

# Appendix B

## Standard Operating Procedures-Laboratories

### Clermont County Water Quality Monitoring Program

Clermont County  
Office of Environmental Quality

March, 2015

# Table of Contents

SAMPLE HANDLING .....	1
ECOLI .....	6
AMMONIA .....	11
NITRITE NITROGEN .....	16
NITRATE NITROGEN.....	22
ORTHO PHOSPHORUS .....	27
TOTAL PHOSPHORUS.....	33
TOTAL AND TOTAL VOLATIVE SUSPENDED SOLIDS.....	40

## **SAMPLE HANDLING**

The person receiving any samples must ensure: the labels or marked bottles are legible; the samples' markings clearly show identity with the proper WWTP & site codes; the samples date is clearly marked in cases where date confusion could occur; the information on the sample bottles matches the info on the COC; the COC is legible; the COC clearly states the testing requested; the information on the COC, and the sample bottles, matches the info on any Sample Report forms; samples get lab identification numbers on the bottles, the Sample Report forms, or both as necessary; sample info is written on the dry board as necessary for the analyses; the Sample Report forms are distributed to the proper areas; the samples get the proper preservation; no sample is older than the EPA holding time for the analyses requested (be careful that no fecal or E. coli sample is too old); the COC is signed; and that private contractors get a copy of their COC.

### A. MLTS, MLTVS, SVI Samples

1. Check that the date is reasonable; it should be the same date for each sample set. Notify the lab supervisor if older than 7 days.
2. Flag any samples that require other tests: some RAS samples want %TS & %VS.
3. Put the samples at MLTS analysis station.

### B. Sludges

1. Check that the date is reasonable, and the plant and station codes are correct and written on the label. Check that all required information is present. Notify the lab supervisor if older than 7 days
2. For LSL, DSR, & LS2 samples, give the sample a number and record on bottle label. These samples have OEPA required analyses for permit purposes.
3. If the sample requests any tests other than SLGTS & SLGVS, write that information on the dry board & tell the sludge analyst. Tell the lab supervisor if the tests are too odd.
4. Put sample bottle at the sludge analysis station.

### C. Private Contract Samples-- Regularly Scheduled & Unscheduled

1. Check that the correct date and sample time are on the Sample Report form. Make sure all required information is present. Check that this information matches the information on the COC. Put assigned sample number in appropriate space on the Sample Report form.

2. Check that the label information agrees with the Sample Report form. Write the sample number on the label, if the sample has a disposable label.
3. Check that any fecal or E coli samples are < 8 hours old. Tell the bacteriological analyst if you get a sample that is within 30 minutes to its holding time.
4. If more than the regular testing is requested, or if a sample is not scheduled, put this information on the dry board. Notify the lab supervisor if the sample requests any odd testing. The Holding Time requirements of some tests mean that we not accept certain samples without advanced notice.
5. If the samples need SS only, place them at the SS station. Put all other samples in the outlined sample area (on the right as you come in) in 07.
6. For Ammonia samples, put the Sample Report on the Ammonia clipboard at the Ammonia station. For Oil & Grease samples, hang the Sample Report form on the side of the fridge in 05 after preserving the sample. For all other samples, hang the Sample Report forms from the dry board with a magnet.

#### D. System Samples

1. Check the label for all the correct, legible information. Check that sample time is within the last 48 hours. Notify the lab supervisor if the sample came in older than 48 hours. Check that this information matches the information on the COC.
2. Assign the sample a number and write it on the label. The next available number for samples is logged on the clipboard at the sample receiving station. Numbers are 4-5 digits and the first digit(s) correspond to the month. For example the first sample number of January is 1000.
3. Write the information on the Clermont County System Data sheet in 07.
4. Write the information on the dry board for BOD, CBOD, Ammonia, & SS.
5. Write the samples in the outlined sample area (on the right as you come in) in 07.

#### E. LMR Samples

1. Check the bottles & COC for all the correct, legible information.
2. Give the samples numbers. Write these on the labels & on the LMR check off sheet. Get a new LMR check off sheet from the lab supervisor if needed.
3. Write the information on the dry board and note any samples or tests that are not routine.
4. Place the samples in the outlined sample area (on the right as you come in) in 07. Take any E. coli samples back to the bacteriological fridge.

F. Oil & Grease Samples

1. If the sample is taken for analysis by our lab, preserve each sample with 5 mL of 50% HCl per 1000 ml of sample. Mark the label with "HCl". If the sample is taken in a contract lab bottle, proceed to Step 2.
2. Place samples on the Oil shelf in the sample refrigerator in 05.

G. Metals Samples

1. These should already be preserved if they come in a Contract Laboratory lab bottle. Notify the lab supervisor if a sample comes in any other bottle for metals.
2. Place the samples on the table next to the Sample Receiving area.

H. Cyanide Samples

1. If the sample is taken for analysis by our lab, preserve the sample if necessary, with 5 mL 10 N NaOH per 1000 mL of sample. If the sample is taken in a contract lab bottle, proceed to Step 2.
2. Give the sample a unique lab id number. Write it on the label.
3. Place samples on the CN shelf in the sample refrigerator in 05.

I. Ammonia, PTOT, & TKN Samples

1. If the sample is to be analyzed in our lab, preserve these with conc. H<sub>2</sub>SO<sub>4</sub>: 2 mL per 1000 mL sample, or to a pH < 2. Any bottles from a contract lab will already have the needed preservative in them, so proceed to Step 2.
2. If you aliquot a larger sample into smaller bottles, label these with all the proper information: identity; date; time; required test; preservation.
3. Store the samples on the correct shelf in the fridge in 05.

J. Nitrate, Nitrite, & Ortho P Samples

1. Filter through a membrane filter.

2. If you aliquot a larger sample into smaller bottles, label these with all the proper information: identity; date; time; required test; preservation.
3. Filtered samples have 48 hour holding times & require preservation by cooling to 6<sup>0</sup> C. These tests can therefore use the same preserved sample. Filter enough for both tests in one bottle.

#### K. END OF THE DAY

1. At about 3:45 P.M. ask each analyst who would handle a sample listed on the dry board if they have run or are aware of the required tests.
2. Check the dry board: analysts usually erase the information on the board as they finish that analysis for a sample. If, at the end of the day, info is still on the dry board, check that that test has been taken care of.
3. Look at the various check off sheets (Week, Month, LMR...) to see if the analyses are marked off as done, or that the samples have been saved for latter analysis.

#### L. Storage

1. All samples are kept close to the analysis area until finished. An extra CCWR EFF bottle is kept in the compositing room until the end of the day.
2. All preserved samples are kept in their area of the fridge in 05 until analysis. During analysis, the analyst keeps track of them, usually close to the analysis area.
3. Metals samples are kept on the small table next to the Sample Receiving area until the Contract Laboratory Lab pickup. Pickup usually happens every Monday & Thursday. The lab supervisor must get their COCs after the pickup.
4. Organics (Pesticides, BNAs, VOCs, Phenols), CN samples are kept in the fridge in 05. The lab supervisor must get their COCs after the Contract Laboratory Lab pickup.
5. Miscellaneous (those that are not analyzed that day) samples are kept in the fridge in 08, or in the one in 05.
6. Bacterial samples are kept in the bact fridge analysis. These samples can not be outside the fridge longer than 30 minutes until they are finished.

#### M. Sample Disposal

1. Sludges & MLSS samples go in the fridge in 05 after the initial setup. Any sludge with a sample number goes on the top shelf. Operational samples go into a bucket at the bottom. These sludges poured down the sink and the containers are thrown in the dumpster. The top shelf samples are discarded by the lab supervisor after that month's EPA DMR is finished.
2. Regular daily samples are discarded down the sink in the compositing room after all analyses are finished.

3. Any special sample is discarded as in #2 once all special testing is either done, or the sample has been saved for future analysis. The preserved samples are discarded by the analyst when they have been analyzed. LMR samples must be checked off the sheet before discarding
4. Bacterial samples are discarded in the fecal room once they have been set up.
5. Any samples that go to an outside lab are discarded by them.

#### N. Sample Tracking

1. The lab supervisor regularly runs a tracking program in the SW Monitoring software: WW NPDES Monitoring Requirement Tracking. This report compares the data entered into the SW database to the requirements for that sample site. It states what data is missing for the CCSD sites.
2. Since the report in #1 only monitors data in the SW software, the lab supervisor then checks the various check off sheets against the contents of the fridges to ensure that samples have been preserved & saved for future analysis. He checks all CCSD sample, contract lab samples, & the LMR or other special samples.
3. After the checks in #1 & #2, the lab supervisor checks any missing samples to see if any testing was not requested by the COCs, or was missed by the lab. Further sampling & testing, if required, is scheduled.
4. From the check in #2, the lab supervisor monitors the various holding times to ensure timely analysis.
5. Early in each month, the lab supervisor makes up a chart listing all metals & organics sampling required for that month by the CCSD WWTPs. This information is also on the Monthly & Quarterly check off sheets. He then fills in the chart to ensure that all testing is done.
6. When any sample is preserved & saved for a latter test, the analyst who saves it must mark the Weekly or Monthly check off sheets to show that those samples have been saved for that test. We do this with a small "s" & the sample date.

Revision 6  
Revised 6/22/2015  
Hannah Lubbers

## Standard operating procedure for simultaneous determination of *Escherichia coli* (*E. coli*), fecal coliform and total coliform using the IDEXX Quanti-tray/2000 system.

### BACKGROUND

Improperly treated water may contain microorganisms that are pathogenic. Water can be analyzed for the presence of an indicator organism from the coliform group of bacteria. Fecal coliform and specifically *Escherichia coli* tests are used in an attempt to more accurately determine the extent of human wastes in water.

### 1 DEFINITIONS

- 1.1 Total coliform – Rod shaped gram-negative bacteria which ferment lactose and contain the enzymes  $\beta$ -D-galactosidase. They are abundant in the feces of warm-blooded animals and include bacteria that are naturally present in soil and water. They are not the cause of sickness, but their presence is used to indicate contamination in water quality.
- 1.2 Fecal Coliform - A group comprising many anaerobic, gram-negative, rod shaped bacteria that develop blue colonies within 24 hours at  $44.5 \pm 2^\circ$  C on an M-FC type medium containing lactose. Coliform bacteria include genera that originate in feces (e.g. *Escherichia*) as well as genera not of fecal origin (e.g. *Klebsiella*, *Citrobacter*).
- 1.3 E. Coli - A gram-positive bacteria possessing the enzyme  $\beta$ -D-glucosidase, which cleaves the nutrient indicator and produces fluorescence under a long wavelength (366nm) UV light. Found primarily in the gut and feces of warm-blooded animals. Most *E. Coli* strains are harmless, but some can cause food poisoning in humans. Of the several types of bacteria in the total coliform group, *E. Coli* does not typically reproduce in soil and water environments. Their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination.
- 1.4 Most Probable Number (MPN) – A statistical method for determining bacterial density based on the Poisson distribution.

### 2 SCOPE AND APPLICABILITY

- 2.1 The Colilert Quanti-tray/2000 method, approved by the USEPA in 2000, describes the process for the collection and analysis of total coliform and *E. coli* bacteria in 100 mL samples of water and wastewater.
- 2.2 The detection limit for this test ranges from 1 Most Probable Number (MPN) per 100 mL of sample to > 2419 MPN per 100 mL sample.
- 2.3 This procedure may be used to determine fecal coliform by using colilert-18 and incubating at 44.5 C temperature only if permitted by your EPA regulatory agency. A written document stating the permission should be included with this SOP in order to use colilert-18 for fecal coliform determination.

### 3 SAMPLE COLLECTION, HANDLING AND PRESERVATION

3.1 Ice or refrigerate bacteriological samples at a temperature less than 10°C during transit to the laboratory. Samples are stored at 4°C once at the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Take care that storage vessels are not totally immersed in water during transit.

3.2 Examine samples as soon as possible after collection. Do not exceed 6 hours holding time and process within 2 hours.

#### **4 APPARATUS AND MATERIALS**

4.1 Quanti-tray sealer

4.2 Watt fluorescent lamp

4.3 Incubator at 35.0 ± 0.5° C (Total Coliforms and *E. Coli*)

4.4 Incubator at 44.5 ± 0.5° C (Fecal Coliforms)

4.5 Colilert-18 reagent

#### **5 HEALTH AND SAFETY WARNINGS**

5.1 Lab Safety – Due to various hazards, safety glasses must be worn at all times while in the laboratory. Hands must be washed thoroughly before performing any bacteriological work; during the analysis if there is contact with a contaminated surface or sample, and after the analysis. Avoid inhalation of dust from media reagent ampoules when they are opened. Do not look directly into the UV light.

5.2 Chemical Hygiene – Please refer to the MSDS file for any questions concerning an analysis reagent or standard's toxicity and the necessary safety precautions.

5.3 Waste Disposal – All contaminated articles (Quanti-Trays, sample bottles, media ampoules) are disposed in biohazard bags separately from general waste. Biohazard bags are kept in the bacteriological areas for this purpose. Biohazard bags are autoclaved for 45 minutes and disposed with the general waste.

5.4 Pollution Prevention – Be respectful of sterility issues in the bacteriological area; use a bacterial disinfectant to remove residue/contamination from countertops. Wash hands thoroughly BEFORE and AFTER performing tasks in the bacteriological area.

#### **6 INTERFERENCES**

6.1 Antifoam solution may be added if excessive foaming occurs after the addition of colilert-18 to the sample.

6.2 Samples may contain material that affects the color of the sample. Compare inoculated trays to a control tray containing only water. Some water samples containing humic material may have an innate color and a control blank of the same water sample may be required for comparison to the inoculated sample.

6.3 Test samples should be incubated for the full term. Test sensitivity maybe affected by taking the samples out of the incubator too soon ending with false negatives

6.4 Autofluorescent plasticware or glassware may produce false positives. Check sample containers prior to sampling and processing.

## **7 METHOD CALIBRATION AND PREVENTATIVE MAINTENANCE**

7.1 No calibration is necessary for this method. A sealer check is conducted monthly to ensure that equipment seals properly.

7.2 Prompt cleaning after a spill will help to maintain the proper performance of the 2X sealer. Any troubleshooting or repairs other than cleaning must be referred to an IDEXX service center.

7.3 Ensure power supply is off, sealer is unplugged and unit has completely cooled down for at least 90 minutes. Remove input tray shelf. Loosen the four quarter-turn fasteners and remove the access panel.

7.4 Use mild detergent, diluted bleach or isopropyl alcohol to clean all accessible surfaces inside the sealer and the lower roller assembly. DO NOT use abrasive materials. DO NOT use caustic cleaners. Use ONLY alcohol on cool sealer.

7.5 Dry interior and roller assembly with paper towels or soft cloth. Fasten access panel and reattach tray shelf. Sealer is now ready for use.

## **8 SAMPLE PREP AND ANALYSIS**

8.1 Wipe down the counters with bacteriological disinfectant before and after the analysis.

8.2 Turn on the sealer and allow it to warm up. The sealer is ready when the green light turns on.

8.3 Label the Quanti-tray with sample name, dilutions if any, analysis date, analysis run (E. coli, fecals)

8.4 Pour water sample into 120 mL container and aseptically adjust volume to the calibrated 100 mL line.

8.5 Open one colilert-18 media reagent ampule and add contents to the sample container.

8.6 Shake until media is dissolved. Allow foam to settle.

8.7 Use one hand to hold a Quanti-tray with the well side facing the palm.

8.8 Squeeze the upper part of the Quanti-tray so that it bends toward the palm.

8.9 Gently pull the foil tab to separate the foil away from the tray.

8.10 Pour 100 mL sample into the tray. Tap small wells 2-3 times to release air bubbles.

8.11 Place the sample filled tray onto the sealer rubber insert with the well side (plastic) facing down.

8.12 Slide insert into front of sealer until it takes hold and pulls the rest of the way through.

8.13 Incubate all trays for 18-22 hours at  $35.0^{\circ}\text{C} \pm 0.5$  for E. coli or  $44.5^{\circ}\text{C} \pm 0.5$  for fecal coliforms.

## 9 INTERPRETATION

9.1 Count both large and small yellow wells. These yellow wells can be used to determine Total Coliforms.

9.2 If less yellow than the comparator, the sample is negative.

9.3 If the sample has a yellow color equal to or greater than the comparator, the presence of total coliforms is confirmed.

9.4 If the sample is yellow, but lighter than the comparator, it may be incubated an additional 4 hours (but no more than 22 hours total). If the sample is total coliform positive, the color will intensify. If it does not intensify, the sample is negative.

9.5 If yellow is observed, check vessel for fluorescent by placing a 6-watt, 365-nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. If the fluorescence is greater or equal to the fluorescence of the comparator, the presence of E. coli is confirmed.

9.6 Count both large and small fluorescing wells.

9.7 If no wells fluoresce, it is negative for E. coli or fecal coliforms

9.8 Colilert-18 results are definitive at 18-22 hours. In addition, positives for both total coliforms and E. coli observed before 18 hours and negatives observed after 22 hours are also valid.

9.9 Use MPN chart to calculate results.

9.10 Report results on a bench sheet.

## 10 DILUTIONS

10.1 A knowledge of the source water to be sampled, seasonal variability, storm events, or known influences can be helpful when dilutions need to be made.

10.2 If it is unknown if or how much a sample needs to be diluted, Take two samples at the sampling site and perform a 1:2 and a 1:10 dilution from one bottle and process the other bottle as a whole sample.

10.3 1:2 dilutions are made by pipetting 50 mL of sample with a 10 mL sterile disposable pipet into a new sample bottle and then fill the bottle to the 100 mL line with sterile distilled or deionized water from a dedicated carboy or known clean supply. Process the sample as you would a whole sample.

10.4 1:10 dilutions are made by pipetting 10 mL of sample with a 10 mL sterile disposable pipet into a new sample bottle and then fill the bottle to the 100 mL line with sterile distilled or deionized water from a dedicated carboy or known clean supply. Process the sample as you would a whole sample.

10.5 Multiply the MPN results from 1:2 diluted samples by 2, and multiply the results from 1:10 samples by 10 to get the correct results from the diluted samples.

## 11 QUALITY CONTROL

- 11.1 A positive and negative control must be run on each **lot** of media before the first use.
- 11.2 The IDEXX-QC fecal coliform kit provides a positive and negative control. The viable organisms are each contained in a colored disc, which resides on top of a cotton plug and silica gel desiccant (tiny yellow spheres) in the vial.
- 11.3 The vials must be stored unopened at or below -10° C. The reagents are stable through the expiration date when stored at this temperature.
- 11.4 All steps must be performed at room temperature.
- 11.5 Remove vials from freezer. Equilibrate at room temperature for 10-15 minutes.
- 11.6 Open a vial and aseptically (with a flame sterilized tweezer) transfer a colored disc to an appropriately labeled vessel of 100 mL of sterile, nonbuffered, oxidant-free water in a sterile vessel.
- 11.7 **NOTE:** The disc may occasionally stick to the vial. If this occurs use sterile tweezers to gently remove the disc.
- 11.8 Swirl the sample and allow to stand for 10-15 minutes. The disc should completely dissolve. After it is dissolved, mix by inverting 10 times.
- 11.9 Use within 30 minutes of hydration. Process the controls as you would a normal sample.

## **12 WASTE MANAGEMENT**

- 12.1 Samples, reference materials and equipment known or suspected to have viable bacteria attached or contained must be sterilized prior to disposal.
- 12.2 The wastes generated in this method are not hazardous. The water can be discarded in the laboratory sink and Quanti-Trays are autoclaved and then can be discarded with the paper trash.

## **13 REFERENCES**

- 13.1 IDEXX Laboratories, Inc. Westbrook, ME 04092. Instruction manuals for use of:  
Colilert, Quanti-Tray/2000, Quanti-Tray Sealer, IDEXX-QC fecal coliform.
- 13.2 Standard Methods for the Examination of Water and Wastewater. 9223 B.,  
APHA, 22nd Edition, 2012.

**ANALYSIS: AMMONIA, NH<sub>3</sub>-N** - Electrode method

**Method & Source:** Potentiometric, Ammonia-Selective Electrode; Standard Methods, 18<sup>th</sup> Ed., 1992; 4500-NH<sub>3</sub> F.

**Container:** Glass or plastic

**Preservative:** 2 mL conc. H<sub>2</sub>SO<sub>4</sub> per liter of sample and 4<sup>o</sup> C.

**Holding Time:** 28 days at pH < 2

**Range of Procedure:** 0.017 - 1400 mg/L

**Minimum Detection Limit:** 0.017 mg/L

**Minimum Reportable Detection Limit:** 0.100 mg/L

**I. Reagents**

- A. Sodium Hydroxide 10N: Add 400.0 g NaOH pellets to 600 mL DI water. Stir until the pellets dissolve. Let this solution get hot as the NaOH dissolves. Dilute to 1 L. Store in a plastic bottle with screw top.
- B. Ammonia Stock: Add 0.3819 g Ammonium chloride (NH<sub>4</sub>Cl) to a 100 mL volumetric flask and dilute to volume with DI water. Label "Ammonia Stock". Make fresh monthly. Store in any reagent type bottle.
- C. Ammonia Check Stock: Make as B above from a different lot of dry NH<sub>4</sub>Cl. Label "Ammonia Check Stock". Make fresh monthly. Store in any reagent type bottle.
- D. Indicating Base: Ammonia pH-adjusting ISA, Orion catalog #951211 or equivalent; consists of an indicator that turns blue at a pH of >11 & 5 N or 10 N NaOH.
- E. Sulfuric Acid, concentrated. Purchased.
- F. Sodium Sulfite, NaSO<sub>3</sub>. Purchased.

**CAUTION:** This procedure uses chemicals that OSHA defines as "Particularly Hazardous. Sodium hydroxide and Sulfuric Acid are highly acute toxins. They must be handled using the "Safe Work Practices" listed in the CHP. If using these chemicals for the first time, the analyst must have written permission from the proper authority.

**II. Pre-analysis Preparation**

- A. Warm or cool all samples to exactly 20<sup>o</sup> C as measured by the metal thermometers. While warming take care to stir frequently for accurate temperature measurement.
- B. Any sample with residual chlorine must be de-chlorinated. Stir the sample in a beaker so that the chlorine evaporates. If stirring does not work, add Sodium sulfite (I. F.) carefully to remove the chlorine. Measure the chlorine residual with a DPD kit from the CBOD station.
- C. Mix the electrode's filling solution by gently pulling up on the electrode's wire twice while holding the electrode body.

D. Soak the tip of the electrode at least 15 minutes in the 1000 ppm ammonia stock before analysis. Since the electrode is stored in this solution, do this step after changing the electrode's membrane.

**III. Procedure**

1. In 150 mL beakers, prepare 100 mL of blank, standards, and samples or dilutions thereof. Put a magnetic stir bar in each beaker. See endnote 2. See endnote 6 for meter problems.

1a. Use graduated cylinders for DI water and samples.  
 1b. Use autopipets, 100 mL graduated cylinder, DI water, and the following table for stock measurement:

<u>mL of stock (I.B.)</u>	<u>mg/L standard</u>	
0.1	-	1.0
2.0	-	20.0
1.0 *check	-	10.0

\* use Ammonia Check Stock (I.C) for the 10.0 mg/L check standard.

2. We leave the Hach sensION2 meter on. If it is off, press the Exit button to turn it on.

2a. The meter must be in the ISE mode for ammonia. Press the ISE/mV key to get to that mode if needed.

3. Rinse electrode with DI water and blot dry. Pull the wire twice. Put the 1.0 mg/L standard beaker on the stirplate. Place the tip of the electrode in the standard. Turn stirrer on a slow speed.

3a. Water level must cover membrane but not be over the screw thread line of the tip of the electrode.

3b. Place a plastic insulator pad between the beaker and the stirplate to protect the sample from heat generation.

4. Press the Cal button. A ? & flashing units will appear in the display.

4a. If the units are not mg/L, use the arrow keys to scroll through the units to mg/L.

5. Press "READ/ENTER". CAL, & Standard 1 will appear on the display. The display will read 1 if the last first standard was a 1.

5a. The ? will be flashing if the meter needs to be calibrated.

5b. Press a number key to change the standard value. The flashing underscore shows where the next number will go.

6. Add 1 mL of 10 N NaOH (I.A) to the standard.

6a. Use an autopipet.

6b. pH of standard + base solution must be >11. See endnote 4.

7. Press "READ/ENTER". When the electrode

7a. The reading should stabilize in less than 5

reading is stable, the meter will beep 3 times as it sets the 1.00 standard. The meter will automatically switch to Standard 2 on the display.

minutes. If it takes longer, report this to the supervisor or the chemist.

7b. Record the time that the 1.00 standard is set on the data sheet.

7c. Check the 1.00 standard every 2 hours. It must read between 0.950 and 1.05, or be re-set.

8. Remove the electrode from the beaker, rinse it with DI, and blot it dry. Place electrode tip in the 20.0 mg/L standard. While stirring, add 1 mL of 10 N NaOH (I.A).

8a. Use an autopipet to add NaOH.

8b. The previous value for Standard 2, 20 mg/L, will be displayed.

9. Press "READ/ENTER". When the electrode reading is stable, the meter will beep 3 times as it sets the 20.00 standard.

9a. The reading should stabilize in less than 5 minutes. If it takes longer, report this to the supervisor or the chemist.

10. Press Exit. The Store & ? icons will appear. Press READ/ENTER to store the calibration curve.

10a. If you press Exit, the calibration will be lost, & the meter will return to the Reading mode.

11. Remove, rinse, and blot dry the electrode. Place tip of electrode in the 10.0 mg/l Check Standard. While stirring, add 1 ml of 10 N NaOH (I.A). Press READ/ENTER. When the reading is stable, the meter will display a lock in lower left corner of the screen, & beep 3 times. Record result on data sheet.

11a. Add NaOH with an autopipet.

11b. Record 10.0 Check standard value in the appropriate QC computer database. If the value is out of the control limits, it is invalid. The calibration must be re-done from step 1.

12. Check the slope by pressing the Review button. Arrow up through the standards to the slope. Press Exit to return to the Reading screen.

12a. An acceptable slope must be between -54 to -60.

12b. If the slope is not acceptable, repeat the calibration.

12c. Record the slope on the data sheet.

13. Remove, rinse, and blot dry electrode. Place tip of electrode in a sample. While stirring, add 1 mL of 10 N NaOH (I.A). Press READ/ENTER. When the reading is stable, the meter will display a lock in lower left of the screen. The meter will also

13a. Add NaOH with an autopipet.

13b. The meter displays the sample's ammonia concentration in the units of calibration.

13c. For acidified samples, use the I.D Indicating Base, not the 10 N NaOH. Add enough to turn the sample light blue.

beep 3 times. Record result on data sheet.

13d. pH of sample + base solution must be >11. See endnote 4.

13e. See endnote 3 for cleaning guide.

14. Repeat step 13 for all samples.

15. Analyze a duplicate and a spike on every tenth sample: QC number 1. Check that the QC is in control in the appropriate QC databases. Re-analyze any QC that is out of control.

15a. If the 2<sup>nd</sup> QC works, then analyze the rest of the samples. If the 2<sup>nd</sup> QC does not work, recalibrate the meter with the 1.0 mg/l standard. Then redo QC again. If the QC works, then re-analyze all the samples that had been done after the previous QC and proceed with the rest of the test. If the QC does not work for the 3<sup>rd</sup> time, consult the supervisor.

14. When all samples have been analyzed for the day, rinse electrode and place in dilute ammonia stock solution to store until the next day's use. Leave the meter turned on.

15. Enter all the QC into the appropriate databases. Enter the results in the lab software. Record the results of any contract sample on their Sample Transfer Sheets.

#### IV. Calculations

Range = duplicate 1 - duplicate 2

Spike % Recovery =  $\frac{\text{spike value (mg/L)} - \text{avg. of duplicates (mg/L)}}{\text{spike amount added (mg/L)}} \times 100$

#### ENDNOTES:

1. Surfactants can cause the membrane to clog. Dilute any sample suspected of containing surfactants.
2. We normally analyze 100 mL of plant effluents and 25 mL dilutions of plant influents. IWPT samples are highly variable; anywhere from 1 mL to 100 mL may be analyzed. All samples, regardless of the dilution used, must be brought up to 100 mL total volume.
3. Cleaning tip: the strong base used in every beaker of this procedure will precipitate onto the surface of the beaker and the stir bars if the base is allowed to sit very long in the beaker. If this precipitation occurs, it will not come off in the glassware washer. The beakers and stir bars will have to be acid cleaned and hand scrubbed to get them clean. To prevent this from happening, simply rinse the beakers 2 or 3 times in hot water shortly after the analysis is complete. Never allow a beaker to sit with base in it for more than 10 minutes.

4. Basic Theory: In acidic or neutral solutions, ammonia ( $\text{NH}_3$ ) is present as ammonium ion ( $\text{NH}_4^+$ ). In a basic solution with a pH of  $>11$ , all the ammonium ions are converted to ammonia gas ( $\text{NH}_3$ ). The ammonia gas diffuses across the electrode membrane where it reacts with the water in the electrode filling solution to form  $\text{NH}_4^+$  and  $\text{OH}^-$ . The electrode detects the concentration of the  $\text{OH}^-$  which is directly proportional to the concentration of  $\text{NH}_4^+$  and converts the concentration to mg/L of ammonia as displayed on the meter. Greater detail can be found in Orion's Ammonia Electrode Instruction Manual.
5. If samples are to be held past 24 hours, they must be acidified to a pH  $< 2$  with concentrated Sulfuric acid. This converts the  $\text{NH}_3$  to  $\text{NH}_4^+$  where it is stable for 28 days.
6. If the meter is not working properly, consult the Troubleshooting section of the manual.
7. If the meter is working correctly, but the check standard or the slope is not acceptable, or if the numbers seem odd, change the membrane. Consult the Orion Ammonia Electrode Instruction Manual for this procedure.
8. Always calibrate starting with the lowest standard first.

Revision 4  
William Johnson  
3-17-2011

ANALYSIS: NITRITE NITROGEN (NO<sub>2</sub>-N)

Method & Source: Spectrophotometric; Standard Methods, 20<sup>th</sup> Ed., 1998; 4500 B

Container: Glass or Plastic

Preservative: 4° C.

Holding Time: 48 hours maximum

Range of Procedure: 0.0034 - 0.5000 mg/L NO<sub>2</sub>-N

A. Reagents

A.1. Nitrite Stock & Check Stock Solution: Dissolve 0.2463 g of anhydrous Sodium nitrite (dry in oven one hour & then desiccate for at least 24 hours) in DI water in a 500 mL volumetric flask. Dilute to volume. Make a 2nd stock solution from a different lot number of Sodium nitrite. Preserve each with 1 mL of chloroform. Label one "Nitrite Stock", and label the other "Nitrite Check Stock". Stock = 100 mg/L NO<sub>2</sub>-N.

A.2. Nitrite Standard & Check Standard Solution: Pipet 1000 uL Stock (A.1.) into a 200 mL volumetric flask. Dilute to volume with DI water. Label this "Standard". Into another 200 mL volumetric flask, pipet 1000 uL "Check Stock" (A.1.) and dilute to volume. Label this "Check Standard". Make both fresh for each day. Solution = 0.5 mg/L NO<sub>2</sub>-N.

A.3. Buffer-Color Reagent: Do this in a hood. To a 250 mL beaker, add 160 mL DI water, then 20 mL conc. Phosphoric acid (H<sub>3</sub>PO<sub>4</sub>), and then 2.0 g sulfanilamide. After the sulfanilamide is completely dissolved, add 0.2 g N-(1-Naphthyl) ethylenediamine dihydrochloride. Stir until dissolved. Dilute the solution to volume in a 200 mL volumetric flask. Store in amber bottle in the refrigerator. This solution is stable for only one month.

CAUTION: This procedure contains chemicals that are defined by OSHA as "Particularly Hazardous". The finished buffer-color reagent contains Phosphoric acid--a reproductive toxin. Chloroform is a carcinogen and a reproductive toxin. They must be handled using the "Safe Work Practices" listed in the CHP. If using these chemicals for the first time, the analyst must have written permission from the proper authority.

B. Procedure

1. Filter samples. Use Whatman 40 ashless filter papers. Samples filtered for Ortho P can be

1.a If the sample is still turbid, refilter through 0.45um membrane filters.

used.

2. Adjust sample pH to between 5 & 9 with 1N HCl or NH<sub>4</sub>OH.

2.a Samples pH adjusted for the nitrate test need no further adjustment for nitrites.

3. Use only the nitrite tubes & caps marked with an N. These must be kept separate from all other tubes & caps.

4. Pipet blank, 0.056, 0.278 & 0.5 mg/L standards, 0.222 mg/L check standard, and samples into Hach tubes. Make the standards from the right column in chart A below (Section F. Charts) using the A.2 dilution of Stock

3.a Use volumetric pipets from the "general use" drawer.

3.b Use 9 mL DI water for blank.

3.c Dilute samples if the final concentration is expected to be >0.600 mg/L.

4. For sample QC, do a duplicate (precision) and a spike (accuracy) on every 10th sample, or on QC # 1.

4.a Measure out 3 identical dilutions. Pipet a spike into one tube.

4.b Use chart B (Section F.) below for spike values.

To each flask add 0.4 mL of color reagent (A.3.). Mix well. Allow to stand undisturbed for at least 15 minutes.

5.a Use a blue gun auto-pipet.

5.b Color is stable for 4 hours.

6. Turn on the DR4000 spec; set wavelength reading at 540 nm.

6.a. Use the tube module and the Hach Tube adaptor.

7. Read the samples after at least 15 minutes. Put each tube, starting with the blank in the tube holder. Put the Hach logo pointing out towards you. Close the lid of the module before reading any tube.
8. Use the ZERO soft key to zero the blank.
9. Read and record the absorbance of each tube.

Enter the absorbances of the standards into the computer standard curve database. Determine the r-Factor of the standard curve. Enter the sample and checks absorbances and dilution factors to get their concentrations from the standard curve.

11. Record the initial and final concentrations of the samples, the curves r-factor, the concentration of the check standard, and any sample QC on the data sheet.
12. In the proper QC database, enter the r-Factor, the concentration of the check standard, and any sample QC. All values must be acceptable for the test to be valid.
13. Clean the tubes & caps separately from any other tubes & caps. Do not mix them. Do not add the dirty caps to the dirty beaker in the compositing room.

### C. Calculations

1. For final results, multiply the initial concentration by the sample's dilution factor (from Chart A). Report 3 significant figures.
  - 1.a The dilution factor is the inverse of the dilution; for example, a sample with a dilution of 1/50 has a dilution factor of 50/1.
2. Average the initial concentration values of the duplicates. Multiply this average by the dilution factor to get the final result.
3. Record the duplicate range on the bottom of the data sheet and enter the range into the computer QC database.
  - 3.a Range = Duplicate 1 - Duplicate 2
4. Spike calculations:  
% recovery =  $\frac{A - D}{D} \times 100$

C

where: A = initial concentration of the spike

C = concentration of added spike

D = average concentration of the duplicates

5. Record the % recovery on the bottom of the data sheet and enter it into the computer QC database.
6. The sample QC (Range & % Recovery) must be acceptable for the test to be valid.

#### D. Notes

1. If any of the quality control is outside the control parameters of the graphs in the computer QC database, then the entire test must be re-done.

#### E. Discussion

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate (by *Nitrosomonas* bacteria) and in the reduction of nitrate (under anaerobic conditions in a WWTP). Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems, and natural waters. Nitrite can enter a water supply system through its use as a corrosion inhibitor in industrial process water. Nitrite is the actual etiologic agent of the illness known as methemoglobinemia in infants. Nitrous acid, which is formed from nitrite in acidic solution, can react with secondary amines to form nitrosamines, many of which are known to be carcinogens. The toxicologic significance of nitrosation reactions in vivo and in the natural environment is the subject of much current concern and research.

#### F. Principle

Nitrite ( $\text{NO}_2^-$ ) is determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with N-(1-naphthyl)-ethylenediamine dihydrochloride. The color system obeys Beer's law up to 180 ug N/L with a 1-cm. light path at 543 nm. Higher nitrite concentrations can be determined by diluting a sample.

G. Charts

Chart A

In a Hach Tube:				
Dilution	mL Sample or A.2 Solution	mL DI Water	Dilution Factor	Standards' Concentration
1/18	0.5	0.5 + 8	18	
1/9	1	8	9	0.056 mg/L
2/9	2	7	4.5	
3/9	3	6	3	
4/9	4	5	2.25	0.222 mg/L
5/9	5	4	1.8	0.278 mg/L
6/9	6	3	1.5	
7/9	7	2	1.29	
8/9	8	1	1.12	
9/9	9	0	1	0.500 mg/L

Chart B

In a Hach tube:	
MI of Nitrite A.2 Solution Used	Concentration of Added Spike, mg/L
0.5	0.028
1	0.056
2	0.111
3	0.167
4	0.222
5	0.278

Revised W. Johnson  
9.29.2009 Revision 2

ANALYSIS: NITRATE, NITROGEN (NO<sub>3</sub>-N)

METHOD & SOURCE: Colorimetric, Brucine; Methods For Chemical Analysis of Water and Wastes, USEPA 1983, Method 352.1

CONTAINER: Glass or Plastic

PRESERVATIVE: 2 mL conc. H<sub>2</sub>SO<sub>4</sub> per liter of sample & 4 degrees Celsius

HOLDING TIME: 48 hours

RANGE: 0.1 – 1.6 mg/L

A. REAGENTS:

A.1 Sulfuric Acid Solution: Carefully add 1500 mL conc. H<sub>2</sub>SO<sub>4</sub> to 375 ml DI water in a 2 L beaker. Stir and then let cool. Store in a tightly-stoppered 2 L reagent bottle.

A.2 Brucine-Sulfanilic Acid Solution: Dissolve 2 g brucine sulfate [C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>]<sub>2</sub> H<sub>2</sub>SO<sub>4</sub> 7H<sub>2</sub>O] and 0.2 g sulfanilic acid (NH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub>H H<sub>2</sub>O) in 130 mL hot D.I. water. Add 6 mL conc. HCl, cool, mix and dilute to 200.00 mL with DI water. Store in a dark bottle at 4° C. This solution is stable for several months; the pink color that develops slowly does not effect its usefulness. Mark bottle with warning: Caution: Brucine Sulfate is toxic; take care to avoid ingestion. Wears gloves, glasses & coat.

A.3 Potassium Nitrate Stock Solution: Dissolve 0.7218 g anhydrous KNO<sub>3</sub> (stored in desiccator) in DI water and dilute to 1000 mL in a volumetric flask. Preserve with 2 mL chloroform per L. This solution is stable for at least 6 months. 1.0 mL = 0.1 mg NO<sub>3</sub>-N. Make a Check Nitrate Stock as above from a different batch of KNO<sub>3</sub>

A.4 Potassium Nitrate Standard Solution: Pipet 1000 uL of the stock solution (1.3) into a 100 mL volumetric flask. Dilute to volume with DI water. Prepare fresh daily. 1.0 mL = 0.001 mg NO<sub>3</sub>-N. Make a Check Nitrate Standard Solution from the Check Nitrate Stock as above.

A.5 Acetic Acid (1 + 3): Add 3 volumes of DI water to 1 volume glacial acetic acid.

A.6 Sodium Hydroxide (1N): Dissolve 40 g of NaOH pellets in DI water. Cool and dilute to 1 L.

A.7 Sodium Arsenite Solution: Dissolve 1 g of sodium arsenite in 200 mL DI water.

A.8 Spike Solution: Into a 50 mL volumetric flask, pipet appropriate amount of stock (1.3), as listed in the table below, to achieve desired spike amount in the tube. Dilute to volume with DI water. Add exactly 500 uL of this spike solution to the sample that is to be spiked.

mL stoc		final mg/L
<u>in 50 mL</u>	<u> </u>	<u>spike in tube</u>
5		0.5

4		0.4
3		0.3
2		0.2
1		0.1

**CAUTION:** This procedure contains chemicals that OSHA defines as "Particularly Hazardous": Sodium arsenite and Nitrate reagent pillows are highly acute toxins and carcinogens; Acetic acid is a reproductive toxin; Sodium hydroxide, Brucine sulfate and Sulfuric acid are highly acute toxins; Chloroform is a carcinogen and a reproductive toxin. They must be handled using the "Safe Work Practices" listed in the CHP. If using these chemicals for the first time, the analyst must have written permission from the proper authority.

2. PROCEDURE:

2.1 Fill water bath to one inch below top clips.  
 Turn on "High."

2.1a Use DI water to fill bath.

2.2 Filter samples to remove turbidity.

2.2a Use Whatman grade 40 filters. If turbidity remains, filter again using membrane filters.

2.3 Check for residual chlorine. If any, neutralize it with sodium arsenite (A.7).

2.3a Add sodium arsenite after filtering.

2.4 Adjust pH of samples, including DI water, to between 6.5 and 7.5.

2.4a Use acetic acid (A.5) or NaOH (A.6) as necessary. If the samples have been acidified, begin pH adjustment with 6N or 10N NaOH. Wear gloves, glasses & coat.

2.5 Pipet 10 mL of blank, standards samples, or appropriate amount diluted to 10 mL, into large test tubes placed in a plastic test tube rack. On every 10th sample, do a spike and a duplicate. Sludges should have separate QC numbers.

2.5a Use volumetric pipets for all additions.

2.5b Use DI water for blank.

- 2.5c 1 mL A.4 + 9 mL DI water = 0.1 mg/L;
- 5 mL A.4 + 5 mL DI water = 0.5 mg/L;
- 9 mL A.4 + 1 mL DI water = 0.9 mg/L;
- 5 mL Check A.4 = Check 5 mg/L.

2.5d IMPORTANT: Space tubes evenly throughout the rack so the even flow of bath water among the tubes achieves uniform heating of all tubes.

2.5e To correct for color or turbidity after membrane filtration, run a duplicate sample to which all reagents

except the brucine have been added.

2.5f For spike, add exactly 500 uL of spike solution (A.8).

2.6 Place rack in an ice water bath.

2.6a Use enough water in ice bath to cover liquid level in tubes.

2.7 Add 10 mL of H<sub>2</sub>SO<sub>4</sub> (A.1) to each tube. Gently swirl each tube until acid is completely mixed. Wear gloves, glasses, and coat.

2.7a Use 2 additions of 5 mL each. Use the nitrate auto-pipet. Auto-pipet tips should have been previously hand cleaned and dried.

2.7b Thorough mixing is important.

2.8 Allow all tubes to come to thermal equilibrium before continuing to next step.

2.8a Temp. = < 20° C.

2.9 With tube rack still in the ice bath, add 500 uL brucine-sulfanilic acid solution (1.2) to each tube. Gently swirl each tube until brucine is thoroughly mixed.

2.9a Use auto-pipet. Wear gloves, glasses and coat.

2.9b Thorough mixing is extremely important.

2.10 Remove rack from ice bath and allow tubes to come to about 20° C. Cover each tube tightly with aluminum foil.

2.10a Caution: Placing very cold tubes into the hot water bath may cause the tubes to crack.

2.11 Place rack of covered tubes in 100° C water bath and time for exactly 25 minutes. Keep the lid on the bath while the tubes are heating.

2.11a The water bath must be 100° ± 1 the entire time while heating. Timing and temperature control are critical. Uneven heating of the samples and standards during the reaction time will result in erratic values. The necessity for absolute control of temperature during the critical color development period cannot be too strongly emphasized.

2.12 Turn on spectrophotometer. Set the wavelength to 410 nm.

2.12a Put the sipper module in the spec.

2.12b Allow at least 20 to 30 minutes warmup.

2.13 After exactly 25 min. in the hot water, quickly remove rack and place it in the ice bath for about 3 minutes.

2.13a Usually, 3 min. in the ice bath will bring the samples to room temperature.

2.14 Remove rack from ice bath. Allow tubes to come to room temperature.

2.15 Read and record on the data sheet the absorbance readings of all standards and samples.

2.16 Between all the readings in Step 2.15, run 1 sip of DI water.

2.17 Enter the absorbance values of the standards in the Nitrate standard curve in the SW Monitoring program. Get the concentration values of the samples from the standard curve. Enter the dilution factors to get the final concentrations. Enter the spike and duplicate results, and the standard curve QC, in the QC databases.

2.18 Do a standard curve and a known every three months.

#### FOR SLUDGES:

2.19 Shake the sample well. Filter the sludge through a #40 size filter. Filter at least 20 mL. Use the acidified liquid sludges and the prepared dry sludges for filtering.

2.20 Adjust the pH of the filtrate to between 6.5 and 7.5.

2.21 If the filtrate becomes hazy or turbid after pH adjustment, then filter it through a membrane

2.15a This cleans out the tubing so the gooey nitrate solution does not carry over to the next sample.

2.17a To correct samples for turbidity or color, subtract the absorbance of the sample without brucine from the absorbance of the sample with brucine. Use the final answer absorbance to find the concentration of the sample.  
2.17b If the initial concentration of a diluted sample is >1.6 mg/L or <0.10 mg/L, re-do the sample at a different dilution. If the initial concentration of the undiluted sample is <0.10 mg/L, report this as the final result.

2.18a For the standard curve, use 0.1, 0.5, 0.9, 1.2, 1.4, 1.6, and 2.0 mg/L standards. For standards >1 mg/L, use a concentrated standard solution: dilute 1 mL of stock solution (1.3) to 50 mL in volumetric flask with DI water. Then 6 mL = 1.2 mg/L standard, 7 mL = 1.4 mg/L standard, etc.

2.19a To prepare dry sludge, weigh about 10 g into a 150 mL beaker. Record weight on data sheet. Add a stir bar and 50 ml of DI water. Stir for 30 minutes. Transfer liquified sludge to a 100 mL volumetric flask and dilute to volume with DI water. Rinse beaker at least 3 times. Other volumes of flasks can be used to concentrate or dilute the sludge more. The volumetric flask's size, 100 mL, is the initial volume of the sludge for the final calculation.

2.20a Use the same pH adjusting chemicals as for the normal samples as in 2.4a.

filter.

2.22 To check the sample concentration, use the Hach Nitrate Tubes with the Hach spec. The tube measure 0.3 mg/L to 30 mg/L. Use appropriate dilutions to measure the nitrate level of the sludge.

2.22a The Hach tubes are in 07.

2.22b For example, the undiluted filtrate tests >30 mg/L, so a 1/10 dilution is tested. The 1/10 dilution gives a result of 23 mg/L. Multiply this by the dilution factor to give the concentration of the undiluted filtrate: 23 mg/L x 10 = 230 mg/L.

2.23 Divide the concentration from Step 2.22 by 0.5. Use this as the dilution to do on the undiluted filtrate in order to get a readable sample in the nitrate tube. Round this number to a dilution that can be easily set up.

2.23a The result from Step 2.22b is 230 mg/L. 230 divided by 0.5 is 460; round this number to 500. So, do a 1/500 dilution on the undiluted filtrate for the actual nitrate test.

2.24 Continue with the regular analysis at step 2.4 through 2.17.

NOTE: This procedure is extremely sensitive to contamination. Make sure all glassware (reagent bottles, beakers, pipets, pipet tips, test tubes, etc.) is hand cleaned, thoroughly rinsed and then dried before use.

### 3. CALCULATIONS

#### 3.1 Liquid sludge:

$$\text{mg/kg nitrate} = \frac{\text{final concentration, mg/l}}{\% \text{ total solids as a decimal}}$$

#### 3.2 Dry sludge:

$$\text{mg/kg nitrate} = \frac{\text{final concentration, mg/L} \times \text{Initial Volume, mL}}{\text{weight, g} \times \% \text{ total solids as a decimal}}$$

Revision 3  
William Johnson  
3-5-2010

ANALYSIS: ORTHO PHOSPHORUS

METHOD & SOURCE: Colorimetric, Ascorbic Acid, Single Reagent; Standard Methods, 18<sup>th</sup> Ed. 1992; 4500-P E & B.

CONTAINER: Glass or Plastic

PRESERVATIVE: 4° C.

HOLDING TIME: 48 hours

RANGE OF PROCEDURE: 0.01 - 1.0 mg/L P & 0.03 - 3.1 mg/L PO<sub>4</sub>

A. Reagents

1. Sulfuric Acid Solution, 5N: In a 1 L beaker, in a hood, mix 200 ml of DI water and 70 mL of conc. H<sub>2</sub>SO<sub>4</sub> with stirring. Dilute to 500 mL with DI water. Always add acid to water. Store in a glass or plastic bottle. Wear rubber gloves, face shield, safety glasses, lab coat and rubber apron.
2. KAT Solution: Dissolve 1.3715 g of Antimony Potassium Tartrate, K(SbO)C<sub>4</sub>H<sub>4</sub>O<sub>6</sub> 1/2 H<sub>2</sub>O, in 400 mL DI water in a 500 mL volumetric flask, dilute to volume with DI water. Store at 4° C in a dark, glass-stopper bottle.
3. Ammonium Molybdate Solution: Dissolve 20 g (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> 4H<sub>2</sub>O in 500 mL DI water in a 1 L beaker. Store in a plastic bottle at 4° C.
4. Ascorbic Acid, 0.1M: Dissolve 1.76 g ascorbic acid in DI water; dilute to volume in a 100 mL volumetric flask. Store in a plastic bottle at 4° C. Make fresh weekly. One can make any multiple of 100 mL of this.
5. Combined Reagent: For 100 mL, mix 50 mL of 5N H<sub>2</sub>SO<sub>4</sub> (A.1), 5 mL of KAT solution (A.2), 15 mL of ammonium molybdate solution (A.3), and 30 mL of ascorbic acid solution (A.4). Measure with graduated cylinders and autopipets. Mix after addition of each reagent. All reagents must reach room temperature before they are mixed and must be mixed in the order given. If turbidity forms, shake and let stand for a few minutes until the turbidity disappears before proceeding. This solution is stable for only 4 hours.
6. Phosphorus Stock Solution (double strength): In a 1 L volumetric flask, dissolve 0.4394 g of Potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) in DI water. Dilute to volume with DI water. 1.0 mL = 0.10 mg P.  
Notes: 1. The KH<sub>2</sub>PO<sub>4</sub> should have been previously dried for 2 hours at 105° C. Store in the desiccators; 2. Make a Check Phosphorus Stock from a different batch of KH<sub>2</sub>PO<sub>4</sub> that has been dried & desiccated as above; 3. We make the stock twice as strong as the reference stock.
7. Standard Phosphorus Solution: Dilute 10.00 mL of stock P solution (A.6) to 1000 mL with DI water. 10 ml stock diluted to 1000 mL = 1 mg/L P. 1 mL std = 1.0 ug P. Note: Make a Check Standard Solution as above from the Check Stock.

8. Sample Blank Color Reagent: Mix 50 mL 5N H<sub>2</sub>SO<sub>4</sub> (A.1), and 15 mL of ammonium molybdate solution (A.3). Measure with graduated cylinders and autopipets. Mix after addition of each reagent. All reagents must reach room temperature before they are mixed.

**CAUTION:** This procedure contains chemicals that OSHA defines as "Particularly Hazardous". They must be handled using the "Safe Work Practices" listed in the CHP. If using these chemicals for the first time, the analyst must have written permission from the proper authority.

## B. Procedure

1. Bring all samples and reagents to room temperature. Filter the samples that need PO<sub>4</sub>DIS, unless they are LMR (OEQ) samples, in which case they are already filtered. Turn on the spec.
  - 1a. PO<sub>4</sub>DIS samples must be filtered through a membrane filter.
  - 1b. Total Ortho P samples are analyzed unfiltered.
2. Dilute any samples that will be too concentrated to give an initial concentration < 1.0 mg/L. Set up the dilutions as in E. Pipet 9 mL of this into a Hach tube. For samples too colored or turbid, set up a sample blank.
  - 2a. Use volumetric pipets for all measuring.
  - 2b. A Hach Phosphorus kit may be used to determine a suitable dilution of the sample to be analyzed.
3. On QC #1, do a spike and a duplicate. For the duplicate, set up another tube as in Step 4.
  - 3a. Use volumetric pipets for all measuring.
4. Spike a sample as in C. Use the same dilution as used for the sample and duplicate.
5. Make standards: add Standard Phosphorous Solution (A.7) and DI water to a labeled Hach tube. Make a 0.3 mg/L Check Standard from the Standard Check Phosphorous Solution plus DI water.
  - 5a. Use volumetric pipets for all measuring.
  - 5b. Use 9 ml DI water for the blank.
  - 5c. 0.111 mg/L = 1 mL A.7 + 8 mL DI; 0.556 mg/L = 5 mL A.7 + 4 mL DI; 1.0 mg/L = 9 mL A.7.
  - 5d. 0.333 mg/L = 3 mL A.7 Check + 6 mL DI.
6. To one tube at a time, add 1.45 mL Combined Reagent, cap and mix for 15 to 20 seconds. DO not add the Combined Reagent to the sample blanks; add 1.45 mL of the Sample Blank Color Reagent. Allow 10 min of color development.
  - 6a. Use the Oxford autopipet in the PO<sub>4</sub>DIS drawer.
  - 6b. Wear gloves for this step.
  - 6c. The upper time limit for this color development is 30 min from the addition of the

combined buffer.

7. Set the spec's wavelength at 880 nm. For the HACH DR14000V spec, use the "Spec Setup" instructions.
- 7a. Put the tube module in the spec.
8. On the HACH DR14000V enter the blank and standards using the "B. Standard Curve" instructions.
- 8a. Read through the tubes.
9. On the HACH DR14000V, set the absorbance to 0.00 with the blank. Read the absorbances of the standards and the samples; record these values on the data sheet.
10. Enter the absorbances of the standards in the PTOT Standard Curve database. Enter the rest of the absorbances to get the initial concentrations; enter the dilution factors to get the final concentrations. Record these concentrations and the r-Factor on the data sheet.
11. Enter the r-Factor, the 0.333 mg/L check standard's concentration, and the range and spike recovery of any sample QC in the appropriate QC databases. All values must be acceptable for the test to be valid.
12. Enter the samples' concentrations in the Data Entry database and on any sample transfer sheets, if necessary.

### C. Spiking

1. Spikes can be made in a volumetric flask, with 9 mL then pipetted into the tube, or directly in a Hach tube.
2. When spikes are made in a 50 mL volumetric flask, the concentration of the added spike is 0.02 times the volume of the Standard P Solution used. (3 mL/50 mL = 0.06 mg/L; 10 mL/50 mL = 0.2 mg/L)

For spikes made directly in a tube, the concentration will be the volume of the Standard P Solution times 0.111 (1/9). This is the same as making the standards. (See B.5)

### D. Calculations

1. Use the initial and final concentration values from the Standard Curve.
2. Multiply concentration value by dilution factor if applicable. Report final results in three significant figures.
3. For the phosphate ( $\text{PO}_4$ ) concentration, multiply the phosphorus result by 3.06.

4. Duplicate range in wastewater samples = sample mg/L - duplicate mg/L

5. Spike recovery in wastewater samples:

$$\% \text{ recovery} = \frac{\text{spiked sample's mg/L} - \text{avg. of duplicates in mg/L} *}{\text{concentration of added spike}}$$

\*If the sample was analyzed 1/1, and the spike was diluted, multiply the average of the duplicates by the dilution factor:

E. Dilutions

1. Dilutions can be set up in 50 mL volumetric flasks or directly in the Hach tube. The flask dilutions are made by adding a volume to a flask and diluting it to volume with DI water. The Hach tube dilutions are made by adding a volume to a tube, then adding enough DI water to bring the level to 9 mL. Examples are 1 + 8, 2 + 7, 4 + 5.
5. Use these charts as a guide:

In a 50 mL Volumetric Flask:			
Dilution	mL Used	Dilution Factor	Approximate Hach Tube Dilution
1/50	1	50	---
3/50	3	16.7	0.5/9
1/10	5	10	1/9
1/5	10	5	2/9
2/5	20	2.5	4/9
1/2	25	2	4.5/9
3/5	30	1.67	5/9
1/1	50	1	9/9

In a Hach Tube:			
Dilution	mL Sample	mL Di Water	Dilution Factor
1/18	0.5	0.5 + 8	18
1/9	1	8	9
2/9	2	7	4.5
3/9	3	6	3
4/9	4	5	2.25
5/9	5	4	1.8
6/9	6	3	1.5
7/9	7	2	1.29
8/9	8	1	1.12
9/9	9	0	1

Revision 3 William Johnson 3.5.2010

ANALYSIS: TOTAL PHOSPHORUS

METHOD & SOURCE: Standard Methods, 18<sup>th</sup> Ed., 1992; Persulfate Digestion Method 4500-P C; Ascorbic Acid Single Reagent 4500-P E.

CONTAINER: Glass or Plastic

PRESERVATIVE: 4<sup>o</sup> C & 2 mL conc. H<sub>2</sub>SO<sub>4</sub> per liter of sample

HOLDING TIME: 28 days

RANGE OF PROCEDURE: 0.01 – 1.00 mg/L P & 0.03 - 1.5 mg/L PO<sub>4</sub>

A. Reagents

1. Sulfuric Acid Solution, 5N: In a 1 L beaker mix 230 mL of DI water and 70 mL of conc. H<sub>2</sub>SO<sub>4</sub> while stirring. Dilute to 500 mL with DI water. Always add acid to water. Store in a glass or plastic bottle. Wear rubber gloves, face shield, safety glasses, lab coat and rubber apron. Do this procedure in the hood.
2. KAT Solution: Dissolve 1.3715 g of Antimony Potassium Tartrate, K(SbO)C<sub>4</sub>H<sub>4</sub>O<sub>6</sub> 1/2 H<sub>2</sub>O, in 400 mL DI water in a 500 mL volumetric flask, dilute to volume with DI water. Store at 4<sup>o</sup> C in a dark, glass-stoppered bottle.
3. Ammonium Molybdate Solution: Dissolve 20 g (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> 4H<sub>2</sub>O in 500 mL DI water in a 1 L beaker. Store in a plastic bottle at 4<sup>o</sup> C.
4. Ascorbic Acid, 0.1M: Dissolve 1.76 g of ascorbic acid in a 100 mL volumetric flask diluted to volume with DI water. Store in a plastic bottle at 4<sup>o</sup> C. Make fresh weekly.
5. Combined Reagent: For 100 mL, mix 50 mL 5N H<sub>2</sub>SO<sub>4</sub> (A.1), 5 mL KAT solution (A.2), 15 mL ammonium molybdate solution (A.3), and 30 mL ascorbic acid solution (A.4). Measure with graduated cylinders and autopipets. Mix after addition of each reagent. All reagents must reach room temperature before they are mixed and must be mixed in the order given. If turbidity forms, shake and let stand for a few minutes until the turbidity disappears before proceeding. This solution is stable for only 4 hours.
6. Sulfuric Acid Solution, 11N: Slowly add 77.5 mL of concentrated Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) to 150 mL of DI water in a 500 mL beaker. When cool, dilute to 250 mL with DI water. Always add acid to water. Wear rubber gloves, face shield, safety glasses, lab coat and rubber apron. Do this procedure in the hood.
7. Phosphorus Stock Solution (double strength): In a 1 L volumetric flask, dissolve 0.4394 g of Potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) in DI water. Dilute to volume with DI water. 1.0 mL = 0.10 mg. P. Note: The KH<sub>2</sub>PO<sub>4</sub> should have been previously dried for 2 hours at 105<sup>o</sup> C. Store in the desiccator.

8. Standard Phosphorus Solution (double strength): Dilute 10.00 mL of stock P solution (A.7) to 1000 mL with DI water.

10 mL stock diluted to 1000 mL = 1 mg/L P

1 mL std = 1.0 ug P

0.2 mg/L std.: 10 mL std =  $\frac{0.010}{0.050} \times 1 \text{ mg/L} = 0.2 \text{ mg/L}$

9. Sodium Hydroxide, 1N: In a 500 mL beaker, dissolve 10 g NaOH in 150 mL DI water. Cool and dilute to 250 mL with DI water. Mix this in the hood. Wear rubber gloves, impervious clothing, face shield, and safety glasses.

10. Adenosine Monophosphate (AMP) Stock: Place 0.1120 g AMP in a 100 mL volumetric flask dilute to volume with DI water. AMP dissolves very slowly. Let sit overnight and it will probably be dissolved by morning. After it is completely dissolved, store at 4<sup>o</sup> C. 1 mL = 0.1 mg P.

11. AMP Standard Solution: Place 1000 µL of stock AMP (A.10) in a 100 mL volumetric flask dilute to volume with DI water. 1 mL = 0.001 mg P.

12. Sample Blank Color Reagent: Mix 50 mL of 5N H<sub>2</sub>SO<sub>4</sub> (A.1), and 15 mL of ammonium molybdate solution (A.3). Measure with graduated cylinders and autopipets. Mix after addition of each reagent. All reagents must reach room temperature before they are mixed.

CAUTION: This procedure contains chemicals that OSHA defines as "Particularly Hazardous" as defined by OSHA. Sodium hydroxide, Antimony potassium tartrate and Sulfuric acid are highly acute toxins. They must be handled using the "Safe Work Practices" listed in the CHP. If using these chemicals for the first time, the analyst must have written permission from the proper authority.

## B. Procedure—Hot Plate Digestion

1. Bring all samples and reagents to room temperature. Put the hot plate in the hood.

2. Place a blank, 3 standards, a check standard and the desired aliquots of sample into clean, labeled 250 mL, narrow-mouthed, erlenmeyer flasks, and dilute to about 50 mL with DI water. Use DI water for the blank. On every tenth sample (on QC #1), do a duplicate and spike.

1a. Remove ammonium molybdate solution, KAT solution, AMP, and any ascorbic acid solution from the refrigerator.

2a. Use volumetric pipets for all measuring.

2b. Use the Standard Phosphorus Solution (A.8) for the standards:

3.00 ml = 0.06 mg/l;

25.00 ml = 0.50 mg/l;

50.00 ml = 1.00 mg/l.

2c. A Hach Phosphorus kit may be used to determine

a suitable dilution of the sample to be analyzed.  
2d. 0.3 mg/l check standard = 15.00 mL of AMP  
standard (A.11).

3. To each flask add 1.0 mL of 11N H<sub>2</sub>SO<sub>4</sub>, 0.4 g of ammonium persulfate and 6-8 boiling chips. Place each flask on a hot plate, set at medium heat and boil GENTLY to reduce volume to 5-10 mL. Remove from heat and allow to cool to room temperature. Rinse down sides of each flask with some DI water. Digest in a hood. Do not allow samples to digest to dryness.

3a. Use autopipet for H<sub>2</sub>SO<sub>4</sub>.

3b. Use a loosely packed 0.5 g reagent spoon to add the ammonium persulfate.

3c. On large hot plate, set at 300 F. On small plate, set at 4. All hot plates must be placed in the hood for this digestion.

3d. If samples digest to dryness, they make choking fumes that look like smoke.

3e. While samples are digesting, make the ascorbic acid (A.4) solution if needed.

4. Cool the digested flasks to room temperature. Add two drops of phenolphthalein indicator to each flask and adjust pH to  $7.0 \pm 0.2$  using 6 N and 1 N NaOH as required.

4a. pH is approx. 7.0 when the solution turns VERY FAINT pink with the addition of a drop 1N NaOH.

Too much pink color will interfere in the final color.

4b. If pH is overshoot (color is too pink), use 1N H<sub>2</sub>SO<sub>4</sub>.

4c. Turn the spec on and allow to warm up.

5. Transfer the contents of each digestion flask to 50 mL volumetric flask. Add at least one DI water rinse and more if space permits. Dilute each volumetric to volume with DI water. Sludge samples will need to be transferred to larger volumetrics, (i.e. 200, 250, 500) depending on desired dilution.

5a. A dispo transfer pipet may be used to add the last few drops of water.

5b. It is best to rinse at least 3 times, but do not overfill. If volumetrics are overfilled, consult JB about salvaging the sample.

6. Mix each volumetric flask well (Invert and shake at least 3 times) and transfer the contents to a 125 mL erlenmeyer flask.

6a. For sludges: measure 50 mL of sample from the volumetrics with a 50 mL graduated cylinder; pour the sample into a 125 mL flask.

### C. Procedure—Autoclave Digestion

1. Bring all samples and reagents to room

1a. Remove ammonium molybdate solution, KAT

temperature.

2. Place a blank, 3 standards, a check standard and the desired aliquots of sample into clean, labeled 125 mL, narrow-mouthed, erlenmeyer flasks, and dilute to about 40 mL with DI water. Use DI water for the blank. On every tenth sample (on QC #1), do a duplicate and spike. You can use 250 mL flasks for digestion if you do not have enough 125 mL flasks.

3. To each flask add 1.0 mL of 11N H<sub>2</sub>SO<sub>4</sub>, and 0.4 g of ammonium persulfate. Mix each flask. Place each flask in the autoclave. Run the autoclave for a 30 minute cycle with Slow Exhaust. Remove the flasks from the autoclave and cool to room temperature. Rinse down sides of each flask with some DI water.

4. Cool the digested flasks to room temperature. Add two drops of phenolphthalein indicator to each flask and adjust pH to  $7.0 \pm 0.2$  using 6 N and 1 N NaOH as required.

5. Transfer the contents of each digestion flask to a 50 mL volumetric flask. Rinse the flask least once with DI water and more if space permits. Dilute the flask to volume with DI water. Sludge samples will need to be transferred to larger volumetrics, (i.e. 200, 250, 500) depending on desired dilution.

6. Mix each sample well and transfer the contents to a 125 mL erlenmeyer flask.

solution, AMP, and any ascorbic acid solution from the refrigerator.

2a. Use volumetric pipets for all measuring.

2b. Use the Standard Phosphorus Solution (A.8) for these standards:

3.00 mL = 0.06 mg/L;

25.00 mL = 0.50 mg/L;

Use the Phosphorus Stock (A.7) for this standard:

0.500 mL = 1.00 mg/L.

2c. A Hach Phosphorus kit may be used to determine a suitable dilution of the sample to be analyzed.

2d. 0.3 mg/L check standard = 15.00 mL of AMP standard (A.11).

3a. Use autopipet for H<sub>2</sub>SO<sub>4</sub>.

3b. Use a loosely packed 0.5 g reagent spoon to add the ammonium persulfate.

3c. You can set the flasks on a metal tray with holes.

3d. Be sure to fill the autoclave with DI water, & close the drain valve.

4a. pH is approx. 7.0 when the solution turns VERY FAINT pink with the addition of a drop 1N NaOH.

Too much pink color will interfere in the final color.

4b. If pH is overshoot (color is too pink), use 1N H<sub>2</sub>SO<sub>4</sub>.

4c. Turn the spec on and allow to warm up.

5a. A dispo transfer pipet may be used to add the last few drops of water.

5b. It is best to rinse at least 3 times, but do not overfill. Consult JB about salvaging the sample in cases of overfilling.

6a. You can use the same flask used for digestion.

6b. For sludges: measure 50 mL of sample from the volumetrics with a 50 mL graduated cylinder; pour the sample into a 125 mL flask.

7. Because the autoclave digestion does not significantly reduce the volume of the samples & standards, we can not digest more than a 40 mL volume. We must have volume to add the pH adjusting chemicals, and the DI water rinses without going over 50 mL in our final dilution. (See Step 5 above.) The 1.00 standard is set up from the stock (A.7), instead of the standard (A.8), to stay under the 50 mL limit. Also, this digestion does not allow a 1/1, or 50/50 dilution. The most concentrated dilution is 40/50. Contact JB if this is a problem.

#### D. Spec Reading

- |   |  |
|---|--|
| 1. Prepare the combined reagent (in A.5) Using an auto-pipet, add 8.0 mL combined reagent to each flask, mix well and allow to stand undisturbed for a minimum of 10 minutes. | 1a. Read samples after a minimum of 10 minutes color development but no longer than 30 minutes. Color is no good after 30 minutes. |
| 2. Set the spec's wavelength at 880 nm.   | 2a. Allow the spec at least 30 minutes warm up.  |
| 3. Use the sipper module. Sip each sample & record its absorbance.  | 3a. Remember to zero the Hach spec before sipping the digested blank.  |
4. Rinse the sipper module with DI water after reading all the samples. Be careful not to overfill the module's waste beaker.
5. Enter the absorbances of the three standards in the PTOT Standard Curve database. Enter the samples' absorbances to get the initial concentrations; enter the dilution factors to get the final concentrations. Record these concentrations and the r-Factor on the data sheet.
6. Enter the r-Factor, the 0.3 mg/L check standard's concentration, and the range and spike recovery of any sample QC in the appropriate QC databases. All values must be acceptable for the test to be valid.

#### E. Calculations

1. Use the initial and final concentration values from the Standard Curve.
2. Multiply concentration value by dilution factor if applicable. Report final results in three significant figures.
3. If phosphate ( $\text{PO}_4$ ) concentration is needed, multiply the phosphorus result by 3.06.
4. Duplicate range in wastewater samples = sample mg/L - duplicate mg/L

5. Spike recovery in wastewater samples:

$$\% \text{ recovery} = \frac{\text{spiked sample's mg/L} - \text{avg. of duplicates in mg/L}}{\text{mg/L of std. added}}$$

6. Concentration of Phosphorus in sludge samples:

$$\text{mg/kg P} = \frac{\text{conc (mg/L)**} \times 0.05 \times 1000 \times \text{DF***}}{\text{grams of sludge added} \times \% \text{ TS*}}$$

\* % TS expressed as a decimal

\*\* concentration in mg/L as calculated by the computer from the graph (do not enter any dilution factors in the computer for sludges)

$$\text{*** DF (dilution factor)} = \frac{\text{final volume (mL) in volumetric flask}}{50}$$

Report results in three significant figures.

7. Duplicate range in sludge samples = sample mg/kg - duplicate mg/kg

8. Spike recovery in sludge samples:

$$\% \text{ recovery} = \frac{B - A}{C} \times 100$$

where: A =  $\frac{\% \text{ TS* of sludge} \times \text{avg. mg/kg of duplicates} \times \text{grams of spiked sample}}{1000}$

B = mg P in spike = conc (mg/L)\*\* x 0.05 x DF\*\*\*

C = mL std. added in spike

1000

\* % TS expressed as a decimal

\*\* concentration in mg/L as calculated by the computer from the graph (do not enter any dilution factors in the computer for sludges)

$$*** \text{ DF (dilution factor)} = \frac{\text{final volume (mL) in volumetric flask}}{50}$$

Revised 3/5/2009  
Revision 5  
William Johnson

## TOTAL SUSPENDED SOLIDS & TOTAL VOLATILE SUSPENDED SOLIDS

Method & Source: Total Suspended Solids Dried at 103<sup>o</sup>-105<sup>o</sup> C and Total Volatile Suspended Solids Ignited at 550<sup>o</sup> C; Standard Methods, 18th Ed., 2540D.

Container: Plastic or Glass

Preservation: 4<sup>o</sup> C.

Holding Time: 7 days

Range of Procedure: >1.0 mg/L for TSS; >1.5 mg/L for TVSS

### A. Preparation of papers

1. Filter papers must be pre-washed and pre-dried before they can be used in the analysis.

1.a. Use 4.7 cm diameter glass fiber filter papers - Whatman grade 934AH, or Gelman type A/E.

2. Attach the 3-place manifold for the magnetic funnels to a 4 L filtering flask which is attached to the catch filtering flask and the desiccant tube which is attached to the vacuum pump on the countertop.

3. Place filter papers in 500 mL beaker with DI water and swirl with glass rod. Repeat 3 times.

3. a. Use the house DI water.  
b. Dump out the DI water after each swirling.

4. Place a filter paper on each of the 3 funnel receptacles. Turn on the vacuum and make sure papers are in the center. Attach the funnels.

4. a. Place papers on the receptacle with wrinkled side up.  
b. This step dries the papers so that that they do not stick to the supports.

5. Remove the filter papers from the filtering manifold and place on a tray.

5. a. Use tweezers to handle the filter papers from this step on.

6. When the tray is full, place it in a drying oven and dry for one hour.

6. a. The oven must be stabilized at 104<sup>o</sup> +/- 1<sup>o</sup> C.

7. Remove papers from tray and store in the smaller desiccators until used. They must cool a minimum of 1 hour before use.

7. a. DO NOT cover the bottom of the small desiccators with papers. Stack the papers neatly so that the holes in the floor of the desiccators are uncovered & the desiccant can work.

8. If TVSS (Total Volatile Suspended Solids) is to be done, then fire the papers in the muffle furnace after rinsing and drying. Then cool and store in the

8. a. Fire for 20 minutes in a preheated muffle furnace at 500 ± 50<sup>o</sup> C.  
8. b. When removing the papers from the muffle

cabinet desiccators.

furnace, you must wear a face shield and heavy gloves in addition to the lab coat.

## B. Set up Procedure

1. Warm all samples to 20° C.
2. Attach a magnetic funnel receptacle to a 4 L filtering flask that is attached to a catch filtering flask and a desiccant tube which is attached to the vacuum pump.
  - 2.a. Turn on the drying oven and adjust to  $104^{\circ} \pm 1^{\circ}$  C.
3. Remove pre-numbered aluminum pans from the drawer and line them up on the stainless steel tray.
  3. a. Make sure that all numbers are legible.
4. Take tray to the analytical balance. With tweezers, remove a paper from the desiccators, weigh it, record weight on data sheet, and place paper on the first aluminum pan. Continue until all needed papers are weighed. If TVSS is to be done, then use pre-fired papers and separate data sheets made especially for TSS & TVSS. . For every day of analysis, weigh a paper that will be used as an analysis blank.
  4. a. Papers must have been prepared as stated in section A.
  4. b. Calibrate the balance first. Record the calibration in the balance QC record book. If the balance has already been calibrated for the day, then just recheck it with the 1.0000 gram class "S" weight.
  4. c. Papers may be removed from the small desiccators 4 at a time for weighing.
  4. d. Double check that the support numbers agree from aluminum pan to data sheet.
5. Return tray to filtering apparatus. Place a paper on the funnel receptacle, turn on vacuum, center the paper, and attach the funnel.
  5. a. Place paper on the receptacle with wrinkled side up.
  5. b. Use tweezers whenever handling the filter papers throughout the rest of this procedure.

6. Shake sample vigorously, in an arc of one foot for 10 seconds at least 25 times, and quickly measure desired volume into appropriately sized graduated cylinder. Record mL filtered on the data sheet. Pour the sample into the funnel. Rinse graduate twice with DI water and filter this rinse water through the same paper as the sample. Allow all the liquid to filter, and then rinse down sides of funnel through the same filter paper. After all liquid has completely filtered, keep the vacuum on for an additional minute to ensure complete filtration. Remove funnel, wipe bottom of funnel with a paper towel, remove paper with tweezers and return it to its proper aluminum support.

7. Put the tray with the aluminum supports and the filter papers from the samples and the blank into the drying oven and dry for one hour.

8. After drying, put the tray into a cabinet desiccator. Allow to completely cool for at least one hour.

9. Put tray by the analytical balance and weigh all the papers including the blank. Record each weight on the data sheet.

10. If TVSS is to be done, then put the TVSS papers in the muffle furnace and fire for 20 minutes. Remove papers and cool for 1 hour in the desiccators. Then weigh again.

6. a. Shaking is very important. Make the sample as homogeneous as possible, then measure quickly before any settling occurs.

6. b. Measure out enough sample to give between 0.0010 to 0.2000 grams of solids on the paper after filtering - filter no more than 1000 mL.

6. c. Amt. measured   cylinder to use		< 25	
mL	25 mL	26 to 50 mL	50
mL		51 to 100 mL	100 mL
101 to 250 mL		250 mL	251 to 500 mL
500 mL		501 to 1000 mL	1000 mL

6. D.If all the sample will not filter through the paper, pour the leftover sample from the funnel back into the graduate. Do not wipe off the funnel. Weigh out another paper, place it on the funnel receptacle, and pour the leftover sample back into the funnel, filter and rinse as usual. Indicate on the data sheet that there are more than one filter papers for that sample; this is usually indicated with an arrow.

6. e. Repeat the procedure in steps 5 & 6 until all samples have been filtered.

6. f. Lab coat, gloves, and safety glasses must be worn when working with wastewater.

7. a. Oven must be stable at  $104^{\circ} \pm 1^{\circ}$  C. Record oven temperature on the chart on the oven door.

8. a. The papers must be allowed to cool for an entire hour. So if some other hot thing is put into the desiccators during the cooling hour then the timing must begin again.

9. a. First, recheck the balance with the 1.0000 gram class "S" weight.

10. A. Muffle furnace must be preheated to  $500^{\circ} \pm 50^{\circ}$  C.

10. b. Wear a face shield and heavy gloves in addition to the lab coat when working in front of the hot muffle furnace.

11. Do calculations. Enter blank difference into the QC computer database. Check all results for compliance. If QC is OK and there are no violations, then papers and samples may be discarded.

11. a. Before discarding samples check to see if anyone else needs them.

11. b. Any sample that violates its OEPA limits must be reported to a supervisor or designated person as soon as possible.

11. c. If blank QC is out of control see note 7 below.

### C. Calculations

1. a. For samples that use just one filter paper:

$$\frac{(A - B) \times 1,000,000}{C} = \text{mg/L Total Suspended Solids (TSS or SS)}$$

1. b. For samples that use more than one filter paper:

$$\frac{[(A-B) \text{ of 1st paper}] + [(A-B) \text{ of 2nd paper}] + \text{the rest of papers} \times 1,000,000}{C}$$

A = weight (grams) of filter paper plus dried solids

B = weight (grams) of filter paper before use

C = mL of sample filtered

2. a. For samples that use just one filter paper:

$$\frac{(A - D) \times 1,000,000}{C} = \text{mg/L Total Volatile Suspended Solids (TVSS)}$$

2. b. For samples that use more than one filter paper:

$$\frac{[(A-D) \text{ of 1st paper}] + [(A-D) \text{ of 2nd paper}] + \text{the rest of papers} \times 1,000,000}{C}$$

D = weight (grams) of filter paper plus fired solids

3. Range of duplicates = 1st duplicate (grams) - 2nd duplicate (grams)

### D. Notes

1. The temperature at which the residue is dried has a very important bearing on results, because weight losses due to volatilization of organic matter, mechanically occluded water, water of crystallization, and gases from heat-induced chemical decomposition as well as weight gains due to oxidation, depend on temperature and time of heating.

Residues dried at  $103^{\circ}$  to  $105^{\circ}$  C may retain not only water of crystallization but also some mechanically occluded water. Loss of  $\text{CO}_2$  will result in conversion of bicarbonate to carbonate. Loss of organic matter by volatilization usually will be very slight. Because removal of occluded water is marginal at this temperature, attainment of constant weight may be very slow.

We have found that in this lab, for our types of wastewater, one hour of drying and one hour of cooling leads to constant weights. If in doubt, heat, cool and weigh repeatedly until a constant weight is obtained.

When the solids are fired at  $550^{\circ} \pm 50^{\circ}$  C., most of the loss is due to volatilization of the organic matter that is present in the solids. The determination of volatile solids is useful in the control of wastewater treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge, and industrial wastes.

2. Exclude large floating particles or submerged agglomerates of nonhomogeneous materials from the sample to be filtered if it is determined that their inclusion would not be representative of the whole sample. For example, an effluent sample is fairly clear but contains 2 bugs. When sample is poured out into the graduated cylinder, one bug comes out. These bugs would not be representative of the whole sample and they should be removed from the graduate before filtering or from the paper after filtering.
3. After the filter papers have been prepared, never touch them with your hands. Skin oils may adhere to the filter papers causing false results.
4. Results for residues high in oil or grease may be questionable because of the difficulty of drying to constant weight in a reasonable time. In this lab these types of samples should be dried for a total of at least 75 minutes.
5. Whenever samples are handled differently for any reason, write out the circumstances and what was done differently on the data sheet.
6. Filter papers must be pre-rinsed to remove any loose particles that can go through the paper before the paper is weighed.
7. If the difference of the blank's before weight minus its after weight is out of control as indicated in the computer database, then the entire day's results are not acceptable. Save all samples and re-do them the next day

Revised William Johnson  
Revision 4  
3.5.2010