 8141 Tables 5,6,7. Our intralaboratory generated data is expected to achieve similar results. Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QC Office.

REFERENCES

EPA 8141B SW- 846 Revision 2A, 1999 Methods for the Chemical Analysis of Waters and Wastes.

Note: All *italicized items* are an indication of a variation from the method.

Approved by Susann K. Thomas Date 9/27/04

TABLE 7. PERCENT RECOVERY OF 27 ORGANOPHOSPHATES BY SOXHLET EXTRACTION

Compound	Percent Recovery		
	Low	Medium	High
Azinphos methyl	156	110 +/- 6	87
Bolstar	102	103 +/- 15	79
Chlorpyrifos	NR	66 +/- 17	79
Coumaphos	93	89 +/- 11	90
Demeton	169	64 +/- 6	75
Diazinon	87	96 +/- 3	75
Dichlorvos	84	39 +/- 21	71
Dimethoate	NR	48 +/- 7	98
Disulfoton	78	78 +/- 6	76
EPN	114	93 +/- 8	82
Ethoprop	65	70 +/- 7	75
Fensulfonthion	72	81 +/- 18	111
Fenthion	NR	43 +/- 7	89
Malathion	100	81 +/- 8	81
Merphos	62	53	60
Mevinphos	NR	71	63
Monocrotophos	NR	NR	NR
Naled	NR	48	NR
Parathion, ethyl	75	80 +/- 8	80
Parathion, methyl	NR	41 +/- 3	28
Phorate	75	77 +/- 6	78
Ronnel	NR	83 +/- 12	79
Sulfotep	67	72 +/- 8	78
TEPP	36	34 +/- 33	63
Tetrachlorvinphos	50	81 +/- 7	83
Tokuthion	NR	40 +/- 6	89
Trichloroate	56	53	53

NR = Not recovered.

Standard Operating Procedure
Edward S. Babcock & Sons
Date Effective: 04/15/06

METHOD #: 8081A
TITLE: ORGANOCHLORINE PESTICIDES BY GAS CHROMATOGRAPHY
INSTRUMENTATION: Gas Chromatography

1.0 SCOPE AND APPLICATION

1.1 Method 8081 is used to determine the concentrations of various organochlorine pesticides in extracts from solid and liquid matrices, using fused-silica, open-tubular, capillary columns with electron capture detectors (ECD).. The compounds listed below are determined by a dual-column analysis system. Analytes in parenthesis are not certified by NELAP.

1.2 Aqueous Range: Single peak pesticides: 0.01ppb – 5ppb
Chlordane/Toxaphene: 0.1/1ppb – 40ppb
Nonaqueous Range: Solid single peak pesticides: 2 ug/kg – 150 ug/kg
Sludge single peak pesticides: 6 ug/kg – 500 ug/kg
Solid Chlordane/Toxaphene: 7/40 ug/kg – 1333 ug/kg
Sludge Chlordane/Toxaphene: 20/100 ug/kg – 4000 ug/kg
Low end of range depends on the RL. The pesticide RL varies among analytes. See attached LIMS information for individual RL's.

Compound	CAS Registry No.
Aldrin	309-00-2
alpha-BHC	319-84-6
beta-BHC	319-85-7
gamma-BHC - Lindane	58-89-9
delta-BHC	319-86-8
(Chlorobenzilate)	510-15-6
(alpha-Chlordane)	5103-71-9
(gamma-Chlordane)	5103-74-2
Chlordane - not otherwise specified	57-74-9
(DBCP)	96-12-8
4,4'-DDD	72-54-8
4,4'-DDE	72-55-9
4,4'-DDT	50-29-3
(Diallate)	2303-16-4
Dieldrin	60-57-1
Endosulfan I	959-98-8
Endosulfan II	33213-65-9
Endosulfan sulfate	1031-07-8
Endrin	72-20-8
Endrin aldehyde	7421-93-4
(Endrin ketone)	53494-70-5
Heptachlor	76-44-8
Heptachlor epoxide	1024-57-3

Hexachlorobenzene	118-74-1
Hexachlorocyclopentadiene	77-47-4
(Isodrin)	465-73-6
(Kepone)	143-50-0
Methoxychlor	72-43-5
Toxaphene	8001-35-2

Analytes in parenthesis are not certified by NELAP

1.3 Several multi-component mixtures (i.e., Chlordane and Toxaphene) are listed as target analytes. When samples contain more than one multi-component analyte, a higher level of analyst expertise is required to attain acceptable levels of qualitative and quantitative analysis. The same is true of multi-component analytes that have been subjected to environmental degradation or degradation by treatment technologies. These result in "weathered" multi-component mixtures that may have significant differences in peak patterns than those of standards.

1.4 The dual-column option is used. This option allows a hardware configuration of two analytical columns joined to a single injection port. The option allows one injection to be used for dual-column analysis. Analysts are cautioned that the dual-column option may not be appropriate when the instrument is subject to mechanical stress, many samples are to be run in a short period, or when contaminated samples are analyzed.

1.5 This method is restricted to use by, or under the supervision of, analysts experienced in the use of gas chromatographs (GC) and skilled in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.6 Extracts suitable for analysis by this method may also be analyzed for organophosphorus pesticides (Method 8141). Some extracts may also be suitable for triazine herbicide analysis, if low recoveries (normally samples taken for triazine analysis must be preserved) are not a problem.

1.7 The following compounds may also be determined using this method. Analytes in parenthesis are not certified by NELAP

Compound	CAS Registry No.
(Alachlor)	15972-60-8
(Captafol)	2425-06-1
(Chloroneb)	2675-77-6
(Chloropropylate)	5836-10-2
Chlorothalonil	1897-45-6
(DCPA)	1861-32-1
(Dichlone)	117-80-6
(Dicofol)	115-32-2
(Etridiazole)	2593-15-9
(Halowax-1000)	58718-66-4
(Halowax-1001)	58718-67-5
(Halowax-1013)	12616-35-2
(Halowax-1014)	12616-36-3
(Halowax-1051)	2234-13-1
(Halowax-1099)	39450-05-0
(Mirex)	2385-85-5
(Nitrofen)	1836-75-5

(PCNB)	82-68-8
(Permethrin) (cis + trans)	52645-53-1
(Perthane)	72-56-0
Propachlor	1918-16-7
(Strobane)	8001-50-1
(trans-Nonachlor)	39765-80-5
(Trifluralin)	1582-09-8

2.0 SUMMARY OF METHOD

2.1 A measured volume or weight of sample (approximately 1 L for liquids, 5g for sludge, or 15g for solids) is extracted using the appropriate matrix-specific sample extraction technique.

2.2 Liquid samples are extracted at neutral pH with methylene chloride using Method 3510 (separatory funnel) to a final volume of 10mL.

2.3 Solid samples are extracted with hexane-acetone (1 – 1) using Method 3550 (ultrasonic extraction) to a final volume of 5 mL.

2.4 A variety of cleanup steps may be applied to the extract, depending on the nature of the matrix interferences and the target analytes. Florisil cleanups are performed on samples that appear dirty or have historically required one.

2.5 After cleanup, the extract is analyzed by injecting a 2 uL sample into a gas chromatograph with a narrow- or wide-bore fused-silica capillary column and electron capture detector (GC/ECD).

3.0 INTERFERENCES

3.1 Sources of interference in this method can be grouped into three broad categories.

3.1.1 Contaminated solvents, reagents, or sample processing hardware.

3.1.2 Contaminated GC carrier gas, parts, column surfaces, or detector surfaces.

3.1.3 Compounds extracted from the sample matrix to which the detector will respond.

3.1.4 Interferences co-extracted from the samples will vary considerably from waste to waste. While general cleanup techniques are referenced or provided as part of this method, unique samples may require additional cleanup approaches to achieve desired degrees of discrimination and quantitation.

3.2 Interferences by phthalate esters introduced during sample preparation can pose a major problem in pesticide determinations.

3.2.1 These materials may be removed prior to analysis using Method 3640 (Gel Permeation Cleanup) or Method 3630 (Silica Gel Cleanup).

3.2.2 Common flexible plastics contain varying amounts of phthalate esters which are easily extracted or leached from such materials during laboratory operations.

3.2.3 Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled.

3.2.4 Interferences from phthalate esters can best be minimized by avoiding contact with any plastic materials and checking all solvents and reagents for phthalate contamination. Exhaustive cleanup of solvents, reagents and glassware may be required to eliminate background phthalate ester contamination.

3.3 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with hot water, and rinses with tap water and organic-free reagent water. Drain the glassware and dry it, or rinse with the appropriate solvent (hexane for solids, methylene chloride for liquids). Store dry glassware in a clean environment.

3.4 The presence of elemental sulfur will result in broad peaks that interfere with the detection of early-eluting organochlorine pesticides. Sulfur contamination should be expected with sediment samples. Method 3660 is suggested for removal of sulfur. Since the recovery of Endrin aldehyde (using the TBA procedure) is drastically reduced, this compound must be determined prior to sulfur cleanup.

3.5 Waxes, lipids, and other high molecular weight materials can be removed by Method 3640 (gel-permeation cleanup).

3.6 Other halogenated pesticides or industrial chemicals may interfere with the analysis of pesticides. Certain co-eluting organophosphorus pesticides are eliminated by Method 3640 (gel-permeation cleanup - pesticide option). Co-eluting chlorophenols may be eliminated by using Method 3630 (silica gel), Method 3620 (Florisil), or Method 3610 (alumina). Polychlorinated biphenyls (PCBs) also may interfere with the analysis of the organochlorine pesticides. The problem may be most severe for the analysis of multicomponent analytes such as Chlordane, Toxaphene, and Strobane. If PCBs are known or expected to occur in samples, the analyst should consult Methods 3620 and 3630 for techniques that may be used to separate the pesticides from the PCBs.

3.7 The following compounds may coelute using the dual-column analysis scheme. In general, the HP-5 column resolves fewer compounds that the HP-1701.

ZB-1701 Permethrin/Heptachlor epoxide
Endosulfan I/alpha-Chlordane
Perthane/Endrin
Endosulfan II/Chloropropylate/Chlorobenzilate
4,4'-DDT/Endosulfan sulfate
Methoxychlor/Dicofol

SPB-608 Chlorothalonil/beta-BHC
delta-BHC/DCPA/Permethrin
alpha-Chlordane/trans-Nonachlor

Nitrofen, Dichlone, Carbophenothion, Dichloran exhibit extensive peak tailing on both columns. Simazine and Atrazine give poor responses on the ECD detector. Triazine compounds should be analyzed using Method 8141 (NPD option).

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph - An analytical system complete with gas chromatograph suitable for on-column and split-splitless injection and all required accessories including autosampler, analytical columns, gases, electron capture detectors (ECD), and data system.

4.1.1 Primary Gas Chromatograph: HP 5890.or HP 6890
4.1.2 Detector: Electron Capture Detector.
4.1.3 Turbochrome/ Data Capture
4.1.4 Primary column: ZB-1701 30 m X 0.25 mm ID, 0.25 µm phase thickness.
4.1.5 Confirmatory Detector: Electron Capture Detector.
4.1.6 Confirmatory Column: SPB-608 30m x 0.25mm ID, 0.25 µm thickness.
4.1.7 Column Conditions:

4.1.7.1 Injector Temp: 220°C
4.1.7.2 Detector Temp: 320°C
4.1.7.3 Initial Temp: 120°C
4.1.7.4 Initial Hold: 4 min.
4.1.7.5 Rate: 5°C/min.
4.1.7.6 Final Temp: 280°C
4.1.7.7 Final Hold Time: 4 min
4.1.7.8 He Carrier head pressure set to 180kPa.
4.1.7.9 N_2 Make-up set to 60 mL/min.

All results are reported from the primary column and confirmed using the secondary column, unless analytical conditions and quality control samples indicate that the secondary column results are more accurate. See ESB SOP Q20 for details. If in any case the secondary column is used for quantification, the analyst must document reasoning for doing so.

5.0 REAGENTS

5.1 Reagent grade or pesticide grade chemicals are used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Solvents used in the extraction and cleanup procedures and for standards include n-hexane, methylene chloride, MtBE, and acetone.

5.3 Organic-free reagent water: Nanopure

5.4 STOCK STANDARD SOLUTIONS – Stock standards are purchased from a certified manufacturer. Pesticides are in a 90% Hexane/ 10% Acetone mix. Solutions are stored in sealed vials, protected from light, at 4^{o}C or room temperature. Manufacturer expiration dates are observed until vial is opened. Once vial is opened, standards are kept for up to six months. Stock standards can be replaced sooner if comparison with laboratory fortified blanks, or QC samples indicate a problem.

5.4.1 SURROGATE STANDARD SOLUTION – Decachlorobiphenyl is used for the surrogate standard fortifying solution. Solutions are stored in sealed vials, protected from light, at 4°C. A 2ppm solution is prepared by diluting 200µL of stock into 100 mL of methanol. Solutions are replaced after 6 months or sooner when ongoing QC indicates a problem. Spike samples and QC with 150uL for a final concentration of 20ppb in solid samples, 60ppb in sludges and 0.3ppb in aqueous samples.

5.5 CALIBRATION STANDARDS, LABORATORY FORTIFIED BLANK SOLUTION, LABORATORY FORTIFIED SAMPLE MATRIX SOLUTION - Solutions are stored in sealed vials, protected from light, at -10°C. Intermediate Standard Solutions are replaced after 6 months or sooner if QC samples indicate a problem. Working Standards are prepared fresh.

5.5.1 Calibration standards are made from a source separate from the LFB and LFSM. The lowest calibration standard is at or below the method reporting limit. The rest of the calibrators bracket the expected working range of the samples. Calibration concentrations are 1ppb, 25ppb, 50ppb, 100ppb, 250ppb, and 500ppb for single peak compounds and 100ppb, 500ppb, 1000ppb, 2000ppb, 3000ppb, and 4000ppb for multipeak compounds.

5.5.1.1 Single Peak Compounds:

5.5.1.1.1 Intermediate Standard: 1000ppb pesticide standard diluted from individual stock standard vials into 90% hexane/10% acetone.

5.5.1.1.2 Working Standards: Dilute the following aliquots of 1000ppb Intermediate standard including surrogate into 10 mL of hexane: 10uL, 250uL, 500uL, 1000uL, 2.5 mL, and 5.0mL.

5.5.1.2 Multiple Peak Compounds:

5.5.1.2.1 Intermediate Standard: (Analyte 4ppm/ Surrogate 0.2 ppm) Standard diluted by taking 0.4 mL of analyte stock at 100ppm and 2 uL of surrogate at 100ppm into 10 mL of hexane.

5.5.1.2.2 Working Standards: Dilute the following aliquots of Intermediate standard into 10 mL of hexane: 1 mL, 0.75 mL, 0.5 mL, 0.25 mL, 0.125 mL, and 0.025 mL.

5.5.2 LFB, MS, ICV

5.5.2.1 Pesticides Spiking Standard (1.25ppm)- Dilute 0.5 mL of 250ppm stock standard into 100 mL of methanol. Spike 400 uL into 1L of sample or Nanopure for a final concentration of 50 ppb in the extract. Spike 200 uL into 5g or 15g of sample or blank sand for a final concentration of 50ppb in the extract.

5.5.2.2 Chlordane or Toxaphene (10ppm)- Dilute 1 mL of 1000ppm stock standard into 100 mL of methanol. Spike 200 uL into 1L of sample or Nanopure for a final concentration of 2000 ppb in the extract. Spike 1 mL into 5g or 15g of sample or blank sand for a final concentration of 2000ppb in the extract.

5.5.3 Endrin/DDT Degradation Check (50ppb/100ppb) – Dilute 500uL of stock standard (1ppm/2ppm) into 10 mL of 90% hexane/10% acetone.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Grab samples are collected in glass containers. Conventional sampling practices are followed; however, the bottle must not be prerinsed with sample before collection.

6.2 SAMPLE PRESERVATION

6.2.1 Samples are taken from nonchlorinated sources.

6.2.2 Samples are iced or refrigerated at 4-C from the time of collection until extraction.

6.2.3 Preservation study results indicate that most of the target analytes present in the samples are stable for 7 days for aqueous samples and 14 days for nonaqueous sample when stored under these conditions.

6.3 Extracts are stored under refrigeration in the dark and analyzed within 40 days of extraction.

7.0 PROCEDURE

7.1 Sample extraction - In general, water samples are extracted at a neutral pH with methylene chloride using a separatory funnel (Method 3510) SOP O05, Solid samples are extracted with hexane-acetone (1:1 sonication extraction (Method 3550) SOP O51.

7.2 Extract cleanup - Cleanup procedures may not be necessary for a relatively clean sample matrix, but most extracts from environmental and waste samples will require additional preparation before analysis. The specific cleanup procedure used will depend on the nature of the sample to be analyzed and the data quality objectives for the measurements.

7.2.1 If a sample is of biological origin, or contains high molecular weight materials, the use of Method 3640 (GPC cleanup - pesticide option) is recommended. Frequently, one of the adsorption chromatographic cleanups (alumina, silica gel, or Florisil) may also be required following the GPC cleanup.

7.2.2 Method 3610 (alumina) may be used to remove phthalate esters.

7.2.3 Method 3620 (Florisil) may be used to separate organochlorine pesticides from aliphatic compounds, aromatics, and nitrogen-containing compounds.

7.2.4 Method 3630 (silica gel) may be used to separate single component organochlorine pesticides from some interflerants.

7.2.5 Elemental sulfur, which may be present in certain sediments and industrial wastes, interferes with the electron capture gas chromatography of certain pesticides. Sulfur should be removed by the technique described in Method 3660.

7.3 Calibration

7.3.1 The method requires a five point calibration for a linear (first order) model, six point calibration for a quadratic (second order) model, and seven point for a polynomial (third order) model. The lab generally calibrates with 6-7 standards.

7.3.2 A calibration is performed for single–component analytes whenever a CCV indicates a new calibration is needed.

7.3.3 Each run begins with a low level calibration check for each multi-component analyte. This is intended to demonstrate that the pattern is recognizable, the analyst is familiar with the retention times on each column and that if any multi-component were present, it would be detected. A full calibration is performed for each multi-component analyte *detected in the sample*.

7.3.4 Separate calibration standards are used for each multi-component target analyte (e.g., Toxaphene and Chlordane). Analysts evaluate the specific Toxaphene standard carefully. Some Toxaphene components, particularly the more heavily chlorinated components, are subject to dechlorination reactions. As a result, standards from different vendors may exhibit marked differences which could lead to possible false negative results or to large differences in quantitative results.

7.3.5 For calibration verification see section 7.4.3.

7.3.6 A 2 uL injection volume of each calibration standard is used.

7.3.7 Calibration factors - The calibration factor for each analyte at each concentration, the mean calibration factor, and the relative standard deviation (RSD) of the calibration factors, are calculated using the formula below for the calculation of response factors.

7.3.7.1 Calculate the calibration factor for each analyte at each concentration as:

$$CF = \frac{\text{Peak Area (or Height) of the Compound in the Standard}}{\text{Concentration of the Compound Injected}}$$

7.3.7.2 Calculate the mean calibration factor for each analyte as:

$$\text{mean CF} = \bar{CF} = \frac{\sum_{I=1}^{n} CFi}{n}$$

where n is the number of standards analyzed.

7.3.7.3 Calculate the standard deviation (SD) and the RSD of the calibration factors for each analyte as:

$$SD = \left[\frac{\sum_{I=1}^{n} (CFi - \overline{CF})^2}{n-1}\right]^{0.5}$$

$$RSD = \frac{SD}{\overline{CF}} \times 100$$

7.3.8 A linear curve must have a correlation coefficient, $r \geq 0.99$. A nonlinear curve must have a coefficient of determination, $r^2 \geq 0.99$. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range (20% RSD or less), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.3.9 If due to the long list of compounds every analyte cannot meet the above criteria, a calibration can be accepted if the mean of the RSD values of all analytes in the calibration is $\leq$ 20%. If the mean criteria is used instead of individual %RSD or coefficients, reportable results must be qualified for analytes outside 20% criteria.

7.3.10 All calibrations are verified by the analysis of an ICV standard (usually the LCS) made from a noncalibration source. The calibration factor for each single component analyte should not exceed a +/- 15 percent difference from the mean calibration factor calculated for the initial calibration. If this criterion is exceeded for any analyte, calculate the average percent difference across all analytes. If the average of the responses is within the +/- 15% limit then the calibration has been verified. If average criteria is used, all reportable results for target analytes must be qualified if that analyte was outside 15% criteria. See Q06 for additional details.

7.4 Gas chromatographic analysis of sample extracts

7.4.1 The breakdown of DDT and endrin should be measured before samples are analyzed and at the beginning of each 12 hour shift. DDT and endrin are easily degraded in the injection port. Breakdown occurs when the injection port liner is contaminated with high boiling residue from sample injection or when the injector contains metal fittings. Check for degradation problems by injecting a standard containing only 4,4'-DDT and endrin. Presence of 4,4'-DDE, 4,4'-DDD, endrin ketone or endrin indicates breakdown. If degradation of either DDT or endrin exceeds 15%, take corrective action before proceeding with calibration.

Calculate percent breakdown as follows:

$$\%\ \text{breakdown of DDT} = \frac{\text{sum of degradation peak areas (DDD + DDE)}}{\text{sum of all peak areas (DDT + DDE + DDD)}} \times 100$$

$$\%\ \text{breakdown of endrin} = \frac{\text{sum of degradation peak areas(aldehyde+ketone)}}{\text{sum of all peak areas(endrin+aldehyde+ketone)}} \times 100$$

7.4.1.1 Injector maintenance and recalibration should be completed if the breakdown is greater than 15% for either compound.

7.4.2 The same GC operating conditions used for the initial calibration is employed for samples analyses.

7.4.3 The calibration is verified at the beginning of each run by the analysis of a midpoint calibration check for all single-component analytes. A low level calibration check for each multi-component analyte is also injected, see section 7.3.3. Every 10 samples, and at the end of the run, analysts use 2 different concentration mixtures of single-component analytes for calibration verification. If multi-component analytes are detectable in a sample then a calibration was performed with that run and a calibration check for Toxaphene, and/or Chlordane is analyzed at the end of the run to bracket results.

7.4.3.1 The calibration factor for each single component analyte should not exceed a +/- 15 percent difference from the mean calibration factor calculated for the initial calibration. If a non-linear calibration model or a linear model not through the origin has been employed for the initial calibration, consult Sec. 7 of Method 8000 for the specifics of calibration verification.

$$\% \text{ Difference} = \frac{\overline{CF} - CF(V)}{\overline{CF}} \times 100$$

7.4.3.2 *85% or more of all single-component analytes in an individual CCV must fall within the acceptance criteria of +/-15%. All reportable results for target analytes must be qualified if that individual analyte was outside CCV criteria. See Q06 for additional details.* If the results still do not agree, a new calibration curve is prepared.

7.4.3.3 If the average of the responses is not within the +/- 15%, check the instrument operating conditions, if necessary, restore them to the original settings, and inject another aliquot of the calibration verification standard. If the average response still exceeds 15%, a new calibration is performed.

7.4.3.4 A standard at the reporting limit is analyzed at the end of the run or after every 20 samples. This is used to evaluate instrument sensitivity should calibration checks show a low bias. This standard should have a signal greater then the method blank.

7.4.4 Retention time windows - Absolute retention times are used for compound identification. Retention time windows are crucial to the identification of target compounds.

7.4.1.1 Before establishing the retention time windows, make sure the gas chromatographic system is operating within optimum conditions.

7.4.1.2 Retention time window studies are performed as stated in Method 8000 section 7.6. Windows are set per analyte, per analyst discretion. The experience of the analyst weighs heavily in the interpretation of chromatograms.

7.4.5 Each analyte in each standard must fall within its respective retention time window. If not, the gas chromatographic system must either be adjusted so that a second analysis of the standard does result in all analytes falling within their retention time windows, or a new initial calibration is performed and new retention time windows established.

7.4.6 Inject a 2 uL aliquot of the concentrated sample extract. Record the resulting peak height or area.

7.4.7 Tentative identification of an analyte occurs when a peak from a sample extract falls within the absolute retention time window. All results are reported from the primary column and confirmed using the secondary column, unless analytical conditions and quality control samples indicate that the secondary column results are more accurate. See ESB SOP Q20 for details. The primary column for all of

the analytes specified in section 1.1 and 1.6 is column A. If in any case the secondary column is used for quantification, the analyst will document her reasoning for doing so.

7.4.8 When using the external calibration procedure, determine the quantity of each component peak in the sample chromatogram that corresponds to the compounds used for calibration purposes, as follows. Proper quantitation requires the appropriate selection of a baseline from which the peak area or height can be determined.

7.4.8.1 For aqueous samples

$$\text{Concentration (ug/L)} = \frac{(Ax)(Vt)(D)}{(\overline{CF})(Vi)(Vs)}$$

where:

Ax = Area (or height) of the peak for the analyte in the sample.
Vt = Total volume of the concentrated extract (uL).
D = Dilution factor, if the sample or extract was diluted prior to analysis. If no dilution was made, D = 1. The dilution factor is always dimensionless.

$\overline{CF}$ = Mean calibration factor from the initial calibration (area/ng).
Vi = Volume of the extract injected (uL). The injection volume for samples and calibration standards is the same. For purge-and-trap analysis, Vi is not applicable and therefore is set at 1.
Vs = Volume of the aqueous sample extracted in mL. If units of liters are used for this term, multiply the results by 1000.

Using the units specified here for these terms will result in a concentration in units of ng/mL, which is equivalent to ug/L.

7.4.8.2 For non-aqueous samples

$$\text{Concentration (ug/kg)} = \frac{(Ax)(Vt)(D)}{(\overline{CF})(Vi)(Ws)}$$

where Ax, Vt, D, CF, and Vi are the same as for aqueous samples, and

Ws = Weight of sample extracted (g). The wet weight or dry weight may be used, depending upon the specific application of the data. If units of kilograms are used for this term, multiply the results by 1000.

Using the units specified here for these terms will result in a concentration in units of ng/g, which is equivalent to ug/kg.

7.4.8.3 If the responses exceed the calibration range of the system, dilute the extract and reanalyze. Peak height measurements are recommended over peak area integration when overlapping peaks cause errors in area integration.

7.4.9 Each sample analysis must be bracketed with an acceptable initial calibration, calibration verification standard(s), or calibration standards interspersed within the samples. The results from these bracketing standards must meet the calibration verification criteria. When a calibration verification standard fails to meet the QC criteria, all samples that were injected after the last standard that last met the QC criteria must be evaluated to prevent misquantitations and possible false negative results, and re-injection of the sample extracts may be required. However, if the standard analyzed after a group of samples exhibits a response for an analyte that is above the acceptance limit, i.e., > 15%, and the analyte was not reportable in the specific samples analyzed during the analytical shift, then the extracts for those samples do not need to be reanalyzed, as the verification standard has demonstrated that the analyte would have been detected were it present. If the analyte was reportable then the sample must be reanalyzed or qualified. In contrast, if the standard analyzed after a group of samples exhibits a response for an analyte that is below the acceptance limit, ND results may be accepted if the RL check response is greater than the MB response. Reportable results must be reanalyzed or qualified.

7.4.10 Sample injections may continue for as long as the calibration verification standards and standards interspersed with the samples meet instrument QC requirements.

7.4.11 If the peak response is less than 2.5 times the baseline noise level, the validity of the quantitative result may be questionable. The analyst should consult with the source of the sample to determine whether further concentration of the sample is warranted.

7.4.12 Identification of mixtures (i.e. Chlordane and Toxaphene) is based on the characteristic "fingerprint" retention time and shape of the indicator peaks; and quantitation is based on the height or area under the characteristic peaks as compared to the height or area under the corresponding calibration peaks of the same retention time and shape generated using either external calibration procedures.

7.4.13 If compound identification or quantitation is precluded due to interference (e.g., broad, rounded peaks or ill-defined baselines are present) cleanup of the extract or replacement of the capillary column or detector is warranted. Rerun the sample on another instrument to determine if the problem results from analytical hardware or the sample matrix.

7.4.14 Watch for possible carry over. Studies have shown that no carry over occurs after injection of the high calibration standard. Rerun samples at or above the reporting limit that following a sample at this concentration. Be mindful of carry over that may happen during the extraction procedure.

7.4.15 Raw results are sent to LIMS. Post-extraction dilutions are entered in the dilution column.

7.5 Quantitation of multi-component analytes - Multi-component analytes present problems in measurement. See EPA Method 8081A 7.6 for suggestions for handling Toxaphene, Chlordane, BHC, and DDT.

7.6 Suggested chromatographic system maintenance - When system performance does not meet the established QC requirements, corrective action is required, and may include one or more of the following.

7.6.1 GC injector ports can be of critical concern, especially in the analysis of DDT and Endrin. Injectors that are contaminated, chemically active, or too hot can cause the degradation ("breakdown") of the analytes. Endrin and DDT breakdown to endrin aldehyde, endrin ketone, DDD, or DDE. When such breakdown is observed, clean and deactivate the injector port, break off at least 30 cm of the column and remount it. Check the injector temperature and lower it to 205-C, if required. Endrin and DDT breakdown are less of a problem when ambient on-column injectors are used.

7.6.2 Metal injector body - Turn off the oven and remove the analytical columns when the oven has cooled. Remove the glass injection port insert (instruments with on-column injection). Lower the injection port temperature to room temperature. Inspect the injection port and remove any noticeable foreign material.

7.6.2.1 Place a beaker beneath the injector port inside the oven. Using a wash bottle, serially rinse the entire inside of the injector port with acetone and then toluene, catching the rinsate in the beaker

7.6.2.2 Prepare a solution of a deactivating agent (Sylon-CT or equivalent) following manufacturer's directions. After all metal surfaces inside the injector body have been thoroughly coated with the deactivation solution, rinse the injector body with toluene, methanol, acetone, then hexane. Reassemble the injector and replace the columns.

7.6.3 Column rinsing - The column should be rinsed with several column volumes of an appropriate solvent. Both polar and nonpolar solvents are recommended. Depending on the nature of the sample residues expected, the first rinse might be water, followed by methanol and acetone. Methylene chloride is a good final rinse and in some cases may be the only solvent required. The column should then be filled with methylene chloride and allowed to stand flooded overnight to allow materials within the stationary phase to migrate into the solvent. The column is then flushed with fresh methylene chloride, drained, and dried at room temperature with a stream of ultrapure nitrogen.

8.0 QUALITY CONTROL

Note: See also ESB SOP Q01 for general QC requirements

8.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, determination of surrogate compound recoveries in each sample and blank, analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and PT samples.

8.2 Laboratory Reagent Blanks -- Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a laboratory reagent blank (LRB) is analyzed. This blank is analyzed every 20 samples per matrix type. The analyst analyzes Nanopure water for aqueous batches and sand for nonaqueous batches. If within the retention time window of any analyte of interest the LRB produces a peak above the reporting limit, that would prevent the determination of that analyte determine the source of contamination and eliminate the interference before processing samples. Sample results at or above the reporting limit must be accompanied with a qualifier if the method blank exceeds a concentration greater than 1/10 the measured raw concentration of the sample. Blank results below the reporting limit are considered to be ND and will not require a note

8.3 INITIAL DEMONSTRATION OF CAPABILITY

8.3.1 Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made.

8.3.2 A representative fortified concentration of 10 to 50 times the method detection limit or midrange is used. A 10ppb sample concentration is prescribed by the method for single component analytes and 50ppb for multiple but this value may not fall within the working range of the instrument. A sample concentrate (in methanol) containing each analyte at 1000 times selected concentration was prepared. With a syringe, add 1 mL of the concentrate to each of at least four 1-L aliquots of reagent water for aqueous studies and onto sand for nonaqueous studies, analyze each aliquot according to procedures beginning in Section 11. *Past studies were performed at the following: Chlordane- less than or equal to the RL. Toxaphene- at the RL.*

8.3.3 For each analyte the mean recovery value of four of these samples must fall in the proper acceptance range. For aqueous samples: 70 - 130% with a RSD $\leq$ 20%. For poorly performing analytes, the criteria from Table 12 of EPA Method 8081A, is utilized. For analytes not found in Table 12, the range is set to equal that of a similar poor performing analyte. For nonaqueous samples; 70 - 130% with a RSD $\leq$ 20%. For poorly performing analytes, the criteria from Table 9 of EPA Method 8081A is utilized. For analytes not found in the table, the range is set to equal that of a similar poor performing analyte. Since data is compared to performance data developed from single-laboratory data, certain analytes may be outside the limits however, the majority should be within the acceptance limits (per 8000B sec 8.4.7.).

8.4 ASSESSING SURROGATE RECOVERY

8.4.1 When surrogate recovery from a sample or method blank is not within historically generated acceptance limits, check calculations to locate possible errors, fortifying solutions for degradation, contamination or other obvious abnormalities, and instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

8.4.2 If a blank extract reanalysis fails the recovery criterion, the problem must be identified and corrected before continuing.

8.4.3 If sample extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract. If sample extract reanalysis continues to fail the surrogate recovery criterion, report all data for that sample as suspect.

8.4.4 See LIMS for most current historical limits.

8.5 ASSESSING LABORATORY PERFORMANCE - LABORATORY FORTIFIED BLANK

8.5.1 A laboratory fortified blank (LFB) and duplicate are analyzed with every twenty samples or one per sample set (all samples extracted within a 24-h period) whichever is greater. The analyst fortifies Nanopure water for aqueous batches and sand for nonaqueous batches. Acceptance ranges are generated from historical data . See LIMS for most current limits.

8.5.2 A LCS is acceptable if 80% or more of the LCS analytes fall within the laboratory prescribed acceptance criteria. If this criteria is not met the batch is judged to be out of control, and the source of the problem should be identified and resolved before continuing analyses.

8.6 An MDL study is completed whenever major equipment or procedural changes are made. Standards are spiked at a concentration 2.5-5 times the estimated MDL into Nanopure water for aqueous studies and onto sand for nonaqueous. A minimum of seven replicates is analyzed. See QA Manual for calculation. Results must within 2.2 times the reporting limit. *Past studies were performed at midrange.*

8.7 Demonstration of Continuing Proficiency: On an annual basis, each analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 40% for Organic analyses. Performance Evaluation Studies are performed twice a year.

8.8 ASSESSING METHOD PERFORMANCE - LABORATORY FORTIFIED SAMPLE MATRIX

8.8.1 The laboratory adds a known concentration to a minimum of 5% of the routine samples per matrix type or one sample concentration per set, whichever is greater.

8.8.2 Acceptance ranges are generated from historical data. See LIMS for most current limits.

8.8.3 A MS is acceptable if 80% or more of the analytes fall within the laboratory prescribed acceptance criteria.

8.8.4 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control, the recovery problem encountered with the dosed sample is judged to be matrix related, not system related.

8.9 QC For Florisil Clean Ups

8.9.1 Perform a florisil method blank in first clean up batch following florisil activation to demonstrate that there is no contamination.

8.9.2 With each use of activated florisil, carry a laboratory fortified blank through the clean up process.

9.0 SAFETY

9.1 The following compounds have been tentatively identified as known or suspected carcinogens: 4,4-DDT, 4,4-DDD, the BHC's and the PCB's.

9.2 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis.

9.3 See SOP S02 – Compressed Gas Cylinder Handling
SOP S03 – Spill Control Policy

10.0 DEFINITIONS

10.1 See SOP Q15 – SOP Definitions

11.0 CORRECTIVE ACTION FOR OUT OF CONTROL / UNACCEPTABLE DATA

11.1 See SOP Q06 – Corrective Action

12.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

12.1 See SOP S05 – Neutralization Procedure for Acid and Alkaline Wastes
SOP S06 – Disposal of Chlorinated Solvents
SOP S07 – Pollution Prevention

13.0 METHOD PERFORMANCE

13.1 See Method 8081B Tables 9 – 16.

13.1.1 Our intralaboratory generated data is expected to achieve similar results. Refer to MDL studies and laboratory control charts maintained in the QC Office.

14.0 REFERENCES

14.1 EPA 8081B Methods for the Chemical Analysis of Waters and Wastes.
14.2 (SW-846 Prop. Update IV, January 1998)

Note: All *italicized items* are an indication of a variation from the method.

Approved by Susann K. Thomas Date 12/12/05

Edward S. Babcock
Standard Operating Procedure
Data Review and Validation
Effective Date: 05/01/06

1.0 Analyst Review:

1.1 The analysts review raw data for the following:
- 1.1.1 Results are calculated correctly. (Final results on the data page must be calculated, not copied from Element.)
- 1.1.2 Batch QC meets acceptance criteria.
- 1.1.3 Dilution or concentration factors were correctly applied and chosen appropriately.
- 1.1.4 Proper units were used.
- 1.1.5 Results are reported accurately.
- 1.1.6 Additional reporting requirements were met (i.e. QC report, notes, special requirements).

1.2 Once the analyst feels the data is acceptable, he/she enters the data into Element and reviews the entered data for the following:
- 1.2.1 Raw and final results on the instrument report forms match the data in Element.
- 1.2.2 Dilution factors and initial/final sample volumes are correct.
- 1.2.3 MRL, initials of the chemist, date and time are correct..
- 1.2.4 All red data in Element is corrected or properly qualified.

1.3 The analyst fills out the Peer Review Checklist using the following directions:
- 1.3.1 Fill out the Checklist completely. If a check space is not applicable, write NA in the space. Be sure the batch number, date and your initials are on the checklist. Include all necessary raw data to support the answers on the checklist. **Checklist answers must be true and correct!**
- 1.3.2 Please complete the comment section with every batch as necessary. Please include the following at a minimum if applicable: use and justification of secondary column data, deviations from the method and the circumstances involved, MB, LCS, Initial Calibration, CCV or ICV failures, and the reasons for the QC failure if known
- 1.3.3 If QC failures render a batch unacceptable, the analyst must get Supervisor approval to turn in that batch. This approval must be documented on the peer review sheet.

1.4 Once the data is verified as acceptable, change the status to “Needs Peer Review” and turn data into the peer reviewer.

2.0 Peer Review:

Analyst results are verified by a designated peer analyst prior to release of data to the supervisor. Any major problems are brought to the supervisor's attention immediately. All data packages should be able to stand on their own. The peer reviewer performs the following functions:

2.1 Check raw data for the following:
- 2.1.1 Calculations are correct. Spot check.
- 2.1.2 The batch meets acceptance criteria.
- 2.1.3 Proper documentation concerning QC failures, method deviations, elimination of calibration points, etc.
- 2.1.4 All comments or initials are properly dated.

2.2 Check the Peer Review Checklist for the following:
- 2.2.1 Raw data matches notations made on the checklist.
- 2.2.2 QC failures are marked appropriately.
- 2.2.3 The Peer Review Checklist is filled out completely
- 2.2.4 Initials and signatures are dated
- 2.2.5 All the necessary support data is present.
- 2.2.6 Follow up actions are being taken. If it says that RE was created, double check in Element that it was done.

2.3 Check the data in Element for the following:
- 2.3.1 Raw data matches Element entries.
- 2.3.2 All samples and analytes have answers.
- 2.3.3 The bench sheet matches the data in Element.
- 2.3.4 Units and reporting limits are correct.
- 2.3.5 All red data is qualified correctly. Check that the qualifiers are correct for the problem associated with the data. Remember that there may be some issues that require qualifiers but do not turn red.

2.4 You must return the batch to the reporting chemist and ask for additional data if data is missing. Do not sign or submit incomplete data packages. If you are sending the data back to the analyst, change the status to "pending".

2.5 Once the data is verified, set the data status to "Analyzed", sign and date the Peer Review Checklist as the Peer Reviewer. Organic data is submited to the supervisor. Inorganic data is returned to the analyst.

3.0 Supervisory Review

<u>Inorganics and Microbiology</u>

3.1 Query reviewable reports under the following departments in Project Management/Query Work Status and then print.

- 3.1.1 Inorganics: Inorganics and Soil-ag
- 3.1.2 Microbiology: Bateriological

3.2 Pick up reviewable list from printer.

3.3 Pull up each sample report in Data Entry Review by 'work order"under Laboratory and review on screen.

3.4 Examine also the report. Click on the printer button. Choose a data review format (e.g. rev_InorgSuperRev.ppt). Click preview button to view report.

3.5 Data is examined for the following:

3.5.1 All tests have answers.
3.5.2 The correct units and reporting limits are entered.
3.5.3 Customers name and sample ID make sense with the answers.
3.5.4 Qualifiers are appropriate
3.5.5 Answers relate to each other properly. For intra-sample quality control the following procedures for checking correctness of analyses are applicable to water samples for which relatively complete analyses are made. These include pH, conductivity, total dissolved solids (TDS), major cationic and anionic constituents, demand parameters, nutrients and trace organic contaminants. Use the criteria below.
3.5.6 Calculations are correct. The following calculations appear on the last pages of the report in the Inorganic Supervisor Review: Aggressive Index, Langlier Index, Total Anions, and Electrochemical Balance. Other calculations must be manually calculated such as: TDS by summation, carbon dioxide, chloramines, Cr3 and any total, inorganic, or organic nitrogen that did not have –Calc in their name. All calculations must be manually entered on the data review screen. Click "Edit" and enter results on the appropriate line. When finished move curser off last entry and click "Save"

Mineral Balances

Equation (1)
me/L (Ca+Mg+Na+K) = total cations

Equation (2)
me/L (Total Alkalinity+SO4+Cl+NO3-N+F) = total anions

Equation (3)
Calculated TDS by summation = mg/L (Ca+Mg+Na+K+Cl+SO4+NO3+F+SiO2)+ (0.6)(Total Alkalinity as CaCO3)

The measured TDS may be higher than the calculated TDS because a significant contributor may not be in the calculation.

Equation (4)
Acceptance Criteria: % Difference =

$$\frac{100 \times (\text{total cations} - \text{total anions})}{(\text{total cations} + \text{total anions})}$$

% Difference should be $\pm$ 5%

Equation (5)
EC(Calc)= (total cations + total anions) x 50

(Both the total anion and total cations should be 1/100 of the measured EC value.) The calculated EC is expected to be within 10% of the measured EC. Thus:

Equation (6)

$0.9\ \mu \leq$ calculated EC $\leq \mu\ 1.1$

If the ratio of TDS to conductivity falls below 0.55, the lower ion sum is suspect and reanalyzed. If the ration is above 0.7, the higher ion sum is suspect and reanalyzed. If reanalysis causes no change in the lower ion sums, an unmeasured constituent, such as nitrite or organic acids may be present at significant levels. If poorly disassociated calcium and sulfate ions are present, the TDS may be higher than the EC. The acceptance criterion is as follows:

Equation (7)

$$\frac{\text{Measured TDS}}{\text{Measured EC}} = 0.55 \text{ to } 0.7 \text{ and/or } \frac{\text{Calculated TDS}}{\text{Calculated EC}} = 0.55 \text{ to } 0.7$$

Reference: Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WEF, 18th edition

Demand Ratios
A general rule of thumb is:
BOD = 0.4 to 0.6 of COD
TOC = 0.4 (approx) of COD
TOC = 0.6 (approx) of BOD

Nutrient Relationships
Total Nitrogen = Organic Nitrogen + Inorganic Nitrogen
Inorganic Nitrogen = NO3-N + NO2-N + NH3-N
Kjeldahl Nitrogen = Organic Nitrogen + NH3-N
Organic Nitrogen = Kjeldahl Nitrogen - NH3-N
The above nitrogen relationships are checked to ensure proper calculations have been performed.

Trace Organic Contaminants:
TOX = Volatile Organic Halogens + Non-volatile Organic Halogens
Volatile Organic Halogens = Polar + Non-polar Volatile Organic Halogens
Non-volatile Organic Halogens = Polar + Non-polar Non-volatile Organic Halogens
Non-polar Volatile Organic Halogens are measured from EPA Methods 502.2, 601, 8010, 524, 624, 8240, or 8260 analysis.
Non-polar Non-volatile Organic Halogens are measured from EPA Method 525, 625, or 8270 analysis.

From the above relationships, the following is performed:
TOX · Non-polar (Volatile + Non-volatile) Organic Halides

Microbilogy :
Total Coliform ≥ Fecal Coliform
Fecal Streptococcus ≥ Enterococcus

Organics

3.6 Pull up batch in Data Entry Review. Review paperwork and data on screen by examining the following:
- 3.6.1 QC recovery and results appear correct in relation to raw results.
- 3.6.2 Batch is acceptable.
- 3.6.3 Flags are appropriate.
- 3.6.4 Correct units and Reporting Limits are used.
- 3.6.5 Sample ID makes sense with result.

Data Approval

3.7 If data looks acceptable:
- 3.7.1 Click on top left corner of screen to highlight entire screen. Right click. Choose Update Status. Choose "update to Reviewed".
- 3.7.2 If any lines are not acceptable or not reportable, highlight line and right click "update to Cancelled".
- 3.7.3 Click Done to close.

3.8 If something needs to be edited:
- 3.8.1 Under Laboratory-Data Entry/Review, choose batch to edit "batch" or sample to edit "sample". Click edit.
- 3.8.2 Columns with blue headers are the only ones that can be edited.
- 3.8.3 Click save to save changes.
- 3.8.4 Click on Done.

3.9 If something needs to be redone:
- 3.9.1 Under Laboratory pick Update status, then sample, proceed to section 3.9.3.
- 3.9.2 Or in Data/Review simply proceed to section 3.9.3.
- 3.9.3 Highlight line of analysis and right click- choose create re-extract.
- 3.9.4 New line is added as Re with status of available.
- 3.9.5 Update statue of original result to "pending" if necessary.

3.10 When approving an invoice that has redos, follow the steps in 3.5 except do not mark all analytes.
- 3.10.1 Once the RE is ready for supervisor review, compare the results and document on each batch (the original and the RE batch) which result will be taken and the reasons why
- 3.10.2 Approve only the entry lists that are acceptable. (Cancel all others)
- 3.10.3 If an entire entry list is unacceptable use the mouse to change the Approval Status in the upper right hand corner.
- 3.10.4 Update to cancelled.

4. Quality Assurance Review

4.1. Log In Review (prior to sample analysis) checks the following:

4.1.1. The list of analyses on the chain of custody is compared to the Log-In Sheet to ensure accuracy.
4.1.2. All vital information has been accurately transferred into LIMS.
4.1.3. Appropriate projects and client specific requests are included in the comments.

4.2. Project Manager Review

4.2.1. Client reports for special projects will be reviewed by a project manager.
4.2.2. State forms will be reviewed by a project manager.
4.2.3. The project manager checks the following:
4.2.3.1. All of the appropriate analyses and analytes are on the final report.
4.2.3.2. All required QC is attached, QC is acceptable, qualifiers make sense, etc.
4.2.3.3. Adds J flags if requested
4.2.3.4. Checks the comment section in the LIMS review report. Ensures that client special requests have been followed such as different reporting limits, analyte lists, qualifiers, etc.

5. Final Review

5.1. The final reviewer checks the reports for any apparent mistakes prior to signing.

Approved by Susann K. Thomas Date 04/04/06

E.S. BABCOCK QUALITY CONTROL DATA
STANDARD OPERATING PROCEDURE
Effective Date____09/08/06_____

Any regulation or method criteria more stringent than the QC specified here will supercede the following requirements for that particular analysis. When methods are analyzed simultaneously, the most stringent requirements of the combined methods will be followed.
If an uncertified matrix is analyzed under a particular method, per client request, extra QC may not be analyzed for that matrix. Normal QC for the certified matrix will validate the batch.
All QC data is reviewed by the analyst and Peer Reviewer. The Supervisor, Quality Assurance Manager, or other designee may also review the data.

The following applies primarily to Inorganic and Organic analyses. See ESB SOP B01 for microbiology quality control procedures.

1.0 Internal Quality Control:

Note: Acceptance Limits - Statistical ranges are provided in the LIMS or analytical SOP for all QC samples. Limits may be set by the method or generated historically depending on specific method requirements. Ranges are monitored regularly by the QA Manager for trends and updated as necessary. See ESB SOP Q03 for more details.

1.1 Laboratory Control Samples-LCS (Laboratory Fortified Blanks-LFB or Blank Spikes-BS):

1.1.1 A laboratory control is analyzed with each batch of samples or 1 per 20 samples whichever is more frequent and reported per matrix type. The control is a standard from a noncalibration source that is processed in the same manner as a sample and is used to determine batch acceptance.

1.1.1.1 Matrix type refers to one of following possible groups: water, liquid (including extracts), solid/soil/sludge, or gas. If possible it is preferred to group sludge separately from the others solids and CAM/TCLP extracts separately from other liquids. If the water and liquid procedures are identical, one LCS may be performed for both matrices as long as it still represents a frequency of 5%.

1.1.2 For specific analyses it may be impractical to perform a LCS (i.e. settleable solids, dissolved oxygen, sulfide etc.). See method SOPs for specifics.

1.1.3 The laboratory fortifies LCS samples with all reportable components with the following exceptions:

1.1.3.1 The method specifies specific spiking components.

1.1.3.2 Components that interfere with each other may be excluded or handled separately.

1.1.3.3 Test methods with a long list of target analytes will spike only a core group of compounds. This core group of spiking compounds represents all chemistries, elution patterns and masses. The core group must consist of the following number of spiking compounds:

# of target analytes	# of spiking compounds in LCS mix
1-10	100%
11-20	80%(at least 10 compounds)
>20	16 compounds

1.1.3.4 Every two years a set of 4 LCS's is analyzed containing all other NELAP analytes per method, per matrix. Since historical data is unavailable for these analytes, results are simply noted.

1.1.4 LCS results are compared to the "True" value.

1.1.5 Percent Recovery is calculated as indicated in the laboratory Quality Assurance Manual.

1.1.6 Quality control charts are available in LIMS indicating the percent recoveries, mean, standard deviation, and the upper and lower control limits (UCL and LCL).

1.1.7 Percent recoveries are compared to control limits. If the percent recovery for the Laboratory Control Sample does not fall within the acceptance range, corrective actions must be taken. See SOP Q06 for further corrective action requirements. If it is still unclear how to proceed, discuss the problem with the supervisor or QA Manager.

1.2 Blanks:

1.2.1 A method or reagent blank – (MB or LRB) is analyzed with each batch of samples or 1 per 20 samples, whichever is more frequent and reported per matrix type.

1.2.1.1 Matrix type refers to one of following possible groups: water, liquid (including extracts), solid/soil/sludge, or gas. If possible it is preferred to group sludge separately from the others solids and CAM/TCLP extracts separately from other liquids. If the water and liquid procedures are identical, one MB may be

performed for both matrices as long as it still represents a frequency of 5%.

1.2.2 In some cases a method blank is not appropriate and will not be analyzed, (e.g. pH, flashpoint, etc.).

1.2.3 A batch is acceptable if method blanks meet acceptance criteria stated in each analytical SOP. Blanks cannot be more negative than batch acceptance criteria.

1.2.4 If the blank is unusually high or negative, troubleshooting must be performed to eliminate the source of contamination before continuing with the analysis. See SOP Q06 for further corrective action requirements. If it is still unclear how to proceed discuss the problem with the supervisor or QA Manager.

1.2.5 Method blank results greater than or equal to batch acceptance criteria, must not exceed a concentration greater than 1/10 of the measured raw concentration of any sample in the associated sample batch.

1.2.6 Travel Blanks: If provided, travel blanks must be analyzed whenever a drinking water sample has a result at or above the reporting limit or a liquid sample has a reportable result less than10 times the reporting limit. Certain commonly occurring analytes (i.e. PCE, TCE, THMs, HAAs, MtBE) may be considered an exception. See specific method SOP. If analyte is present in the travel blank at or above the method reporting limit, attach the proper qualifier (NTBcv) to corresponding sample analytes. If analyte is not present in the travel blank at or above the method reporting limit, attach NTBnd to the sample or analyte where applicable.

1.3 Matrix Spikes-MS (or Laboratory Fortified Sample Matrix-LFSM):

1.3.1 Matrix spikes are performed on appropriate analyses. (Check the SOP for the analysis in question to determine if a matrix spike is to be performed.)

1.3.2 Matrix spikes are performed at a frequency that meets data quality objectives or specified test method requirements.

1.3.3 The laboratory fortifies matrix spikes with all reportable components with the following exceptions:

1.3.3.1 The method specifies specific spiking components.

1.3.3.2 Components that interfere with each other may be excluded or handled separately.

1.3.3.3 Test methods with a long list of target analytes will spike only a core group of compounds. This core group of spiking compounds represents all chemistries, elution

patterns and masses. The core group must consist of the following number of spiking compounds:

# of target analytes	# of spiking compounds in MS mix
1-10	100%
11-20	80%(at least 10 compounds)
>20	16 compounds

1.3.4 Every two years a set of 4 MS's or 2 MS/MSD's is analyzed containing all other NELAP analytes per method, per matrix. Since historical data is unavailable for these analytes, results are simply noted.

1.3.5 Samples for matrix fortification are chosen at random, rotated among clients. Samples that pose unusual, obvious matrix problems however, are rejected as an unrepresentative choice for the batch.

1.3.6 Percent Recovery is calculated by computer as indicated in the Quality Assurance Manual.

1.3.7 Percent recoveries are compared to laboratory acceptance ranges. If recoveries do not fall within the control limits, laboratory personnel must check for errors in the analysis. See SOP Q06 for further corrective action requirements. If it is still unclear how to proceed discuss the problem with the supervisor or QA Manager.

1.3.8 Quality Control Charts are available in LIMS indicating the percent recoveries, mean, standard deviation, UCL, and LCL.

1.4 Precision Data (Matrix Spike Duplicates-MSD, Sample Duplicates-DUP, or Laboratory Control Duplicates - LCSD):

1.4.1 In general, precision data is a measurement of the reproducibility of results within the sample matrix.

1.4.2 Using good laboratory technique, duplicate results from a homogeneous sample should agree closely.

1.4.3 Results from a non-homogeneous sample might not agree as well, but if a representative sample is taken, the results should still be fairly close. The larger the sample taken from a non-homogeneous matrix, the more representative the sample is likely to be.

1.4.4 Samples for duplication are chosen at random, rotated among clients. Samples that pose unusual, obvious matrix problems however are rejected as an unrepresentative choice for the batch.

1.4.5 Precision data is obtained from duplicate spikes or from duplicate runs of the sample or Lab Control. Duplicate sample runs are used

when analyte is normally present at high enough concentrations for precision analysis. (Check the SOP for the analysis in question to determine if a matrix spike is to be performed.) If for some reason the required sample volume is unavailable, precision data may be taken from a LCSD.

1.4.6 Precision data is performed at a frequency that meets data quality objectives or specified test method requirements.

1.4.7 Relative percent difference (RPD) and UCL are calculated by computer as indicated in the Quality Assurance Manual.

1.4.8 Quality control charts are available in LIMS for precision data indicating the RPD, mean, and UCL.

1.4.9 If recoveries do not fall below the UCL, laboratory personnel should review laboratory techniques used in the procedure, check for oddities in the matrix, and ensure that the precision results were the best possible using the prescribed method. See SOP Q06 for further corrective action requirements. If it is still unclear how to proceed discuss the problem with the supervisor or QA Manager.

1.4.10 Duplicate samples may be analyzed for confirmation purposes as is the case for organic analyses of drinking water samples having a result at or above the reporting limit, or liquid samples having a reportable result less than ten times the reporting limit. If the result is confirmed by duplicate analyses, attach N-DUP or Nconf to the sample analyte. Certain commonly occurring analytes like PCE, TCE, MtBE, HAAs, and THMs are generally not confirmed by reanalysis. Results supported by historical data are also generally not reanalyzed but may be qualified (N-HST).

1.4.11 Where replicate sample results are available, see ESB SOP Q24 for reporting guidance and retest policy.

1.5 Surrogates

1.5.1 If the method requires the addition of surrogate, a known amount is added to all client and QC samples. Surrogate compounds must be added for all organic chromatography methods except when the matrix precludes its use or when surrogate is unavailable. Surrogates are also used for Inorganic Chromatography analysis if required by the method.

1.5.2 Percent Recovery is calculated by computer as indicated in the Quality Assurance Manual.

1.5.3 Percent recoveries are compared to laboratory acceptance ranges. If recoveries do not fall within the control limits see SOP Q06 for further corrective action requirements. If it is still unclear how to proceed discuss the problem with the supervisor or QA Manager.

1.5.4 Quality control charts are available in LIMS indicating the percent recoveries, mean, standard deviation, and the upper and lower control limits (UCL and LCL).

1.6 Internal Standards

1.6.1 Internal standard calibration is used per method requirements. In such cases internal standard is added to all client and QC samples in the batch.

1.6.2 Internal standard methods normally have protocol for evaluating the internal standard recovery in QC and Sample data. Two variations on this evaluation process are most common. Compare the mean value of the internal standard from the calibration standard and/or compare the daily calibration verification internal standard to the internal standards in the QC and Sample data.

1.6.3 Internal standard response is calculated by chromatography software.

1.6.4 Internal standard performance is monitored and evaluated based on method specified criteria. If internal standards do not satisfy requirements, see SOP Q06 for further corrective action requirements. If it is still unclear how to proceed discuss the problem with the supervisor or QA Manager.

2.0 External Quality Control:

2.1 Semiannual Check Samples:

2.1.1 Every six months certified samples are purchased from an outside source such as Environmental Resource Associates.

2.1.2 The results of these tests are used to check the accuracy of the analyses within the lab.

2.1.3 The Quality Control Manager or Supervisor has a copy of the certified results. Upon completion of analysis, each analyst verifies their result with the QA Department.

2.2 Proficiency Testing Samples (PT):

2.2.1 Check samples are purchased on a periodic basis from a NIST certified vendor to check for the accuracy of analyses in the laboratory. The results from these samples are reported to the governing and/or certifying agencies.

2.2.2 The values and ranges for these samples are completely unknown to anyone in the laboratory.

2.2.3 The vendor provides final reports to the lab and governing and/or certifying agencies. These reports give the results and acceptance ranges. This report is posted so that every analyst can view his/her performance. A written response is made to the certifying agency regarding follow up investigations of unacceptable results.

2.3 Blind Samples:

2.3.1 Blind samples are spiked samples or duplicate samples that are logged in as if they were normal client samples.

2.3.2 Blind samples either come from the management or an outside source such as a client or an agency.

2.3.3 Analysts do not know that blind samples are QC samples. If the sample comes from an outside source, the laboratory management might not know that the blind samples are QC samples.

2.3.4 If it becomes necessary to investigate an analytical issue,. the laboratory management shall periodically log in blind samples as a diagnostic tool.

3.0 Instrument Calibrations

3.1 Number of Calibration Points:

3.1.1 The minimum number of calibration points is determined by the method.

3.1.2 If the method does not specify the number of calibration standards required, a minimum of 2 points is used.

3.1.3 For 500 and 600 series methods a three point calibration is recommended for linear curves. More than a three point calibration is recommended for nonlinear curves.

3.1.4 For 8000 series methods, a five point calibration is required for a linear (first order) model, six point calibration for a quadratic (second order) model, and seven point for a polynomial (third order) model.

3.1.5 The analyst has the option to use linear or nonlinear integration and to force, include, or ignore the origin, depending on method requirements.

3.1.6 The analyst may decide to exclude a calibration point if it appears to be invalid, under the following conditions:

- The curve will still contain the minimum number of points required without the excluded point.
- A standard at or below the reporting limit is included.
- No sample result is reported above the high calibrator unless qualified.
- The analyst documents reasoning for eliminating a calibrator.

Note: If a point is eliminated, it is recommended that the analyst verify that portion of the curve with a QC sample.

Note: The analyst may replace the above calibrator with a standard injected later in the run (e.g. extra CCV) as long as the new calibration can be validated with method prescribed ICVs and CCVs.

3.2 Calibration Levels:

3.2.1 The lowest calibration standard is above the analyte MDL.

3.2.2 The low standard must be at or below the reporting limit.

3.2.3 Other calibration standards include those that are at or below the regulatory limit, if known.

3.2.4 Standards are made at levels that typically bracket expected concentrations but do not exceed instrument linearity.

3.3 Calibration Acceptance: Calibrations are accepted based on individual method requirements. Calibration curves or response factors are printed on hard copy or stored electronically. If the method does not specify acceptance criteria, a linear curve must have a correlation coefficient (r) as specified below and a nonlinear curve must have a coefficient of determination (r^2) as specified below.

	Linear	Nonlinear
Inorganic methods	$r \geq 0.995$	$r^2 \geq 0.99$
500,600 methods	$r \geq 0.99$	$r^2 \geq 0.98$
8000 methods	$r \geq 0.99$	$r^2 \geq 0.99$

Note: The run may be reprocessed based on a later calibration as long as the reprocessed run contains method required QC that is acceptable under the new calibration.

3.4 Initial Calibration Verification Standard-ICV: Initial calibrations are validated by quality control samples obtained from a noncalibration source and analyzed during the analytical run. This may be either the LCS or a separate ICV as long as it represents a second source for every analyte of interest. Results must be within ICV acceptance ranges specified in the analytical SOP. If the ICV is not acceptable see SOP Q06 for corrective action requirements. If it is still unclear how to proceed discuss the problem with the supervisor or QA Manager.

3.5 Initial calibration is used directly for quantitation.

3.6 Initial calibrations are performed daily or when continuing calibration checks are out of the control limits as specified by the method or SOP.

3.7 Continuing Instrument Calibration Checks-CCV

3.7.1 If an initial calibration is not performed, a continuing calibration check is analyzed to verify the existing calibration prior to sample analysis.

3.7.2 Laboratory policy requires that every result reported from an instrumental analysis be bracketed with working QC. This may be a continuing calibration check.

3.7.3 If continuing calibration checks are outside acceptance criteria, see SOP Q06 for further corrective action requirements. If it is still unclear how to proceed discuss the problem with the supervisor or QA Manager.

3.8 A calibration check standard at the reporting limit may be analyzed at the end of an automated run. This standard is used to validate ND results should the LCS, ICV, or CCV be biased low, by demonstrating that the instrument signal at the RL is greater than the method blank signal.

3.9 Data reported outside the calibration range is flagged as such (NOcal).

3.10 Data associated with an unacceptable calibration coefficient or response factor is not reported.

4.0 Method Detection Limit Studies

4.1 A Method Detection Limit Study-MDL is completed when there is a significant change in the test method or instrument type.

4.1.1 A column of a different phase constitutes a significant change. A new MDL study is required unless the new column proves to be more sensitive than its confirmation column.

4.1.2 A second instrument of the same type will be compared to the original instrument by analysis of a RL standard. If the sensitivity of the new instrument is the same or better than the sensitivity of the old instrument, a MDL study is not necessary. If it is not as sensitive, a MDL study will be performed on the new instrument.

4.1.3 If an instrument is torn down and moved, the RL standard after the move will be compared to the RL standard prior to the move. If it is not as sensitive, a MDL study will be performed on the instrument once it is up and running in the new location.

4.1.4 Sensitivity is measured by comparing the signal to noise ratio of an analyte in one RL standard versus another. The RL standard with the higher S/N ratio demonstrates a system that is more sensitive.

4.2 Standards are generally spiked at or near the reporting limit (2.5 – 5 times the estimated MDL per Appendix B of 40CFR-136) or at a method prescribed level into a quality system matrix free of target analytes or interferences. A minimum of seven replicates are carried thru all sample processing steps of the analytical method. See QA Manual for calculation. If the procedure for solid and sludge matrices is identical, except for initial volume, the study is

performed using solid preparation volumes. Sludge MDLs are then calculated by applying a preparation factor to the solid MDL.

4.3 Results must be below the reporting limit for Inorganic analyses or $\leq$ 2.2 times the reporting limit for Organic analyses. If the MDL is greater then the reporting limit but within the 2.2 times criteria for Organic analyses, the MDL will be entered into LIMS as equivalent to the RL or, if possible, the RL will be raised.

4.4 An analyst may exclude a data point from the study as long as it is proven to be a statistical outlyer. This is determined using the Grubbs Test. A 95% confidence level is used to determine if a data point may be rejected. The analyst must document this proof with the raw data. All data is reported from the primary column or detector.

4.5 Method MDLs may be set equal to or greater than the statistically derived MDL. Often it is set at ½ the reporting limit or 2.2 times the statistically derived MDL. If an analysis is performed on more than one instrument or column, the higher of the two MDLs is generally entered as the default value into LIMS.

4.6 New MDLs are entered into the LIMS system. The RL date is changed to reflect the date of entry. All versions of analyses may be updated from the Master or primary analysis with the following exceptions:

4.6.1 UCMR, ML, and SIM products have specific RLs and MDLs.
4.6.2 CAM and TCLP analyses have RLs that relate to the regulatory limits. See ESB SOP E01 and E02.
4.6.3 Client products may have specific RLs. Check with Project Managers.
4.6.4 zz analyses are inactive and should not be altered.

4.7 An additional MDL study may be performed for common laboratory contaminants. This study consists of the analysis of standards over a period of several days, injected into several instruments, if applicable.

4.8 LOD Verification: The MDL may also be defined as the Limit of Detection or LOD. On a yearly basis, the validity of the LOD shall be confirmed by qualitative identification of the analyte(s) in a QC sample in each quality system matrix containing the analyte at no more than 2-3X the LOD for single analyte tests and 1-4X the LOD for multiple analyte tests. Analyte response must be greater than method blank response to verify the Level of Detection or MDL for each column or instrument. An LOD study is not required for any component for which spiking solutions or quality control samples are not available or when test results are not to be reported to the LOD.

5.0 Reporting Limits

5.1 Reporting limits are established based on MDL studies, regulation, or client requirements.

5.2 The RL may also be defined as the Limit of Quantitation (LOQ). A yearly LOQ verification study must be performed if the LOD has not been verified as described in section 4.8.

5.2.1 The validity of the LOQ is confirmed by successful analysis of a QC sample containing the analytes of concern in each quality system matrix 1-2 times the claimed LOQ. A successful analysis is one where the recovery of each analyte is within the established test method acceptance criteria or client data quality objectives for accuracy.

5.3 Raising the RL

5.3.1 Inorganics: If a sample is diluted for any reason the RL is raised to reflect the dilution.

5.3.2 Organics:

5.3.2.1 If a sample is diluted due to a chromatographic interference that prevents peak integration, the RL is raised to reflect the dilution.

5.3.2.2 If a sample is diluted because it is over calibration range, the RL is raised to reflect the dilution. .

5.3.2.3 If the above does not apply to all target analytes in one sample, report other analytes from the original injection with original reporting limits.

5.3.3 If a raw result is below the RL but rounds up to a reportable value (see ESB SOP Q04 for rounding rules) then Element will report that result as a hit for that analyte.

6.0 Demonstration of Capability (DoC):

The laboratory evaluates the Precision and Bias of a Standard Method for each analyte of concern for each quality system matrix.

6.1 Initial Demonstration (IDoC):

6.1.1 A demonstration of capability must be completed each time there is a change in instrument type, personnel, or test method. A work cell is an exception, see section 6.1.5.

6.1.1.1 A column of a different phase constitutes a significant change. A new demonstration study is required.

6.1.1.2 A second instrument of the same type does not require a new study.

6.1.2 The study is completed by the analysis of four replicates of a QC sample diluted in a clean quality system matrix at a concentration specified in the analytical SOP. This level is taken from individual method requirements or in the absence of such requirements set at 1 – 4 times the LOQ or RL. If analytes do not lend themselves to spiking, QC or PT samples are used.

6.1.3 An analyst may exclude a data point from the study as long as it is proven to be a statistical outlyer. This is determined using the Grubbs Test. A 95% confidence level is used to determine if a data point may be rejected. The analyst must document this proof with the raw data. All data is reported from the primary column or detector.

6.1.4 Results are compared to acceptance ranges specified in the SOP. If limits for specific analytes are not found in the determinative method, acceptance criteria may be taken from limits provided by Performance Testing Studies. The analyst must successfully complete the study prior to analysis of client samples. All work performed during the training period, prior to a successful DoC, must be co-initialed by the trainer.

6.1.5 If the analyst is joining a cell, the DoC consists of a cell change form. The analyst must turn in valid LCS and method blank data from four consecutive batches of the training period. LCS and MB results must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than 20% for Inorganic analyses and less than 40% for Organic analyses. The trainer must co-initial all work performed during the training period.

6.2 Demonstration of Continuing Proficiency (DoCP):

6.2.1 On an annual basis, each analyst or work cell must complete a Demonstration of Continuing Proficiency for every certified analytical procedure performed that year unless they or a member of their cell has performed an IDoC or cell change form that year.

6.2.2 The demonstration may consist of one of the following:

6.2.2.1 Valid LCS data from four consecutive LCS samples.

6.2.2.2 Results from a successful Proficiency Testing Study.

6.2.2.3 Successful analysis of a blind performance sample on a similar test method using the same technology (e.g. 524, 624, and 8260).

6.2.2.4 If one of the above is not practical, the analysis of authentic samples that have been analyzed by another trained analyst with statistically indistinguishable results.

6.2.3 LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20% for Inorganic analyses or 40%

for Organic analyses (allowing for one marginal exceedance if applicable).

7.0 Evaluation of Selectivity

7.1 The laboratory shall evaluate selectivity by following the checks established within the method, which may include mass spectral tuning, second column confirmation, ICP inter-element interference checks, chromatography retention time windows, sample blanks, spectrochemical absorption, or electrode response factors.

Approved by Susann K. Thomas Date 08/28/06

CORRECTIVE ACTION FOR CHEMICAL ANALYSIS
Edward S. Babcock & Sons
STANDARD OPERATING PROCEDURE
Effective Date___09/08/06_____

The following is a description of Edward S. Babcock & Sons Corrective Action Protocol for quality control samples. Please consult the analytical SOP for method specific requirements that may supercede the requirements below. After following these procedures, if it is still unclear how to proceed, discuss the problem with the supervisor or QA Manager.

1.0 Laboratory Control/ Laboratory Fortified Blank/ Blank Spike:

1.1 When a LCS is out of the laboratory acceptance criteria, the batch is considered to be out of control.

1.1.1 Recheck the calibration. This may be done with the analysis of a QC sample from a third source. If the standard shows the calibration to be off, recalibrate or restandardize depending upon the method and rerun samples.
1.1.2 For instrumental analyses, check for instrument setup problems.
1.1.3 Check blanks to determine if there is contamination in either the glassware or reagents.

1.2 If the above does not correct the problem, remake the laboratory control carefully to be certain that the procedure is followed correctly. A new LCS may be considered as part of the original batch as long as 24 hours has not been exceeded from the start of processing of the first sample and the start of processing of the new LCS. If a new LCS is processed, conditions must be identical to those conditions under which the samples were prepared and analyzed.

1.3 If the problem is still not corrected, check with the supervisor for advice before proceeding with the analysis.

1.4 If the noncompliant LCS is discovered after the analysis is completed, an out of control qualifier must be placed on the LCS (QLout). Batch data may be accepted if:

1.4.1 The problem does not appear to have affected the entire run AND one of the following is true:
1.4.1.1 A MS or MSD from a noncalibration source, fell within the LCS laboratory acceptance criteria (QL-MS) Samples may be released without a qualifier.
1.4.1.2 There is reasonable evidence (documented with the raw data) to suspect that the spiking solution was made incorrectly. If possible evaluate recovery based on the new spiking level. Samples may be released without a qualifier. If this is not possible place QLout and QFspk on the LCS.

1.4.1.3 Management deems the run reportable. In this case each sample in the batch is reported with the proper qualifier attached (NLOhi, or NLOlo).

1.4.2 If it is uncertain whether the problem affected the entire run, the batch must be reanalyzed. In some cases however additional sample may not be available for reanalysis. In this case, either the tests are canceled, the client is informed and given the option to resample or results are reported with the proper qualifier (NLOhi, or NLOlo).

1.5 Organic determinations with 11 or more compounds in the LCS:

1.5.1 If a large number of analytes are in the LCS, it becomes statistically likely that a few will be outside control limits. This may not indicate that the system is out of control, therefore corrective action may not be necessary.

1.5.2 Upper and lower marginal exceedance (ME) limits can be established to determine when corrective action is necessary. A ME is defined as being beyond the LCS control limit (3 standard deviations), but within the ME limits. ME limits are between 3 and 4 standard deviations around the mean. ME 4s limit minimum is 10%.

1.5.3 One marginal exceedance is allowed in an LCS containing 11-16 analytes (QLpas). If more analytes exceed the LCS control limits, or if any one analyte exceeds the ME limits, the LCS fails and corrective action is necessary (QLout).

1.5.4 If the LCS fails then one of the following must happen:

1.5.4.1 A MS or MSD from a noncalibration source, falls within the LCS laboratory acceptance criteria and may replace the LCS (QL-MS).

1.5.4.2 An LCSD may be used in place of the LCS unless the LCSD is also required by the method.

1.5.4.3 The batch is reanalyzed or re-extracted with acceptable QC. Contact the client to determine if re-extraction past holding time is an acceptable option.

1.5.4.4 If management deems the batch reportable, results are reported with the proper qualifiers.

1.5.4.4.1 All samples with results at or above the reporting limit are flagged with a qualifier on the sample analytes signifying a low or high bias (NLOlo or NLOhi).

1.5.4.4.2 Samples that are ND:

1.5.4.4.2.1 If the LCS is bias high, a qualifier is not necessary as long as <u>all</u> analytes in the sample are ND.

1.5.4.4.2.2 If the LCS is bias low, *the RL check must be acceptable for that analyte and sample surrogate compounds must be acceptable otherwise a qualifier must be placed on the sample analytes (NLOlo).*

1.5.5 Marginal exceedances must be random. If the same analyte exceeds the LCS control limit repeatedly (2 batches out of the most recent 3 batches), it is an indication of a systemic problem. The source of the

error must be located and corrective action taken. To ensure random behavior, marginal exceedance allowance to the LCS is monitored on the peer review sheet.

2.0 Method Blank

2.1 When a method blank is out of the laboratory acceptance criteria stated in the SOP, the batch validity is called into question.

2.2 Examine the calibration zero point and/or baseline for contamination.

2.3 Examine scale tare weights.

2.4 Examine the blank source for possible contamination.

2.5 If the above does not correct the problem, remake the method blank carefully to be certain that the procedure is followed correctly. A new MB may be considered as part of the original batch as long as 24 hours has not been exceeded from the start of processing of the first sample and the start of processing of the new MB. If a new MB is processed, conditions must be identical to those conditions under which the samples were prepared and analyzed.

2.6 Wherever possible, the analyst must rerun samples associated with an invalid method blank result. Invalid blanks must be accompanied with the qualifier (QBLK)

2.7 Report results that management deems acceptable. Sample results above the reporting limit must be accompanied with the proper qualifier (NBLK) if the method blank associated with the sample:

2.7.1 - exceeds a concentration greater than 1/10 of the measured raw concentration of the sample (The raw concentration is the value prior to dilution or concentration factor.)

2.7.2 - does not satisfy method requirements (if stricter), see SOP.

Note: The blank value noted in the NBLK qualifier is the raw blank result. If the sample being qualified was diluted, in addition to NBLK, the analyst must also attach the qualifier NBLKd which includes the sample raw result.

Note: Method blank results below batch acceptance criteria (i.e. <RL or ½ RL) are considered to be ND and will not require a note. If batch acceptance is ½ the RL and this value is <MDL, method blanks will be considered ND below the MDL.

2.8 Sample results that require a J-flag will be reviewed by the Project Manager to determine where qualifiers are necessary (i.e. PblkJ). If results are less than ten times the level detected in the method blank, the proper qualifier (NBLK) is placed on the sample.

2.9 Travel Blanks: See ESB SOP Q01 section 1.2.6.

3.0 Spikes and Duplicates

3.1 When a spike or duplicate is out of the laboratory acceptance criteria, that sample result is called into question (not batch acceptance).

3.2 If the sample background is 4 times or greater than the fortification concentration, the spike is considered invalid and follow up is not necessary. Attach the proper qualifier to the spike (QM-4X).

3.3 Duplicates must meet the RPD criteria unless the sample concentration is $\leq$ 10 times the reporting limit. If the sample concentration is $\leq$ 10 times the reporting limit, the analysis should be reviewed whenever the difference between the sample and the duplicate is greater than the reporting limit. Attach the proper qualifier to the duplicate (QRPDl). The above may not be appropriate for some analyses. See specific method SOP for details.

3.4 When an MS/MSD or DUP fails to meet the laboratory prescribed acceptance criteria for either percent recovery or RPD, a follow-up must be performed unless:

- 3.4.1 Sections 3.2 or 3.3 apply.
- 3.4.2 The sample is obviously non-homogeneous and any attempts to reproduce the same sample aliquot is unlikely (attach QRPDo and QFnoH to QC and NRPDh to samples).
- 3.4.3 MS recovery and RPD are acceptable (attach qualifier QMSD) unless the analyst is using the MSD to satisfy MS frequency requirements specified by the method.
- 3.4.4 Sample matrix problems are **obvious** (i.e. interfering peaks, a physical attribute of the sample that hinders normal analysis). Attach QMint to the QC and NMint to the sample.
- 3.4.5 There is reasonable evidence (documented with the raw data) to suspect that the spiking solution was made incorrectly. If possible evaluate recovery based on the new spiking level. A qualifier is not necessary. If this is not possible attach qualifiers QMout and QFspk.

3.5 For MS/MSD results over calibration range:

- 3.5.1 If the result is acceptable – report with (QOcal) qualifier
- 3.5.2 If the result is unacceptable - Dilute and rerun.

3.6 Any analyte falling outside the acceptance criteria must be flagged (QMout or QRPDo. The sample used for the spike or duplicate must be flagged. NMout, NMoRo, or NRPDo.

3.7 Ideally, the spike or duplicate should be reanalyzed during the initial analytical run and the final (post follow-up) data entered into the computer. If

the retry is still out of acceptance criteria in the original batch, attach QFob to the QC and NMout, NRPDo, NMoRo, or NRPDa to the sample.

3.8 If sample is unavailable for follow up or past the holding time, attach the appropriate qualifiers to the QC (QMout, QRPDo, QRPDa, QMoRo, and QFnes or QFhte) and to the sample (NMout, NRPDo, NMoRo, or NRPDa).

3.9 If, due to the nature of the analysis, the follow-up is not performed during the initial run:

3.9.1 Enter the original data for the sample and QC and attach the qualifier- ?SUS to the sample used for the MS or DUP, QRPDo, QMoRo, or QMout and QFinP to the MS or DUP.

3.9.2 Create a re-extract for that sample. Explain in "Comments" that a re-extract was created for QC follow up purposes. Create a separate batch for the re-extract sample and the MS or DUP.

3.9.3 Enter the data from the re-analyzed QC and sample with the new batch. If the follow-up QC still does not pass MS/MSD or duplicate criteria, attach the proper qualifier to the QC (QFini and one of the following: QMint, QMout, QMSD, QRPDo, QRPDa, QRPDl) and to the sample (NMout, NRPDo, NMoRo, or NRPDa).

3.9.4 The ?SUS and QFinP qualifiers must be removed from the original data. Tag the original QC with QFpas or QFini. The original sample result must be tagged with NMout, NRPDo, NMoRo, or NRPDa if the follow up did not correct the problem.

3.10 If sample matrix is considered to be the cause of the QC failure, attach QMint to the QC and NMint to the sample. The analyst **must** however have valid reasons to suspect matrix. For example a MS and MSD attempted twice that fails % recovery criteria both times but passes precision criteria. See also section 3.4.4.

4.0 Initial Calibration

4.1 Acceptance is based on the Correlation Coefficient for linear curves, the Coefficient of Determination for nonlinear curves, or the percent RSD when response factors or calibration factors are used. See specific methods for requirements.

4.2 If the above criteria are not met, analysis cannot begin until the problem has been isolated and a valid calibration has been performed.

4.3 In addition, if Initial Calibration Verification (ICV) samples are not within specified laboratory acceptance criteria, the ICV may be reanalyzed. If the

ICV is still out, a new calibration must be performed. Sample analysis must not begin prior to resolving this problem. In some cases an autosampler run may be initiated prior to the end of a shift and then reviewed the next day. In this case all samples must be rerun with a valid calibration. If this is not possible, samples must either be canceled or reported with the proper qualifier (NCALh or NCALl).

4.4 Under the following circumstances results may be reported:

4.4.1 If the failed initial calibration check has a high bias, samples that are non-detects may be reported without a qualifier.

4.4.2 If the failed initial calibration check has a low bias, samples that have a result greater than the maximum regulatory result may be reported with a qualifier.

4.5 Organic analyses with ≥seven analytes

4.5.1 Analytes appearing in the calibration standards that are not part of the LIMS reporting lists will be noted with parentheses as **nontarget** analytes. These analytes are not evaluated for calibration acceptance since their results are not reported to clients. If a special request is made to report these analytes then calibration acceptance will be evaluated.

4.5.2 If all of the individual **target** analytes do not meet calibration acceptance criteria, a calibration may be accepted if the mean of the RSD values of **all** analytes (target and nontarget) in the calibration is ≤ 20%. If mean criteria is used instead of individual %RSD or coefficients, reportable results must be qualified for analytes outside original method criteria. If the mean is not acceptable, then a new calibration must be made before sample analyses.

4.5.3 If the ICV average is unacceptable, the system must be recalibrated. If any samples were analyzed they must be rerun.

4.5.4 An ICV is acceptable if the average of **all** the ICV analyte responses (% recovery or % difference between calibration average RF and ICV RF) is within 85-115% acceptance criteria or if every individual **target** analyte meets method acceptance criteria specified in the SOP.

4.5.4.1 Individual analytes falling outside the acceptance criteria must be noted on the analyst's run.

4.5.4.2 If analyte average is used for acceptance criteria, analytes outside original method criteria must be qualified as specified below:

4.5.4.2.1 All samples with results at or above the reporting limit are flagged with a qualifier signifying a low or high bias on specific analytes (NCALl or NCALh).

4.5.4.2.2 Samples that are ND for specific analytes:

4.5.4.2.2.1 If the ICV is bias high, a qualifier is not necessary.

4.5.4.2.2.2 If the ICV bias is low, and the reporting limit standard analyzed at the end of the run or batch of 20 samples shows a signal

4.5.4.2.2.2.1 - greater than the method blank then results do not require a qualifier.

4.5.4.2.2.2.2 - less than the method blank then results may not be reported and sample analysis must be repeated after the system has been brought under control. If reanalysis is not possible then results are reported with the (NCALl) qualifier and the RL is raised to the level of the ICV.

4.5.5 If any one or more analytes are outside laboratory acceptance criteria for two consecutive batches, a follow-up must be performed to determine the cause. See method SOP for specific exceptions.

5.0 Continuing Calibration Checks

5.1 If a calibration check (CCV) falls outside the acceptance criteria, it must be reanalyzed immediately.

5.2 If it is still out of range, determine the cause of the problem. Make necessary corrections and perform either two consecutive successful calibration verification checks or a new calibration. Any analyzed samples that were not bracketed by working QC must be reanalyzed.

5.3 If a continuing calibration verification check at the end of the run is out of range during an automated analysis, it is likely that the problem will be discovered after the run is completed. Upon discovery, if the instrument has been idle and it is believed that conditions of the instrument have not changed, the analyst may reinject the continuing calibration verification check. If this check is successful, the run is considered valid. If this check is not successful, the analyst must take corrective action and perform either two consecutive successful calibration verification checks or a new calibration. After that, any analyzed samples that were not bracketed by working QC must be reanalyzed.

5.4 Any data deemed reportable by management, that is associated with a failed continuing calibration check, must be flagged with the proper qualifier (NCALh or NCALl on specific analyte).

5.5 Under the following circumstances results may be reported:

5.5.1 If the failed continuing calibration check has a high bias, samples that are non-detects may be reported without a qualifier.

5.5.2 If the failed continuing calibration check has a low bias, samples that have a result greater than the maximum regulatory result may be reported with a qualifier.

5.6 Organic analyses with ≥seven analytes-

5.6.1 Analytes appearing in the calibration standards that are not part of the LIMS reporting lists will be noted with parentheses as **nontarget** analytes. These analytes are not evaluated for calibration acceptance since their results are not reported to clients. If a special request is made to report these analytes then calibration acceptance will be evaluated.

5.6.2 If all of the individual **target** analytes do not meet CCV acceptance criteria, a run may still be accepted as long as 85% or more of the target analytes in an individual CCV fall within method acceptance criteria. Target analytes outside method acceptance criteria must be qualified. See section 4.5.4.2.1 and 4.5.4.2.2 for qualifier details.

6.0 Surrogate Recovery

6.1 Surrogate recovery is acceptable if compound response falls within laboratory prescribed acceptance limits. If samples are spiked with multiple surrogate compounds, the following acceptance criteria applies:

# of surr. compounds in spike	# of surr. compounds allowed outside acceptance limits
1 – 2	0
3 – 4	1*
5 or more	see specific SOP

* Examine the entire run. If the same surrogate compound is out in more than 20% of the samples, perform maintenance prior to analyzing the next batch.

6.2 If there is reasonable evidence (documented with the raw data) to suspect that the spiking solution was made incorrectly. Evaluate recovery based on the new spiking level and report results without a qualifier. If this is not possible, qualify the surrogate compounds with QFspk for QC surrogates and NSspk for sample surrogates.

6.3 If surrogate recovery is unacceptable, the sample must be re-extracted assuming enough sample is available.

6.4 Whenever possible, re-extractions are performed within the holding time. However, they may be performed past the holding time since the reanalysis is to confirm if matrix interference is present. The original result, bearing a confirmed qualifier (NSdup) is reported to the client unless the follow up

shows it to be erroneous. If the reanalysis is reported it must be accompanied with the proper qualifier (N_HTc), clearly stating that it was analyzed past the regulatory holding time.

6.5 If re-extraction is not possible or if re-extraction does not correct the problem, results must be accompanied by the proper qualifier on the surrogate compound (NSdup, NShi, NSlo), if management deems the sample reportable.

6.6 On QC samples if some of the surrogates are out but it is still considered acceptable based on the above criteria use the qualifier QSpas on the surrogate compound that is out. If surrogate recovery is not acceptable on a QC sample, use QSout on the surrogate compound that is out. Surrogate failures on QC samples do not make the QC "out of control". Percent recovery and RPD alone are used to determine QC sample acceptability unless otherwise specified in the method SOP. However, method blanks however with low surrogate recoveries may indicate a problem. Please consult your supervisor.

7.0 Internal Standard

7.1 When internal standard performance is outside method specified criteria, the sample chromatogram is examined for possible interferences and the extract is reinjected if necessary.

- 7.1.1 If the reinjected result is compliant, results are reported from the second injection.
- 7.1.2 If the re-injection does not produce compliant results a calibration check is injected or the internal standard performance of the closest calibration check sample is examined.
  - 7.1.2.1 If the calibration check internal standard is valid, the sample is reextracted (if sample is available).
  - 7.1.2.2 If the calibration check internal standard is not valid, the instrument must be recalibrated and all samples not bracketed by working QC must be reanalyzed.

7.2 If re-extraction is not possible or it does not correct the problem, the appropriate qualifier (NIS) must be placed on the client's report, for samples that management deems reportable.

8.0 Carry Over

8.1 If during the course of an analysis, a sample is analyzed containing elevated levels of the analyte of interest, the analyst must reanalyze samples with a result falling between the reporting limit and ten times the reporting limit that followed the contaminating sample.. If the sample will be reported on a J-flag report all results greater than the MDL must be evaluated for possible carry over contamination.

8.2 Carry over studies are performed on most analyses.

8.2.1 The level at which carry occurs is proven by the carry over study. The carry over study consists of analyzing standards at increasing concentrations until carry over is observed in a blank at the reporting limit.

8.2.2 The standard level which produced carry over in the blank is noted by the analyst as the minimum level at which proceeding samples may need to be reanalyzed in future runs.

8.3 Whenever possible, follow-ups are performed within the holding time. However, they may be performed past the holding time since the reanalysis is to investigate if carry over is present. If reanalysis confirms the presence of the analyte, the original (within holding time) result must be reported. If the analyte is not present in the follow-up the reanalysis result is reported accompanied by the proper qualifier ((N-HTe or N-HTa), clearly stating that it was analyzed past the regulatory holding time.

8.4 If the sample is unavailable for reanalysis, the analyst must document with the raw data reasons why the sample can not be reanalyzed. Results may be reported with the appropriate qualifier (Nco).

8.5 If possible carry over has caused a drinking water analyte to be near or above the maximum contaminant limit (MCL), the result must be verified or the analysis must be canceled and the client notified to resample.

9.0 Extra Quality Control Samples

9.1 When the analyst performs QC samples at a frequency that exceeds SOP requirements which are based on method, NELAP, and ESB laboratory criteria, the extra QC may be treated in the following manner:

9.1.1 All QC is reported, however it must be accompanied with the proper QC qualifier if it is out of laboratory acceptance criteria.

9.1.2 Data associated with out of control QC (that is considered to be extra) does not require a sample qualifier.

10.0 Qualifier Note

10.1 If a LCS or MB qualifier is necessary, it must be placed on samples associated with the corresponding **preparation** batch. ICV and CCV qualifiers must be placed on samples associated with the corresponding **analytical** batch or run. MS, duplicate, surrogate or internal standard qualifiers are placed on the **sample** in question only.

10.2 Definitions:

10.2.1 **Batch**: environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents.

10.2.2 A **preparation batch** is composed of one to twenty environmental samples of the same NELAP-defined matrix, meeting the above-mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours.

10.2.3 An **analytical batch or run** is composed of prepared environmental samples (extracts, digestates or concentrates) which

are analyzed together as a group. An analytical batch can include prepared samples originating from various environmental matrices and can exceed twenty samples.

11.0 Calculation of QC Results

11.1 Convert results into final units prior to calculation.

11.2 Always use the maximum number of digits to calculate QC results, percent recovery, RPD etc.

11.3 QC results may round up to be acceptable. For example: If 95% is the lower limit acceptance criteria

11.3.1 $\geq$94.6% is acceptable

11.3.2 $\leq$94.5% is not acceptable

Approved by Susann K. Thomas Date 08/28/06

Edward S. Babcock & Sons
STANDARD OPERATING PROCEDURE
Pollution Prevention and Waste Management
Effective Date_____09/08/06_____

1.0 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel will use pollution prevention techniques to address their waste generation. In some cases when wastes cannot be feasibly reduced at the source, recycling is utilized.

2.0 Chemical Volume Reduction
- 2.1 Wherever permitted, methods are used that require smaller volumes of reagents, thereby reducing the potential hazards to both the analyst and the environment involved.
- 2.2 A chemical inventory is maintained in order to monitor that chemical volumes are kept to a minimum. Whenever supplies are ordered, the smallest quantity needed is purchased. Quantities are based on expected usage during shelf life.
- 2.3 Actual reagent preparation volumes are based on anticipated usage and reagent stability.

3.0 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions. The laboratory has the responsibility to protect the air, water, and land by minimizing and controlling releases from fume hoods and bench operations. The laboratory must attempt to comply with the letter and spirit of sewer discharge permits and regulations, and with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal.

4.0 Excess reagents, samples, digests, and extractions are characterized and disposed of in an acceptable manner.
- 4.1 Acid and alkaline wastes are neutralized prior to sewer disposal. More concentrated acid and base solutions are hauled away by an independent waste hauler. See SOP S05
- 4.2 Waste generated from the Chemical Oxygen Demand analysis containing chromate, mercury, and silver is poured into a COD Only drum which is hauled away by an independent waste hauler.
- 4.3 Waste generated from the Kjeldahl analysis containing mercury is poured into a KjN Only drum which is hauled away by an independent waste hauler.
- 4.4 Chlorinated solvents are recycled whenever possible. When this is not feasible, the waste is sparged continuously until the contents of the drum has

an acceptable level of residual solvents. The contents are then neutralized and sewered. See SOP S06.

References:

"The Waste Management Manual for Laboratory Personnel," available from the American Chemical Society

"Less Is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C., 20036. (202) 872-4477.

Approved by Susann K. Thomas Date 08/28/06