

**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 mmddyinitials" 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 “FILTEREDmmddyinitials” in front of the “needs to be filtered” note.

- 3.7.1 For metals filtering: Acid rinse filtration apparatus with 1:1 HNO<sub>3</sub>. 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  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  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  $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$  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  $\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$  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<sub>2</sub>SO<sub>4</sub> per liter to pH < 2 and refrigerated to 4° 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 °C 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 (NH<sub>4</sub>)/ 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<sub>2</sub>SO<sub>4</sub>. 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  $\frac{1}{2}$  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 **NO<sub>3</sub>** 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 (**DH<sub>2</sub>S**) - 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<sub>3</sub>, 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

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

Approved by Susann K. Thomas Date 08/28/06

**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  $35^{\circ}\text{C} \pm 0.5^{\circ}$ . Each broth tube which shows  $\text{CO}_2$  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  $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$  for  $24 \pm 2$  hours in the fecal water bath. If  $\text{CO}_2$  formation is observed in the EC tube after the incubation period, the sample is considered positive for fecal coliform bacteria. If  $\text{CO}_2$  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  $44.5^{\circ}\text{C} \pm 0.2^{\circ}$
- 6.8 Incubators at  $35^{\circ}\text{C} \pm 0.5^{\circ}$

#### 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<sup>2</sup> 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:  $6.8 \pm 0.2$
- 7.1.2.2 Brilliant Green Bile:  $7.2 \pm 0.2$
- 7.1.2.3 EC Media and EC + MUG:  $6.9 \pm 0.2$
- 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  $35.0 \text{ }^\circ\text{C} \pm 0.5 \text{ }^\circ\text{C}$ . 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  $7.1 \pm 0.2$ . 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°C until manufacturers due date. Reference cultures are rehydrated in lauryl sulfate broth at  $35^\circ\text{C} \pm 0.5^\circ\text{C}$  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
<i>Enterobacter aerogenes</i>	+	+	-	-	NA
<i>Pseudomonas aeruginosa</i>	-	-	-	-	NA
<i>Escherichia coli</i>	+	+	+	+	+
<i>Enterococcus faecalis</i>	NA	NA	NA	NA	-

## 8.0 Sample Collection, Preservation and Storage

- 8.1 Samples are collected in sterile 120 mL containers containing  $\text{Na}_2\text{S}_2\text{O}_3$  for chlorine removal.
- 8.2 Samples must be kept cold,  $<10^\circ\text{C}$  from sampling until analysis. If a sample arrives  $<30^\circ\text{C}$  and on ice it is assumed that the cooling process has begun. If a sample arrives  $>30^\circ\text{C}$  (even if it is on ice) or  $>10^\circ\text{C}$  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  $24 \pm 2$  hours, examined, and then incubated for an additional  $24 \pm 3$  hours, if needed at  $35^\circ\text{C} \pm 0.5^\circ$ .
- 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  $1 \times 10^{-3}$  mL at the beginning of the series and ending at  $1 \times 10^{-6}$  mL. Semi-solid raw influents usually are started at  $1 \times 10^{-5}$  mL and end at  $1 \times 10^{-8}$  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  $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ .  $24 \pm 2$  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  $\text{CO}_2$  formation inside the Durham tube. The negative broth tubes are re-incubated for another  $24 \pm 3$  hours. After the total  $48 \pm 3$  hours of incubation shows no  $\text{CO}_2$  formation in the Durham tubes, the test is completed and the sample is considered negative for coliform bacteria. The broth tubes which do contain  $\text{CO}_2$  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  $\text{CO}_2$  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  $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for a total of  $48 \pm 3$  hours. If gas formation is present in their Durham tubes when examined after  $24 \pm 2$  or  $48 \pm 3$  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  $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$  for only  $24 \pm 2$  hours in the fecal water bath. Tubes are placed in the water bath within 30 minutes of inoculation. If  $\text{CO}_2$  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  $\text{CO}_2$  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  $24 \pm 2$  hours. If the fecal water bath has maintained a constant temperature of  $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ , 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  $24 \pm 2$  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  $\text{CO}_2$  is streaked on an eosin methylene blue agar dish. The streaking method must ensure presence of some discrete colonies after a  $24 \pm 2$  hour incubation period at  $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . 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  $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for  $24 \pm 2$  hours. If no gas production is observed, reincubate for an additional  $24 \pm 3$  hours. Formation of gas within this lauryl sulfate broth tube within the  $48 \pm 3$  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:

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

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
0-0-0	< 2	—	—	4-2-0	22	9.0	56
0-0-1	2	1.0	10	4-2-1	26	12	65
0-1-0	2	1.0	10	4-3-0	27	12	67
0-2-0	4	1.0	13	4-3-1	33	15	77
1-0-0	2	1.0	11	4-4-0	34	16	80
1-0-1	4	1.0	15	5-0-0	23	9.0	86
1-1-0	4	1.0	15	5-0-1	30	10	110
1-1-1	6	2.0	18	5-0-2	40	20	140
1-2-0	6	2.0	18	5-1-0	30	10	120
2-0-0	4	1.0	17	5-1-1	50	20	150
2-0-1	7	2.0	20	5-1-2	60	30	180
2-1-0	7	2.0	21	5-2-0	50	20	170
2-1-1	9	3.0	24	5-2-1	70	30	210
2-2-0	9	3.0	25	5-2-2	90	40	250
2-3-0	12	5.0	29	5-3-0	80	30	250
3-0-0	8	3.0	24	5-3-1	110	40	300
3-0-1	11	4.0	29	5-3-2	140	60	360
3-1-0	11	4.0	29	5-3-3	170	80	410
3-1-1	14	6.0	35	5-4-0	130	50	390
3-2-0	14	6.0	35	5-4-1	170	70	480
3-2-1	17	7.0	40	5-4-2	220	100	580
4-0-0	13	5.0	38	5-4-3	280	120	690
4-0-1	17	7.0	45	5-4-4	350	160	820
4-1-0	17	7.0	46	5-5-0	240	100	940
4-1-1	21	9.0	55	5-5-1	300	100	1300
4-1-2	26	12	63	5-5-2	500	200	2000
				5-5-3	900	300	2900
				5-5-4	1600	600	5300
				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 Relevance:** 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°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  $20 \pm 1^\circ\text{C}$  (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  $\text{KH}_2\text{PO}_4$ , 21.75 g  $\text{K}_2\text{HPO}_4$ , 33.4 g  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$  and 1.7 g  $\text{NH}_4\text{Cl}$  in 500 ml Nanopure water and dilute to 1 liter. PH should be 7.2.
- 10.2. Magnesium Sulfate solution: Dissolve 22.5 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in Nanopure water and dilute to 1 liter.
- 10.3. Calcium Chloride solution: Dissolve 27.5 g  $\text{CaCl}_2$  or 36.5 g  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$  in Nanopure water and dilute to 1 liter.
- 10.4. Ferric Chloride solution: Dissolve 0.25 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  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<sub>1</sub> and D.O<sub>2</sub> 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<sub>1</sub> reading.
  - 11.2.7. Determine the initial dissolved Oxygen (see Dissolved Oxygen procedure SOP I24) of samples using DO<sub>1</sub>. bottles.
    - 11.2.7.1. If the DO<sub>1</sub> is considerably lower (1 mg/L) than the other DO<sub>1</sub>'s and blanks then redo that sample with a lesser volume.
    - 11.2.7.2. If the DO<sub>1</sub> 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<sub>2</sub>) 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<sub>2</sub>. samples for 5 days, determine the final Dissolved Oxygen (see Dissolved Oxygen procedure) on the D.O<sub>2</sub>. bottles.
- 11.3. Other BOD Products
- 11.3.1. BOD-dissolved - dissolved BOD: Filter sample through 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 – carbonaceous 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:

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

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:

$$\text{BOD mg/L} = \frac{(\text{D.O}_1 - \text{D.O}_2) - (\text{B}_1 - \text{B}_2) f}{P}$$

or

$$\text{BOD mg/L} = \frac{(\text{D.O}_1 - \text{D.O}_2) - (\text{B}_1 - \text{B}_2) f}{W} \times 100$$

Where:

D.O<sub>1</sub>. = D.O. of diluted sample immediately after preparation.

D.O<sub>2</sub>. = D.O. of diluted sample after a 5 day incubation at 20°C.

B<sub>1</sub>. = D.O. of seeded blank immediately after preparation.

B<sub>2</sub>. = 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 recal” 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°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°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.

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**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 digester, 150°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)

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- 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 is 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

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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 18<sup>th</sup> 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  $\mu\text{mho/cm}$

**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  $\mu\text{mhos/cm}$  (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  $\mu\text{mhos/cm}$ .

**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  $\mu\text{mho/cm}$  (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° 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° 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°C using a water bath. Warm tap water of approximately 27°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  $\frac{1}{4}$  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  $\mu\text{mho/cm}$ . 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:

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

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

1413 $\mu$ mhos/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:

Sample Reading ( $\mu$ mho's) X	1413	
		Standard Reading ( $\mu$ mho's)

### 14.0 Reporting:

- 14.1 Conductivity is reported as  $\mu$ mhos/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  $\mu$ mho/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  $\mu$ mhos/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

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**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**

<u>ANALYTE:</u>	<u>CAS #</u>	<u>MCL (mg/L)</u>
Chloride Cl	7782-50-5	250/500/600*
Nitrate NO <sub>3</sub>	14797-55-8	45
Sulfate SO <sub>4</sub>	14808-79-8	250/500/600*

**Non NELAP Analytes:**

Fluoride F	7782-41-4	2.0
Nitrite NO <sub>2</sub>		3.29
Phosphate PO <sub>4</sub>		
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.

	<u>RL, mg/L</u>
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  $\mu\text{L}$  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 times 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 ( $\text{NaHCO}_3$ ) and 0.763 g of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) 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. $\text{H}_2\text{SO}_4$ 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 <sub>3</sub>	50ppm	90-110
SO <sub>4</sub>	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 <sub>3</sub>	50	100ppm	90-110
SO <sub>4</sub>	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<sub>3</sub>, SO<sub>4</sub>): 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.

<u>Std #1</u>			<u>Std #5</u>		
	<u>mL into 1L</u>			<u>mL into 200mL</u>	
Cl	1 ppm	1.0	Cl	50ppm	10
NO <sub>3</sub>	1 ppm	1.0	NO <sub>3</sub>	50ppm	10
SO <sub>4</sub>	0.5ppm	0.5	SO <sub>4</sub>	50ppm	10
<u>Std #2</u>			<u>Std #6</u>		
	<u>mL into 50mL</u>			<u>mL into 50mL</u>	
Cl	10ppm	0.5	Cl	75ppm	3.75
NO <sub>3</sub>	10ppm	0.5	NO <sub>3</sub>	60ppm	3.0
SO <sub>4</sub>	10ppm	0.5	SO <sub>4</sub>	100ppm	5.0
<u>Std #3</u>			<u>Std #7</u>		
	<u>mL into 50mL</u>			<u>mL into 50mL</u>	
Cl	20ppm	1.0	Cl	100ppm	5.0
NO <sub>3</sub>	20ppm	1.0	NO <sub>3</sub>	75ppm	3.75
SO <sub>4</sub>	20ppm	1.0	SO <sub>4</sub>	150ppm	7.5
<u>Std #4</u>			<u>Std #8</u>		
	<u>mL into 50mL</u>			<u>mL into 200mL</u>	
Cl	35ppm	1.75	Cl	250ppm	50
NO <sub>3</sub>	35ppm	1.75	NO <sub>3</sub>	100ppm	20
SO <sub>4</sub>	35ppm	1.75	SO <sub>4</sub>	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:

<u>Std #1</u>	<u>mL into 50ml</u>	<u>Std #4</u>	<u>mL into 40mL</u>
PCBSA 1ppm	0.5	PCBSA 50ppm	20
<u>Std #2</u>	<u>mL into 40mL</u>	<u>Std #5</u>	<u>g into 1L</u>
PCBSA 5ppm	2	PCBSA 100ppm	0.1
		*same as the Int. Std.	
<u>Std #3</u>	<u>mL into 50mL</u>		
PCBSA 10ppm	5		

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

9.5.6.1 The standards below for Cl,NO<sub>3</sub>, and SO<sub>4</sub> are made from 1000ppm stock solution into a graduated cylinder and brought up to volume of 100ml with nanopure.

		<u>mL of 1000ppm stock</u>
Cl	50ppm	5
NO <sub>3</sub>	50ppm	5
SO <sub>4</sub>	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<sub>3</sub>, SO<sub>4</sub>) : 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 <sub>3</sub>	High	20 ppm	100 uL of 1000ppm stock
SO <sub>4</sub>	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  $\mu\text{m}$  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<sub>4</sub>, 100 mg/L for NO<sub>3</sub>) 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<sub>2</sub><sup>-</sup> as N  
NO<sub>3</sub><sup>-</sup> as N or as NO<sub>3</sub> if desired by the client  
H(PO<sub>4</sub>)<sub>2</sub><sup>-</sup> 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)  
$$\text{mequiv. of OH} + \text{CO}_3 + \text{HCO}_3 + \text{SO}_4 + \text{Cl} + \text{NO}_3 = \text{TA}$$
- 12.5.2 Electrochemical Balance (ECB)  
$$\text{Total Cations (TC)} - \text{Total Anions (TA)}$$
- 12.5.3 Total Dissolved Solids by Summation (TDSSUM)  
$$\text{mg/L of } 0.6(\text{Total Alkalinity}) + \text{Na} + \text{K} + \text{Ca} + \text{Mg} + \text{SO}_4 + \text{Cl} + \text{NO}_3 + \text{F} + \text{SiO}_3 = \text{TDSSUM}$$

## 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<sub>2</sub> 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 NO<sub>2</sub>-N/L as proven by a yearly curve. The reporting limit is 0.10 mg NO<sub>2</sub>-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 soluble 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°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  $\text{NCl}_3$  imparts a false red color when color reagent is added.
- 4.4 The following ions cause precipitation:  $\text{Sb}^{3+}$ ,  $\text{Au}^{3+}$ ,  $\text{Bi}^{3+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{PtCl}_6^{2-}$ , and  $\text{VO}_3^{2-}$ .
- 4.5 Cupric ion may cause low results by catalyzing decomposition of the diazonium salt.
- 4.6 Chlorine converts  $\text{NO}_2$  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 manufacturer'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:  
$$\frac{(\text{Spiked sample} - \text{sample})}{1} \times 100$$

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

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**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<sub>2</sub> B

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

Approved by  
08/23/06

Susan K. Thomas

Date

**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: 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  $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$  or 364 g  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  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  $\text{NaN}_3$  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  $\text{C}_7\text{H}_6\text{O}_3$

- 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  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  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  $\text{KH}(\text{IO}_3)_2$  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  $\text{Na}_2\text{S}_2\text{O}_3$  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  $\text{DO}_1$  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  $\text{DO}_2$  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.  $\text{DO}_1$ : Take a probe reading.
- 10.2.2.2.3.  $\text{DO}_2$ : 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.



**pH**  
Edward S. Babcock & Sons  
STANDARD OPERATING PROCEDURE  
(EPA 9040B, 9045C)  
(SM 4500-H<sup>+</sup> 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  $\frac{1}{4}$  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  $\pm 0.1$  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  $\pm 0.1$  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 every analytical batch and at a minimum of once for every 20 samples per matrix type. The results are compared to acceptance ranges  $\pm 0.1$  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°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:
- | <u>Ec reading</u> | <u>Na in Molarity</u> |
|-------------------|-----------------------|
| 2300              | 0.1                   |
| 11,500            | 0.5                   |
| 23,000            | 1.0                   |
| 46,000            | 2.0                   |



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

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

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**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**

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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

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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<sub>2</sub>SO<sub>4</sub>, 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<sub>2</sub>SO<sub>4</sub>. 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<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O] 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<sub>4</sub>H<sub>4</sub>O<sub>6</sub>·1/2H<sub>2</sub>O 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<sub>2</sub>PO<sub>4</sub> previously dried in a 105°C oven and stored in a desiccator.

9.6.2 Intermediate 1000ppm: Weigh up 4.394g KH<sub>2</sub>PO<sub>4</sub> 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 PO<sub>4</sub>-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<sub>2</sub>PO<sub>4</sub> previously dried in a 105°C oven and stored in a desiccator.

9.6.5 LCS Intermediate 500ppm: Dilute 2.197g KH<sub>2</sub>PO<sub>4</sub> 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.





































































































































































































































































































































































