## Appendix A
### Standard Operating Procedures

List of Standard Operating Procedures (SOP’s)

University of Rhode Island Watershed Watch

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Date: August 2008
Revision: 4
Author: Marie Esten
1.0 PURPOSE AND DESCRIPTION

LAB SAFETY IS EVERYBODY’S JOB! Please be sure to familiarize yourself with these general procedures, as well as the specific handling requirements included in the Standard Operating Procedure (SOP) for each analysis/process. Further general information regarding University of Rhode Island standards for health and safety are found in SOP 001a – University Safety and Waste Handling Document.

2.0 HEALTH AND SAFETY

2.1 Emergency Numbers:

**EMERGENCIES: 874-2121**

**UNCONTAINED SPILLS: 874-2618**

**GENERAL HEALTH AND SAFETY INFORMATION**

**URI SAFETY AND RISK: 874-2618**

2.2 General Health and Safety Information

1. Eye protection, gloves and lab coats are REQUIRED in this laboratory when working with chemicals.

2. Closed-toed shoes are REQUIRED whenever you are working in the lab. Even if you are just entering data.

3. Know where the accident and safety equipment is:
   - First–aid kit:
     1. Behind the sink in room 018.
     2. On top of the refrigerator in room 002.
     3. On top of the refrigerator in room 019.
   - Safety shower: next to the entry door of each laboratory.
   - Eye wash: at main sink in each laboratory.
   - Yellow Spill pads:
     1. Under the laboratory bench to the right of blue oven in room 018.
     2. Next to the main sink in room 002.
     3. On top of the refrigerator in room 019.
4. Report ANY accidents IMMEDIATELY to Linda Green (874-2905) or Elizabeth Herron (874-4552). If neither are available, first contact staff in the Cooperative Extension Water Quality Suite (room 001), Dr. Art Gold (874-2903) or department secretary (874-2026). Report all emergencies to 874-2121.

5. If you spill anything hazardous on yourself immediately flush it with water for 15 minutes. Report all emergencies to 874-2121. Uncontained spills should be reported to 874-2618.

6. Concentrated acids and bases are ALWAYS handled in a fume hood, with the door below face level.

7. Wastes are ALWAYS stored in the lab in which they are generated (i.e. acetone waste in the fluorometer lab, autoanalyzer waste in the instrument lab, etc.). Do not transport wastes from one lab to another. All wastes must have a University of Rhode Island (URI) Safety and Health label on them.

8. Liquid wastes must have secondary containment adequate for the full volume of waste.

9. When waste containers are nearing full (~80% for high volume wastes or 90% for low volume wastes), coordinate with Linda Green or Elizabeth Herron to have them removed. Be sure all waste containers are properly and completely labeled at all times.

10. Whenever possible use a cart to transport water samples and/or reagents.

11. All chemicals must be properly labeled and stored at all times. Hazardous labels must indicate in English what harm the chemical represents (i.e. corrosive).

12. Liquid chemicals can not be stored above eye level.

13. If you have any safety or environmental questions, call URI Safety and Risk at 874-2618.

14. All URI Watershed Watch (WW) staff including student technicians are required to successfully complete URI’s “Environmental Awareness and Initial Waste Training” course within their first 6 months of employment. Subsequently, all URIWW staff including student technicians are required to successfully complete URI’s annual refresher course: “Prudent Practices and Laboratory Waste Management”. Certificates attesting to successful completion are posted in URIWW laboratories.
UNIVERSITY OF RHODE ISLAND DEPARTMENT OF SAFETY & RISK MANAGEMENT

STANDARD OPERATING PROCEDURE


POLICY: This policy provides for the disposal of hazardous materials and chemical waste in accordance with all Federal and State regulations.

APPLICABILITY: Faculty, Staff, Employees, Students, Guests. This policy applies to URI Kingston Campus, Narragansett Bay Campus (GSO), W. Alton Jones Campus, and Providence CCE Campus. Further, it applies to all departments and organizations on each of these campuses. All laboratory chemicals, oil-based paint, paint solvents, photographic chemicals, preservatives used for biological specimens, and any hazardous waste material listed or identified in Title 40 Code of Federal Regulations, Part 261, or any materials which exhibits the characteristics of ignitability, corrosivity, reactivity, or toxicity is covered by this policy.

PURPOSE: To provide for the proper disposal of hazardous materials and chemical waste according to Federal and State hazardous waste disposal regulations. Failure to comply may result in civil, criminal, or administrative penalties for the University.

PROCEDURE:

Federal law requires that once a chemical is declared to be hazardous chemical waste, it must be removed from the campus within ninety (90) days. The University of Rhode Island is not permitted to treat or dispose of hazardous waste locally. It is illegal to dispose of hazardous chemical waste by evaporation, disposal down the sink drain to the sewer, or throwing it in the trash. Personnel from the Department of Safety and Risk Management collect, transport, and store hazardous chemical waste prior to final disposal by a licensed hazardous materials vendor.

All faculty must establish laboratory waste management programs for the laboratories they teach. The waste should be collected at the end of each class and each student must be made responsible for disposing of the waste material to the appropriate containers. Principal Investigators are responsible for the waste generated in the research laboratories. A copy of the procedure that the Chemistry Department uses to instruct its teaching assistants is attached to this policy. Labels are available from the Department of Safety & Risk Management.

Departments are encouraged to share chemicals to reduce waste. Buy the minimum amount required to reduce acquisition and disposal costs. This will insure that you have fresh reagents. If you have good quality chemicals that you wish to donate, you may contact Andy Clapham, the Chemistry Department stockroom manager, at 874-5084 (e-mail: Aclapham@chm.uri.edu).
The Department of Safety and Risk Management picks up waste chemicals from individual laboratories. Please call the Hazardous Materials and Chemical Waste Unit (874-2618) to arrange a pick-up. In the event of an emergency, please call S&RM’s main office number (874-2618). If you need to leave a voice mail, please speak clearly leaving your name, phone number, building name, room number, and a brief description of the waste to be picked up. If you have more than five (5) containers, the attached “Request for Hazardous Waste Disposal” form must be faxed in advance to the Department of Safety and Risk Management (Fax Number: 789-5126). All containers of chemicals to be picked up MUST be accompanied by a completed, typed form.

ALL CONTAINERS MUST HAVE A PROPER LABEL. Responsibility for establishing the identity of any unknown materials rests with the department wishing to dispose of it. “unknowns” may cost the University in excess of $100 for each analysis. Costs for unknowns may be charged back to departments in the future.

Other Considerations:

- For removal of radioactive materials, please contact the Radiation Safety Office at 874-6126.
- For removal of infectious and biological waste (also called biohazard or medical waste), including broken or intact hypodermic needles or syringes that are contaminated by chemicals or that have been used in chemical laboratories, please call the Department of Safety and Risk Management (874-2618).
- For disposal of potentially explosive materials such as picric acid, silanes, nitro compounds, and ethers, contact the Department of Safety and Risk Management (874-2618) for assistance.
- Disposal of all hazardous chemicals down the sink to the sewer is prohibited by Federal law. In addition, these substances are explicitly prohibited:
  
  Metals: Arsenic, Cadmium, Chromium, Copper, Lead, Mercury, Nickel, Silver, Zinc
  
  Materials with pH<2 or >12.5, volatile organic compounds, Cyanide, sulfate, sulfide, oil, or grease.
  
  Also prohibited are: Solids or viscous substances in quantities or of such size capable of causing obstruction to the flow in the sewers.

UNDER NO CIRCUMSTANCES SHOULD ANY LABORATORY GLASSWARE OR ANY CHEMICAL CONTAINER EVER BE PLACED IN THE ALUMINUM OR GLASS RECYCLING BINS.
1.0 PURPOSE AND DESCRIPTION

There are three types of water in the URI Watershed Watch (URIWW) laboratories: tap water, deionized water and Ultrapure water. Each type of water is used for specific purposes.

1.1 Tap Water

Tap water is found at each sink and is used for the initial rinsing of labware.

The University of Rhode Island is located in the Kingston Water District; water is stored in the water tower on Flagg Road.

1.2 Deionized Water (DI)

Deionized water (DI) is tap water that has had most ions removed. The deionizing process is done centrally for the building. Each laboratory has at least one DI water tap. In the URIWW laboratory there are two DI water taps: one at the main sink and another at the sink in the central laboratory bench.

DI water is used for rinsing labware between pH and other laboratory measurements, rinsing labware after acid-soaking and for preparing certain reagents. It is obtained by turning on the tap.

Do not leave the tap running. Do not leave the hose hanging in the sink when not in use, coil it up.

1.3 Ultrapure Water.

Ultrapure water is made from the further processing of DI water to remove almost all ions. Each laboratory has one Aries Vaponics filtration unit. The one in the URIWW laboratory is located at the main sink. This water is sometimes referred to as “Millipore water” since that was the brand of the purifying units in Woodward Hall (the location of the URIWW laboratory prior to moving to the Coastal Institute building).

This water is used for final rinsing of all critical labware, such as total phosphorus/total nitrogen (TP/TN) vials, beakers and volumetric flasks.

It is also used for making up critical reagents such as nutrient standards. It should not be wasted.
2.0 METHOD USED TO OBTAIN ULTRAPURE WATER

1. Turn on the switch on the top right of the Aries Vaponics unit.

2. Observe the digital number on the front right of the unit. Once the number reaches approximately 17 megaohms the water is ready for use.
   a. This should only take a few seconds.
   b. If it takes longer contact Linda Green as the cartridges may need to be replaced.

3. Open the tap by turning the handle coming out of the right side of the unit near the hose up.

4. When making reagents let at least 500 ml water flow into the sink before collecting water for use.
   a. This allows the system to flush out any possible contamination collected in the hose.
   b. It is not necessary to allow the system to flush when rinsing labware.

5. Control the flow of water by the handle only. Do not try to slow the flow by pinching the hosing. The back pressure can damage the Aries Vaponics unit.

6. The flow of water from the unit is fairly slow; therefore the two 20 gallon carboys next to the purifying units are routinely filled with Ultrapure water for general use.
   a. When filling a carboy, do not forget that water is flowing and leave the laboratory.
      Washing the floor with Ultrapure water is expensive!!!!
   b. One of the carboys leaks from the valve a bit, tighten the top cap when the carboy is not in use to stop the drip.

7. Do not leave the hose from the Ultrapure unit hanging in the sink. It will pick up contaminants. Coil it up and place it over the tap when not in use.

8. Turn off the switch on the top right of the Aries-Vaptronics unit when done.

2.1 Quality Assurance/Maintenance

2.1.1 Daily Quality Assurance

Each time that the Aries Vaponics unit is used the built-in digital Ohm meter will be checked. The Ohm meter should read approximately 17 megaohms. If the observed value is less than 17 megaohms contact Linda Green or Elizabeth Herron to replace the filtration cartridges.

The final filter will be replaced every year. The replacement date for the filtration cartridges as well as the final filter will be noted on the cartridge and filter, respectively.
2.1.2 Annual Metals Testing

Annually, water obtained from the Aries Vaponics units in Rooms 002 and Room 019 will be tested for lead, cadmium, chromium, copper, nickel and zinc. These contaminations will be at a level of less than 0.05 mg/L per contaminant and less than 0.1 mg/L when added together.

Corrective Action
If the annual metals testing reveals metal value(s) at a level greater than acceptable then the water will not be used for microbiological purposes until another sample is taken and returns an acceptable value.

2.1.3 Monthly heterotrophic plate count

Monthly a sample of water from Room 002 and 019 will be analyzed for heterotrophic plate count. The test results will be less than 500 CFU/ml.

Corrective Action
If the monthly heterotrophic plate count(s) return a value greater than 500 CFU/ml then the affected water source(s) will not be used for microbiological purposes until another sample is taken and returns an acceptable value.
1.0 PURPOSE AND DESCRIPTION

Clean labware and sampling containers are necessary to obtain accurate results for the assays analyzed in the laboratory. Proper cleaning procedure is imperative to maintain the necessary level of cleanliness.

2.0 HEALTH AND SAFETY

Glassware & plasticware are soaked in a bath of dilute (10%) hydrochloric acid which is kept in the fume hood. Wear apron, heavy gloves and goggles when working with the acid baths. Aprons, gloves and goggles are stored in vicinity of the main sink and fume hood.

3.0 METHOD DESCRIPTION

3.1 Overview

1. Labware is washed in non-phosphate detergents. Do not use any commercially available detergent. The laboratory uses Liqui-Nox brand. It is stored under the sink in a 1 gallon container. Pour a small amount into labeled squeeze bottles, dilute with DI water.

2. Labware is acid soaked, large (500 ml) plastic bottles are not. Bacteria bottles are not acid-soaked. Ask if you have any questions.

3. Tap water and deionized (DI) water can be obtained at each sink from the appropriately labeled tap.

4. The Aries Vaponics system, referred to as Ultrapure water, is to the left of the main sink in the main University of Rhode Island Watershed Watch (URIWW) laboratory. To produce Ultrapure water, turn on at the switch, wait until the readout reaches approximately 17 megaohms and open the valve. Refer to SOP 002 - Laboratory Water for further details.

5. In the URIWW laboratory, water samples are collected from lakes, ponds, streams and estuaries, which are generally referred to as “clean lakes” samples. Samples are also collected from septic systems for which there are dedicated “ISDS” bottles. There are dedicated sample bottles for clean lakes samples and ISDS samples. The bottles are always washed separately in tubs or buckets dedicated (and labeled) for each. Do not wash clean lakes sample bottles in ISDS tubs.

6. Clean labware for clean lakes and ISDS samples are stored in separate areas of the laboratory in labeled boxes.
7. In addition, there are glass vials used for total phosphorus/total nitrogen (TP/TN) digestion only. These vials have dedicated “label-soaking” and “soapy soak” tubs in the autoanalyzer room (room 018). These glass vials are not washed/soapy soaked with general labware. SOP 016 – Total Phosphorus and Nitrogen Analysis provides information on how to clean TP/TN vials.
   
   a. As with the water sample bottles, there are dedicated TP/TN vials for clean lakes samples and ISDS samples.
   
   b. The bottles are always washed separately in tubs or buckets dedicated (and labeled) for each. Do not wash clean lakes sample TP/TN vials in ISDS tubs.
   
   c. There are separate storage locations for ISDS and clean lakes TP/TN vials.

8. Since there are multiple students working in the laboratory, it is important to keep track of which cleaning step has been completed to ensure that no steps are missed. Using scrap paper label the labware as:

   a. Clean lakes or ISDS
   
   b. Date
   
   c. The step the labware is in its washing regimen when leaving for soaking or drying, etc.

3.2 Labware Cleaning Procedure

3.2.1 Summary

The following is a general summary of the steps involved in cleaning labware. Do not place any bottle caps in any acid bath.

1. Empty (non-hazardous) contents of bottles down the drain and hazardous contents in the appropriate waste containers.

2. Remove labels. This is expedited by soaking bottles in hot tap water. Label-soaking is a separate step, using a separate tub from soapy water soak/wash. We have discovered that some labels are high in phosphorus.

3. Wash in soapy water in the appropriate tub. Use brush to scrub inside of bottles.

4. Rinse with tap water.

5. Soak for at least 24 hours in tub of 10% hydrochloric acid.

6. Rinse thoroughly with with DI water, inside and out.

7. Fill labware with DI or Ultrapure water and allow to soak for at least 24 hours.

8. Rinse the inside and outside with Ultrapure water.

9. Air dry, inverted.

10. Put away in appropriate location.
3.2.2 Full Description of Labware Cleaning Procedure.

All labware including sample bottles, with the exception of beakers used for pH and alkalinity analysis are soaked in acid after the soapy water soak and rinse. Plasticware used for filtered samples is also acid-soaked after soapy water wash and rinse.

TP/TN digestion vials have further cleaning requirements after the acid soaking, which is detailed in SOP 016 – Total Phosphorus and Nitrogen Analysis.

1. Always wear an apron, heavy gloves and goggles when washing labware. This safety equipment is stored in the vicinity of the sinks and fume hood in each laboratory.

2. Empty (non-hazardous) contents of bottles down the drain and hazardous contents into appropriate, labeled waste containers.

3. Rinse labware with tap water.

4. To remove labels fill a 5 gallon bucket, or the sink with (preferably) hot water and place labware into the water. The hot water expedites label removal. Note that hot water is often unavailable in the summer.

5. Remove all traces of the labels as they are high in phosphorus. Rinse the outside of the labware where the label was. Empty any water from the labware.

6. Dump out, rinse and re-use the bucket or fill another bucket with tap water and a squirt of detergent. Add labware and soak for at least a 1/2 hour. Use an appropriately sized brush to scrub bottles and labware.

7. Rinse labware 3 times with tap water, inside and out.

8. Put cleaned labware into a labware carrying tub and carry it to the acid baths, which are inside the fume hood (room 002).

9. Remove the top from the acid-soaking tub. Carefully put labware into the tub minimizing splashing. Remember the tub contains 10% hydrochloric acid. Make sure the labware is completely covered by the acid solution. Label the tub with the date and time the labware was placed into the tub. Soak the labware for at least 24 hours.

10. After acid soaking, carefully remove labware, emptying acid back into the bath. Put the labware into a labware carrying tub and bring it to a sink. Do not carry labware dripping with acid to the sink.

11. Rinse each piece of labware 3 times, inside and out, with DI water.

12. Fill each piece of labware with DI water and allow it to soak for at least 24 hours, be sure to label the piece of labware with the date of filling.

13. After soaking, empty out and discard the soaking water and rinse 3 times with Ultrapure water.

14. Invert to air dry on drying rack or on clean paper towels. Volumetric flasks are stored filled with Ultrapure water with parafilm across the opening so they do not have to be allowed to air dry.

15. After drying, graduated cylinders are stored with either parafilm or aluminum foil across the top. Bottles are stored with caps loosely attached before storing in boxes in the appropriate area of the laboratory.
3.2.3 Plasticware & All Bottle Caps Cleaning Procedure

Plasticware is used to hold samples for pH, alkalinity, chlorophyll filtration, total suspended solids (TSS), biological oxygen demand (BOD) and/or microbial analysis. This plasticware is never soaked in acid to minimize the potential for inadvertently acidifying the water samples.

Bottles utilized in the microbiological assays are autoclaved after cleaning. The procedure for autoclaving plastic bottles for microbial analysis can be found in SOP 005 - Bottle Autoclaving Procedure.

Bottle caps, especially those for glass bottles, are never soaked in acid because it may damage the surface of the cap.

Plasticware and bottle caps are cleaned using the procedure outlined below:

1. Follow steps 1 through 7 of the labware cleaning procedure.
2. Follow steps 12 through 14 of the labware cleaning procedure.
1.0 PURPOSE AND DESCRIPTION

The autoclave is used to sterilize materials used for bacterial analysis. The procedure outlined below is for the autoclave in Coastal Institute only. This autoclave is sometimes referred to as the “NRS autoclave”.

2.0 HEALTH AND SAFETY CONSIDERATIONS

Be aware that materials that have been autoclaved will be extremely hot when the cycle is completed. Wear insulated gloves when handling materials.

3.0 AUTOCLAVE OPERATION

3.1 Overview & Maintenance

Before operating the autoclave please take a few minutes to read through the operator’s manual.

It is very important that the unit and area around it be kept clean, and that materials to be autoclaved are appropriate for this unit and properly packaged.

The door to the autoclave should be kept open when it is not in use, with the power cord unplugged.

Please complete the log sheet with each run (a sample log sheet is attached below). THE EXACT MATERIALS PLACED INTO THE AUTOCLAVE MUST BE RECORDED (ie: filter assemblies, pipettes, media, etc.). Also be sure that required maintenance has been performed. Elizabeth Herron (room 001F, 4-4552) is responsible for maintaining the unit, contact her if there are any questions regarding the operation or maintenance of the autoclave.

3.2 Operation

The instructions below are to be used for the autoclave in the Kingston Coastal Institute only.

1. Place items to be sterilized on the appropriate tray (see operators manual), and put into the autoclave, leaving the door open.

2. Make sure that the maximum temperature recording thermometer is in the autoclave and the mercury is shaken down below 100 °C.

3. Ensure that the water reservoir is filled to BOTTOM of the safety value. If not, add deionized (DI) water, being sure not to immerse the safety valve.

4. Plug the power cord in, and turn the main switch to START.
5. Fill the autoclave with water by turning the multi-purpose value (always turn this valve clockwise) to FILL. The water should reach most of the width of the “indicator channel” at the front of the chamber. Turn the multi-purpose valve to STE to stop the flow of water and prepare the unit for sterilization.

6. Close the door, and lock it by turning the handle clockwise. The door should be secure with a good seal, but only “hand tight”.

7. Set the temperature at 250 °F / 121 °C with the thermostat knob. This is the temperature most often used for sterilization. If you are in doubt of the correct autoclave temperature or time, refer to the appropriate method SOP. If you are autoclaving waste materials, refer to SOP 006 – Waste Autoclaving Procedure, for correct temperatures and autoclave times.

8. Set the STE timer to the correct sterilization period. Note: this unit takes about 30 minutes to get up to temperature/pressure so be sure to factor that into your time (set the time for the TOTAL time period = +/- 30 minute “heat up” + sterilization time needed (usually ≥ 15 minutes).

9. Check on the unit after about 25 minutes to be sure that it is reaching temperature / pressure. Small bursts of steam may be evident from the water reservoir during the run; this is normal and necessary. Record the unit pressure on the Autoclave Use log. It is necessary to record the maximum pressure while the unit is running because the maximum pressure needle is broken (as of 2008).

10. If the unit does not reach the set temperature contact Elizabeth Herron to arrange for professional servicing of the unit. Until the unit is repaired, it should not be used to sterilize materials.

11. When the timer reaches 0, the run is complete.

12. Turn the multi-purpose knob to EXH + DRY.

13. Follow the procedure below based on desired cycle (with or without drying)

Cycle without drying:
1. When the pressure gauge reads 0, turn the main switch to STOP, and the multi-purpose switch to 0.
2. Carefully open the door – steam will escape so do not put any body parts near the edge of the door while opening it.
3. Remove the load using insulated gloves and unplug the unit if you are not running any more loads immediately.
4. Record the temperature on the maximum temperature recording thermometer and the run time on the autoclave run sheet. The thermometer should read at least 121 °C. If it doesn’t contact Elizabeth Herron and assume the materials taken out of the autoclave are not sterile.

Cycle with drying:
1. When the pressure gauge reads 0, carefully open the door slightly to let the steam escape. Leave the door closed, but not locked down with the locking screw.
2. Set the timer for 20 – 30 minutes; the drying indicator lamp will light.
3. After the time has elapsed, turn the multi-purpose valve to 0, the main switch to STOP.
4. Open the door and remove the load using insulated gloves.

5. Unplug the unit if you are not running any more loads immediately.

6. Record the temperature on the maximum temperature recording thermometer and the run time on the autoclave run sheet. The thermometer should read at least 121 °C. If it doesn’t contact Elizabeth Herron and assume the materials taken out of the autoclave are not sterile.

### 3.3 Quality Assurance/Quality Control

#### 3.3.1 Monthly Spore Testing

Each month Global Autocalve Compliance or a similar firm will provide URIWW with spore strips. These strips have active spores on them. The strips are placed into the autoclave and a normal sterilization run completed. The strips are then packaged and sent back to the firm providing the strips for analysis. The strips must exhibit no growth; this provides assurance that the autoclave is efficiently sterilizing materials.

**Corrective Action**

Spore strip testing must exhibit no growth. If the spore strips are found to exhibit growth after being sterilized then an additional round of spore strip testing should be completed. While awaiting the results of the second spore strip testing round the autoclave will not be used. If the second round of strips also exhibit growth the autoclave must be serviced and will not be used to sterilize materials until serviced and spore strip testing exhibits no growth.
### 4.0 DOCUMENTATION

**Autoclave Use / Maintenance Log**

To be completed for EACH run. This is CRITICAL to ensure that we perform maintenance at correct intervals. If you have ANY questions, please see Elizabeth Herron (Rm 001 F, 4-4552)

<table>
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<th>Date</th>
<th>Person/lab</th>
<th>Materials autoclaved or maintenance performed</th>
<th>Max. Temp. (C°)</th>
<th>Max. Pressure (PSI)</th>
<th>Run duration (Mins)</th>
<th>Comments</th>
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</tbody>
</table>
1.0 PURPOSE AND DESCRIPTION

Plastic bottles must be sterilized prior to use in microbiological assays.

2.0 HEALTH AND SAFETY

Be aware that materials that have been autoclaved will be extremely hot when the cycle is completed. Wear insulated gloves when handling materials.

3.0 AUTOCLAVE OPERATION

3.1 Preparation

Clean the white HDPE plastic sampling bottles as per SOP 003 - General Labware Cleaning Procedure.

Adjust the shelves of the autoclave, if necessary.

Stand bottles in a metal tray, rest the caps on top of each bottle; do not engage the threads of the cap.

Note: Until the bottles are sterile it doesn’t matter if the tops/lids fall on the ground. Once they have been autoclaved, you must beware of the ever-present bacteria in our surroundings and re-autoclave bottles and tops if they are opened or fall onto the ground.

3.2 Using Autoclave in the Kingston Coastal Institute Building

1. Ensure that the water reservoir is filled to the bottom of the safety valve. If not, add deionized (DI) water, being sure not to immerse the safety valve.

2. Plug the power cord in, and turn the main switch to START.

3. Fill the autoclave with water by turning the multi-purpose value (always turn this valve clockwise) to FILL. The water should reach most of the width of the indicator channel at the front of the chamber. Turn the multi-purpose valve to STE to stop the flow of water and prepare the unit for sterilization.

4. Make sure that maximum temperature recording thermometer is in the autoclave. The mercury should be shaken down below 100 °C.

5. Close the door, and lock it by turning the handle clockwise. The door should be secure with a good seal, but only hand tight.

6. Set the temperature for 250 °F / 121 °C with the thermostat knob.
7. Set the STE timer to the correct sterilization period. If the autoclave is cold set it for 55 minutes, if the autoclave is warm set for 45 minutes. Check on the unit after about 20 or 25 minutes to be sure that it is reaching the correct temperature / pressure. Small bursts of steam may be evident from the water reservoir during the run; this is normal and necessary. **Record the unit pressure on the Autoclave Use log.** It is necessary to record the maximum pressure while the unit is running because the maximum pressure needle is broken (as of 2008).

8. When the timer reaches 0, the run is complete.

9. Turn the multi-purpose knob to EXH + DRY.

10. When the pressure gauge reads 0, turn the main switch to STOP, and the multi-purpose switch to 0.

11. Carefully open the door – steam will escape so do not put any body parts near the edge of the door while opening it.

12. Allow to cool or remove the load immediately. Be sure to use insulated gloves when handling the tray. It will be hot!

13. Record the temperature on the maximum temperature recording thermometer and the run time on the autoclave run log (attached below). If the temperature on the recording thermometer does not read at least 121 °C contact Elizabeth Herron.

14. Unplug the unit if you are not running any more loads immediately.

15. **Do not tighten** bottle caps until the bottles are cooled to room temperature or they will warp.

16. Place a “STERILE” label over the cap so it connects to the bottle shoulder to indicate that the bottle has been sterilized.

### 3.3 Using Autoclave in the Basement of Woodward Hall.

1. Seal the door by pressing the button; wait for SEALED DOOR light to come on.

2. Set EXPOSURE TIME for 20 minutes.

3. Set EXHAUST TIME for 5 minutes.

4. Select WRAPPED setting (temperature should be set for 121 °C).

5. Press START.

6. The autoclave will print out a record of the sterilizing event. Write your initials, item being sterilized, room number and phone extension on the tape (so that you can be contacted in case there is a problem).

7. The cycle will take about ½ hour to complete. You will be unable to open the door unless the autoclave has cooled to a safe temperature. Be sure to use insulated gloves when handling the tray. It will be hot!

8. Once the bottles are cool to the touch, tighten the lids and put a “STERILE” label from the cap to the shoulder of the bottle (in effect sealing it).
# Autoclave Use / Maintenance Log

To be completed for EACH run. This is CRITICAL to ensure that we perform maintenance at correct intervals. If you have ANY questions, please see Elizabeth Herron (Rm 001 F, 4-4552)

<table>
<thead>
<tr>
<th>Date</th>
<th>Person/lab</th>
<th>Materials autoclaved or maintenance performed</th>
<th>Max. Temp. (C°)</th>
<th>Max. Pressure (PSI)</th>
<th>Run duration (Mins)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>
1.0 PURPOSE AND DESCRIPTION

This procedure is for autoclaving used media plates (mTec, mE, and EIA) and IDEXX trays using either the autoclave in the basement of Woodward Hall or in the Kingston Coastal Institute.

2.0 HEALTH AND SAFETY

Used media plates constitute a biological hazard, therefore wear plastic gloves when handling the used media plates.

Be aware that materials that have been autoclaved will be extremely hot when the cycle is completed. The media may still be liquid when removed from the autoclave. Spilling liquid media onto any part of the body will cause a burn. If this occurs bathe the effected body part under cool water for 5-10 minutes. Refer to SOP 001a – University Safety and Waste Handling Document. Wear insulated gloves when handling materials.

3.0 AUTOCLAVE OPERATION

3.1 Preparation

Used plates and trays are stored in autoclavable plastic bags in the labeled red waste bin beneath the 35 °C incubator in room 019 of the Coastal Institute. The bags are autoclaved when they appear half full. Do not wait until the bag is completely full.

3.2 Using Autoclave in the Kingston Coastal Institute Building

1. Adjust the shelves of the autoclave if necessary. Ensure there is adequate volume in the tray to handle the number and volume of plates/trays.

2. Wearing gloves, remove the half filled bag of plates/trays and place them in an autoclave tray.

3. Ensure that the water reservoir is filled to the bottom of the safety valve. If not, add deionized (DI) water, being sure not to immerse the safety valve.

4. Plug the power cord in, and turn the main switch to START.

5. Fill the autoclave with water by turning the multi-purpose value (always turn this valve clockwise) to FILL. The water should reach most of the width of the indicator channel at the front of the chamber. Turn the multi-purpose valve to STE to stop the flow of water and prepare the unit for sterilization.

6. Make sure that maximum temperature recording thermometer is in the autoclave. The mercury should be shaken down below 100 °C.
7. Close the door, and lock it by turning the handle clockwise. The door should be secure with a good seal, but only hand tight.

8. Set the temperature for 250 °F / 121 °C with the thermostat knob for media plates (mTec, mE, and EIA) or 134 °C for IDEXX trays.

9. Set the STE timer to the correct sterilization period. If the autoclave is cold set it for 60 minutes, if the autoclave is warm set for 50 minutes. Check on the unit after about 20 or 25 minutes to be sure that it is reaching the correct temperature / pressure. Small bursts of steam may be evident from the water reservoir during the run; this is normal and necessary. Record the unit pressure on the Autoclave Use log. It is necessary to record the maximum pressure while the unit is running because the maximum pressure needle is broken (as of 2008).

10. When the timer reaches 0, the run is complete.

11. Turn the multi-purpose knob to EXH + DRY.

12. When the pressure gauge reads 0, turn the main switch to STOP, and the multi-purpose switch to 0.

13. Carefully open the door – steam will escape so do not put any body parts near the edge of the door while opening it.

14. Allow to cool or remove the load immediately. Be sure to use insulated gloves when handling the tray. It will be hot!

15. Record the temperature on the maximum temperature recording thermometer and the run time on the autoclave run log (attached below). If the temperature on the recording thermometer does not read at least 121 °C for media plates and 134 °C for IDEXX trays contact Elizabeth Herron.

16. Unplug the unit if you are not running any more loads immediately.

17. If you were autoclaving plates, once they are cool enough to handle, bring the waste to the dumpster and carefully discard the plates, bag and all. The media may still be liquid, so be careful not to spill any on you – it will HURT! You must bring the bags to the dumpster, the janitors will not dispose of them.

18. If you are autoclaving IDEXX trays, once they are cool enough, drain bags down the sink with running water and then discarded them in the dumpster. You must bring the bags to the dumpster, the janitors will not dispose of them.

19. Place a new autoclave bag into the red waste container.

3.3 Autoclaving Using Autoclave in Woodward Hall

1. In the autoclave room, adjust the shelves of the autoclave if necessary. Ensure there is adequate volume in the tray to handle the number and volume of plates/trays.

2. Wearing gloves, remove the half filled bag of plates/trays and place them in an autoclave tray.

3. Close the door all the way.

4. Seal the door by pressing the button; wait for SEALED DOOR light to come on.

5. Set EXPOSURE TIME for 20 minutes (for both trays and plates).
6. Set EXHAUST TIME for 5 minutes (for both trays and plates).

7. Select the UNWRAPPED setting. Temperature should be set for 132 °C for both trays and plates.

8. Press START.

9. The autoclave will print out a record of the sterilizing event. Write your initials, item being sterilized, building and room number and phone extension on the tape (so that you can be contacted in case there is a problem).

10. The cycle will take about ½ hour to complete. You will be unable to open the door unless the autoclave has cooled to a safe temperature. Be sure to use the insulated gloves when handling the tray. It will be hot!

11. If you were autoclaving plates, once they are cool enough to handle, bring the waste to the dumpster and carefully discard the plates, bag and all. The media may still be liquid, so be careful not to spill any on you – it will HURT! You must bring the bags to the dumpster, the janitors will not dispose of them.

12. If you are autoclaving IDEXX trays, once they are cool enough, drain bags down the sink with running water and then discarded them in the dumpster. You must bring the bags to the dumpster, the janitors will not dispose of them.

13. Clean the autoclave tray with soap and hot water.

14. Place a new autoclave bag into the red waste container.
Standard Operating Procedure 007
(Prior number URIWW-SOP-4A)

Ambient Waters Microbiological Procedure
University of Rhode Island Watershed Watch

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1.0 PURPOSE AND DESCRIPTION

The purpose of this method is to determine the concentration of *Escherichia coli* (*E. coli*) and fecal coliform bacteria within an ambient water sample. Samples are collected in sterile bottles, an aliquot of sample is filtered and the resulting filter placed into a media dish and incubated. After incubation the number of bacteria colonies are counted. This procedure is utilized for ambient water (lakes, ponds, rivers, etc.) only. Analysis of ISDS/septic samples is completed using SOP 008 - ISDS Microbiological Procedure. This method is applicable to undiluted samples in the range of <1 to 80 colonies/100 mL and samples diluted to return values in this range.

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

Samples to be analyzed are a potential biological hazard. All personnel shall wear a laboratory coat, gloves and goggles when performing this procedure to protect them from accidental exposure.

Wastes in the form of used media plates are also a potential biological hazard and will be disposed of following autoclaving. The procedure for sterilization of plates is found in SOP 006 - Waste Autoclaving Procedure.

Wastes and materials pose a burn hazard immediately following autoclaving. Never move materials that have been autoclaved without the proper insulated autoclave gloves. In addition, when opening the front door on the autoclave, steam will escape as soon as the door is cracked. Any exposed body parts near this steam could be burned. Specific information on autoclaving procedures can be found in the following SOPs: SOP 004 - General Autoclave Operation, SOP 005 - Bottle Autoclaving Procedure and SOP 006 - Waste Autoclaving Procedure.

The ultraviolet light associated with the UV light Box can potentially harm the eyes. Therefore, never look directly at the light for any extended period of time.

Ethanol is utilized both as the fuel for the alcohol lamp and to sterilize the filter forceps. The flame from the alcohol lamp is used to sterilize the filter forceps as well as the mouths of test tubes. Remember that ethanol is a flammable substance. Do not leave the alcohol lamp unattended, and be careful not to allow droplets of ethanol to fall into the flame when sterilizing the filter forceps.
Several chemicals are utilized in this SOP. Potassium phosphate monobasic (KH$_2$PO$_4$), magnesium chloride hexahydrate (MgCl$_2$·6H$_2$O), sodium chloride (NaCl) and Phenol red may cause skin irritation after prolonged exposure as well as irritation of eyes, nose and throat if inhaled or allowed to make contact with the eyes. Wear a lab coat, gloves and goggles when handling these chemicals. Material Safety Data Sheets (MSDS) are located in each laboratory for the materials stored in the specific laboratory. MSDS are contained in plastic file folders in rooms 019 and 018 and a notebook in room 002.

2.2 Technician Training/Qualifications

General training in laboratory technique, use of an autoclave and sterile technique as well as specific training on the procedures contained in this method must be completed prior to analyzing samples. Technician training will be provided either by Elizabeth Herron (Laboratory Project Manager – Microbiology) or Linda Green (Laboratory Project Manager – Nutrients).

3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
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<tbody>
<tr>
<td><strong>Coastal Institute in Kingston, room 081</strong></td>
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<tr>
<td>Autoclave and maximum temperature thermometer</td>
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<tr>
<td><strong>University of Rhode Island (URI), Kingston Coastal Institute Watershed Watch (WW) Laboratory, room 002</strong></td>
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<tr>
<td>Autoclave safe white plastic bottles (125 – 500 mL)</td>
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<tr>
<td>2 L autoclave safe flasks</td>
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<td></td>
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<tr>
<td>Insulated autoclave gloves</td>
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<tr>
<td>Metal autoclave tray</td>
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<tr>
<td>Combination hot plate and magnetic stirrer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnetic stirring bar</td>
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<tr>
<td>Microbiology worksheets</td>
<td>Project data sheets are found on the URIWW computer. See Section 8.0 Documentation</td>
<td></td>
</tr>
<tr>
<td>Sodium Hydroxide (NaOH)</td>
<td>Caustic. Causes eye, skin, digestive and respiratory tract burns. Hygroscopic (absorbs moisture from the air).</td>
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<td><strong>Coastal Institute in Kingston, room 018</strong></td>
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<tr>
<td>Balance</td>
<td>Calibration weights in drawer beneath the balance</td>
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<td><strong>Coastal Institute in Kingston, room 019</strong></td>
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<tr>
<td>35 °C Incubator</td>
<td>Thermolyne Type 142300 Incubator</td>
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<tr>
<td>44.5 °C Water bath</td>
<td>Precision fecal coliform bath 66855 Baxter catalog #W3182-2</td>
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<tr>
<td>Petri dish rack (for water bath)</td>
<td>Baxter catalog #W3182-11</td>
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<tr>
<td>UV sterilization box</td>
<td></td>
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<tr>
<td>Required Material</td>
<td>Notes</td>
<td>Re-order information</td>
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<tr>
<td>Vacuum pump and manifold</td>
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<tr>
<td>Pipette-Aid filler/dispenser (electric)</td>
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<td>Fisher catalog # 13-681-15</td>
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<tr>
<td>2 - Glass 400 mL beakers marked URIWW</td>
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<tr>
<td>4 - Side arm filter flasks</td>
<td>500 mL or larger</td>
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</tbody>
</table>

**Coastal Institute in Kingston, room 019, URIWW refrigerator**

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
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</thead>
<tbody>
<tr>
<td>mTEC media plates</td>
<td>less than a month old, stored media side up in a foil lined box in the refrigerator</td>
<td>Fisher catalog # DF0334-15-0</td>
</tr>
<tr>
<td>mTEC media mix</td>
<td></td>
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<tr>
<td>Agar plate with E. coli</td>
<td>QC sample</td>
<td>American Type Culture Collection # 35922</td>
</tr>
<tr>
<td>Tryptic soy broth (TSB) tubes</td>
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<tr>
<td>Agar mix</td>
<td></td>
<td>Fisher catalog #BP1423-500</td>
</tr>
<tr>
<td>Tryptic soy broth mix</td>
<td></td>
<td>Fisher catalog #DF0370-17-3</td>
</tr>
<tr>
<td>Stock KH$_2$PO$_4$ solution</td>
<td>Preparation instructions: Section 5.3.2.5</td>
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<tr>
<td>Stock MgCl$_2$ solution</td>
<td>Preparation instructions: Section 5.3.2.6</td>
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<tr>
<td>Urea substrate</td>
<td>Preparation instructions: Section 5.3.2.3</td>
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<tr>
<td>Sticks - sterilized</td>
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<td>Fisher catalog #01-340</td>
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</tbody>
</table>

**Coastal Institute in Kingston, room 019, Supplies in or on the gray table to the left of countertop**

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
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<tbody>
<tr>
<td>95% Ethanol in a 500 mL plastic bottle</td>
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<tr>
<td>Alcohol lamp</td>
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<td>Fisher catalog #04-245-1</td>
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<tr>
<td>Envirocide or Conflict Disinfectant solution in squirt bottle</td>
<td></td>
<td>Fisher Catalog #04-324-12</td>
</tr>
<tr>
<td>Filter forceps</td>
<td></td>
<td>Fisher catalog #09-753-30</td>
</tr>
<tr>
<td>Small beaker (~ 50 mL)</td>
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<tr>
<td>Indelible marker (Sharpie)</td>
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<tr>
<td>Matches or igniter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnetic filter funnels (9 – autoclaved and stored in sealed autoclave bags until used)</td>
<td>47 mm diameter, Gelman #4242, 300 mL capacity, 50 mL gradations</td>
<td>Fisher #09-735</td>
</tr>
<tr>
<td>Sterile buffer filled Nalgene wash bottles</td>
<td></td>
<td>Fisher catalog #03-409-14C</td>
</tr>
<tr>
<td>Required Material</td>
<td>Notes</td>
<td>Re-order information</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Sterile grid membrane filters</td>
<td></td>
<td>Fisher catalog # 09-719-1B or HAWG 047 S1</td>
</tr>
<tr>
<td><strong>Coastal Institute in Kingston, room 019, Plastic drawers or boxes beneath the lab countertop</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterile plastic Petri dishes</td>
<td></td>
<td>Fisher catalog # 08-757-19</td>
</tr>
<tr>
<td>Sterile 1 mL pipettes</td>
<td></td>
<td>Fisher catalog #13-678-25C</td>
</tr>
<tr>
<td>Sterile 10 mL pipettes</td>
<td></td>
<td>Fisher catalog #13-678-25F</td>
</tr>
<tr>
<td>Sterile 25 mL pipettes</td>
<td></td>
<td>Fisher catalog #13-676-29D</td>
</tr>
<tr>
<td>Pipette sterilizing pouches</td>
<td></td>
<td>Fisher catalog # 01-812-53</td>
</tr>
<tr>
<td><strong>Coastal Institute in Kingston, room 019, Drawer or shelf beneath the lab countertop</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reusable test tubes</td>
<td></td>
<td>Fisher catalog # 14-925J</td>
</tr>
<tr>
<td>Test tube rack</td>
<td></td>
<td>Fisher catalog # 14-809-24</td>
</tr>
<tr>
<td>Aluminum foil</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Coastal Institute in Kingston, room 019, In cabinet beneath the water bath</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whirl-pak™ bags</td>
<td></td>
<td>Fisher catalog # 01-812-5C</td>
</tr>
<tr>
<td>Clear biohazard autoclave bags</td>
<td></td>
<td>Fisher catalog #01-826-5</td>
</tr>
<tr>
<td><strong>Coastal Institute in Kingston, room 019, On shelf above the water bath</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plastic carboy filled with phosphate buffer saline solution</td>
<td>Preparation instructions: Section 5.3.2.1 and 5.3.2.2</td>
<td></td>
</tr>
<tr>
<td><strong>Coastal Institute in Kingston, room 019, In the upper cabinet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td></td>
<td>Fisher catalog #DF0190-17-1</td>
</tr>
<tr>
<td>Phenol red</td>
<td></td>
<td>Sigma catalog #P2417</td>
</tr>
<tr>
<td>Magnesium chloride hexahydrate (MgCl₂·6H₂O)</td>
<td></td>
<td>Fisher catalog #M33-500</td>
</tr>
<tr>
<td>Potassium phosphate monobasic (KH₂PO₄)</td>
<td></td>
<td>Fisher catalog #P382-500</td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td></td>
<td>Fisher catalog # S671-500</td>
</tr>
<tr>
<td>1 N Sodium Hydroxide (NaOH)</td>
<td>Causes eye, skin, digestive and respiratory tract burns. Caustic. Preparation instructions: 5.3.2.4.</td>
<td></td>
</tr>
<tr>
<td><strong>Coastal Institute in Kingston, room 019, In the chemical cabinet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% Ethanol</td>
<td></td>
<td>Fisher catalog # S93231</td>
</tr>
</tbody>
</table>
Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory and the Natural Resources Science department. Temporary replacement of large equipment such as incubators is available through arrangement with other scientists in the department maintaining similar equipment.

4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Autoclavable white plastic bottle - 250 mL</td>
<td>Kept at 4 °C in Sterile Bottles</td>
<td>100 mL</td>
<td>6 Hours</td>
</tr>
</tbody>
</table>

Disposal
Samples are archived for approximately 48 hours. Aqueous samples may only be disposed of after final quality assurance checks are completed and the data found to be acceptable using criteria found in Section 5.2 of this SOP. Aqueous samples are considered a potential biological hazard. Samples may be rinsed down the drain with running water. Personnel will wear gloves, eye protection and a laboratory coat when disposing of samples.

Disposal of used plates should be completed in accordance with SOP 006 - Waste Autoclaving Procedure. Plates are not archived and may be disposed of immediately after counting.

Bottles are cleaned in accordance with SOP 003 - General Labware Cleaning Procedure and SOP 005 - Bottle Autoclaving Procedure.

5.0 METHOD DESCRIPTION

5.1 Scheduling Considerations

Preparation of materials including plates should occur at least one week prior to the sampling day. If it is necessary to prepare additional plates then a Quality Assurance/ Quality Control (QA/QC) check should be completed on the new plates. This procedure is described in Section 5.2.5 of this document. A check of the operation of the UV light box should also be completed at this time.

At least 48 hours (2 days) prior to the sampling event, sampling bottles and sterile phosphate buffered saline solution (PBS) should be prepared.

The day before a sampling event (24 hours) data sheets, QC samples and a final check of equipment should be completed (refer to Section 5.3 for specific details)
5.2 Quality Assurance/Quality Control

5.2.1 Method Detection Limit

The method detection limit (MDL) for fecal coliforms and *E. coli* is related to the dilution factor used to determine the bacteria count. The following equation is utilized:

\[
\text{MDL} = \frac{100 \text{ mL}}{\text{Volume of sample filtered (mL)}} = \frac{\text{Colonies}}{100 \text{ mL}}
\]

Therefore if the volume of sample placed on the plate is 25 mL then the MDL is 4 colonies/100 mL.

The reporting limit (RL) is set at the MDL for this assay. Data are reported to the nearest whole number.

5.2.2 Method Blanks

Method blanks are determined by treating 100 mL of sterile PBS as a sample. The sterile PBS is filtered onto a sterile filter and placed onto a media plate.

There will be 2 method blanks per 100 plates or 2 method blanks per run, whichever is greater. The first blank will be prepared at the beginning of the sample run and the second at the end of the sample run. This is at the least 2% of the plates analyzed. The method blank shall be less than 1 colony/100 mL.

Corrective Action
If the method blank is equal or greater than 1 colony/100 mL then the sample run will be considered contaminated and the samples reanalyzed. The field samples will be out of compliance with holding times if samples need to be reanalyzed. This will be noted on the data sheet.

5.2.3 Sample Replication

Sample replication is completed in one of two ways. Sample replication for projects in areas where it is not necessary to dilute the samples prior to filtering is completed by filtering a second aliquot of the sample and treating it as a regular sample. Sample replication is completed on 1 sample in 4; 25% of the collected field samples. Analysis results for replicate samples should be within 20% relative percent deviation (%RPD). %RPD is calculated as follows:

\[
\%\text{RPD} = \left| \frac{\text{Result of Replicate 1 (mg/L)} - \text{Result of Replicate 2 (mg/L)}}{\text{Average of Result of Replicate 1 (mg/L) and Result of Replicate 2 (mg/L)}} \right| \times 100
\]

Sample replication for projects in areas where it is necessary to analyze each sample at multiple dilutions is completed by comparing final results of samples at different dilutions. Results between dilutions should be within 20%RPD. %RPD is calculated as follows:

\[
\%\text{RPD} = \left| \frac{\text{Result at Dilution 1 (colonies/100 mL)} - \text{Result at Dilution 2 (colonies/100 mL)}}{\text{Average of Result at Dilution 1 (colonies/100 mL) and Result at Dilution 2 (colonies/100 mL)}} \right| \times 100
\]
Corrective Action
If the %RPD is greater than 20% then the deviation is noted on the data sheet. The processing time to determine the sample value is greater than the holding time for the samples. Therefore the samples will not be reanalyzed unless contamination is suspected as shown through a non-compliant method blank.

5.2.4 Positive Plates

Calibration is completed in a qualitative way through an assessment of false negatives. Bacteria from a known plate of *E. coli* are plated at a dilution of 10^-7 using the procedure outlined under Section 5.2.5.1, steps 1 through 6 and 8 only. The inoculated plates are then treated as samples and referred to as positive plates; 2 positive plates are prepared per sample batch. After incubation these plates must exhibit growth.

Corrective Action
The inoculated plates (positive plates) must show growth after incubation. If the plates do not show growth then it is assumed that the run was in error and the batch of samples is reanalyzed. The field samples will be out of compliance with holding times if samples need to be reanalyzed. This will be noted on the data sheet.

5.2.5 Incubators

The temperature of the 35 °C and 44.5 °C incubators are checked twice a day at least 4 hours apart, when in use. Initial and final temperatures for incubations of samples are recorded on the project data reporting sheet (see Section 8.0). The acceptable temperature range for the 35 °C incubator is 35 +/- 0.5 °C. Acceptable temperature for the 44.5 °C incubator is 44.5 +/- 0.2 °C.

Corrective Action
The incubator temperature must remain in the range specified above. If the incubator temperature is found to be outside the acceptable range, contact Elizabeth Herron and adjust the incubator temperature control. Professional maintenance of the incubator may be necessary if adjustment of the temperature control does not rectify the problem.

5.2.6 Germicidal Unit (UV Box)

The UV light box efficiency will be tested quarterly with a UV light meter. Each lamp will be tested individually. A lamp will be replaced if it emits less than 70% of its initial output. All efficiency checks will be recorded on the “Quarterly UV Germicidal Tube Record” data sheet (see Section 8.0 Documentation) and will be available for review.

Corrective Action
If a UV lamp is found to emit less than 70% of its initial output then it will be replaced. The date of replacement will be recorded on the efficiency check data sheet.

5.2.7 Sterility Check on Sample Bottles

Sample bottles after sterilization must be checked for sterility before being placed with the general stock of sterilized bottles. A bottle sterility check will be completed at a frequency of one sample bottle per sterilization run. Sterility checks will be completed by selecting one sterilized sample bottle at random after the sterilization run has been completed. The sample bottle will then be filled with Ultrapure water capped and mixed. Once the water has been
mixed it will be filtered and treated as a sample. The sample bottle sterility check must return a value of zero CFUs. All data will be recorded on the “Bacterial Sample Log & Worksheet: Bacteria Bottle Sterilization Confirmation” data sheet (see Section 8.0 Documentation).

Corrective Action
If the bottle blank returns a value greater than zero CFUs then all the sample bottles sterilized in the run must be re-sterilized and the bottle sterility check completed again after the second sterilization process. No sample bottles will be placed into the general laboratory stock of sterilized bottles until the sterility check returns a value of zero.

5.2.8 QA Check on New Plates

*E. coli* bacterium is the dominant fecal coliform bacteria found in ambient water. Therefore, a maintained TSB tube of *E. coli* is used to assess the quality of new plates. Plates are assessed to determine if they are able to support the growth of *E. coli* by plating bacteria from the known tube of *E. coli* onto 5 plates of media that have been shown to produce colonies during a prior sample run and 5 plates of the new media. Refer to Section 5.2.8.1 for details on the procedure to QA check new plates.

If the laboratory has received bacteria samples from a field site known to produce positive plates, then an aliquot from this sample may be used to check the new plates instead of inoculating a TSB tube from the maintained *E. coli* culture. Preparation of QA check plates using an existing sample is completed using the procedure outlined below in Section 5.2.5.1 skipping steps 1 through 5.

5.2.8.1 Preparation of QC samples for new batch of plates.

1. Obtain the maintained *E. coli* culture from the refrigerator
2. Using a sterile stick, gently touch the *E. coli* culture ONCE.
3. Flame sterilize a TSB tube.
4. Swish the contaminated stick in the TSB tube, re-sterile the neck of the tube, and set the tube cap – do not tighten the cap.
5. Place the inoculated TSB tube in a test tube rack or beaker in the 35 °C incubator for 24 hours.
6. Remove the TSB tube after incubation and use the tube to prepare serial dilutions of $10^{-7}$ and $10^{-8}$.
7. Filter 1.0 mL of the $10^{-9}$ dilution using the sample filtration technique described in Section 5.3.4.2 and 5.3.4.3. Place the filter onto a new media plate and incubate as a sample.
8. Filter 1.0 mL of the $10^{-7}$ dilution using the sample filtration technique described in Section 5.3.4.2 and 5.3.4.3. Place the filter onto a new media plate and incubate as a sample.
9. Repeat Steps 7 and 8 until 6 plates that are known to support bacteria (old batch) and 6 plates from the new batch have filters. Half of the new plates and half of the old plates should contain filters inoculated with the $10^{-7}$ dilution; the other half with the $10^{-8}$ dilution.
10. After incubation, remove the plates and visually inspect them to determine if approximately the same amount of bacteria grew on both the old and new plates.
Corrective Action
New and old plates must exhibit bacteria growth. If new plates do not exhibit bacteria growth, but the old plates do, then the batch of new plates is assumed to be unable to support bacteria and discarded. If both the new and old plates do not exhibit bacteria growth then it is assumed that the plates were not inoculated properly and the inoculation procedure is repeated.

Notes regarding dilutions for QA samples

<table>
<thead>
<tr>
<th>Volume of sample filtered (mL)</th>
<th>Dilution tube preparation</th>
<th>Volume placed on filter (mL)</th>
<th>Calculated Result (# of colonies divided by calculated result = colonies/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1 mL <em>E. coli</em> inoculated TSB sample into 9 mL of PBS</td>
<td>1</td>
<td>1,000 or 10^5</td>
</tr>
<tr>
<td>0.01</td>
<td>1 mL 10^3 sample into 9 mL of PBS</td>
<td>1</td>
<td>10,000 or 10^-4</td>
</tr>
<tr>
<td>0.001</td>
<td>1 mL 10^4 sample into 9 mL of PBS</td>
<td>1</td>
<td>100,000 or 10^-5</td>
</tr>
<tr>
<td>0.0001</td>
<td>1 mL 10^5 sample into 9 mL of PBS</td>
<td>1</td>
<td>1,000,000 or 10^-6</td>
</tr>
<tr>
<td>0.00001</td>
<td>1 mL 10^6 sample into 9 mL of PBS</td>
<td>1</td>
<td>10,000,000 or 10^-7</td>
</tr>
<tr>
<td>0.000001</td>
<td>1 mL 10^7 sample into 9 mL of PBS</td>
<td>1</td>
<td>100,000,000 or 10^-8</td>
</tr>
</tbody>
</table>

5.3 Analysis Method

5.3.1 Preparation – 1 Week Before Scheduled Sampling
1. Check to be sure the UV box works. If the lights flash and turn on when the black button at the left end of the box is depressed – it works. Be sure not to stare at the light as it can burn your retinas! If it doesn’t work contact Elizabeth Herron to have the box repaired.
2. Check to be sure there is an adequate supply of mTEC media plates that are less than 1 month old. If there are not enough plates, more must be prepared. Preparation instructions are found in Section 5.3.1.1.

5.3.1.1 Preparation of mTEC Media Plates

Materials

<table>
<thead>
<tr>
<th>Envirocide or Conflict</th>
<th>Magnetic stirring bar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile Petri dishes</td>
<td>Magnetic stirring and heating plate</td>
</tr>
<tr>
<td>Autoclave</td>
<td>Metal tray</td>
</tr>
<tr>
<td>2 - 2 L autoclave safe flasks</td>
<td>Aluminum foil</td>
</tr>
<tr>
<td>Insulated autoclave gloves</td>
<td>Sterile 25 mL pipette</td>
</tr>
<tr>
<td>Dehydrated mTEC media</td>
<td>Empty foil lined cardboard box</td>
</tr>
<tr>
<td>Deionized (DI) water</td>
<td>Electronic pipette-aid</td>
</tr>
</tbody>
</table>
Procedure

All equipment to come into contact with the Petri dishes must remain sterile. If contamination is introduced the Petri dishes must be discarded.

1 L of media is enough for approximately 175 – 200 plates

1. Weigh out 45.3 g of dehydrated mTEC media into a 2 L Erlenmeyer flask.
2. Add 1 L DI H2O and a magnetic stirring bar.
3. Heat and stir the re-hydrated mTEC media on the stirring/heating plate until the media is fully dissolved (to boiling if necessary).
4. Pour approximately 500 mL of the liquid media into the second 2 L flask.
5. Cover the mouth of the flasks loosely with aluminum foil, and place in metal tray.
6. Autoclave the tray and media for approximately 40 minutes. Check the autoclave often to get the sterilization time correct without over cooking; the autoclave must be at 121 °C for 15 minutes to achieve sterilization. Directions on autoclave use are found in SOP 004 – General Autoclave Operation.
7. While the mTEC is autoclaving, sterilize the table top by wiping down the entire surface with the Envirocide or Conflict and allowing the surface to dry.
8. Set up the electronic pipette aid at the work area. Have a sterilized 25 mL pipette ready, but leave it in its wrapper to maintain sterility.
9. Set out the Petri dishes in rows of 5 to 10, bottom side down, leaving room for the flask along the edge of the countertop.
10. Carefully remove the lids, offsetting the lids from the bottoms so that the dishes are less than half-open. Do not touch the inside of the lid or bottom. If the inside of a dish comes in contact with anything other than the sterile counter top, discard the dish.
11. Remove the tray from the autoclave when the cycle is completed. Be sure to wear the insulated autoclave gloves as the media will be very hot.
12. Place the flask with the boiling hot media into the 44.5 °C water bath for about 5 minutes to allow the media to cool slightly. Be careful not to let the flask tip over! The Petri dish rack and stone may be used to keep the flask upright.
13. Move the slightly cooled flask to the area where the Petri dishes are set-up using the insulated autoclave gloves. Place the hot flask next to the first row of plates (use a tall stool to set the flask on if needed).
14. Remove the 25 mL pipette from its wrapper, being careful not to touch the pipette anywhere but at its neck. Attach the pipette aid to the pipette. Turn the pipette-aid on, and draw up about 25 mL of hot mTEC media from the flask.
15. Dispense 4 to 5 mL of mTEC media into each of the plates in the first row.
16. Repeat until all the media has been used.
17. Once the media has cooled it will form a gelatin-like substance. At this point put the covers back on the Petri dishes completely. Stack the Petri dishes with the bottom (media-side) up in the foil lined box. Label the box with the date and type of media.
18. QC check the plates against old plates using the procedure found in Section 5.2.5.1. If plates are found to be acceptable store them in the refrigerator for up to a month.
5.3.2 Preparation - At least 48 Hours Prior to Sampling Day (As Needed)

1. Autoclave an appropriate number of bottles for sampling. (Review SOP 005 - Bottle Autoclaving Procedure). Put sterile labels and sample labels on the bottles.
2. Make up sterile phosphate buffered saline solution (PBS) as needed; 1L of PBS is enough for approximately 25-50 samples. The solution must be room temperature when used and will need 24 hours to cool. Instructions for preparation of the PBS are located in Section 5.3.2.1.
3. Make sure the water bath and incubator are set to and holding the correct temperatures. The flask with the thermometer in the white incubator should be full of DI water, with the temperature reading 35 +/- 0.5 °C. The water bath should be approximately ¾ full of DI water, and set at 44.5 +/- 0.2 °C.
4. Make sure there is enough urea substrate for plate counting. Approximately 3 mL of urea substrate is needed per plate. Preparation of urea substrate is in Section 5.3.2.3.

5.3.2.1 Preparation of 1L Sterile Phosphate Buffered Saline Solution (PBS)

1. Add the following into a 4 L Erlenmeyer flask:
   a. 1.25 mL Stock KH$_2$PO$_4$ solution (preparation information in Section 5.3.2.5)
   b. 5 mL Stock MgCl$_2$ solution (preparation information in Section 5.3.2.6)
   c. 7 g NaCl
   d. 1 L DI H$_2$O
2. Stir the mixture using a stirring bar and magnetic stirrer until the NaCl is dissolved.
3. Cover the mouth of the flask with aluminum foil and place it on a metal tray.
4. Autoclave the tray and flask for 45 minutes at 121°C (refer to SOP 004 – General Autoclave Operation).
5. Remove the flask and tray using insulated autoclave gloves as the PBS will be very hot.
6. Leave the flask covered and allow it to cool to room temperature before using.

5.3.2.2 Preparation of 4 L Sterile Phosphate Buffered Saline Solution (PBS)

This procedure will prepare enough PBS to fill the safe plastic carboy

1. Add the following to the 6 L plastic carboy
   a. 5 mL Stock KH$_2$PO$_4$ solution (preparation information in Section 5.3.2.5)
   b. 20 mL Stock MgCl$_2$ solution (preparation information in Section 5.3.2.6)
   c. 28 g NaCl
   d. 4 L DI H$_2$O
2. Stir the mixture using a stirring bar and magnetic stirrer until the NaCl is dissolved.
3. Set the carboy cap on the mouth of the carboy, but DO NOT thread. Place in a metal tray.
4. Autoclave the tray and carboy for 60 minutes at 121 °C. The carboy will have to be laid on its side to fit into the autoclave. Use beakers or bottles on either side of the carboy handle to brace it, preventing the carboy from rolling around on the tray (refer to SOP 004 – General Autoclave Operation).
5. After completion of the autoclave cycle, remove the flask and tray using insulated autoclave gloves as the PBS will be very hot.

6. If possible, leave the tray and carboy (with cap set on the mouth) on the cart opposite the autoclave overnight to cool. This is much safer than carrying the hot, loosely capped carboy.

5.3.2.3 Preparation of Urea Substrate

1. Add the following to a 120 mL brown glass bottle
   a. 2.0 g Urea
   b. 10 mg Phenol Red
   c. 100 mL DI H₂O
2. Store excess solution in a labeled bottle in the URIWW Refrigerator in room 002 of the Kingston Coastal Institute.

5.3.2.4 Preparation of 1N NaOH

1. Obtain a 250 mL volumetric flask and fill it approximately ¾ full with ultrapure water.
2. Weight out 10 g of NaOH.
3. Slowly add the NaOH to the volumetric flask while mixing.
4. Remember that once the NaOH starts to dissolve the flask will get hot! Run the flask under cool tap water if necessary, making sure not to get any of the tap water into the flask. Loosely cover the flask top with foil or parafilm while cooling the flask.
5. Allow the flask to cool and add ultrapure water to bring the flask to volume once all the NaOH has dissolved.

5.3.2.5 Preparation of Stock KH₂PO₄ solution

1. Add the following to a 1 L Erlenmeyer flask
   a. 34.0 g KH₂PO₄
   b. 500 mL DI H₂O
2. Adjust to pH 7.2 ± 0.5 with 1N NaOH and dilute to 1 L with DI H₂O
3. Record the initial pH reading as well as that after it has been adjusted to pH 7.2 ± 0.5 on the “Buffered Dilution/Rinse Water KH₂PO₄ Reagent pH Record” data sheet (see Section 8.0 Documentation).
4. Store excess solution in a labeled plastic bottle in the URIWW Refrigerator in room 002 of the Kingston Coastal Institute.

5.3.2.6 Preparation of Stock MgCl₂ solution

Add 81.6 g MgCl₂ · 6H₂O to a 1 L Volumetric flask and dilute to 1 L with DI H₂O.

Store excess solution in a labeled plastic bottle in the URIWW Refrigerator in room 002 of the Kingston Coastal Institute.
5.3.3 Preparation - Day Before Sample Collection

1. Make sure there is an adequate supply of sterile 1 mL, 10 mL and 25 mL pipettes and PBS filled sterile squirt bottles AS WELL AS FILTER ASSEMBLIES. If not, autoclave them.
   a. Pipettes are sterilized in the pipette pouches that are laid on an autoclavable tray.
   b. Squirt bottles are sterilized empty, with foil over the squirt caps. Caps should only be loosely placed in the bottle. Bottles should be placed on an autoclavable tray when placed into the autoclave. Once cooled, sterile squirt bottles can be filled with cool sterile PBS (refer to SOP 004 – General Autoclave Operation).
   c. Filter assemblies are sterilized in autoclavable bags that are laid on an autoclavable tray.

2. Create data sheets from the template found on the WW computers. It is very helpful to include any known dilution information on the data sheet. An example data sheet is located in Section 8.0 Documentation.

3. Prepare the TSB tube for the positive plate using the procedure outlined in Section 5.2.4.1.

5.3.4 Procedure - Day of Sample Collection

5.3.4.1 Initial Preparation

1. Take the appropriate number of mTEC media plates out of the fridge so that they can begin to warm up a little. Allowing the media plates to come to room temperature reduces condensation and ensures that the labels will not rub off.

2. Wipe down the bench in room 019 with Envirocide or Conflict; allow the bench tops to dry.

3. Connect the side arm flasks to the vacuum manifold.

4. Set up the alcohol lamp, beaker with alcohol and filter forceps, membrane filters, Sharpie marker, PBS squirt bottle, etc.

5. Remember to use basic hygienic practices when handling samples. Latex gloves, laboratory coats and eye protection are required.

5.3.4.2 When Samples Arrive At The Laboratory

1. After logging in samples, store them in a cooler with ice packs or the refrigerator located in room 019.

2. Sterilize the filter funnels and filtration bases in the UV box for at least two minutes.
   a. The cardboard has to be over the button in order for the unit to turn on when the door is closed.
   b. The latch must be engaged to keep the door closed. Peek in the side to be sure it is on – don’t stare at the light!

3. Retrieve samples in batches of 4 from the cooler.

4. Label the bottom (half holding the media) of mTEC media plates with the Sharpie. The label should include all the information present on the sample bottle, as well as any necessary sample dilution information.
5. Label at least one plate from each set of 4 for a replicate sample. Select that sample in a random fashion. If multiple dilutions are needed, try to make the replicate one of the anticipated “correct” dilutions.

6. Stack labeled plates from least dilute to most, with most dilute on the top (as applicable).

7. Enter the relevant data in the data sheet to help keep track of the samples.

8. Light the alcohol lamp.

9. Remove the sterilized filter funnels and base from the UV box, being careful to not touch the insides of the funnel or the base. Assemble the funnels and base (they are magnetic, so they will stay together without a clamp). Place one filter funnel setup on each of the side arm flasks, being careful not to touch the inside of the funnel or the base.

10. Squirt a little PBS onto the base of each filter funnel.

11. Remove the filter forceps (which should be soaking in 95% ethanol approximately 1 centimeter deep) and sterilize them by passing them through the flame of the alcohol lamp. Do not hold them in the flame as they will get too hot. Be sure to keep the beaker of 95% ethanol behind or to the side of the alcohol lamp. A flaming drop of alcohol could cause the beaker of ethanol to explode if it is placed in front of the lamp.

12. After lifting off the top of the funnel, place membrane filters on the base of each of the filter funnels using the following procedure:
   a. Using the sterilized filter forceps, carefully remove a filter from the package. The filter should not touch anything but the filter forceps. If the filters are separated by blue liners remove the blue backing, and place the filter with its front liner on the wetted filter base gridded side up. The blue front liner should curl up making it easier to remove.
   b. If a filter is burned or ripped, discard the filter, and place a new one on the filter base.

13. Provided the filter funnels are sterile (i.e. no sample has been introduced yet), the forceps do not need to be re-flamed between each placement of a filter onto each funnel. Touching anything other than the sterile filters with the forceps necessitates re-flaming the forceps prior to continued use.

5.3.4.3 Filtering Samples

1. Set up the samples and media plates so there is one set in front of each of the prepared filter funnels.

2. Loosen the lids on the media plates leaving the lid in place with the labeled bottom facing up.

3. Shake the first sample vigorously (about 15 times in 7 seconds).

4. Pour the sample into the filter funnel
   a. Generally, 100 mL of lake or river water is analyzed per site. The sample is poured directly into the filter funnel.
      i. The volume is determined using the markings on the side of the filter funnel.
      ii. Be sure to complete an entry on the data sheet for each sample including any dilution (volume) or replicate information as well as sample date and location.
b. If a particular location has a history of high levels of fecal coliforms or if there is reason to suspect that high levels may be present due to events such as large rain storms, several sets of diluted plates should be prepared.

Preparation of dilutions

i. Ambient water samples that must be diluted are generally diluted to 1, 10 and 100 mL.

ii. Prepared dilutions should be filtered sequentially in a single filter funnel in the following order: 1 mL, 10 mL and 100 mL. This will ensure there is no cross-contamination. Remember, only one filter and one dilution per plate.

iii. A sterile 1 mL or 10 mL pipette is used to dispense the appropriate volume. Approximately 10 mL of PBS should be added to the funnel prior to adding sample volumes less than or equal to 10 mL to ensure adequate dispersal of bacteria throughout the filter.

iv. See Elizabeth Herron for guidance on appropriate sample dilution values as they may change over time.

5. Repeat steps 3 and 4 until all 4 filter funnels have sample in them.
6. Open the valve of each of the filter funnel setups, and turn on the vacuum pump.
7. After all the samples have filtered through, use the squirt bottle containing PBS to rinse the inside of each funnel approximately three times to wash any stray bacteria onto the filter. Do not touch the tip of the squirt bottle to the inside of any of the funnels. This will contaminate other samples.

8. Turn off the vacuum pump.
9. Flame sterilize the forceps and shut off the valve for the first funnel.
10. Carefully remove the filter from the first funnel with the sterilized forceps.
11. Carefully place the filter onto the media of a labeled Petri dish, grid side up, so there are no bubbles apparent. Touch the outer edge of the filter with the forceps until it is completely flat. If it appears there are bubbles in the media pick up the filter and lay it onto the media again.

12. Put the cover back on the plate, invert the plate and set it aside.
13. If additional dilutions or replicates will be completed on the sample, place a sterile filter on the empty filter base and add the next dilution or replicate. The forceps do not have to be re-sterilized for this step provided they have not made contact with anything but the sterile media or a more dilute sample.

14. Repeat steps 9 through 13 for the rest of the filter funnels.
15. Once all dilutions and replicates for the first set of 4 samples have been filtered, place the plates with filters into the 35 °C incubator for 2 hours. Plates should be inverted and stacked no higher than 2.

16. Remove the filter funnels and base replacing them with sterilized ones from the UV box for the next set of samples. Put the non-sterile filter funnels into the UV box and turn the UV lights on by closing the lid and depressing the button.

17. Repeat the procedure until all of the samples have been processed.
18. After 2 hours in the incubator place up to 8 to plates into a Whirl-pak™ bag, whirl shut being careful not to trap too much air. Tie bags onto shelves in the Petri dish rack using the wire ties of the bag. Be sure that the plates are inverted on the rack.

19. Transfer the plates to the 44.5 °C water bath for 22 hours. Air trapped in the Whirl-pak™ bags may cause the test tube rack to float. Place the large granite slab onto the rack to keep it from floating, if necessary.

5.3.4.4 Clean-up

1. Discard the filtrate from the side arm flasks down the sink drain. Clean the side arm flasks with warm water and the angled bottle brush. They can be placed on the top of the cart to dry.
2. Clean the filter funnels with warm water and a brush, then place them on the rack hanging from the right side of the 35 °C incubator to dry.
3. Pour the alcohol from the beaker with the forceps back into the labeled plastic container and close tightly. This alcohol is re-used. Alcohol will evaporate very quickly if not stored in a sealed container.
4. Wipe down the alcohol lamp, beaker with forceps, alcohol container, filters, pipette aid and anything else that has come into contact with sample with Envirocide or Conflict and put everything back where it is stored.
5. Thoroughly wipe the counter, tabletops and incubator handles with Envirocide or Conflict.
6. Be sure the UV box is off and the door is slightly ajar.

5.3.4.5 Counting The Plates

1. After 20 - 22 hours, remove the Whirl-pak™ bags from the water bath.
2. Organize the plates according to the data sheet for ease of data entry.
3. Count and record the number of yellow or yellow-green colonies on each membrane filter. There is a digital hand-held counter available.
   a. Establish a system for counting (left to right, top to bottom, etc.) but be consistent!
   b. The ideal range for a plate is 20-80 colonies. Higher or lower plate counts can be used if necessary, with the average of replicate plates used when possible.
   c. Record the number of yellow and yellow-green colonies in the column marked “24 hour count” – this is the fecal coliforms value.
4. Once all the plates have been counted, place a filter pad in the lid of the first plate using forceps. Pipette enough urea substrate media onto the pad to saturate the pad, being careful not to create a big puddle. Approximately 3 mL of urea should be sufficient.
5. With the forceps, remove the membrane filter from the mTEC media, and place it onto the saturated filter pad grid side up. Leave it on the pad for 15 – 20 minutes.
6. Repeat for the remaining filters.
7. After 15 – 20 minutes, starting with the first plate where the filter was placed on the urea saturated filter pad, count the number of yellow and yellow-brown colonies again exactly as discussed in step 3. Record this number in the column labeled “# Urea Negative”.
   a. This is the number of *E. coli* colonies on the plate.
b. The other types of fecal coliform bacteria will have been turned a reddish – purple color by being in contact with the urea substrate media.

8. Count the colonies on the remaining plates.

5.3.5 Disposal

5.3.5.1 Used Plates

Place all of the used plates into a clear autoclavable bag located in the labeled red container. Never fill the autoclavable bags more than half full with plates. If necessary, obtain another autoclavable bag. Follow the instructions in SOP 006 – Waste Autoclaving Procedure for using either the Woodward Hall or the Kingston Coastal Institute autoclave to dispose of plates. Place the cooled bag into the dumpster (do not put in a trash can as the janitors will NOT dispose of it).

5.3.5.2 Sample Bottles

Samples may only be disposed of after final quality assurance checks are completed and the data found to be acceptable using criteria found in Section 5.2 of this SOP. Wearing gloves, remove the sample bottles from the refrigerator in room 019. Empty samples down the drain, and wash bottles with hot soapy water in room 002 as discussed in SOP 003 - General Labware Cleaning Procedure.

6.0 CALCULATIONS

Fecal coliforms and E. coli are reported in terms of the number of bacteria per 100 mL. Fecal coliforms and E. coli counts of 0 are reported as <1 colonies/100 mL.

6.1 Calculation of fecal coliforms Results

Select the membrane filter with the number of colonies closest to the acceptable range (20 – 80 colonies) from the 24 hour count column on the data sheet, and calculate the count per 100 mL according to the general formula shown below. Results are reported as the number of fecal coliforms colonies per 100 mL to the nearest whole number.

\[
\text{fecal coliforms colonies} = \frac{\text{number of yellow colonies counted}}{100 \text{ mL}} \times 100 \text{ mL}
\]

6.2 Calculation of E. coli results:

Select the membrane filter with the number of colonies closest to the acceptable range (20 – 80 colonies) from the # Urea Negative column on the data sheet, and calculate the count per 100 mL according to the general formula shown below. Results are reported as the Number of E. coli colonies per 100 mL to the nearest whole number.

\[
E. \text{coli} = \frac{\text{number of yellow colonies counted}}{\text{Volume in mL of sample filtered}} \times 100 \text{ mL}
\]
7.0 REFERENCES

Methods referenced: Microbiological Examination (9000), Recreational Waters (9213-D) and Membrane Filtration Technique for Members of the Coliform Group (9222 A.)
# 8.0 DOCUMENTATION

Example Data Sheet

### Bacterial Sample Log & Worksheet: Shickasheen (8/20/04)

mTEC Membrane Filtration Method

<table>
<thead>
<tr>
<th>Monitoring Location</th>
<th>Setup Date</th>
<th>Dil. (mLs)</th>
<th>24 Hr Count</th>
<th># urea neg</th>
<th>Total fecal (per 100mL)</th>
<th>Total E.coli (per 100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mud Brook</td>
<td></td>
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<td>Shick - a @ Rte 2</td>
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<td>Shick - b @ Miskiania</td>
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<tr>
<td>Shick - c @ Barber Outlet</td>
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<td>Shick - d @ Rte 138</td>
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<tr>
<td>Shick @ Potter - new dam</td>
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</tbody>
</table>

### Understanding the URIWW Bacterial Data Sheet

**Location:** The name of the waterbody or specific site from which the sample was collected.

**Date Setup:** The date on which the sample was filtered and placed on the media. This SHOULD be the same data as the sample date (the usual hold time for samples < 6 hours). In the event that it is not the same as the sample date, the sample date should be written in parenthesis next to the location identification.
Dil. (mLs): The volume of sample filtered, reported in milliliters. Typically 100 mL are filtered, although if the bacteria levels are expected to be high smaller volumes may be filtered as well.

24 Hr Count: The number of yellow and yellow-brown colonies counted on that filter after a 24 hour total incubation period. This number corresponds to the number of fecal coliform bacteria for that volume of water.

# urea neg.: The number of yellow and yellow-brown colonies counted on that filter after being placed on a urea substrate media saturated pad for 15 minutes. This number corresponds to the number of *E. coli* bacteria for that volume of water.

Total fecal coliforms (per 100 mL): The number of fecal coliform bacteria per 100 mL (the standard method of reporting fecal coliform bacteria). This was determined by dividing the 24 Hr Count by the Dil. (mLs) and multiplying by 100.

Total *E. coli* (per 100 mL): The number of *E. coli* bacteria per 100 mL (the standard method of reporting *E. coli* bacteria). This was determined by dividing the # urea neg. by the Dil. (mLs), and multiplying by 100.
**URI Watershed Watch Laboratory**

**Quarterly UV germicidal Tube Record**

<table>
<thead>
<tr>
<th>Date</th>
<th>tube #</th>
<th>intensity</th>
<th>Date</th>
<th>tube #</th>
<th>intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td></td>
<td></td>
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<td>6</td>
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</table>

Initial intensity readings

<table>
<thead>
<tr>
<th>date</th>
<th>tech's initials</th>
<th>tube #</th>
<th>reading (right side)</th>
<th>reading (center)</th>
<th>reading (left side)</th>
<th>average reading</th>
<th>intensity reduction</th>
<th>comments / notes</th>
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</table>

Wear protective gear when measuring UV lamp intensity!

This form is intended to be used to maintain a quarterly record of the intensity of the germicidal UV labs in the sterilizing box in room 19. The intensity of EACH of the SIX tubes must be measured on a quarterly basis, with each tube measured four inches from each end, as well as in the center, with the average of those three readings used. Once this sheet is full it should be replaced with a new blank sheet. The full sheet should be returned to the URIWW Micro Laboratory Binder in Room 019; blank sheets are also located in this binder. Any maintenance activity should also be recorded on this sheet. See Elizabeth with questions.
Bacterial Sample Log & Worksheet: Bacteria Bottle Sterilization Confirmation

### mTEC Membrane Filtration Method

<table>
<thead>
<tr>
<th>Monitoring Location</th>
<th>Setup Date</th>
<th>Dil. (mls)</th>
<th>24 Hr Count</th>
<th>Total Fecal (per 100ml)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch #4</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Batch #5</td>
<td>100</td>
<td></td>
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</table>

**Enterolert IDEXX Method**

**Notes:** "-" = Quanti-tray used, no small wells

Final count determined by using the correct table

(51 well Quanti-Tray lg wells only, Quanti-Tray / 2000 with small wells)

<table>
<thead>
<tr>
<th>Enterococci</th>
<th>Setup Date</th>
<th>Dil. (mls)</th>
<th># lg pos. wells</th>
<th># sm pos. wells</th>
<th>Table Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch #4</td>
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<tr>
<td>Batch #5</td>
<td>100</td>
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<tr>
<td>IDEXX Blank</td>
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<td>URIWW Sterilized</td>
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</tbody>
</table>
## URI Watershed Watch Laboratory

### Buffered Dilution/rinse Water KH$_2$PO$_4$ reagent pH Record

<table>
<thead>
<tr>
<th>Date</th>
<th>Tech's Initials</th>
<th>Meter Letter (A or B)</th>
<th>Calibration$^2$</th>
<th>Initial pH</th>
<th>Volume 1N NaOH added (mL)</th>
<th>Final pH</th>
<th>Comments / Notes</th>
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</table>

This form is intended to be used to maintain a record of pH and adjustment for the phosphate reagent for the fecal coliform dilution/rinse water. When new reagent is made up, the initial pH, amount of NaOH added, and final pH should be recorded on this sheet. Once this sheet is full it should be replaced with a new blank sheet. The full sheet should be returned to the URIWW Micro Laboratory Binder in Room 019; blank sheets are also located in this binder. Check the Laboratory QAPP, SOP 010 - Alkalinity and pH procedures, for details regarding the pH and alkalinity procedures.

Calibration and Maintenance Information:

1. Each time the pH probe is used the filling solution level in the probe should be checked. The level should be within 1/4 inch of the top of the electrode. If it isn't, add enough filling solution to bring it up to that point.

2. The meter should have a slope (Electrode Efficiency) of at least 96% after calibration. If the meter doesn't initially calibrate correctly, change the probe filling solution and replace the pH buffers, then recalibrate the probe. If this doesn't solve the problem replace the probe. See Linda or Elizabeth with questions.
# Standard Operating Procedure 008
(Prior number URIWW-SOP-4B)

**ISDS Microbiological Procedure**

University of Rhode Island Watershed Watch

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1.0 PURPOSE AND DESCRIPTION

The purpose of this method is to determine the concentration of fecal coliform bacteria within an Individual Sewage Disposal System (ISDS) sample. Samples are collected in sterile bottles, an aliquot of sample is filtered and the resulting filter placed into a media filled Petri dish and incubated. After incubation, the number of bacteria colonies are then counted, and the concentration calculated. This procedure is utilized for ISDS samples only. This method is applicable to undiluted samples in the range of <1 to 80 colonies/100 mL and samples diluted to return values in this range. Analysis of ambient water (lake, ponds, rivers, etc.) is completed using SOP 007 – Ambient Waters Microbiological Procedure.

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

Samples to be analyzed are a potential biological hazard. All personnel shall wear a lab coat, gloves and goggles when performing this procedure to protect them from accidental exposure.

Wastes in the form of used media plates are also a potential biological hazard and will be disposed of following autoclaving. The procedure for sterilization of plates is found in SOP 006 - Waste Autoclaving Procedure.

Wastes and materials pose a burn hazard immediately following autoclaving. Never remove materials from the autoclave without the proper insulated autoclave gloves. In addition, when opening the front door on the autoclave, steam will escape as soon as the door is cracked. Any exposed body parts near this steam could be burned. Specific information on autoclaving procedures can be found in the following SOPs: SOP 004 - General Autoclave Operation, SOP 005 - Bottle Autoclaving Procedure and SOP 006 - Waste Autoclaving Procedure.

The ultraviolet light associated with the UV light Box can potentially harm the eyes. Therefore, never look directly at the light for any extended period of time.

Ethanol is utilized both as the fuel for the alcohol lamp and to sterilize the filter forceps. The flame from the alcohol lamp is used to sterilize the filter forceps as well as the mouths of test tubes. Remember that ethanol is a flammable substance. Do not leave the alcohol lamp unattended, and be careful not to allow droplets of ethanol to fall into the flame when sterilizing the filter forceps.

Several chemicals are utilized in this SOP. Potassium phosphate monobasic (KH₂PO₄), magnesium chloride hexahydrate (MgCl₂·6H₂O), sodium chloride (NaCl) and Phenol red may cause skin irritation after prolonged exposure as well as irritation of eyes, nose and throat if inhaled or allowed to make contact with the eyes. Wear a lab coat, gloves and goggles when
handling these chemicals. Material Safety Data Sheets (MSDS) are located in each laboratory for the materials stored in the specific laboratory. MSDS are contained in plastic file folders in rooms 019 and 018 and a notebook in room 002.

### 2.2 Technician Training/Qualifications

General training in laboratory technique, use of an autoclave and sterile technique as well as specific training regarding procedures specific to this method must be completed prior to analyzing samples using this method. Technician training will be provided either by Elizabeth Herron (Laboratory Project Manager – Microbiology) or Linda Green (Laboratory Project Manager – Nutrients).

### 3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
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</thead>
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<td><strong>Coastal Institute in Kingston, room 081</strong></td>
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</tr>
<tr>
<td>Autoclave and maximum temperature thermometer</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>University of Rhode Island (URI), Kingston Coastal Institute Watershed Watch (WW) Laboratory, room 002</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoclave safe white plastic sample bottles</td>
<td>125 – 500 mL</td>
<td></td>
</tr>
<tr>
<td>Autoclave safe flasks</td>
<td>2 L</td>
<td></td>
</tr>
<tr>
<td>Autoclave gloves (Insulated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metal autoclave tray</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination hot plate and magnetic stirrer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnetic stirring bar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Hydroxide (NaOH)</td>
<td>Caustic. Causes eye, skin, digestive and respiratory tract burns. Hygroscopic (absorbs moisture from the air).</td>
<td></td>
</tr>
<tr>
<td><strong>URI – WW Computer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiology worksheets</td>
<td>Project data sheets are found on the URIWW computer. See Section 8.0 Documentation</td>
<td></td>
</tr>
<tr>
<td><strong>Coastal Institute in Kingston, room 018</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balance</td>
<td>Calibration weights in drawer beneath the balance</td>
<td></td>
</tr>
<tr>
<td><strong>Coastal Institute in Kingston, room 019</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35° C Incubator</td>
<td>Thermolyne Type 142300 Incubator</td>
<td>Thomas catalog #6118L50</td>
</tr>
<tr>
<td>44.5° C Water bath</td>
<td>Precision Fecal coliform bath 66855</td>
<td>Baxter catalog #W3182-2</td>
</tr>
<tr>
<td>Petri dish rack for water bath</td>
<td></td>
<td>Baxter catalog #W3182-11</td>
</tr>
<tr>
<td>UV Sterilization box</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vacuum pump and manifold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required Material</td>
<td>Notes</td>
<td>Re-order information</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>----------------------------------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Pipette-Aid filler/dispenser (electric)</td>
<td></td>
<td>Fisher catalog # 13-681-15</td>
</tr>
<tr>
<td>2 - Glass 400 mL beakers marked URIWW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 - Side arm filter flasks</td>
<td>500 mL or larger</td>
<td></td>
</tr>
<tr>
<td>Thermolyne Vortex Mixer</td>
<td></td>
<td>Fisher catalog #12-814</td>
</tr>
</tbody>
</table>

**Coastal Institute in Kingston, room 019, URIWW refrigerator**

| mTEC media plates | Must be less than a month old, stored media side up in a foil lined box in the refrigerator | Fisher catalog # DF0334-15-0 |
| mTEC media mix    |                                                                                              |                      |
| Agar plate with E. coli | QC sample                                                                                   |                      |
| Blank media plates |                                                                                              |                      |
| Tryptic soy broth tubes |                                                                                             |                      |
| Agar mix          |                                                                                              | Fisher catalog #BP1423-500 |
| Tryptic soy broth mix |                                                                                             | Fisher catalog #DF0370-17-3 |
| Stock KH₂PO₄ solution | Preparation instructions: Section 5.3.2.4                                                    |                      |
| Stock MgCl₂ solution | Preparation instructions: Section 5.3.2.5                                                    |                      |
| Sticks - sterilized |                                                                                              | Fisher catalog #01-340 |

**Coastal Institute in Kingston, room 019, Supplies in or on the gray table to the left of countertop**

| 95% Ethanol in a 500 mL plastic bottle |                                                                      |                      |
| Alcohol lamp                          |                                                                      | Fisher catalog #04-245-1 |
| Envirocide or Conflict Disinfectant solution in squirt bottle |                                                                      | Fisher Catalog #04-324-12 |
| Filter forceps                        |                                                                      | Fisher catalog #09-753-30 |
| Small beaker (~ 50 mL)                |                                                                      |                      |
| Indelible marker (Sharpie)            |                                                                      |                      |
| Matches                                |                                                                      |                      |
| Magnetic filter funnels (9 – hanging from drying rack on incubator) | 47 mm diameter, Gelman #4242, 300 mL capacity, 50 mL gradations      | Fisher #09-735 |
| Sterile buffer filled Nalgene wash bottles |                                                                      | Fisher catalog # 03-409-14C |
| Sterile grid membrane filters         |                                                                      | Fisher catalog # 09-719-1B or HAWG 047 S1 |
## Required Material

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal Institute in Kingston, room 019, Plastic drawers or boxes beneath the lab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>countertop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterile plastic Petri dishes</td>
<td></td>
<td>Fisher catalog # 08-757-19</td>
</tr>
<tr>
<td>Sterile 1 mL pipettes</td>
<td></td>
<td>Fisher catalog #13-678-25C</td>
</tr>
<tr>
<td>Sterile 10 mL pipettes</td>
<td></td>
<td>Fisher catalog #13-678-25F</td>
</tr>
<tr>
<td>Sterile 25 mL pipettes</td>
<td></td>
<td>Fisher catalog #13-676-29D</td>
</tr>
<tr>
<td>Pipette sterilizing pouches</td>
<td></td>
<td>Fisher catalog # 01-812-53</td>
</tr>
<tr>
<td>**Coastal Institute in Kingston, room 019, Drawer or shelf beneath the lab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>countertop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reusable test tubes</td>
<td></td>
<td>Fisher catalog # 14-925J</td>
</tr>
<tr>
<td>Test tube rack</td>
<td></td>
<td>Fisher catalog # 14-809-24</td>
</tr>
<tr>
<td>Aluminum foil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>**Coastal Institute in Kingston, room 019, In cabinet beneath the water bath</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whirl-pak™ bags</td>
<td></td>
<td>Fisher catalog # 01-812-5C</td>
</tr>
<tr>
<td>Clear biohazard autoclave bags</td>
<td></td>
<td>Fisher catalog #01-826-5</td>
</tr>
<tr>
<td>**Coastal Institute in Kingston, room 019, On shelf above the water bath</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plastic carboy filled with phosphate buffer saline solution</td>
<td>Preparation instructions: Section 5.3.2.1 and 5.3.2.2</td>
<td></td>
</tr>
<tr>
<td>**Coastal Institute in Kingston, room 019, In the upper cabinet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenol red</td>
<td>Sigma catalog #P2417</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>Fisher catalog # S671-500</td>
<td></td>
</tr>
<tr>
<td>Magnesium chloride hexahydrate (MgCl₂·6H₂O)</td>
<td>Fisher catalog # M33-500</td>
<td></td>
</tr>
<tr>
<td>Potassium phosphate monobasic (KH₂PO₄)</td>
<td>Fisher catalog # P382-500</td>
<td></td>
</tr>
<tr>
<td>1 N Sodium Hydroxide (NaOH)</td>
<td>Causes eye, skin, digestive and respiratory tract burns. Caustic. Preparation instructions: Section 5.3.2.3.</td>
<td></td>
</tr>
<tr>
<td>**Coastal Institute in Kingston, room 019, In the chemical cabinet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% Ethanol in a 2 L glass bottle</td>
<td>Available through the Agricultural Experiment Station office in Woodward Hall</td>
<td></td>
</tr>
</tbody>
</table>

Equipment is maintained by the Watershed Watch Laboratory and the Natural Resources Science department. Temporary replacement of large equipment such as incubators is available through arrangement with other scientists in the department maintaining similar equipment.
4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Autoclavable 125 or 250 mL white plastic bottles. Sample bottle size is project dependent</td>
<td>Kept at 4 °C in sterile bottle</td>
<td>100 mL</td>
<td>6 Hours</td>
</tr>
</tbody>
</table>

Disposal

ISDS bacterial samples are not archived; samples are disposed of after filtering as they degrade rapidly. Once outside the 6 hour holding time, bacteria in ISDS samples die off quickly, resulting in reduced counts. Due to the potential presence of pathogens, the samples are considered a biological hazard and must be handled carefully. Samples may be rinsed down the drain with running water. Personnel will wear gloves, eye protection and a laboratory coat when disposing of samples.

Disposal of used plates should be completed in accordance with SOP 006 - Waste Autoclaving Procedure. Plates are not archived and may be disposed of immediately after counting.

Bottles are cleaned in accordance with SOP 003 - General Labware Cleaning Procedure and SOP 005 - Bottle Autoclaving Procedure.

5.0 METHOD DESCRIPTION

5.1 Scheduling Considerations

The information provided below is a basic outline of tasks that must be completed prior to the sampling day. Step-by-step instructions to complete each task are provided under Section 5.2 and 5.3.

Preparation of materials including sterile dilution tubes and media plates should occur at least one week prior to the sampling day. If it is necessary to prepare new media plates then a Quality Assurance/Quality Control (QA/QC) check should be completed on the new plates prior to use. This procedure is described in Section 5.2.5 of this document. An operations check of the UV light box should also be completed at this time.

At least 48 hours (2 days) prior to the sampling event sample bottles and sterile phosphate buffered saline solution (PBS) should be prepared.

The day before a sampling event (24 hours) data sheets, QC samples and a final check of equipment should be completed (Refer to Section 5.3 for specific details)
5.2 Quality Assurance/Quality Control

5.2.1 Method Detection Limit

The method detection limit (MDL) for fecal coliforms is related to the dilution factor used to determine the bacteria count. The following equation is utilized:

\[
\text{MDL} = \frac{100 \text{ mL}}{\text{Volume of sample filtered (mL)}} = \frac{\text{Colonies}}{100 \text{ mL}}
\]

Therefore, if the volume of sample placed on the plate is 25 mL then the MDL is 4 colonies/100 mL. The reporting limit (RL) is set at the level of the MDL. Final data are reported to the nearest whole number.

5.2.2 Method Blanks

Method blanks are determined by treating 100 mL of sterile buffer (PBS) as a sample. The sterile PBS is filtered onto a sterile filter and placed onto a media plate.

There will be 2 method blanks per 100 plates or 2 method blanks per run, whichever is greater. The first blank will be prepared at the beginning of the sample run and the second at the end of the sample run. This is at the least 2% of the plates analyzed or approximately 6% of the field samples analyzed, assuming each sample is prepared at three dilutions. The method blank shall be less than 1 colony/100 mL.

Corrective Action

If the method blank is equal to or greater than 1 colony/100 mL then the sample run will be considered contaminated and the data flagged accordingly on the data sheet. Since ISDS samples degrade rapidly once outside the holding time it is not possible to reanalyze the samples.

5.2.3 Sample Replication

Each sample is analyzed at multiple dilutions. The relative percent deviation (%RPD) is determined by comparing plates from the same sample but at different dilutions. Only plates that exhibit the ideal number of colonies (20-80 colonies per plate) are compared. Results between dilutions should be within 20%RPD. %RPD is calculated as follows:

\[
\%\text{RPD} = \left| \frac{\text{Result at Dilution 1 (colonies/100 mL)} - \text{Result at Dilution 2 (colonies/100 mL)}}{\text{Average of Result at Dilution 1 (colonies/100 mL) and Result at Dilution 2 (colonies/100 mL)}} \right| \times 100
\]

Corrective Action

If the %RPD is greater than 20% then the deviation is noted on the data sheet. The processing time to determine the sample value is greater than the holding time for the samples and ISDS samples degrade rapidly. Therefore, the samples will not be reanalyzed.

5.2.4 Positive Plates

Calibration is completed in a qualitative way through an assessment of false negatives. Bacteria from a known plate of *Escherichia coli* (*E. coli*) are plated at a dilution of $10^{-7}$ using the procedure outlined under Section 5.2.5.1, steps 1 through 6 and 8 only. The inoculated plates
are then treated as samples and referred to as positive plates; 2 positive plates are prepared per sample batch. After incubation these plates must exhibit growth.

Corrective Action
The inoculated plates (positive plates) must exhibit growth after incubation. If the plates do not exhibit growth then it is assumed that the run was in error and the data are flagged accordingly on the data sheet. The time required for sample analysis is greater than the sample holding time; therefore, the samples will not be reanalyzed.

5.2.5 Incubators

The temperature of the 35 °C and 44.5 °C incubators are checked twice a day at least 4 hours apart, when in use. Initial and final temperatures for incubations of samples are recorded on the project data reporting sheet (see Section 8.0). The acceptable temperature range for the 35 °C incubator is 35 +/- 0.5 °C. Acceptable temperature for the 44.5 °C incubator is 44.5 +/- 0.2 °C.

Corrective Action
The incubator temperature must remain in the range specified above. If the incubator temperature is found to be outside the acceptable range, contact Elizabeth Herron and adjust the incubator temperature control. Professional maintenance of the incubator may be necessary if adjustment of the temperature control does not rectify the problem.

5.2.6 Germicidal Unit (UV Box)

The UV light box efficiency will be tested quarterly with a UV light meter. Each lamp will be tested individually. A lamp will be replaced if it emits less than 70% of its initial output. All efficiency checks will be recorded on the “Quarterly UV Germicidal Tube Record” data sheet (see Section 8.0 Documentation) and will be available for review.

Corrective Action
If a UV lamp is found to emit less than 70% of its initial output then it will be replaced. The date of replacement will be recorded on the efficiency check data sheet.

5.2.7 Sterility Check on Sample Bottles

Sample bottles after sterilization must be checked for sterility before being placed with the general stock of sterilized bottles. A bottle sterility check will be completed at a frequency of one sample bottle per sterilization run. Sterility checks will be completed by selecting one sterilized sample bottle at random after the sterilization run has been completed. The sample bottle will then be filled with Ultrapure water capped and mixed. Once the water has been mixed it will be filtered and treated as a sample. All data will be recorded on the “Bacterial Sample Log & Worksheet: Bacteria Bottle Sterilization Confirmation” data sheet (see Section 8.0 Documentation).

Corrective Action
If the bottle blank returns a value greater than zero CFUs then all the sample bottles sterilized in the run must be re-sterilized and the bottle sterility check completed again after the second sterilization process. No sample bottles will be placed into the general laboratory stock of sterilized bottles until the sterility check returns a value of zero.
5.2.8 QA Check on New Plates

*E. coli* bacterium is the dominant fecal coliform bacteria found in ambient water. Therefore, a maintained plate of *E. coli* is used to assess the quality of new plates. Plates are assessed to determine if they are able to support the growth of *E. coli* by plating bacteria from the known plate of *E. coli* onto 6 plates of media that have been shown to produce colonies during a prior sample run and 6 plates of the new media. Refer to Section 5.2.5.1 for details on the procedure to QA check new plates.

If the laboratory has received bacteria samples from a field site known to produce positive plates, then an aliquot from this sample may be used to check the new plates instead of inoculating a TSB tube from the maintained *E. coli* culture. Preparation of QA check plates using an existing sample is completed using the procedure outlined below in Section 5.2.5.1 skipping steps 1 through 5.

5.2.8.1 Preparation of QC samples for new batch of plates.

1. Obtain the maintained *E. coli* culture from the refrigerator
2. Using a sterile stick, gently touch the *E. coli* culture ONCE.
3. Flame sterilize a TSB tube.
4. Swish the contaminated stick in the TSB tube, re-sterile the neck of the tube, and set the tube cap – do not tighten the cap.
5. Place the inoculated TSB tube in a test tube rack or beaker in the 35 °C incubator for 24 hours.
6. Remove the TSB tube after incubation and use the tube to prepare serial dilutions of 10^-7 and 10^-8.
7. Filter 1.0 mL of the 10^-6 dilution using the sample filtration technique described in Section 5.3.4.2 and 5.3.4.3. Place the filter onto a new media plate and incubate as a sample.
8. Filter 1.0 mL of the 10^-7 dilution using the sample filtration technique described in Section 5.3.4.2 and 5.3.4.3. Place the filter onto a new media plate and incubate as a sample.
9. Repeat Steps 7 and 8 until 6 plates that are known to support bacteria (old batch) and 6 plates from the new batch have filters. Half of the new plates and half of the old plates should contain filters inoculated with the 10^-7 dilution; the other half with the 10^-8 dilution.
10. After incubation, remove the plates and visually inspect them to determine if approximately the same amount of bacteria grew on both the old and new plates.

Corrective Action

New and old plates must exhibit bacteria growth. If new plates do not exhibit bacteria growth, but the old plates do, then the batch of new plates is assumed to be unable to support bacteria and discarded. If both the new and old plates do not exhibit bacteria growth then it is assumed that the plates were not inoculated properly and the inoculation procedure is repeated.
5.3 Analysis Method

5.3.1 Preparation – 1 Week Before Scheduled Sampling

1. Check to be sure the UV box works. If the lights flash and turn on when the black button at the left end of the box is depressed – it works. Be sure not to stare at the light as it can burn your retinas! If it doesn’t work contact Elizabeth Herron to have the box repaired.

2. Check that enough sterile dilution tubes are prepared for the anticipated number of samples to be analyzed. The project data sheet will provide a count of the number of dilution tubes needed. If there are not enough dilution tubes available then using SOP 004 – General Autoclave Operation, prepare more. Dilution tubes are autoclaved in the autoclavable bags.

3. Check to be sure there is an adequate supply of mTEC media plates that are less than 1 month old. If there are not enough plates, more must be prepared. Preparation instructions are found in Section 5.3.1.1.

5.3.1.1 Preparation of mTEC Media Plates

Materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Envirocide or Conflict</td>
<td>Magnetic stirring bar</td>
</tr>
<tr>
<td>Sterile Petri dishes</td>
<td>Magnetic stirring and heating plate</td>
</tr>
<tr>
<td>Autoclave</td>
<td>Metal tray</td>
</tr>
<tr>
<td>2 - 2 L autoclavable flasks</td>
<td>Aluminum foil</td>
</tr>
<tr>
<td>Insulated autoclave gloves</td>
<td>Sterile 25 mL pipette</td>
</tr>
<tr>
<td>Dehydrated mTEC media</td>
<td>Empty foil lined cardboard box</td>
</tr>
<tr>
<td>Deionized (DI) water</td>
<td>Electronic pipette-aid</td>
</tr>
</tbody>
</table>

Procedure

Note: All equipment that contacts the Petri dishes must remain sterile. If contamination is introduced the Petri dishes must be discarded.

Note: 1 L makes approx. 175 – 200 plates

1. Weigh out 45.3 g of dehydrated mTEC media into a 2 L Erlenmeyer flask.
2. Add 1 L DI H₂O and a magnetic stirring bar.
3. Heat and stir the re-hydrated mTEC media on the stirring/heating plate until the media is fully dissolved (to boiling if necessary).
4. Pour approximately 500 mL of the liquid media into the second 2 L flask.
5. Cover the mouth of the flasks loosely with aluminum foil, and place in metal tray.
6. Autoclave the tray and media for approximately 40 minutes. Check the autoclave often to get the sterilization time correct without over-cooking; the autoclave must be at 121 °C for 15 minutes to achieve sterilization. Directions on autoclave use are found in SOP 004 – General Autoclave Operation.
7. While the mTEC media is autoclaving, sterilize the table top by wiping down the entire surface with the Envirocide or Conflict and allowing the surface to dry.

8. Set up the electronic pipette-aid at the work area. Have a sterilized 25 mL pipette ready but leave it in its wrapper to maintain sterility.

9. Set out the sterile Petri dishes in rows of 5 to 10, bottom side down, leaving room for the flask along the edge of the countertop.

10. Carefully remove the lids, offsetting the lids from the bottoms so that the dishes are less than half-open. Do not touch the inside of the lid or bottom. If the inside of a Petri dish comes into contact with anything other than the sterile counter top, discard the Petri dish.

11. Remove the tray from the autoclave when the cycle is complete. Be sure to wear the insulated autoclave gloves as the media will be very hot.

12. Place the flask with the boiling hot media into the 44.5 °C water bath for approximately 5 minutes to allow the media to cool slightly. Be careful not to let the flask tip over! The Petri dish rack and stone may be used to keep the flask upright.

13. Move the slightly cooled flask to the area where the Petri dishes are set-up using the insulated autoclave gloves. Place the hot flask next to the first row of plates (use a tall stool to set the flask on if needed).

14. Remove the 25 mL pipette from its wrapper, being careful not to touch the pipette anywhere but at its neck. Attach the pipette aid to the pipette. Turn the pipette aid on, and draw up about 25 mL of hot mTEC media from the flask.

15. Dispense 4 to 5 mL of mTEC media into each of the plates in the first row.

16. Repeat until all the media has been used.

17. Once the media has cooled it will form a gelatin like substance. At this point put the covers back on the Petri dishes completely. Stack the Petri dishes with the bottom (media-side) up in the foil lined box. Label the box with the date and type of media.

18. QC check the plates against old plates using the procedure found in Section 5.2.5.1. If plates are found to be acceptable store them in the refrigerator for up to a month.

### 5.3.2 Preparation - At least 48 Hours Prior to Sampling Day (As Needed)

1. Autoclave an appropriate number of bottles for sampling. (Review SOP 005 - Bottle Autoclaving Procedure). Put sterile labels and sample labels on the bottles.

2. Make up sterile phosphate buffered saline solution (PBS) as needed; 1 L of PBS is enough for approximately 25-50 ISDS samples. The solution must be room temperature when used and will need 24 hours to cool. Instructions for preparation of the PBS are located in Section 5.3.2.1.

3. Make sure the water bath and incubator are set to and holding the correct temperatures. The flask with the thermometer in the white incubator should be full of DI water, with the temperature reading 35 +/- 0.5 °C. The water bath should be approximately ¾ full of DI water and set at 44.5 +/- 0.2 °C.
5.3.2.1 Preparation of 1L Sterile Phosphate Buffered Saline Solution (PBS)

1. Add the following into a 4 L Erlenmeyer flask:
   a. 1.25 mL Stock KH₂PO₄ solution (preparation information in Section 5.3.2.4)
   b. 5 mL Stock MgCl₂ solution (preparation information in Section 5.3.2.5)
   c. 7 g NaCl
   d. 1 L DI H₂O
2. Stir the mixture using a stirring bar and magnetic stirrer until the NaCl is dissolved.
3. Cover the mouth of the flask with aluminum foil and place it on a metal tray.
4. Autoclave the tray and flask for 45 minutes at 121°C (Refer to SOP 004 – General Autoclave Operation)
5. Remove the flask and tray using insulated autoclave gloves as the PBS will be very hot.
6. Leave the flask covered and allow it to cool to room temperature before using.

5.3.2.2 Preparation of 4 L Sterile Phosphate Buffered Saline Solution (PBS)

This procedure will prepare enough PBS to fill the safe plastic carboy

1. Add the following to the 6 L plastic carboy
   a. 5 mL Stock KH₂PO₄ solution (preparation information in Section 5.3.2.4)
   b. 20 mL Stock MgCl₂ solution (preparation information in Section 5.3.2.5)
   c. 28 g NaCl
   d. 4 L DI H₂O
2. Stir the mixture using a stirring bar and magnetic stirrer until the NaCl is dissolved.
3. Set the carboy cap on the mouth of the carboy, but DO NOT thread. Place on a metal tray.
4. Autoclave the tray and carboy for 60 minutes at 121°C. The carboy will have to be laid on its side to fit into the autoclave. Use beakers or bottles on either side of the carboy handle to brace it, preventing the carboy from rolling around on the tray. (Refer to SOP 004 – General Autoclave Operation)
5. After completion of the autoclave cycle, remove the flask and tray using insulated autoclave gloves as the PBS will be very hot.
6. If possible, leave the tray and carboy (with cap set on the mouth) on the cart opposite the autoclave overnight to cool. This is much safer than carrying the hot, loosely capped carboy.
5.3.2.3 Preparation of 1N NaOH

1. Obtain a 250 mL volumetric flask and fill it approximately ¾ full with ultrapure water.
2. Weight out 10 g of NaOH.
3. Slowly add the NaOH to the volumetric flask while mixing.
4. **Remember that once the NaOH starts to dissolve the flask will get hot!** Run the flask under cool tap water if necessary, making sure not to get any of the tap water into the flask. Loosely cover the flask top with foil or parafilm while cooling the flask.
5. Allow the flask to cool and add ultrapure water to bring the flask to volume once all the NaOH has dissolved.

5.3.2.4 Preparation of Stock KH$_2$PO$_4$ solution

1. Add the following to a 1 L Erlenmeyer flask
   a. 34.0 g KH$_2$PO$_4$
   b. 500 mL DI H$_2$O
2. Adjust to pH 7.2 ± 0.5 with 1N NaOH and dilute to 1 L with DI H$_2$O.
3. Record the initial pH reading as well as that after it has been adjusted to pH 7.2 ± 0.5 on the “Buffered Dilution/Rinse Water KH$_2$PO$_4$ Reagent pH Record” data sheet (see Section 8.0 Documentation).
4. Store excess solution in a labeled container in the URI WW Refrigerator in room 019 of the Kingston Coastal Institute

5.3.2.5 Preparation of Stock MgCl$_2$ solution

Add 81.6 g MgCl$_2$ · 6H$_2$O to a 1 L Volumetric flask and dilute to 1 L with DI H$_2$O.

Store excess solution in a labeled container in the URI WW Refrigerator in room 019 of the Kingston Coastal Institute

5.3.3 Preparation - Day Before Sample Collection

1. Make sure there is an adequate supply of sterile 1 mL, 10 mL and 25 mL pipettes and PBS filled sterile squirt bottles AS WELL AS FILTER ASSEMBLIES. If not, autoclave them.
   a. Pipettes are sterilized in the pipette pouches in an autoclavable tray.
   b. Squirt bottles are sterilized empty, with foil over the squirt caps. Caps should only be loosely placed in the bottle. Bottles should be placed on an autoclavable tray when placed into the autoclave. Once cooled, sterile squirt bottles can be filled with cool sterile PBS. (Refer to SOP 004 – General Autoclave Operation).
   c. Filter assemblies are sterilized in autoclavable bags on an autoclavable tray.
2. Create data sheets from the template found on the WW computers. It is very helpful to include any known dilution information on the data sheet. An example data sheet is located in Section 8.0 Documentation.

3. Prepare the TSB tube for the Positive plate using the procedure outlined in Section 5.2.4.1.

5.3.4 Procedure - Day of Sample Collection

5.3.4.1 Initial Preparation

1. Set up sterile test tubes by placing them into the test tube rack. Remember to touch only the outside of each tube. A large piece of aluminum foil can be used to cover the top of the tubes. Sterilize the aluminum foil in the UV box.

2. Sterilize a beaker in the UV box for 2 minutes.

3. Fill the beaker with sterile PBS.

4. Using a sterile pipette and the electric filler/dispenser, pipette 9 mL of PBS into the appropriate number of test tubes.

5. Fill the sterile squirt bottles with PBS if necessary.

6. Take the appropriate number of mTEC media plates out of the fridge so that they can begin to warm up a little. This reduces condensation and ensures that the labels will not rub off.

7. Wipe down the benchtops in room 019 with Envirocide or Conflict; allow the bench tops to dry.

8. Connect the side arm flasks to the vacuum manifold.

9. Set up the alcohol lamp, beaker with alcohol and filter forceps, membrane filters, Sharpie marker, PBS squirt bottle, etc

10. Remember to use basic hygienic practices when handling ISDS samples. Latex gloves, lab coats and eye protection are required.

5.3.4.2 When Samples Arrive At The Laboratory

1. After logging in samples, store them in a cooler with ice packs or the refrigerator located in room 019.

2. Sterilize the filter funnels and funnel base in the UV box for at least two minutes.
   a. The cardboard has to be over the button in order for the UV box to turn on when the door is closed.
   b. The latch must be engaged to keep the door closed. Peek in the side to be sure the light is on – don’t stare at the light!

3. Organize the samples. For ease of filtering, try to setup samples so that each ISDS site is grouped together, with components moving from most to least contaminated. The more contaminated samples are more difficult to filter, therefore filter these first.
4. Label the bottom (half holding the media) of mTEC media plates with a Sharpie. The label should include all the information present on the sample bottle, as well as the sample dilution.

5. Stack labeled plates from least to most dilute, with most dilute on the top.

6. Enter the relevant data on the data sheet to help keep track of the samples.

7. Light the alcohol lamp.

8. Set the sterile filter funnels on the side arm flasks, being careful not to touch the inside of the funnel or the base.

9. Squirt a little PBS onto the base of each filter funnel.

10. Remove the filter forceps (which should be soaking in 95% ethanol only up to the tips) and sterilize them by passing them through the flame of the lamp. Do not hold them in the flame as they will get too hot. Be sure to keep the beaker of 95% Ethanol and the alcohol lamp separated. The beaker of ethanol could explode if it comes into contact with an open flame.

11. After lifting off the top of the funnel, place membrane filters on the base of each of the filter funnels using the following procedure:
   a. Using the sterilized filter forceps, carefully remove a filter from the package. The filter should not touch anything but the filter forceps. If the filters are separated by blue liners remove the blue backing, and place the filter with its front liner on the wetted filter base gridded side up. The blue front liner should curl up making it easier to remove.
   b. If a filter is burned or ripped, discard the filter, and place a new one on the filter base.

12. Provided the filter funnels are sterile (i.e. no sample has been introduced yet), the forceps do not need to be re-flamed between placement of a filter on each funnel. Touching anything other than the sterile filters with the forceps necessitates re-flaming the forceps prior to continued use.

5.3.4.3 Filtering Samples

1. Set up samples and media plates so there is one set in front of each prepared filter funnel.

2. Loosen the lids on the media plates – but leave the lid in place with the labeled bottom facing up.

3. Shake the first sample vigorously (about 25 times in 7 seconds).

4. If this is to be a serial dilution sample, carefully draw up 1 mL of homogenized sample using the pipette-aide and a sterile 1 mL pipette.
   a. Carefully discharge the sample into a prepared test tube containing 9 mL of PBS.
   b. Rinse the pipette by drawing in about 1 mL of diluted sample, then discharging back into the test tube, being sure to blow out the entire sample.
   c. Use the Vortex mixer to thoroughly mix the sample.
d. Then remove 1 mL of diluted sample, add it to the next tube, etc. until all of the
dilutions for the sample are prepared. Refer to Section 6.0 - Calculations for
information on preparation of serial dilutions.

e. The number and value of dilutions to be run per sample is found on the project
data sheet. Dilutions for a new sample or a new project should be determined by
consultation with Elizabeth Herron.

f. Once the most diluted sample dilution tube has been thoroughly mixed, pipette 1
mL of this dilution into the appropriate filter funnel. Approximately 10 mL of PBS
should have already been added to the filter funnel to pre-wet the filter.

g. Place the 1 mL pipette into the next lowest dilution tube for that sample.

5. If a serial dilution is not necessary filter the volume of sample noted on the project data
sheet.

6. Repeat steps 3, 4 and 5 until each filter funnel has a sample in it.

7. Open the valve of each of the filter funnel setups, and turn on the vacuum pump.

8. After all the samples have filtered through, use the sterile squirt bottle filled with PBS to
rinse the inside of each funnel approximately three times to wash any stray bacteria onto
the filter. Do not touch the tip of the squirt bottle to the inside of any of the funnels, this
will contaminate other samples.

9. Turn off the vacuum pump.

10. Flame sterilize the forceps and shut off the valve for the first funnel.

11. Carefully remove the filter with the sterilized forceps.

12. Carefully place the filter onto the media of a labeled Petri dish, grid side up, so no
bubbles are apparent. Touch the outer edge of the filter with your forceps until it is
completely flat. If it appears there are bubbles in the media pick up the filter and lay it
into the media again.

13. Put the cover back on the plate, invert the plate and set it aside.

14. If additional dilutions will be completed on the sample, place a sterile filter on the empty
filter base corresponding to the sample and add the next diluted sample. The forceps do
not have to be re-sterilized provided they have not made contact with anything but the
sterile media or a more dilute sample.

15. Repeat steps 10 through 14 for the rest of the filter funnels.

16. Once all dilutions and replicates for the first set of samples have been filtered, place the
plates with filters into the 35 °C incubator for 2 hours. Plates should be inverted, and
stacked no higher than 2.

17. Remove the filter funnels and base replacing them with sterilized ones from the UV box
for the next set of samples. Put the non-sterile filter funnels and bases into the UV box
and turn the UV light on.

18. Repeat this procedure until all of the samples have been processed.

19. After 2 hours in the incubator place up to 8 to plates into a Whirl-pak™ bag, whirl shut
being careful not to trap a large amount air. Tie the bags onto shelves in the Petri dish
rack using the wire ties of the bag. Be sure that the plates are inverted on the rack.
20. Transfer the plates to the 44.5 °C water bath for 22 hours. Air trapped in the Whirl-pak™ bags may cause the test tube rack to float. If necessary, place the large granite slab onto the rack to keep it from floating.

5.3.4.4 Clean-up

1. Discard the filtrate from the side arm flasks down the sink drain, then clean the side arm flasks with warm water and the angled bottle brush. They can be placed on the top of the cart to dry.

2. Clean the filter funnels with warm water and a brush, then place them on the rack hanging from the right side of the 35 °C incubator to dry.

3. Pour the alcohol from the beaker with the forceps back into the labeled plastic container and close tightly. This alcohol is re-used. Alcohol evaporates very quickly if not stored in a sealed container.

4. Wipe down the alcohol lamp, beaker containing the forceps, alcohol container, filters, pipette-aid and anything else that has come into contact with sample with Envirocide or Conflict and put everything back where it is stored.

5. Thoroughly wipe the counter, tabletops and incubator handles with Envirocide or Conflict.

6. Be sure the UV box is off and the door is slightly ajar.

5.3.4.5 Counting The Plates

1. After 20 - 22 hours, remove the Whirl-pak™ bags from the water bath.

2. Organize the plates according to the data sheet for ease of data entry.

3. Count and record the number of yellow or yellow-green colonies on the membrane filters. There is a digital hand-held counter available.
   a. Establish a system for counting (left to right, top to bottom, etc.) but be consistent!
   b. The ideal range for a plate is 20-80 colonies. Higher or lower plate counts can be used if necessary. Ideally, the results from different dilutions of the same sample are averaged.

5.3.5 Disposal

5.3.5.1 Used Plates

Place all of the used plates into a clear autoclavable bag located in the labeled red container. Never fill the autoclavable bags more than half full with plates. If necessary, obtain another autoclavable bag. Follow the instructions in SOP 006 – Waste Autoclaving Procedure for plate disposal using either the Woodward Hall or the Kingston Coastal Institute autoclave. Place the cooled bag into the dumpster (do not put in a trash can as the janitors will NOT dispose of it).
5.3.5.2 Sample Bottles

Samples may be disposed of after filtering. Wearing gloves, empty samples down the drain and then wash the bottles with hot soapy water in room 002. Wash bottles in accordance with SOP 003 – General Labware Cleaning Procedure.

6.0 CALCULATIONS

Fecal coliforms are reported in terms of the number of bacteria per 100 mL. Select the membrane filter with the number of colonies closest to the acceptable range (20 – 80 colonies) from the 24 hour count column on the data sheet, and calculate the count per 100 mL according to the general formula shown below. Results are reported as the number of fecal coliforms colonies per 100 mL to the nearest whole number. Fecal coliforms counts of 0 are reported as <1 colonies/100 mL.

\[
\text{Fecal coliforms colonies} = \frac{\text{Count}}{100 \text{ mL}} \quad \text{Yields}
\]

Notes regarding dilutions:

<table>
<thead>
<tr>
<th>Volume of ISDS sample filtered (mL)</th>
<th>Dilution tube preparation</th>
<th>Volume placed on filter (mL)</th>
<th>Calculated Result (# of colonies divided by …)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>None</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>None</td>
<td>10</td>
<td>0.1</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>0.1</td>
<td>1 mL ISDS sample into 9 mL of PBS</td>
<td>1</td>
<td>(10^{-3})</td>
</tr>
<tr>
<td>0.01</td>
<td>1 mL (10^3) sample into 9 mL of PBS</td>
<td>1</td>
<td>(10^{-4})</td>
</tr>
<tr>
<td>0.001</td>
<td>1 mL (10^4) sample into 9 mL of PBS</td>
<td>1</td>
<td>(10^{-5})</td>
</tr>
<tr>
<td>0.0001</td>
<td>1 mL (10^5) sample into 9 mL of PBS</td>
<td>1</td>
<td>(10^{-6})</td>
</tr>
</tbody>
</table>

The convention is to report a sample with 23 colonies from a \(10^{-4}\) plate as \(2.3 \times 10^5\) colonies/100 mL. Run multiple dilutions bracketing the dilution anticipated to produce the best result (20 - 80 colonies per plate).

7.0 REFERENCES


Methods referenced: Microbiological Examination (9000), Recreational Waters (9213-D) and Membrane Filtration Technique for Members of the Coliform Group (9222 A.)
8.0 DOCUMENTATION

Example Project Data Sheet

Sample Date: ____________

<table>
<thead>
<tr>
<th>Community: Block Island</th>
<th>36</th>
<th>Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyst – set-up:</td>
<td>69</td>
<td>Plates</td>
</tr>
<tr>
<td>Analyst – counts:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubator temp. start:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubator temp. end:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waterbath temp. start:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waterbath temp. end:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>Setup Date</th>
<th>Dilution</th>
<th>Vol. Filtered</th>
<th>(Yields)</th>
<th>24 Hr. Count</th>
<th>Count per 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BI - 3  Rea</td>
<td>STE – 1</td>
<td></td>
<td>$10^2$</td>
<td>1</td>
<td>$10^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3  Rea</td>
<td>STE – 1</td>
<td></td>
<td>$10^3$</td>
<td>1</td>
<td>$10^4$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3  Rea</td>
<td>STE – 1</td>
<td></td>
<td>$10^4$</td>
<td>1</td>
<td>$10^5$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3  Rea</td>
<td>RTE</td>
<td></td>
<td>$10^2$</td>
<td>1</td>
<td>$10^3$</td>
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<td>RTE</td>
<td></td>
<td>$10^3$</td>
<td>1</td>
<td>$10^4$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3  Rea</td>
<td>AXE</td>
<td></td>
<td>$10^2$</td>
<td>1</td>
<td>$10^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3  Rea</td>
<td>AXE</td>
<td></td>
<td>$10^1$</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3  Rea</td>
<td>AXE</td>
<td></td>
<td>$10^2$</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3  Rea</td>
<td>AXE</td>
<td></td>
<td>$10^3$</td>
<td>1</td>
<td></td>
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<tr>
<td>Lab Positive</td>
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<tr>
<td>Lab Positive</td>
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<tr>
<td>Lab Positive</td>
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<tr>
<td>Start Blank</td>
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<td></td>
<td></td>
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<td>1</td>
<td></td>
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</tr>
<tr>
<td>End Blank</td>
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<td></td>
<td></td>
<td>100</td>
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</tbody>
</table>
Understanding the URIWW ISDS Bacterial Data Sheet

Site: Project ID – site #, then homeowner ID
Location: The ID of the component of the septic system from which the sample was collected.
Setup Date: The date on which the sample was filtered and placed on the media plate. This SHOULD be the same date as the sample date (the usual hold time for samples < 6 hours). ISDS samples degrade much more rapidly than do ambient waters, so every effort must be made to setup samples within 6 hours of collection. In the rare event that it is not the same as the sample date, the sample date should be written in parenthesis next to the location identification.
Dilution: The concentration of sample filtered, reported in milliliters. A 1 indicates that this sample was drawn directly from the sample bottle. Anything else indicates that a dilution tube was used.
Vol. Filtered: The volume of sample filtered for that plate. From dilution tubes, this will generally be 1 mL. For dilutions of 1, this could vary from 1 mL all the way up to 100 mL.
(Yields): This is the “final” dilution value used in the total coliform value calculation. This value is referred to as the “calculated result” in the Dilution Notes table.
24 Hr Count: The number of yellow and yellow-brown colonies counted on that filter after a 24 hour total incubation period. This number corresponds to the number of fecal coliform bacteria for the given volume of water.
Count per 100: The number of fecal coliform bacteria per 100 mL (the standard method of reporting fecal coliform bacteria). This is determined by dividing the 24 Hr Count by the Yields column.
This form is intended to be used to maintain a quarterly record of the intensity of the germicidal UV labs in the sterilizing box in room 19. **The intensity of EACH of the SIX tubes must be measured on a quarterly basis, with each tube measured four inches from each end, as well as in the center, with the average of those three readings used.** Once this sheet is full it should be replaced with a new blank sheet. The full sheet should be returned to the URIWW Micro Laboratory Binder in Room 019; blank sheets are also located in this binder. Any maintenance activity should also be recorded on this sheet. See Elizabeth with questions.
**Bacterial Sample Log & Worksheet: Bacteria Bottle Sterilization Confirmation**

### mTEC Membrane Filtration Method

**Analyst - set-up:**

- Incubator temp. start:
- incubator temp. end:

**Analyst - Counts:**

- Waterbath temp. start:
- Waterbath temp. end:

<table>
<thead>
<tr>
<th>Monitoring Location</th>
<th>Setup Date</th>
<th>Dil. (mls)</th>
<th>24 Hr Count</th>
<th>Total fecal (per 100ml)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch #4</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch #5</td>
<td>100</td>
<td></td>
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</tbody>
</table>

### Enterolert IDEXX Method

**Analyst - set-up:**

- Media Batch #:  
- Incubator temp. start:  
- Incubator temp. end:  
- Expiration:  

**Notes:** "-" = Quanti-tray used, no small wells  
Final count determined by using the correct table  
(51 well Quanti-Tray lg wells only, Quanti-Tray / 2000 with small wells)

<table>
<thead>
<tr>
<th>Enterococci</th>
<th>Setup Date</th>
<th>Dil. (mls)</th>
<th># lg pos. wells</th>
<th># sm pos. wells</th>
<th>Table Value</th>
</tr>
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<tbody>
<tr>
<td>Batch #4</td>
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</tr>
<tr>
<td>Batch #5</td>
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<tr>
<td>IDEXX Blank</td>
<td>100</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>URIWW Sterilized</td>
<td>100</td>
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</tr>
</tbody>
</table>
### URI Watershed Watch Laboratory

**Buffered Dilution/rinse Water KH₂PO₄ reagent pH Record**

<table>
<thead>
<tr>
<th>Date</th>
<th>Tech's Initials</th>
<th>Meter Letter (A or B)</th>
<th>Calibration²</th>
<th>Initial pH</th>
<th>Volume 1N NaOH added (mL)</th>
<th>Final pH</th>
<th>Comments / Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

This form is intended to be used to maintain a record of pH and adjustment for the phosphate reagent for the fecal coliform dilution/rinse water. When new reagent is made up, the initial pH, amount of NaOH added, and final pH should be recorded on this sheet. Once this sheet is full it should be replaced with a new blank sheet. The full sheet should be returned to the URIWW Micro Laboratory Binder in Room 019; blank sheets are also located in this binder. Check the Laboratory QAPP, SOP 010 - Alkalinity and pH procedures, for details regarding the pH and alkalinity procedures.

Calibration and Maintenance Information:

1. Each time the pH probe is used the filling solution level in the probe should be checked. The level should be within 1/4 inch of the top of the electrode. If it isn't, add enough filling solution to bring it up to that point.

2. The meter should have a slope (Electrode Efficiency) of at least 96% after calibration. If the meter doesn't initially calibrate correctly, change the probe filling solution and replace the pH buffers, then recalibrate the probe. If this doesn't solve the problem replace the probe. See Linda or Elizabeth with questions.
Standard Operating Procedure 009
(Prior number URIWW-SOP-009)
Total Suspended Solids Analysis
University of Rhode Island Watershed Watch

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1.0 PURPOSE AND DESCRIPTION

The Total Suspended Solids (TSS) Assay allows the determination of the amount of solids contained in an aqueous sample. The final value is expressed as the mass of solids per volume of sample. This method is applicable to Individual Sewage Disposal Systems (ISDS) and ambient water (ponds, lakes and rivers) samples. This method is applicable to undiluted samples in the range of <1 to 500 mg/L TSS and samples diluted to return values in this range.

Samples are analyzed by filtering a known volume of sample through a filter of known weight. The filter and the aluminum weigh dish that the filter rests in are weighed prior to filtering. After filtration, the filter and weigh dish are dried and weighed again. The difference between the two weights is reported as the TSS value.

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

The TSS assay does not utilize any hazardous chemicals. General laboratory good housekeeping procedures should be practiced as outlined in Standard Operating Procedure (SOP) 001 - General Laboratory Safety. Samples obtained from septic systems are sometimes analyzed. These samples should be treated as a potential biological hazard. Eye protection and gloves should be worn and all equipment disinfected after use.

2.2 Technician training/qualifications

General training in laboratory technique, use of an analytical balance and drying oven should be completed prior to analyzing samples using this method.

3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Description</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drying oven</td>
<td>Thelco Laboratory Oven Model 6542</td>
<td>Property of URI Natural Resources Science Dept.</td>
</tr>
<tr>
<td>Balance</td>
<td>Mettler Toledo AB 104 Balance Capable of weighing to 0.5 mg</td>
<td>Property of URI Natural Resources Science Dept.</td>
</tr>
<tr>
<td>Electronic desiccator</td>
<td>Desiccator contains several shelves and an automatic electronic dryer to maintain 30-40% relative humidity.</td>
<td>Fisher Scientific #08-647-30, list price $625.</td>
</tr>
</tbody>
</table>
### Required Material

<table>
<thead>
<tr>
<th>Material</th>
<th>Description</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC filter manifold</td>
<td>Homemade apparatus. Six place manifold with individual stopcocks, attached to a vacuum apparatus connected to a water faucet.</td>
<td>Comparable apparatus commercially available: Fisher Scientific #09-753-39B $950.00</td>
</tr>
<tr>
<td>6 Magnetic filter funnels</td>
<td>47 mm diameter filter funnels, 300 mL capacity, 50 mL gradations Gelman #4242 Plastic elasticized food covers are used to cover filter holders when they are not in use.</td>
<td>Fisher Scientific #09-735 $130/funnel</td>
</tr>
<tr>
<td>Sample bottles</td>
<td>500 mL HDPE wide mouth bottle</td>
<td>Fisher Scientific #02-893-5E pack of 12/$35.00</td>
</tr>
<tr>
<td>Filters</td>
<td>47 mm diameter filters</td>
<td>Millipore AP40 GFF for TCLP #AP4002500</td>
</tr>
<tr>
<td>Aluminum weigh dishes</td>
<td>Dishes are reused</td>
<td>Fisher Scientific # 08-732 Pack of 144/$20</td>
</tr>
<tr>
<td>Aluminum featherweight forceps</td>
<td></td>
<td>Bioquip Products Item #4750 Approximately $4.00 each.</td>
</tr>
<tr>
<td>Aluminum baking trays (approx. 10”X16”)</td>
<td>Capable of holding 4 rows of 6 aluminum weigh dishes</td>
<td>Cookie sheets purchased at local food store</td>
</tr>
<tr>
<td>Squeeze bottle filled with deionized (DI) water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500-1000 mL beaker for DI water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equipment Manufacturers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fisher Scientific Co. 3970 John's Creek Court, Suite 500, Suwanee, GA 30024, phone 1-800-766-7000</td>
<td>Bioquip Products 17803 LaSalle Ave Gardena CA 90248 phone 310-324-0620</td>
<td></td>
</tr>
</tbody>
</table>

Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory. Temporary replacement of equipment may be obtained through arrangement with other scientists in the Natural Resources Science department maintaining similar equipment.

### 4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>500 mL HDPE Bottle</td>
<td>Kept at 4 °C</td>
<td>500 mL</td>
<td>1 week</td>
</tr>
</tbody>
</table>
Disposal

Samples are archived for approximately 1 month. Aqueous samples and filters may only be disposed of after final quality assurance checks are completed and the data found to be acceptable using criteria found in Section 5.2 of this SOP. Aqueous samples are not considered hazardous and may be disposed of by rinsing down the drain. Bottles are cleaned per SOP 003 - General Labware Cleaning Procedure.

Aluminum weighting dishes are saved for re-use and do not have to be washed. Filters may be placed into the garbage.

5.0 METHOD DESCRIPTION

5.1 Scheduling Considerations

Preparations for the analysis of TSS samples should begin the day before analysis is scheduled. Refer to Section 5.3.

5.2 Quality Assurance/Quality Control

5.2.1 Method Detection Limit & Reporting Limit

The Method Detection Limit (MDL) and Reporting Limit (RL) for the TSS analysis are set at 1 mg/L. Data are reported to the nearest whole number.

5.2.2 Method Blanks

The method blank is determined by filtering 200 mL of DI water through a pre-weighed filter and then treating the method blank as a field sample. There shall be one method blank per 24 samples or approximately 4% of the samples analyzed. The method blank shall be less than or equal to 1 mg/L.

Corrective Action

If the method blank is greater than 1 mg/L the method blank is placed back into the desiccator for 5-10 minutes and re-weighed. If the value is still greater than 1 mg/L the deviation is noted on the data sheet.

5.2.3 Sample Replication

Sample replicates are completed by filtering two aliquots of the same sample and processing the resulting filters as two separate samples. Sample replication is completed on 100% of field samples.

Analysis results for replicate samples should be within 30% relative percent deviation (%RPD). %RPD is calculated as follows:

%RPD = \left| \frac{\text{Value of Replicate 1 (mg/L)} - \text{Value of Replicate 2 (mg/L)}}{\text{Average of Result of Replicate 1 (mg/L) and Result of Replicate 2 (mg/L)}} \right| \times 100
Corrective Action
If the %RPD is greater than 30% then the samples are re-analyzed by placing the filter into the desiccator for 5-10 minutes and re-weighing the sample. If the samples are still not within 30% the deviation is noted on the data sheet.

5.2.4 Calibration

Balance calibration is checked using a standard 1 g weight prior to each use. Values returned by the balance should be within 10 percent difference (%D) of the actual calibration value.

Percent difference is calculated as follows:

\[ \%D = \frac{\text{Reported value}}{\text{Known value}} \times 100 \]

Corrective Action
If a deviation of greater than 10%\(\%\)D is noted then the balance will be serviced and re-calibrated. If the balance is found to be outside of the acceptable calibration range then it will not be used in this procedure.

5.2.5 Laboratory Control Standard

Environmental Protection Agency Water Pollution Proficiency Test Study
The laboratory participates in the Environmental Protection Agency Water Pollution Proficiency Test Study yearly. Unknown samples are purchased and analyzed for TSS. The results of the analysis are compared to the actual value and a performance evaluation provided by the vendor. Study results are maintained by the laboratory.

5.2.6 Drying Oven

The temperature of the drying oven is checked daily, when in use. Drying oven temperatures are recorded on the temperature recording data sheet attached to the front of the drying oven. Initial and final temperatures for drying of TSS samples are recorded on the project data sheet. Drying oven temperature must be between 103 and 105 °C.

Corrective Action
The drying oven temperature must remain in the range specified above. If the drying oven temperature is found to be outside the acceptable range, contact Linda Green and adjust the drying oven temperature control. Professional maintenance of the drying oven may be necessary if adjustment of the temperature control does not rectify the problem.

5.3 Analysis Method

5.3.1 Preparation – Filter Preparation - Day Before Samples Arrive or Earlier

1. Print data sheets.
2. Determine the number of filters and aluminum weigh dishes needed. Each sample will be analyzed twice (replicated). One method blank will be analyzed per 24 samples. It is a good idea to have a few extra filters in case of clogging.
3. Aluminum weigh dishes, pans, and desiccator are located in room 018.

4. Place labeled aluminum weigh dishes in sequential order on an aluminum pan. Each pan will hold 24 dishes. Note that the weigh dishes are re-used.

5. Set up the filter manifold in the URIWW laboratory.

6. Move the cart with the filtering manifold in front of the fume hood and attach the outlet hose to the large white vacuum flask and the flask to the vacuum port in fume hood. Be sure that the base of the magnetic filter funnels are well seated on the filtering manifold.

7. Filters are kept in the TSS drawer to the left of the fume hood.

8. Center a filter on the black grid of the magnetic funnel base, gridded side down. Set the top of filter funnel onto the base. The magnets hold the 2 parts together. Set up remaining filters on funnel bases. Six (6) filters can be processed at a time.

9. Close the stopcocks on the manifold under each filter apparatus.

10. Turn on the vacuum port in the fume hood by turning the handle to the marked position. Turn on vacuum to line marked on dial.

11. Pour approximately 50 mL of DI water into each funnel, open the stopcock and allow the water to drain. Repeat this process 2 more times to rinse the filters.

12. Allow vacuum to dry filters.

13. Use forceps (tweezers) to remove each filter from the manifold, closing the stopcock as the filter is removed to shut off the vacuum. Each filter should be placed into a separate aluminum weigh dish.

14. Place aluminum weigh dishes on aluminum pans in sequential order.

15. Place in pre-heated 105 °C oven for at least 1 hour. It is acceptable to leave filters in the drying oven for more than 1 hour. The temperature of the drying oven should range from 105 +/- 5 °C. If the oven is not able to maintain temperature within this range then contact Linda Green or Elizabeth Herron to obtain professional maintenance service of the oven.

16. After filters have been dried store them in the electronic desiccator.

5.3.2 Procedure – Collection Day

5.3.2.1 Preparation of Samples for Filtering

1. Samples are usually collected in 500 mL HDPE wide mouth bottles. They should be kept on ice until delivery to the lab. If possible, do not analyze samples on humid or rainy days because the filters absorb moisture rapidly from the air after removal from the desiccator.

2. Bring samples to room temperature before analysis.

3. Check the calibration of the analytical balance in room 018 using the 1 g weight.
   a. The calibration weights are located in the drawer under the balance. Never touch the calibration weights with anything but forceps. The oils on the fingers may change the weight of the standard.
b. Values returned by the balance should be within 10% of the actual calibration weight value. If a deviation of greater than 10% is noted then the balance will be serviced and re-calibrated. If the balance is found to be outside of the acceptable calibration range then it will not be used in this procedure.

4. Remove aluminum weight dishes holding the clean dried filters from the desiccator immediately prior to weighing.

5. Weigh a sufficient number of previously clean dried filters in their weight dishes to 0.5 mg. Always handle the filters and weight dishes with forceps. Never use fingers.

6. Record the sample number and weight on the data sheet as “initial weight”.
   
a. Remember to have enough dried filters for at least two replicates per sample and one “blank” per 24 samples.

b. It is a good idea to have a few extra filters in case of clogging.

5.3.2.2 Filtering Samples

1. Return to room 002; place a weighed filter grid side down on the bottom of the filter funnel. Set the top half of the filter funnel onto the bottom half. Keep the aluminum weigh dish associated with the filter in front of the appropriate filter funnel.
   
a. Only touch the weigh dishes and filters with forceps.

b. Set-up 6 filters in this manner

2. Vigorously shake the sample bottle a minimum of 10 seconds, uncap and then immediately pour an aliquot of the sample into the magnetic filter funnel. Use the volume markings on the filter funnel to measure sample volume.
   
a. The table below provides information regarding the appropriate volume of ISDS sample to filter as do the project specific data sheets.

b. Use a sample volume of 200 mL for Watershed Watch samples.

c. If the sample volume is less than 50 mL, use a graduated cylinder to measure out the appropriate amount of sample and then pour the sample into the filter funnel.

c. If the sample volume is less than 25 mL, pour approximately 25 mL of DI water into the filter funnel prior to adding the sample. After adding the sample, rinse the graduated cylinder into the filter funnel.

Sample Filter Volume Guide for ISDS Samples

<table>
<thead>
<tr>
<th>ISDS Sample ID</th>
<th>Volume to Filter (mL)</th>
<th>Sample ID</th>
<th>Volume to Filter (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STE-R</td>
<td>10-100</td>
<td>RSFE</td>
<td>200 mL</td>
</tr>
<tr>
<td>RTFE</td>
<td>200</td>
<td>RTE</td>
<td>100 mL</td>
</tr>
<tr>
<td>SFE</td>
<td>200</td>
<td>BFE</td>
<td>150-200 mL</td>
</tr>
<tr>
<td>STE-S</td>
<td>25-100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: DO NOT BE TEMPTED TO USE > 100mL FOR STE-R! If STE-R is thick and chunky use less sample.

3. Turn on the vacuum and open the filter funnel stopcock to filter the sample.
a. If a sample clogs a filter, remove the filter unit from the filter manifold, discard the filter, rinse the funnel and repeat the filtration procedure with a smaller sample volume.

4. Record sample ID and volume filtered on the data sheet.
   a. Be sure that the weigh dish number matches the number on data sheet and the sample ID is correlated with the weigh dish number on the data sheet.

5. When the sample has filtered through, rinse the sidewalls of the filter funnel 2-3 times with DI water from a squeeze bottle.

6. Continue to allow a vacuum on the sample until the filter is dry.

7. When filtering is complete remove the top half of the filter funnel and close the stopcock.

8. Use forceps to carefully remove the filter from the filter holder. Place the filter back into its aluminum weigh dish.

9. Discard any obvious large pieces of debris, such as grass clippings, worms, etc.

10. Check to make sure the sample ID and weight dish number are recorded correctly!

11. Repeat filtering procedure with replicate sample and remaining water samples.

5.3.2.3 Drying and Weighing TSS Samples

1. Dry filters in the aluminum weight dishes on an aluminum pan in a drying oven set at 105 °C for at least 1 hour.

2. Filters can stay overnight in the oven if needed.

3. Once filters are dry, cool them to room temperature in the electronic desiccator (approximately 5-10 minutes).

4. Check the calibration of the balance using the procedure outlined in Section 5.3.2.1

5. Remove samples from desiccator and weigh filters and weigh dishes at once.

6. Record weight in “final weight 1” column on data sheet.

7. Place filters back on the aluminum pan and place them back into the drying oven for at least 1 hour. Repeat the weighing procedure, recording the weight in the “final weight 2” column on the data sheet.
   a. The difference between “final weight 1” and “final weight 2” must be less than or equal to 0.5 mg.
   b. If the difference is greater than 0.5 mg, place the appropriate filters back into the drying oven and complete drying/weighing cycle over. If the difference between “final weight 2” and “final weight 3” is still greater than 0.5 mg complete the cycle again and keep doing it until the difference between the two most recent filter weights is less than 0.5 mg.

5.3.2.4 Cleanup

1. Once all filtering is completed, thoroughly rinse all materials including the filter manifold with clean water.
2. If septic samples were filtered, spray the manifold and counter with disinfectant.

3. Separate the halves of the filter funnels. Wash them in soapy water using a brush to clean all surfaces, including the filter grid. Rinse with tap and then DI water, invert to dry, separated.

6.0 CALCULATIONS

6.1 Calculation if Measured Weights are Recorded in Grams

\[
\text{TSS in mg/l} = \frac{(A - B) \times 1,000,000}{\text{mL sample}}
\]

\[A = \text{final weight (g)} = \text{weigh dish + filter containing dried residue (g)}\]
\[B = \text{initial weight (g)} = \text{clean filter + weigh dish (g)}\]

Final data are reported to the nearest whole number. Values less than 1 mg/L TSS are reported as <1 mg/L TSS.

6.2 Calculation if Measured Weights are Recorded in Milligrams

\[
\text{TSS in mg/l} = \frac{(A - B) \times 1000}{\text{mL sample}}
\]

\[A = \text{final weight (mg)} = \text{weigh dish + filter containing dried residue (mg)}\]
\[B = \text{initial weight (mg)} = \text{clean filter + weigh dish (mg)}\]

Final data are reported to the nearest whole number. Values less than 1 mg/L TSS are reported as <1 mg/L TSS.

7.0 REFERENCES


Method referenced: Total Suspended Solids Dried at 103-105 °C (2540 D).

Personal discussions with Skip Viator, URI Department of Civil and Environmental Engineering.
### 8.0 DOCUMENTATION

Example Data Sheet

**TOTAL SUSPENDED SOLIDS ANALYSIS**

<table>
<thead>
<tr>
<th>Initial Weight Date:</th>
<th>Final Weight 1 Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Weight Analyst:</td>
<td>Final Weight 1 Analyst:</td>
</tr>
<tr>
<td>Oven Temp. Start:</td>
<td>Final Weight 2 Date:</td>
</tr>
<tr>
<td>Oven Temp. End Weight 1:</td>
<td>Final Weight 2 Analyst:</td>
</tr>
<tr>
<td>Oven Temp. End Weight 2:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Location</th>
<th>Sample Date</th>
<th>Volume Filtered (ml)</th>
<th>Initial Weight</th>
<th>Final Weight 1</th>
<th>Final Weight 2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Blank</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Difference between final weight 1 and final weight 2 must not be more than 0.5 mg. Otherwise, re-dry filter again.
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1.0 PURPOSE AND DESCRIPTION

The purpose of this method is to determine the pH and/or alkalinity of both Individual Sewage Disposal System (ISDS) samples as well as ambient water samples (lakes, ponds, streams). The pH of marine samples may also be determined using this method. This method is applicable to samples in the range of 1 to 14 standard units (SU) and <0.1 – 1000 mg CaCO₃.

The pH (hydrogen ion activity) of a sample is a measurement of the intensity of the acidic or basic character of a solution. Alkalinity is the acid-neutralizing capacity of a water sample. The pH of a sample is determined using a pH meter. This instrument consists of a potentiometer, a combination standard glass electrode and reference electrode and a temperature-compensating device. A circuit is completed through the potentiometer when the electrodes are placed in an aqueous sample. The strength of the electromotive force (emf) produced through the circuit is proportional to the pH of the sample.

Alkalinity of a sample is determined by titrating a sample to a specific pH end-point value. The low-alkalinity method is used in this Standard Operating Procedure (SOP).

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

General laboratory good housekeeping procedures should be practiced as outlined in SOP 001 - General Laboratory Safety. Acids (sulfuric acid) and bases (sodium hydroxide) are utilized as part of the procedure outlined in this SOP. Both acids and bases may burn exposed skin and eyes on contact. Sodium carbonate is also utilized in this SOP, this material may cause eye burns on contact and is a skin irritant. Always wear protective clothing in the form of gloves, a lab coat and goggles when working with these chemicals. Further information regarding these chemicals is located in the laboratory MSDS binder found in the laboratory where the chemical of interest is stored. General laboratory information regarding safe handling of chemicals is located in the University Safety and Waste Handling Document – SOP 001a.

Samples obtained from ISDS sites should be treated as a potential biological hazard. All personnel handling ISDS samples should wear a lab coat, gloves and goggles.

2.2 Technician Training/Qualifications

General training in laboratory technique and use of a pH meter must be completed prior to analyzing samples using this method. Technician training will be provided by Linda Green, Laboratory Project Manager – Nutrients or Elizabeth Herron Project Manager – Microbiology.
3.0 REQUIRED MATERIALS

Equipment and Supplies

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fisher Model AR 20 laboratory pH meter</td>
<td>Capable of reading to 0.01 unit with automatic temperature compensation electrode</td>
<td></td>
</tr>
<tr>
<td>Combination pH electrode</td>
<td>Recommended by Fisher Tech Support</td>
<td>Fisher #13-620-185</td>
</tr>
<tr>
<td>Saturated potassium chloride (KCl) solution</td>
<td>This solution is used as the electrode filling solution</td>
<td>Fisher #SP138-500.</td>
</tr>
<tr>
<td>Fisher certified color-coded pH buffers - pH 4.7 &amp; 10</td>
<td>Note the expiration data before using the buffers</td>
<td>pH 7 (yellow) #SB107-500 pH 4.0 (pink) #SB101-500 pH 10 (blue) #SB115-500.</td>
</tr>
<tr>
<td>Magnetic stirrer and magnetic stir bars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mL micro-buret, 0.02 mL subdivisions</td>
<td>NIST traceable, with 3-way plug</td>
<td>Fisher # 17115F-10.</td>
</tr>
<tr>
<td>2 L of 0.02 N H₂SO₄ (Sulfuric Acid)</td>
<td>Preparation instructions: Section 5.4.1.1</td>
<td></td>
</tr>
<tr>
<td>Tubing and stoppers to connect the bottle containing the acid titrant to the buret.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250 mL graduated cylinders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250 mL beakers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Spare Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) laboratory and the Natural Resources Science department.

4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

4.1 pH Only

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient Water</td>
<td>250-500 mL white HDPE or brown glass bottle</td>
<td>Kept at 4 °C</td>
<td>Measured directly in sample bottle</td>
<td>Analyze immediately</td>
</tr>
<tr>
<td>ISDS Sample</td>
<td>125 white HDPE bottle</td>
<td>Kept at 4 °C</td>
<td>Measured directly in sample bottle</td>
<td>Analyze immediately</td>
</tr>
</tbody>
</table>
4.2 pH and Alkalinity

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient Water</td>
<td>500 mL white HDPE bottle</td>
<td>Kept at 4 °C</td>
<td>200 mL</td>
<td>24 Hours</td>
</tr>
<tr>
<td>ISDS Sample</td>
<td>125 mL white HDPE bottle</td>
<td>Kept at 4 °C</td>
<td>35-40 mL</td>
<td>24 Hours</td>
</tr>
</tbody>
</table>

Disposal

Samples are archived for approximately 48 hours. Aqueous samples may only be disposed of after final quality assurance checks are completed and the data found to be acceptable using criteria found in Section 5.2 of this SOP. Aqueous samples associated with ISDS projects are considered a potential biological hazard. Gloves, goggles and a laboratory coat should be worn when disposing of ISDS samples. Both ISDS and ambient samples may be rinsed down the drain with running water.

Bottles are cleaned in accordance with SOP 003 – General Labware Cleaning Procedure.

5.0 METHOD DESCRIPTION

5.1 Scheduling Considerations

A week before alkalinity samples are expected to arrive at the laboratory the amount of titrant (0.02 N H₂SO₄ (sulfuric acid)) available should be checked. Approximately 5 mL of titrant are used per sample. Preparation of titrant is discussed in Section 5.4.1.1.

A check of the status of the pH electrode must be performed at least 48 hours before anticipated sample arrival. The preparation of a new electrode will take at least 24 hours. Data sheets should be printed as well.

5.2 Quality Assurance/Quality Control

5.2.1 Method Detection Limit

The method detection limit (MDL) for the pH analysis is controlled by the pH meter. The meter utilized in this SOP reports pH values to 0.01 SU. The MDL for determination of pH is 1.00 SU. The upper limit of detection is 14.00 SU. The reporting limit (RL) for the pH assay is 1.0 SU and the upper RL is 14.0 SU. Data are reported to 1 decimal place.

The MDL for the alkalinity analysis is controlled by the pH meter as well as the titration method. The MDL is 0 mg/L. Alkalinity of a sample may be reported as 0 mg/L when the initial pH of the sample is at or below the titration endpoint of 4.5 SU. The RL is set at 0.1 mg/L. Data are reported to 1 decimal place.

5.2.2 Method Blanks

Method blanks are not applicable to this method.
5.2.3 Sample Replication

Replicates are completed on at least 10% of samples (1 sample in 10) for both alkalinity and pH assays. The difference between the replicates must be not greater than +/-0.5 S.U. for the pH assay and 25% RPD (relative percent difference) for the alkalinity assay.

%RPD is calculated as follows:

\[
\text{%RPD} = \left| \frac{\text{Result of Replicate 1 (µg/L)} - \text{Result of Replicate 2 (µg/L)}}{\text{Average of Result of Replicate 1 (µg/L) and Result of Replicate 2 (µg/L)}} \right| \times 100
\]

Corrective Action

If the %RPD is greater than 25% for the alkalinity analysis or +/- 0.5 S.U. for the pH assay, then the sample is analyzed again. If the samples are still not within the acceptable range the deviation is noted on the project data sheet. If several replicate pairs are not within the acceptable range then the pH meter calibration should be rechecked and the meter recalibrated if necessary. If the recalibration of the pH meter does not solve errors associated with the alkalinity assay it may be necessary to recheck the titrant.

5.2.4 Calibration and Standards

5.2.4.1 Laboratory Standards

The laboratory participates annually in the Environmental Protection Agency Water Pollution Proficiency Test Study. Unknown samples are purchased and analyzed for pH and alkalinity. The results of the analysis are compared to the actual value and a performance evaluation provided. Study results are maintained by the laboratory.

5.2.4.2 Daily Calibration

The two pH meters utilized in the URIWW laboratory are Fisher Scientific Accumet Research AR20 models. Both meters use AccupHast rugged bulb combination pH and automatic temperature compensation electrodes. According to Fisher Technical Services, the average life of an electrode is 18 - 24 months. If the electrode cannot be calibrated or can be initially calibrated but doesn’t hold calibration, it must be replaced. The serial number on the electrode provides Fisher with the date of manufacture/sale of the electrode. A piece of tape on each electrode contains the electrode installation date.

Calibration of the pH meters is competed each day prior to use. The meters are always left plugged in and in standby mode.

The instruction manuals for the pH meter and electrodes are kept in a drawer below the pH meter.

1. Check the internal filling solution in the electrode to be sure it is within ¼ inch of the top of the electrode. If the solution is below this level, add the saturated potassium chloride filling solution until the solution is at the appropriate level. Leave fill hole open during electrode use.

2. A two point calibration with pH 4 and pH 7 buffers is completed. The buffers are color coded (pH 7 = yellow, pH 4 = pink) and are purchased from Fisher Scientific. Buffers
are stored in bulk in the cabinet above and to the right of the pH meters. Put a small amount of each buffer into a small NON-ACID WASHED beaker and add a magnetic stirrer.

a. Fresh buffer is used for each calibration. DO NOT REUSE BUFFERS, once the pH and/or alkalinity run is completed, dispose of the buffers by rinse them down the sink with plenty of water.

b. Check the expiration date of the buffers before using them. If the expiration date has passed or there is any precipitate in the bottle, DO NOT USE THEM. Dispose of the buffer by rinsing it down the sink and use a newer bottle of buffer.

3. Remove the pH electrode from the storage buffer and rinse with DI water. Rinse water should be collected in a waste container and disposed of by rinsing down the drain.

4. Place the electrode into the pH 4 calibration buffer. The electrode should be immersed approximately 1 inch into the buffer, but must be immersed to at least the liquid junction or white ceramic dot on the side of the glass body of the electrode just above the glass bulb.

5. Turn on the magnetic stirrer. The stirring rod should be rotating rapidly enough to form a smooth current, not hopping around like a jumping bean. This generally means starting the magnetic stirrer at a very low setting and gradually adjusting the speed upward as needed.

6. If the meter is in standby mode (i.e. the screen is dark) touch the screen to activate. Touch it again for the menu screen. Select pH.

7. Touch STD on the pH measure screen to access the standardize screen.

8. Touch clear to delete previous calibrations. If the screen says "Not Standardized" it is already clear and you are ready for the next step.

9. Touch STD again to calibrate the meter with the pH 4 buffer. The word Measuring will flash on the screen until the signal is stable. Once it is, the word Stable will appear, along with a beaker icon and the pH buffer entered value (4.02).

10. Turn off the magnetic stirrer, lift the electrode out of the buffer and rinse the electrode with DI water. Place the 7.00 buffer on the stirrer and set the electrode into the buffer, repeat step 9.

11. Once standardization with the pH 4 and 7 buffers is complete, record the Slope value from the box in the lower part of the pH meter screen, right hand column. This is the Electrode Efficiency and the slope should be 96% or greater. Record this value on the project data sheet.

Corrective Action
If the electrode efficiency is not 96% or greater check the electrode, replace buffers and recalibrate. If the electrode efficiency is still not 96% or greater after re-calibration, prepare a new electrode for use following instructions found under Section 5.2.4.3 or Section 5.3.2.1.

5.2.4.3 New Electrode Preparation

Follow the instructions that come with the electrode. If a previously used and stored electrode is available it can be reactivated using instructions found in Section 5.3.2.1.
5.2.5 Calibration Check/Laboratory Control Standard

A check of the calibration of the pH probe is accomplished at the beginning and end of each pH or alkalinity run by placing the pH probe in the pH 7 buffer. The resulting observation is recorded on the data sheet. The pH meter should read 7.00 +/- 0.2 S.U.

Corrective Action
If the 7 buffer when re-checked at the beginning or end of the pH or alkalinity run is not within the range of 7.00 +/- 0.2 S.U. then re-check the value of the pH 7 buffer again. If the pH 7 buffer is still not within 7.00 +/- 0.2 S.U. then recalibrate the instrument and re-run the affected samples.

5.3 Analysis

5.3.1 Daily Electrode Maintenance

Before use each day:

1. Check to see that the internal filling solution is about ¼ inch below the top of the electrode and that filling hole is OPEN.
2. Re-fill as needed with saturated KCl solution.

5.3.2 Electrode Storage

If it is anticipated that the electrode will be used daily or weekly store the electrode as follows:

1. Store the electrode in pH 4 buffer with the filling hole open.
2. Place the electrode in a 100 mL beaker filled approximately half way with pH 4 buffer. Drape a piece of parafilm around the top of beaker and electrode to slow evaporation of the buffer.
3. On a weekly basis, replace evaporated buffer with water. Replace the storage buffer monthly, if it becomes cloudy or has a precipitate. Do not use this buffer solution for pH calibration.

5.3.2.1 Extended Storage

If it is anticipated that the electrode will NOT BE used daily or weekly store the electrode as follows:

1. Fill the electrode nearly full with filling solution (saturated KCl).
2. Close the filling hole and rinse off the outside of the electrode. Tape a cotton ball over the electrode bulb and store it in its box. (pers. comm. Fisher Tech. Support, 4/98)

Reactivation after Extended Storage
24 hours before the first use of the electrode empty out the KCl filling solution and replace it with new filling solution. Leave the filling hole open and place the electrode in pH 4 buffer.
5.3.3 Procedure – Analysis of pH only

1. Water samples should be allowed to warm to room temperature.
2. Shake the sample bottle to mix well.
3. If the sample will be analyzed for pH only (typically river or estuarine sites), the measurement will take place directly in the sample bottle (brown glass or plastic).
4. Add a magnetic stirring bar to either the sample bottle or beaker. Turn on magnetic stirrer, adjusting to a gentle stirring, not a jumping bean!
5. Immerse the rinsed electrode into the sample to a depth of approximately one inch or at least to cover the white ceramic dot on the side of the glass body just above the glass bulb.
6. Activate the meter and begin measuring pH.
   a. Touch Meas to begin measuring your sample.
   b. The word Stable will appear once the meter recognizes that the measurement is stable and it will beep. Record measurements to 0.01 pH unit on the project data sheet.
7. Raise electrode out of sample and remove the stir bar.
8. Re-cap the sample bottle and place to the side. The remaining sample is utilized for the determination of nutrients.
9. Rinse the electrode with DI water.
10. At the end of the run check the pH meter calibration by immersing the pH probe in the pH 7 buffers and recording the result on the data sheet. Values of the buffer should not change more than +/- 0.2 SU, refer to Section 5.2.5 of this SOP for corrective actions.
11. When all samples have been analyzed put the pH electrode back into the storage buffer. Then touch Mode and then Standby. Do not leave the pH meter (beeping) in the measure mode or the electrode out of solution for an extended period of time.
12. Rinse down the sink any left over calibration buffer.

5.4 Analysis Method pH and Alkalinity

5.4.1 Preparation – 1 Week Before Scheduled Sampling (as needed)

5.4.1.1 Preparation and Standardization of the Alkalinity Titrant

1. Prepared dried anhydrous sodium carbonate (Na$_2$CO$_3$) by placing approximately 2 g of Na$_2$CO$_3$ in the drying oven at 250 °C (480 °F) for 4 hours. After drying, remove the material from the drying oven and place into a desiccator to cool.
   a. Na$_2$CO$_3$ gram formula weight (gfw) = 106 g
   b. Na$_2$CO$_3$ gram-equivalent weight (geqw) = 53 g
2. Prepare a Na$_2$CO$_3$ solution of approx. 0.025 N
   a. Place 1.33 g of Na$_2$CO$_3$ in a 1 L volumetric flask
   b. Dilute with Ultrapure water
   c. \[ N = \frac{\text{weight Na}_2\text{CO}_3 \text{used}}{\text{geqw}} = \frac{1.33 \text{ g}}{53 \text{ g}} = 0.025 \text{ N} \]
   d. Preparation of 500 mL of solution: 0.665 g Na$_2$CO$_3$ to 500 mL Ultrapure water
   e. Preparation of 250 mL of solution: 0.332 g Na$_2$CO$_3$ to 250 mL Ultrapure water
3. Prepare a sulfuric acid (H₂SO₄) solution of approximately 0.02 N
   Dilute 40 mL of 1 N H₂SO₄ to 2 L using Ultrapure water
4. Put the 0.02 N H₂SO₄ solution into the clear glass bottle located on the shelf in room 002, above the pH meters. Place the filled bottle back on the shelf above the pH meters and reattach the buret fill hoses.
5. Drain and fill the 10 mL micro buret several times to ensure that it is filled with the new acid.
6. Using a 5 mL glass pipette, measure 5 mL of sodium carbonate solution into a 50 mL beaker. Add 20 mL of Ultrapure water. Prepare a minimum of 3 beakers.
7. Calibrate the pH electrode as usual (refer to Section 5.2.4.2).
8. Put electrodes into one of the 50 mL beakers. Let the pH equilibrate for only 1-2 minutes.
9. While stirring add 0.02 N sulfuric acid to the beaker.
10. After each 0.5 mL addition, record the amount of acid used and the pH. Do this until the final pH is just above 5.0 SU.
11. Remove the beaker and place aside. Repeat the procedure from step 8 with the remaining beakers. When the procedure has been completed with all the beakers, cover the beakers with watch glasses and use a hot plate to boil the contents of the beakers for 3-5 minutes.
12. Cool the beakers to room temperature using ice bath to hasten the process.
13. Slowly add more acid, 0.1 mL at a time. Record the volume (mL) of acid and resulting pH for each small addition of acid. Continue the addition of acid until the inflection point (maximum pH change per unit acid) has been passed. This step is called the “retitration.” Repeat this procedure for the remaining beakers.
14. Neutralize the solution in each beaker with 1N sodium hydroxide (NaOH) before rinsing down the sink drain, flushing with water for about 1 minute.
15. Calculate the exact normality of the acid using the following equation:
   \[ N = \frac{(A \times B)}{(53.00 \times C)} \]
   where:
   \( A \) = grams sodium carbonate weighed into 1000 mL flask (should be 1.33 g).
   \( B \) = mL sodium carbonate solution used for titration (should be 5 mL).
   \( C \) = mL acid titrated to reach inflection point.
16. Label the clear glass acid bottle with the exact normality of the acid, the date calculated, in which lab notebook the calculations can be found, and your initials. Be sure to save all data.

5.4.2 Procedure - Analysis of pH and Alkalinity

1. Drain off ~20 mL H₂SO₄ through side drain of the alkalinity micro-buret. Empty and fill buret. This rinses the buret and tubing.
2. Zero the acid in the buret by overfilling slightly and draining until the bottom of the meniscus on the buret is at 0.00.
3. Water samples should be allowed to warm to room temperature.
4. Shake the sample bottle to mix well.
5. Use a non-acid washed graduated cylinder to measure the water sample. Pour into a non-acid washed 250 mL beaker. Sample Volumes:
   a. 200 mL for lake samples
   b. 35 - 40 mL for ISDS samples
6. Add a magnetic stirring bar to the beaker. Turn on the magnetic stirrer, adjusting it to a gentle stirring, not a jumping bean!
7. Record the sample volume on the data sheet and the exact normality of the $\text{H}_2\text{SO}_4$ titrant.
8. Immerse the rinsed electrode into the sample to a depth of approximately one inch or at least to the white ceramic dot on the side of the glass body just above the glass bulb.
9. Press pH, then meas to activate the AR 20 meter.
10. Once the meter has stabilized on the pH of the sample record the value as pH.
11. Slowly add acid from the micro-buret to lower the pH to approximately 4.5 (4.3 - 4.7 is acceptable). Pause between additions of acid to allow the meter to stabilize.
   a. When the sample reaches the correct pH, record the volume of acid dispensed and the pH of the sample. Remember to read the buret from the bottom of the meniscus and to record the value to 2 decimal places.
   b. The volume of acid dispensed is “B - volume to pH 4.5” in the alkalinity calculation (Section 6.0 Calculations)
12. Add acid until the sample is at a pH 0.3 units less than that recorded in previous step. Record total volume of acid used. This value is C – “volume to pH 4.2” in the alkalinity calculation.
13. Raise the electrodes out of the sample and rinse the electrode.
14. Re-fill the buret with acid.
15. Rinse the sample down the drain and rinse the beaker with DI water.
16. Repeat the procedure from Step 2 with the next sample.
17. At the end of the run check the pH meter calibration by immersing the pH probe in the pH 7 buffers and recording the result on the data sheet. Values of the buffer should not change more than +/- 0.2 SU, refer to Section 5.2.5 of this SOP for corrective actions.
18. When all samples have been analyzed put the pH electrode back into the storage buffer. Then touch Mode and then Standby. Do not leave the pH meter (beeping) in the measure mode or the electrode out of solution for an extended period of time.
19. Field samples are archived and disposed of in accordance with Section 4.0.
20. Any left over buffer should be rinsed down the drain.

6.0 CALCULATIONS

6.1 pH

The pH of a sample is recorded directly from the pH meter. Values are reported to 1 decimal place by rounding the value obtained from the pH meter. Values less than 1 SU are reported as < 1 SU and values greater than 14 SU are reported as >14 SU.
6.2 Alkalinity

Alkalinity (mg/L) = \( \frac{[(2B - C) \times (N \text{ H}_2\text{SO}_4) \times (50,000)]}{\text{Volume of water sample (mL)}} \)

Where:
B = volume to pH ~4.5
C = volume to pH ~4.2
N = normality of titrant (H\(_2\)SO\(_4\)) (approx. 0.02N)
The exact normality of the H\(_2\)SO\(_4\) is determined through the alkalinity standardization procedure detailed in Section 5.4.1.1.

Final data is reported to 1 decimal place. Values less than 0.1 mg/L CaCO\(_3\) are reported as < 0.1 mg/L CaCO\(_3\).

7.0 REFERENCES


### 8.0 DOCUMENTATION

Data sheet for analysis of pH and alkalinity

**SUPPLEMENTAL COLLECTION - AUGUST 2006**

Please fill-in your initials, and the appropriate value. There should be something in each space.

Remember to note deep sample depth, and pH, alkalinity is not done on deep samples.

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<th>Sample depth (m)</th>
<th>Tech's Initials &amp; date</th>
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<th>Initial pH</th>
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Data sheet for analysis of pH only

**SECOND TRISEASON COLLECTION - JULY 2006**

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<td>X</td>
<td></td>
</tr>
<tr>
<td>Secret - Oak Hill East</td>
<td></td>
<td>0.2</td>
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<td></td>
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</tr>
<tr>
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<td></td>
<td>0.2</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Shunock River @ Babacock</td>
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<td></td>
<td></td>
<td>X</td>
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</tr>
<tr>
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<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S&amp;S @ Keech</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>S&amp;S @ Balcom</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>S&amp;S @ O'Donnel</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Staf Inlet - Downstream</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td>X</td>
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</tr>
<tr>
<td>Stafford - NE Cove</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Watchaug - Perry Healy</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Waterman - Rt 44</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Waterman - Sawmill</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Waterman - Golf Course</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td>X</td>
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</tr>
<tr>
<td>Waterman - Aldrich</td>
<td></td>
<td>0.2</td>
<td></td>
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<td>X</td>
<td></td>
</tr>
<tr>
<td>White Brook Pond Inlet</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Standard Operating Procedure 011
(Prior number URIWW-SOP-6)

Biochemical Oxygen Demand (BOD) Procedure

University of Rhode Island Watershed Watch

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1.0 PURPOSE AND DESCRIPTION

The purpose of this method is to determine the oxygen requirements of Individual Sewage Disposal System (ISDS) or polluted water samples. A sample of water is placed into a specially designed Biochemical Oxygen Demand (BOD) bottle and the dissolved oxygen (DO) concentration is determined. This value is reported as the initial DO value. The bottle is then incubated for five days in the dark at 20 °C. At the end of the incubation period the DO concentration in the bottle is again measured. The difference in DO recorded during the five day period corrected for sample dilution is the BOD. This method is appropriate for undiluted samples ranging from less than 2 to 20 mg/L BOD and samples diluted to return values in this range.

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

ISDS samples should be treated as a potential biological hazard. All personnel handling ISDS samples should wear a laboratory coat, gloves and goggles.

General laboratory good housekeeping procedures should be practiced as outlined in Standard Operating Procedure (SOP) 001 - General Laboratory Safety. The only chemical utilized in this SOP is the Hach BOD nutrient buffer pillow. The chemical inside the pillow may cause irritation to the skin and eyes if exposed. Since the material is in a powder form, inhalation can also cause irritation to the nose, throat and lungs. Always wear protective clothing in the form of gloves, a laboratory coat and goggles when working with this chemical. Further information regarding this chemical may be found in the laboratory Material Safety Data Sheet (MSDS) binder found in the laboratory where the chemical of interest is stored. General laboratory information regarding safe handling of chemicals is located in SOP 001a - University Safety and Waste Handling.

2.2 Technician Training/Qualifications

General training in laboratory technique and the use of the DO meter must be completed prior to analyzing samples using this method. Technician training will be provided by Linda Green, Laboratory Project Manager – Nutrients or Elizabeth Herron Project Manager – Microbiology.
3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubator set at 20 +/- 1 °C</td>
<td>Capable of maintaining +/- 0.02 °C</td>
<td>Fisher Low Temperature Incubator Model 307C Fisher #11-679-25C List price $3400</td>
</tr>
<tr>
<td>DO meter</td>
<td>YSI 5000</td>
<td>Fisher #13-298-21 List price $1300</td>
</tr>
<tr>
<td>BOD DO probe</td>
<td>YSI 5010</td>
<td>Fisher #13-298-23 List price $525</td>
</tr>
<tr>
<td>BOD bottles</td>
<td>Clear 300 mL flared neck with a ground glass stopper</td>
<td>Fisher #09-926 Case of 24 $190</td>
</tr>
<tr>
<td>20 L Carboy</td>
<td>HDPE with spigot in bottom for nutrient buffer water</td>
<td>Fisher #02-963-5C List price $110</td>
</tr>
<tr>
<td>Hach BOD nutrient buffer pillows</td>
<td></td>
<td>Hach Chemical Company #14863-98 25 per box List price $30</td>
</tr>
<tr>
<td>BOD Seed Inoculum</td>
<td></td>
<td>Hach Chemical Company #2471200</td>
</tr>
<tr>
<td>BOD Standard Solution</td>
<td></td>
<td>Hach Chemical Company #1486510</td>
</tr>
<tr>
<td>Aquarium bubbler</td>
<td></td>
<td>Purchased from local pet store - $5</td>
</tr>
<tr>
<td>Deionized (DI) water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pipette filler</td>
<td>Preferably electric</td>
<td>Drummond #4-000-110, Fisher #13-681-15 List price $180</td>
</tr>
<tr>
<td>Glass pipettes</td>
<td>1, 10 and 25 mL</td>
<td></td>
</tr>
<tr>
<td>Graduated cylinders</td>
<td>100 and 250 mL</td>
<td></td>
</tr>
<tr>
<td>Squeeze bottle full of DI water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 beakers</td>
<td>1 for clean water to rinse pipettes between use 1 for dirty rinse water.</td>
<td></td>
</tr>
<tr>
<td>Paper towels, safety goggles, lab gloves</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory and the Natural Resources Science department. Temporary replacement of large equipment such as incubators is available through arrangement with other scientists in the department maintaining similar equipment.
4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (Ambient and ISDS samples)</td>
<td>White 500 mL HDPE</td>
<td>Kept at 4 ºC</td>
<td>500 mL</td>
<td>24 hours max 6 hours ideal</td>
</tr>
</tbody>
</table>

Disposal
Field samples are not archived after sample preparation is complete because the sample holding time is very short and they degrade rapidly once outside the holding time.

Field samples may be disposed of by rinsing down the drain with running water. ISDS samples are considered a potential biological hazard. Technicians disposing of ISDS samples should wear goggles, gloves and a laboratory coat.

5.0 METHOD DESCRIPTION

5.1 Scheduling Considerations

At least 24 hours in advance of expected sample arrival, nutrient buffer water and project data sheets should be prepared. The calibration and status of the DO meter should be checked and it should be confirmed that an appropriate number of BOD bottles are prepared.

5.2 Quality Assurance/Quality Control

5.2.1 Method Detection Limit

The method detection limit (MDL) is 2.0 mg/L BOD. The reporting limit (RL) is 2 mg/L BOD. Final results are reported to the nearest whole number. Ideally, each incubated sample will experience a change in DO of at least 2 mg/L over the 5 day incubation period and contain at least 1 mg/L of DO at the end of the incubation.

5.2.2 Method Blanks

5.2.2.1 Method Blank

The method blank consists of a BOD bottle filled with the nutrient buffer solution, and then treated as a sample; 2 method blanks are completed per sample run.

Corrective Action
The change in dissolved oxygen for the method blank must not be greater than 0.2 mg/L. If the change in dissolved oxygen is greater than 0.2 mg/L then the sample run will be considered contaminated and the data deemed in error. This will be noted on the project data sheet. The samples will not be re-analyzed as they will be substantially outside the acceptable holding time.

5.2.2.2 Field Blank

The field blank consists of a sample bottle filled with laboratory DI water and transported to the field site with the empty sample bottles. The field blank is then returned to the laboratory with the filled sample bottles. The field blank is prepared by filling a BOD bottle with 200 mL of the
field blank water and 100 mL of the nutrient buffer and then treating it as a sample. The number of field blanks analyzed per sample run shall be 1. This will be at least 2% of the field samples collected.

**Corrective Action**

If the BOD for the field blank is greater than 2 mg/L the sample run will be considered contaminated and the data deemed in error. This will be noted on the project data sheet. The samples will not be re-analyzed as they will be substantially outside the acceptable holding time.

5.2.3 Sample Replication

Each sample is analyzed at a minimum of two different dilutions. Some samples are analyzed at three different dilutions because they exhibit highly variable BOD values. Information regarding the number of dilutions to be prepared is located on the project data sheet.

After the five day incubation, an ideal sample will exhibit a decrease in oxygen of at least 2 mg/L and the final DO value will be greater than or equal to 1 mg/L. If each sample dilution exhibits an acceptable change in oxygen and the final oxygen value is greater than 1 mg/L then the samples are considered acceptable and compared to determine the %RPD. Results between dilutions should be within 20%RPD. These values are then averaged and reported as the final BOD value.

Otherwise, only the samples exhibiting the accepted characteristics are used in the %RPD calculation. If only two dilutions were incubated and one is not acceptable then no %RPD is calculated. If no dilution exhibits the ideal characteristics, then the dilution closest to ideal is reported.

%RPD is calculated as follows:

\[
\%\text{RPD} = \left| \frac{\text{Result at Dilution 1 (mg/L)} - \text{Result at Dilution 2 (mg/L)}}{\text{Average of Result at Dilution 1 (mg/L) and Result at Dilution 2 (mg/L)}} \right| \times 100
\]

**Corrective Action**

If the %RPD is greater than 20% for samples that exhibit an acceptable change in oxygen and the final oxygen value is greater than or equal to 1 mg/L then this will be noted on the project data sheet. The sample will not be re-analyzed as it will be outside the sample holding time.

5.2.4 Glucose-Glutamic Acid Check

Three glucose-glutamic acid checks will be completed with each run of BOD samples. The glucose-glutamic acid checks provide assurance that the dilution water is not contaminated in a way that will reduce the microbiological population of the unknown samples. Additionally, the check also ensures that there is an appropriate microbiological community in the unknown sample that will produce an appropriate result. The final BOD value for glucose-glutamic acid check samples should be 400 mg/L +/- 30 mg/L BOD.

**Corrective Action**

If the final BOD for glucose-glutamic acid check samples is not within 400 mg/L +/- 30 mg/L BOD then this deviation will be noted on the project data sheet. The associated samples will not be re-analyzed as they will be substantially outside the sample holding time.
5.2.5 Seed Control

Four seed controls will be completed with each sample run. The seed control assures that the seed bacterial community is viable. The calculated seed control factor should fall between 0.60 and 1.0.

Corrective Action
If the seed control factor does not fall between 0.60 and 1.0 then the associated control is not used in the final calculations for BOD. If more than two of the seed controls produce seed control factors that do not fall between 0.60 and 1.0 then this deviation will be noted on the project data sheet. The associated samples will not be re-analyzed as they will be substantially outside the sample holding time.

5.2.6 Calibration and Standards

5.2.6.1 Laboratory Standards

The laboratory participates yearly in the Environmental Protection Agency Water Pollution Proficiency Test Study. Unknown samples are purchased and analyzed for BOD. The results of the analysis are compared to the actual value and a performance evaluation provided by the vendor. Study results are maintained by the laboratory.

5.2.6.2 Incubator

The incubator temperature at the beginning and end of the BOD incubation is recorded on the project data sheet. The incubator should be set at 20 +/- 1 °C. The temperature is measured using 2 thermometers inside the incubator, not from the reading on the door.

Corrective Action
If the incubator temperature is found to fluctuate greater than 1 °C over the incubation period, then this error is recorded on the project data sheet and the incubator is serviced.

5.2.6.3 Calibration of the DO Meter

The information below pertains to the URIWW YSI 5000 meter only. Detailed instructions are available in the instrument instruction manual found in room 002 in the BOD supply drawer.

Calibration and equipment checks of the DO meter must be completed: 24 hours before samples arrive in the laboratory, on the day of sample collection and on the final day of incubation when the samples are removed from the incubator.

Calibration of the DO meter is completed as follows:

1. Check the condition of the membrane located on the bottom of the probe. There should be no air bubbles under the membrane. If there are air bubbles, change the membrane following the procedure outlined in Step 3.

2. Check the date the membrane was last changed. This information is located on the label sheet on the bottom of the BOD clipboard and on the DO meter. Change the membrane if the current one is greater than two months old using the procedure outlined in Step 3. If the membrane does not need to be replaced, skip to Step 4 of the
procedure. The DO probe should not be used for 24 hours after the membrane is replaced.

3. Changing the membrane
   a. Pull out the stirrer.
   b. Unscrew the membrane cap at the end of the probe and discard.
   c. Use the provided sand paper to lightly sand the bottom of the probe.
   d. Rinse the probe with filling solution.
   e. Fill the new membrane cap with filling solution and screw the cap onto the probe.
   f. Record the date of membrane replacement on the log sheet located on the bottom of the BOD clipboard and on the DO meter.
   g. Re-attach the stirrer.
   h. Rinse the exterior of the probe with DI water.
   i. Place the probe in the storage bottle (BOD bottle). The storage bottle has approximately 1 inch of DI water in it. The probe sits above the water, not in it.
   j. Ideally, let the membrane equilibrate overnight before using.

4. Continue calibration set-up as per instruction manual. The barometric pressure is set at 753 mm Hg, with a maximum calibration of 99% (see p 62 of instruction manual)

5. Turn meter on. Let the meter warm up for at least 30 minutes before calibrating.
   a. The probe is stored in a BOD bottle that is 1/4 full of water.
   b. The probe should be in air, not water. This creates the proper conditions for air saturated calibration.

6. Remove the probe from the BOD bottle. Make sure the end of the probe is dry; if not carefully blot dry with a Kim wipe. Also make sure that there are no air bubbles under the membrane on the end of the DO probe.
   a. If air bubbles are visible under the membrane, the membrane must be replaced.
   b. See Step 3 for the membrane replacement protocol.

7. Put the probe back into the BOD bottle.

8. Press the fourth key on the DO meter. This key is correlated with “Calibrate”.
   a. On the YSI 5000 meter the function of the 4 soft keypads is displayed just above the "soft key" keypad. This display will change when you use the soft keys.
   b. The instruction manual is in the drawer below the meter.

9. Allow the meter to stabilize. It is stable when the meter beeps and an asterisk is visible next to the displayed value.

10. Press the first soft keypad. This key is correlated with “Auto Cal”. The meter will take a moment to stabilize. The meter is now calibrated. Record the DO, temperature, % saturation, date and time on the data sheet.

11. Press the “Mode” keypad to return to the main menu. It may be necessary to press the “Mode” key several times to return to the main menu. The mode button is in the top right of the display.
12. The instrument is now ready for use.

Corrective Action
Corrective action if the DO meter membrane is damaged, contains an air bubble or is greater than two months old is described in the calibration procedure. If the meter is not functioning properly, it can not be used in the BOD analysis. Inform Linda Green or Elizabeth Herron and use an alternate DO meter.

5.3 Analysis Method

5.3.1 Preparation – At least 24 hours prior to sampling day

1. Set up the BOD workstation in room 002 with pipettes, beakers, paper towels, gloves, etc.
2. Print data sheets.
3. Make sure the BOD bottles are clean. The number of BOD bottles necessary is recorded on the project data sheet. Assemble the bottles in numerical order near the BOD work station.
4. Prepare the nutrient buffer (see Section 5.3.1.1).
5. Set-up and check the calibration and operation of the DO meter (see Section 5.2.4.3).

5.3.1.1 Preparation of 19 L of Nutrient Buffer

Prepare 19 L of nutrient buffer water in a 20 L carboy at least 24 hours before the nutrient buffer is needed. The buffer should be air-saturated prior to and during use. Air-saturation of the buffer is achieved by bubbling air through the buffer after it is prepared. An aquarium double-bubbler is available to provide air. Preparation of 19 L of nutrient buffer is completed as follows:

1. Rinse the 20 L carboy with DI water.
2. Begin filling the carboy with DI water.
3. Obtain a white plastic Hach brand BOD nutrient buffer packet. Packets are stored in the cabinet above the pH meter. Shake the packet to suspend the salts.
4. Open one end of the packet with scissors, and pour the contents into the 20 L carboy.
5. Rinse the packet with DI water from a squeeze bottle to be sure all the material is rinsed into the carboy.
6. Fill the carboy to the 19 L mark with DI water.
7. Move the carboy to the BOD workstation. Plug in the aquarium bubblers and suspend the bubblers in the nutrient buffer solution. The bubblers should now be aerating the nutrient buffer solution.
5.3.2 Procedure – Day of Sample Collection

1. Prepare BOD seed (see Section 5.3.2.1).
2. Turn on the DO meter to allow a 30 minute warm-up period.
3. Calibrate the DO meter using the method outlined in Section 5.2.4.3.
4. Allow the samples to warm to room temperature.
5. Prepare the initial method blank by filling the first BOD bottle with nutrient buffer water to 1-2 mm above the base of the ground glass neck.
   a) Record the bottle number on the project data sheet.
   b) Record the initial DO according to Step 7 and place the bottle into the incubating refrigerator.
   a) Open the ampule, it contains 300 mg/L of glucose and 300 mg/L of glutamic acid.
   b) Pipette 3.0 mL of the standard into each of three BOD bottles. Remember to check the 3.0 mL pipette to make sure it is calibrated correctly.
   c) Pipette 4.0 mL of the BOD seed solution into each of the three BOD bottles. Remember to check the 4.0 mL pipette to make sure it is calibrated correctly.
   d) Record the BOD bottle number on the project data sheet.
   e) Fill the remaining volume in each BOD bottle with nutrient buffer water to 1-2 mm above the base of ground glass neck.
   f) Record the initial DO of each bottle according to Step 7 and place the bottles into the incubating refrigerator.
7. Prepare samples:
   For each sample analyze at least 2 replicates, each with a different sample volume. The number of replicates as well as the dilution volumes can be found on the project data sheet. Each replicate requires its own BOD bottle. The sample volume is determined by the source of the sample (refer to the project data sheet).
   a) Record the BOD bottle numbers to be used on the data sheet.
   b) Vigorously shake the first sample bottle for a minimum of 10 seconds, uncap, and immediately remove a sample aliquot using a pipette or graduated cylinder.
   c) Pipette/pour replicate samples directly into each BOD bottle.
      i) Keep a beaker of DI water handy to use for rinsing pipettes between samples.
      ii) It is not necessary to rinse the pipette between replicates of the same sample.
   d) Pipette 4.0 mL of the BOD seed solution into each of the three BOD bottles. Remember to check the 4.0 mL pipette to make sure it is calibrated correctly.
e) Fill the remaining volume in each BOD bottle with nutrient buffer water to 1-2 mm above the base of ground glass neck.

f) Record the initial DO of each bottle according to Step 7 and place the bottles into the incubating refrigerator.

8. Determine initial DO of the blanks, seed controls, glucose-glutamic acid checks and samples according to the following steps: Remember: do not turn off the DO probe between readings.

a) Place the DO probe into the BOD bottle.

b) Switch on the stirrer using the red switch on the top of the DO probe.
   i) The meter takes about 1 minute to equilibrate.
   ii) The DO meter is stable when it emits a beep and an asterisk is present next to the DO reading.

c) While the meter is equilibrating, fill the remaining volume in the next BOD bottle with nutrient solution.

d) The DO meter should have reached stability at this point. Record the DO meter reading to two decimal places on the project data sheet once it has stabilized. This value is the “initial DO reading”.

e) Remove the probe from the BOD bottle. Remember, turn off the stirrer before taking the probe out of the bottle!

f) Rinse the DO probe using a squeeze bottle filled with DI water. Catch the waste water in the wastewater beaker.

g) Place the DO probe into the next bottle and switch on the stirrer.

h) Cap the first BOD bottle with a ground glass stopper. Make sure no air bubbles are trapped below the ground glass stopper.

i) Put the BOD bottle in the BOD incubating refrigerator. The BOD bottle should be water sealed. Water sealing of a BOD bottle is accomplished by using the DI water filled squeeze bottle to completely fill the flared neck of the BOD bottle with water. This ensures that if the volume of the water in the BOD bottle were to change due to changes in temperature that no air would be introduced into the bottle.

j) Keep alternating DO meter readings and filling bottles until all the BOD replicates for a sample are complete.

k) Place the samples into the incubating refrigerator.


10. Once all samples have been prepared, prepare the final method blank (refer to Step 4).

11. All BOD samples should be located in the light-excluding refrigerated incubator. This incubator is set at 20 °C. The time the final sample is placed into the incubator is noted on the project data sheet. Samples are incubated for 5 days.

12. Turn off the DO meter.
13. The water seal of the BOD bottles must be checked and re-filled daily, as needed. The technician that completes this task should record the date and time the water seals were checked and their initials on the project data sheet.

14. Field sample disposal is completed in accordance with Section 4.0.

5.3.2.1 Preparation of BOD Seed

1. Place the contents of one Hach PolySeed capsule into 500 mL of the nutrient buffer water. Do not put the gelatin capsule into the buffer water, throw it away.

2. Aerate and stir the solution for one hour.

3. Decant the supernatant into another clean beaker so that the bran (particles on the bottom of the beaker are left behind). Place a sterile stir bar into this beaker and gently stir for the remainder of the test.

4. The remaining bran can be disposed of by rinsing down the drain.

5. The bacterial solution should be used within six hours of rehydration.

5.3.3 Procedure – End of the incubation (5 days after initial sample preparation)

After the 5 day incubation DO levels in the BOD bottles are recorded in accordance with the following procedure:

1. Allow the DO meter to warm up at least 30 minutes.

2. Calibrate the DO meter (see Section 5.2.4.3).

3. Remove BOD bottles from the incubating refrigerator.

4. Determine the DO value for each BOD bottle using the following procedure:
   a. Place the DO probe into the BOD bottle.
   b. Switch on the stirrer using the red switch on the top of the DO probe.
      i. The meter takes about 1 minute to equilibrate.
      ii. The DO meter is stable when it emits a beep and an asterisk is present next to the DO reading.
   c. Once the DO meter is stable, record the DO meter reading to two decimal places as “final DO reading” on the project data sheet.
   d. Remove the probe from the BOD bottle. Remember, turn off the stirrer before taking the probe out of the bottle!
   e. Rinse the DO probe using a squeeze bottle filled with DI water. Catch the waste water in the wastewater beaker.
   f. Place the DO probe into the next bottle and switch on the stirrer.

5. Turn off DO meter once all samples have been analyzed.

Disposal of incubated samples
Discard the contents of each BOD bottle by rinsing it down the drain with running water. BOD bottles are cleaned by rinsing 3 times with tap water, 3 times with DI water and inverting to dry.
BOD samples are a potential biological hazard. Technicians should wear goggles, gloves and a laboratory coat when disposing of ISDS samples.

6.0 CALCULATIONS

Ideally, samples should exhibit a decrease in DO of 2 mg/L over the 5 day incubation and still retain at least 1 mg/L of DO. If each dilution for a given sample exhibits the ideal decrease in oxygen and an acceptable final DO value, then the result from each acceptable dilution is averaged to produce the final average BOD value. Otherwise, only the sample(s) exhibiting the accepted characteristics are used in determining the final BOD value. If none of the sample dilutions exhibit the desired characteristics then the sample value closest to exhibiting the required changes is reported and this is noted on the project data sheet.

BOD results are reported to the nearest whole numbers. Values less than 2 mg/L BOD are reported as <2 mg/L BOD. Method blanks should exhibit a change in oxygen of less than 0.2 mg/L and field blanks should exhibit a BOD of not greater than 2 mg/L (refer to section 5.2.2 for corrective action information). The glucose-glutamic acid checks should return a BOD value of 400 +/- 30 mg/L. If the checks are not within this range see section 5.2.4 for corrective action information.

The seed control factor should be between 0.60 and 1.0, if the control factor is not within this range see section 5.2.5 for corrective action information.

Seed Control Factor (SCF)

Seed Control Factor = (S1 – S2) * F

S1 = DO of seed control before incubation (mg/L O₂)
S2 = DO of seed control after 5 days incubation at 20 °C (mg/L O₂)
F = Volume of seed in diluted sample / Volume of seed in seed control

Final BOD is calculated as follows:

\[ \text{BOD (mg/l)} = \frac{(D_1 - D_2) - \text{SCF}}{P} \]

D₁ = Initial DO of diluted sample (mg/L O₂)
D₂ = DO of diluted sample after 5 days incubation at 20 °C (mg/L O₂)
SCF = Seed control factor (mg/L O₂)
P = Decimal volumetric fraction of sample used.

For example if 30 mL of sample was diluted to 300 mL, P = 30/300 = 0.10

Dilution Guide

<table>
<thead>
<tr>
<th>Expected BOD (mg/L)</th>
<th>Sample Amount (mL)</th>
<th>Final Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 50</td>
<td>50, 100, 200</td>
<td>300</td>
</tr>
<tr>
<td>50 – 100</td>
<td>10, 25, 50</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>100 – 350</td>
<td>6, 8, 10</td>
<td>300</td>
</tr>
<tr>
<td>350 – 500</td>
<td>1, 3, 5</td>
<td>300</td>
</tr>
<tr>
<td>&gt;= 500</td>
<td>0.5, 1, 2</td>
<td>300</td>
</tr>
</tbody>
</table>

7.0 REFERENCES

Method referenced: Biochemical Oxygen Demand (5210), 5-Day BOD Test (5210 B). #5210.


InterLab “PolySeed ® Application Procedure BOD5 Seed Inoculum” informational pamphlet.
8.0 DOCUMENTATION

Example of project data sheet

<table>
<thead>
<tr>
<th>BOD Bottle</th>
<th>Site</th>
<th>Location</th>
<th>mL Sample</th>
<th>Initial DO</th>
<th>5-Day DO</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>INITIAL</td>
<td>lab nutrient BLANK</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose- Glutamic Acid check</td>
<td></td>
<td>3 mL of standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose- Glutamic Acid check</td>
<td></td>
<td>3 mL of standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose- Glutamic Acid check</td>
<td></td>
<td>3 mL of standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed Control</td>
<td></td>
<td>10 of seed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed Control</td>
<td></td>
<td>15 of seed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed Control</td>
<td></td>
<td>20 of seed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed Control</td>
<td></td>
<td>25 of seed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3 Rea</td>
<td>STE – 1</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3 Rea</td>
<td>STE – 1</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3 Rea</td>
<td>STE – 1</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3 Rea</td>
<td>AXE</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 3 Rea</td>
<td>AXE</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 11 Der</td>
<td>AXE</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 11 Der</td>
<td>AXE</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>STE –S</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>-------</td>
<td>------</td>
<td>----</td>
<td>----</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI - 12 Fla</td>
<td>STE –S</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FIELD</strong></td>
<td>Water</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BLANK</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FINAL</strong></td>
<td>Lab</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>nutrient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BLANK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Standard Operating Procedure 012
(Prior number URIWW-SOP-5)

Chlorophyll-a Analysis –
Welschmeyer Method

University of Rhode Island Watershed Watch

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1.0 PURPOSE AND DESCRIPTION

The purpose of this method is to determine the concentration of chlorophyll-a in ambient water samples from both marine and freshwater systems. The concentration of chlorophyll-a can be used to estimate phytoplankton biomass. Samples are collected and either filtered in the field or upon arrival at the laboratory. The filtered samples are then kept frozen until they are extracted and analyzed using a fluorometer. The value returned by the fluorometer is the raw chlorophyll-a concentration. The final chlorophyll-a concentration is determined through calculation. This method is appropriate for undiluted samples up to 100 µg/L chlorophyll-a and for samples diluted to return values in this range.

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

General laboratory good housekeeping procedures should be practiced as outlined in Standard Operating Procedure (SOP) 001 – General Laboratory Safety. Several chemicals are utilized in this SOP. Acetone is a flammable liquid; it should always be used in the hood. Sodium bicarbonate and magnesium carbonate are also utilized in this SOP, both are considered a possible skin, eye and respiratory tract irritant. The liquid chlorophyll-a standards are flammable as they are prepared in acetone. Always wear gloves, a laboratory coat and goggles when working with any chemical. Further information regarding these chemicals may be found in the laboratory Material Safety Data Sheet (MSDS) binder found in the laboratory where the chemical of interest is stored. General laboratory information regarding safe handling of chemicals is located in SOP 001a – University Safety and Waste Handling.

2.2 Technician Training/Qualifications

General training in laboratory technique and the use of the fluorometer must be completed prior to analyzing samples using this method. Technician training will be provided by Linda Green, Program Director or Elizabeth Herron, Program Coordinator.
### 3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freezer</td>
<td>Maintained at -80 °C</td>
<td></td>
</tr>
<tr>
<td>Walk-in cold room or refrigerator</td>
<td>Maintained at 4 °C</td>
<td></td>
</tr>
<tr>
<td>Balance</td>
<td>Capable of weighing to 0.1 g room 018</td>
<td></td>
</tr>
<tr>
<td>Repipet</td>
<td>Volume range to 20 mL, set to dispense 5 mL</td>
<td>Barnstead/Thermolyne</td>
</tr>
<tr>
<td>90% Acetone</td>
<td>90% acetone is found in the repipet labeled &quot;90% acetone&quot;</td>
<td>Fume hood, room 019a</td>
</tr>
<tr>
<td></td>
<td>Preparation instructions for 90% acetone are found in Section 5.3.2.1.</td>
<td></td>
</tr>
<tr>
<td>Magnetic stirrer and stir bar dedicated to 90% acetone preparation</td>
<td>Located on the laboratory bench in room 002. Stir bar is always left in graduated cylinder used to prepare the 90% acetone.</td>
<td></td>
</tr>
<tr>
<td>Magnetic stirrer and 1&quot; stir bar</td>
<td>Located on side laboratory bench, room 002</td>
<td></td>
</tr>
<tr>
<td>13x100 mm disposable glass test tubes</td>
<td>Stored in room 019A</td>
<td>Fisher # 14-958-10C, case of 1000</td>
</tr>
<tr>
<td>Vial caps</td>
<td>Stored in room 019A</td>
<td>Fisher “Tainer Tops” #02-706-28</td>
</tr>
<tr>
<td>40 place test tube racks</td>
<td>Holes should fit 13x100 mm test tubes</td>
<td></td>
</tr>
<tr>
<td>Deionized (DI) water</td>
<td>10 - 100 mL graduated cylinders</td>
<td></td>
</tr>
<tr>
<td>500 mL graduated cylinder labeled “chl only”</td>
<td>500 mL graduated cylinder labeled “chl only”</td>
<td>Fume hood or center lab bench room 002</td>
</tr>
<tr>
<td>250 mL beaker</td>
<td>Located in the non-acid washed glassware cabinet</td>
<td></td>
</tr>
<tr>
<td>10-25 mL to contain (TC) graduated cylinder</td>
<td>Left in small flask next to bottle of sodium bicarbonate</td>
<td>Pall type A/E glass fiber filter #61630 (preferable) or Millipore AP40 GFF for TCLP #AP4002500 or Filter Housings (holders)</td>
</tr>
<tr>
<td>Required Material</td>
<td>Notes</td>
<td>Re-order information</td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>DI saturated solution of magnesium carbonate (MgCO₃)</td>
<td>Stored in a drawer by the center sink in room 002</td>
<td></td>
</tr>
<tr>
<td>Forceps</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary chlorophyll-a standard</td>
<td>Liquid chlorophyll-a standards. The two standards are approximately 155 µg/l (high standard) and 15.5 µg/l (low standard). They are stored in aluminum foil-covered chlorophyll-a vials in a 4 °C refrigerator in room 019.</td>
<td>Turner Designs # 10-850</td>
</tr>
<tr>
<td>Secondary chlorophyll-a standard</td>
<td>This standard is a dark grey rod and has an indefinite shelf life. It is stored in room 019A next to the fluorometer.</td>
<td>Turner Designs</td>
</tr>
<tr>
<td>Acetone</td>
<td>Located in the flammable storage cabinet in room 002</td>
<td>Fisher Optima #A-929</td>
</tr>
<tr>
<td>Sodium bicarbonate (NaHCO₃)</td>
<td>Located on the top shelf of the glass chemical cabinet.</td>
<td>Fisher, Certified ACS, #S-233</td>
</tr>
<tr>
<td>1N NaHCO₃</td>
<td>Located in the cabinet to the left of BOD incubator room 002 or next to the fume hood. Preparation Instructions in Section 5.3.2.2.</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll-a data sheets</td>
<td>Stored in a drawer in room 019A.</td>
<td></td>
</tr>
<tr>
<td>Filter remover wire</td>
<td>6&quot; aluminum wire with bent tip located in room 019A.</td>
<td></td>
</tr>
<tr>
<td>Kim-wipe tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminum foil</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory and the Natural Resources Science department. Temporary replacement of large equipment such as balances is available through arrangement with other scientists in the department maintaining similar equipment.
4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

Samples for chlorophyll-a analysis may arrive in the laboratory as filters or raw (unfiltered) water samples. Aqueous samples are filtered upon arrival at the laboratory.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass Fiber Filter</td>
<td>Aluminum Foil</td>
<td>Frozen</td>
<td>NA</td>
<td>6 months</td>
</tr>
<tr>
<td>Water</td>
<td>500 mL brown glass or plastic bottle</td>
<td>4 °C</td>
<td>100 mL</td>
<td>24 hours</td>
</tr>
</tbody>
</table>

Sample Disposal
Glass vials used for sample extraction are washed and re-used. Used glass fiber filters are placed on an absorbent material (paper towel) in the hood and allowed to dry. Once filters are dry they are disposed of in the garbage. The 90% acetone extractant is disposed of into the labeled acetone waste container located in the fumehood in room 019A. Once the container is approximately 80% full contact Linda Green or Elizabeth Herron to coordinate a waste removal request. Ensure that the container is appropriately and completely labeled.

5.0 METHOD DESCRIPTION

5.1 Scheduling Considerations

Chlorophyll-a filters must be extracted for 18 - 24 hours prior to analysis using the fluorometer. Check that enough 90% acetone is available for the extraction (5 mL of acetone are needed per sample). Preparation instructions for 90% acetone are in Section 5.3.2.1.

5.2 Quality Assurance/Quality Control

5.2.1 Method Detection Limit

The method detection limit (MDL) is 1.0 µg/L chlorophyll-a as read on the fluorometer. This is equivalent to 0.1 µg/L chlorophyll-a in a 50 mL aliquot of water. The reporting limit (RL) is 2.0 µg/L chlorophyll-a as read on the fluorometer; equivalent to 0.2 µg/L chlorophyll-a in a 50 mL aliquot of water. The upper RL is 50 µg/L chlorophyll-a for the normal calibration and 100 µg/L chlorophyll-a for a high level calibration, assuming no dilution of the sample. Data are reported to 1 decimal place.

5.2.2 Blanks

Method Blank
Method blanks consist of an aliquot of the 90% acetone used to extract the sample filters in a given run. Method blanks are prepared at a ratio of 1 method blank per 38 samples, approximately 3% of samples analyzed. This ratio corresponds to 1 method blank per rack of chlorophyll-a samples. The method blank must be less than or equal to 0.03 µg/L chlorophyll-a as read on the fluorometer.
Filter Blank
Filter blanks consist of an unused filter extracted as a regular sample. Filter blanks are prepared at a ratio of 1 filter blank per 38 samples, approximately 3% of samples analyzed. This ratio corresponds to 1 field blank per rack of chlorophyll-a samples. The filter blank must be less than or equal to 0.03 µg/L chlorophyll-a as read on the fluorometer.

Corrective Actions
If the method blank or filter blank is found to be greater than 0.03 µg/L chlorophyll-a as read on the fluorometer the blank shall be re-analyzed by the fluorometer. If the returned value is still greater than 0.03 µg/L chlorophyll-a as read on the fluorometer then the samples associated with this blank shall be considered suspect and flagged as such on the final data sheet. It is not possible to re-extract the filters, therefore the samples cannot be re-analyzed.

5.2.3 Sample Replication

Each extracted sample is measured on the fluorometer twice. The acceptable relative percent difference (%RPD) between replicate readings of the same sample is 20%. %RPD is calculated as follows:

\[
%\text{RPD} = \frac{|\text{Result of Replicate 1 (µg/L)} - \text{Result of Replicate 2 (µg/L)}|}{\text{Average of Result of Replicate 1 (µg/L) and Result of Replicate 2 (µg/L)}} \times 100
\]

Corrective Action
If the %RPD is greater than 20%, then the sample is re-analyzed by the fluorometer. If the %RPD between the two new readings is still greater than 20%, then the discrepancy is noted on the project data sheet. It is not possible to re-prepare samples as the original field sample is not available and once the filter is extracted it is not possible to re-extract it.

Additionally, sample duplication is completed in the field. At each field site 2 samples are collected, and 2 aliquots from each field sample are filtered, resulting in a total of 4 filters. The %RPD between samples collected must be less than 100%RPD using the average of all the filter results as the denominator in the %RPD calculation.

Corrective Action
If the %RPD is greater then the established goal then the deficiency will be noted in the final data as once the samples are extracted it is not possible to re-extract them.

5.2.4 Calibration

The calibration procedure sets the instrument sample concentration range and sensitivity based on the chosen fluorescent standards. In addition, the direct calibration mode assigns a digital value to the known standard so that subsequent standards or samples can be referenced to the original standards. Two liquid pure chlorophyll-a standards are purchased from Turner Designs at the beginning of each monitoring season. The two standards are approximately 155.0 µg/L chlorophyll-a (high standard) and 15.5 µg/L chlorophyll-a (low standard). The actual concentration of the standard is listed on the certificate of analysis shipped with each set of standards and kept on file in room 002. Standards are stored in aluminum foil-covered chlorophyll-a vials in a 4 °C refrigerator. These standards are used to calibrate the instrument at the beginning of the monitoring season and then used to check the calibration of the instrument each day it is used.
Additionally, a solid secondary standard, also from Turner Designs is read immediately after the initial calibration procedure to determine the formula for calculating chlorophyll-a in a sample. This standard consists of a dark grey rod and has an indefinite shelf life. The secondary standard is also used to check the calibration of the instrument each day it is used.

The percent difference (%D) between the known value of the standard and the instrument reported value of the standard must be not greater than 15%. The calculation of %D is provided below:

\[
\%D = \left| \frac{\text{Known Value} - \text{Reported Value}}{\text{Known Value}} \right| \times 100
\]

Corrective Action

If %D is greater than 15% for a liquid or solid standard then the standard should be re-analyzed. If the %D is still greater than 15% then the deviation should be reported to Linda Green – Laboratory Manager – Nutrients and the instrument re-calibrated. If the instrument does not maintain the calibration of the solid secondary standard it will be considered to be malfunctioning and in need of repair.

5.2.4.1 Initial Calibration Procedure

Chlorophyll-a procedures must be completed in a darkened room. Keep overhead lights off and the door closed to limit the light entering the room. The fluorometer is not turned off except when it is expected that it will not be used for more than 1 month.

Initial Calibration with liquid primary standards

Note: A sufficient quantity of pure chlorophyll-a in 90% acetone is received with each standard to fill 2 vials, so that analysis of each (of two) standards can be completed in duplicate.

1. Remove standards from refrigerator and bring to room temperature. All materials analyzed must be at room temperature. The fluorometric value is temperature dependent. Additionally, condensation may form on the sample vial and affect sample analysis.

2. Pour each liquid standard into 2 vials. Wrap each vial with aluminum foil and label each vial with the contents. The labels should be “High-std A” and “High-std B” for the 2 vials containing the high standard and “Low-std A” and “Low-std B” for the 2 vials containing the low standard.

3. Prepare a blank of 90% acetone, wrap the vial with aluminum foil and label as “Blank”.


5. Press <ESC> to return to previous screen, press <2> to choose calibration procedure. Toggle to the “Direct Concentration” choice.

6. Press <ESC> to return to previous screen, press <3> to choose the units. Toggle to “µg/l”. Press <ESC> twice to return to set-up/Cal screen.

7. Press <2>. The Direct Concentration calibration sequence will appear.
8. When the fluorometer calls for the maximum range press <9> and then enter 500. This sets the maximum linear range of the fluorometer.

9. Key in the number of standards (2), press <ENT>.

10. When the fluorometer calls for the “HiStd Conc”, press <9> then enter the concentration and press <ENT>.

11. Remove the high standard vial from the aluminum foil. Wipe the vial dry, insert it into the cuvette holder and press <*>.

12. Repeat steps 10 - 11 for the low standard, entering its concentration. Press <ENT> when finished.

13. Remove the calibration blank (90% acetone) from the aluminum foil. Wipe the vial dry, insert it into the cuvette holder when prompted and press <ENT>. When the blank has stabilized press <0>. The instrument will read the blank and then return to the Home screen.

14. The initial calibration is now complete.

15. Measure each of the 2 high and 2 low standards and the calibration blank a minimum of 3 times. Record the results on the “Calibration Day Calculations” data sheet as a record of the calibration procedure (see Section 8.0 Documentation). The numbers recorded should be very close in value. If the recorded values exhibit drift then the instrument is in need of maintenance.

Initial Calibration with the Solid Secondary Standard

Calibrate the solid secondary standard after calibration with the liquid standards is complete.

1. Remove the cuvette holder to allow placement of the solid standard.

2. Place the solid standard in the cuvette holder with “L” on the left side.

3. Press <*> to read the value. Record this number as the low standard.

4. Remove the standard from the holder, rotate it 180 degrees and place in the holder again, so that “H” is on the left side.

5. Press <*> to read the value. Record this value as the high concentration.

6. The secondary calibration is now complete.

7. Measure the high and low solid standard a minimum of 3 times. Record the results on the “Calibration Day Calculations” data sheet as a record of the calibration procedure (see Section 8.0 Documentation).

   a. The numbers recorded should be very close in value. If the recorded values exhibit drift then the instrument is in need of maintenance.

   b. The average of the readings for the high solid standard is used to calculate chlorophyll-a concentration (see Section 6.0 Calculations).

8. Remove the standard and replace the cuvette holder. Be sure to insert it correctly, with the metal prong to the left and clear prong to the right.
5.2.4.2 Daily Calibration Procedure

The procedure for calibration outlined below assumes that standards have been previously poured into vials for use in the primary standardization procedure. Each analysis day the liquid primary standards and solid secondary standard are analyzed as standards. Liquid primary standards are only analyzed at the beginning of the run. The solid secondary standard is analyzed at the beginning of the run and at the end of each rack of samples. The daily calibration procedure to be completed prior to sample analysis is presented below:

1. Bring standards to room temperature.
2. Wipe the outside of liquid primary standard vials dry with a Kimwipe.
3. Insert a liquid primary standard into the sample holder and close the lid.
4. Immediately press <*>.
   
   This initiates the following sequence: a 7 second delay for signal stabilization (DLY on display), a 12 second averaging period (AVG on display), then a 5 second display of readout (END on display.)
5. Record the value returned on the Chlorophyll-a Daily Standards record sheet.
   
   The liquid primary standard value should not be greater than 15%D from the actual standard value.
6. Repeat this procedure with the next liquid primary standard and then the standard blank.
7. Check the calibration of the fluorometer using the solid secondary standard using the procedure outlined below:
   
   a. Remove the cuvette holder to allow placement of the solid standard.
   
   b. Place the solid secondary standard in the cuvette holder with “L” on the left side. Press <*> to read the value. Record this number as the low standard.
      
      The solid secondary standard value should not be greater than 15%D from the actual standard value.
   
   c. Remove the standard from the holder, rotate it 180 degrees and place in the holder again, so that “H” is on the left side.
      
      The solid secondary standard value should not be greater than 15%D from the actual standard value.
   
   d. Press <*> to read the value. Record as the high concentration.
   
   e. Remove the standard and replace cuvette holder. Be sure to insert it correctly, with the metal prong to the left and clear prong to the right.
8. The daily calibration procedure is now complete.
5.2.5 Calibration Check/Laboratory Control Standard

The solid secondary standard is used as a calibration check or laboratory control standard. The solid secondary standard is analyzed at the end of each rack of 38 chlorophyll-a samples (3% of the samples analyzed). The resulting observation is recorded on the data sheet. The %D for the calibration check must not be greater than 15%.

**Corrective action**

If the %D for the solid secondary standard is greater than 15% then the calibration check will be considered in error and re-analyzed. If the value is still in error the liquid primary standards will be re-analyzed. If the liquid standard is also in error the instrument will be re-calibrated after discussions with Linda Green (Laboratory Manager – Nutrients) and all the samples analyzed between the acceptable calibration check and the unacceptable calibration check will be re-analyzed by the fluorometer. If the liquid standard is not in error, the deviation of the solid secondary standard will be noted on the data sheet and the analysis of samples will continue.

5.3 Analysis Method

5.3.1 Laboratory Filtration Procedure

Chlorophyll-a samples generally arrive at the laboratory in the form of frozen filters. If chlorophyll-a samples arrive at the laboratory as aqueous samples then they must be filtered upon acceptance into the laboratory. Chlorophyll-a samples are filtered using the following procedure:

1. This procedure must be completed in an area without direct sunlight and the lights turned off.
2. Prepare a filter housing by placing one glass fiber filter, gridded side down, onto the metal screen on the bottom of the filter assembly.
3. Always use the tweezers to handle filters.
4. Place the black rubber gasket on top of the filter, and screw the filter holder back together.
5. Shake the sample bottle well.
6. Use a syringe to draw up approximately 20 mL of sample into the syringe. Use this water to rinse the syringe and then discard the water.
7. Take apart the syringe by pulling the plunger all the way out.
8. Attach the filter holder to the syringe barrel.
9. Mix the sample bottle again and obtain the sample to be filtered by pouring water from the bottle into the syringe (with the filter holder facing down).
10. Note the volume of water poured into the syringe. This volume should be recorded as the “volume filtered”.
11. Shake the bottle of magnesium carbonate, and add four drops of the solution into the water sample in the syringe.
12. Attach the syringe plunger, and slowly push the water through the filter housing with even pressure. Discard the filtered water unless this will be used for nutrient analysis.
13. If the filter becomes clogged, remove the filter and start the procedure over with a smaller amount of sample.

14. After filtering all the water take the filter holder off the syringe and unscrew the two halves.

15. Use forceps to lift out the black rubber gasket.

16. Remove the filter from the housing using the forceps, and place the filter on a piece of blotting paper or paper towel.

17. If the filter breaks while being removed, place all the pieces onto the blotting paper.

18. Fold the filter circle in half with the chlorophyll-a sample on the inside and wrap the blotting paper over the filter.

19. Place the filter and blotting paper on a piece of aluminum foil and cover it loosely with the foil.

20. Repeat the procedure to obtain another filtered sample from the same raw water sample.

21. Securely wrap the piece of aluminum foil around the filters and attach a label with the sampling location, technician’s name, date, volume filtered and the number of filters.

22. Place the aluminum foil packet in a re-sealable plastic bag containing desiccant chips and place into the freezer.

23. Take apart the syringe and filter assembly. Rinse all apparatus with DI water only. Place upside down on a paper towel to dry.

5.3.2 Extraction of Chlorophyll-a Filters – Day Before Analysis

1. Gather the needed supplies. The white test tube racks, disposable test tubes and caps, forceps, data sheets and the repipette containing the buffered 90% acetone solution are all located in room 019A (fluorometer room). The frozen chlorophyll-a filters are located in the URIWW freezer in room 019 or 002.

2. Organize data sheets; blank data sheets are kept next to the fluorometer in room 019A Coastal Institute-Kingston (CIK), URI, referred to as “the chlorophyll-a closet.”
   a. The data sheets are set up to correspond to the sample rack.
   b. A maximum of forty samples and blanks can be accommodated in each rack (and on each data sheet.) *It is recommended that no more than 4 racks be analyzed in one sitting.*

3. Turn on the fume hood fan.

4. Fill in top of the data sheet. Sheets are numbered in consecutive order, 2000-1, 2000-2, etc.

5. If needed, make up buffered 90% acetone solution and place into the labeled Repipette. (Preparation instructions are located in Section 5.3.2.1)
6. Check the calibration of the repipette.
   a. Pump several times to remove air bubbles, then dispense into a “to contain” (TC) graduated cylinder.
   b. Check to see that the volume is 5.0 mL. If not, adjust dispensing volume and re-check.
      i. Please note that this is an update from the previous instructions where the calibration of the repipette was checked using the weight of the 90% acetone.
      ii. 5.0 mL of 90% acetone is approximately 6.15 g.

7. Bring several ziplock bag(s) full of frozen sample filters into the fluorometer lab when ready to set up for extraction. Samples filters are stored in the URIWW freezers in room 019 or 002.

8. Remove foil filter packs from the ziplock bags and sort by date. Set foil packets in chronological order.

9. Enter information from sample label onto the chlorophyll-a analysis data sheets. Be sure to read and enter the information carefully. Initial and date the chlorophyll “Storage Location” data sheet corresponding to the monitoring location being prepared for analysis (see Section 8 Documentation).
   a. While most foil packets will contain only one filter per packet, some may contain more, and should have been labeled as such.
   b. Location, date, volume filtered and sample depth must be entered for each filter. Rack ID number and setup date must be indicated on the data sheet.

10. Turn out the room lights. The filters must not be exposed to light.

11. Once the data sheet has been completed for a row of 10 samples, using the forceps, remove the filter(s) from the aluminum foil and place one filter in the bottom of each glass vial.
   a. Do not touch the filter with anything but the forceps!
   b. The vial containing the filter should then be placed in the test tube rack matching the position identified on the data sheet.

12. Continue until the rack has been filled. The second to last spot should contain an unused filter, for a filter blank. The last spot on the rack should contain an empty vial. This vial will be filled with the 90% acetone used to extract the filters in the rack and considered a method blank.

13. Dispense 5 mL of buffered 90% acetone into each vial using the Repipette, and secure a snap cap onto the top of each vial.

14. Vigorously shake each vial, making sure that the filter remains completely submerged in acetone at the end.

15. Cover the rack completely with aluminum foil. Tape the data sheet to the rack it corresponds to.

16. Place the covered, labeled racks into the URIWW refrigerator in room 019. Allow 18-24 hours for complete extraction of chlorophyll-a from the filter.
17. Complete the procedure with the rest of the chlorophyll-a filters being prepared.

18. Turn on the fluorometer if it is not already on. Typically the fluorometer is left on unless it will not be used for more than 1 month. Let the instrument warm up for at least 1 hour prior to use if it was necessary to turn the instrument on.

**Clean-up**

1. Place desiccant chips from ziplock bags into the bottle under the fume hood. The desiccant chips will be regenerated later. Desiccant bottles are stored on cabinets in room 002, labeled as to whether they need to be regenerated or not.

2. Clean off work surface.

3. Put empty ziplock bags in the labeled box for later re-use or discard if warranted.

4. Save aluminum foil for recycling.

**5.3.2.1 Preparation of 500 mL of 90% Acetone**

Preparation of reagents used in the analysis of chlorophyll-a should be completed in room 002. Prepare 500 mL of 90% acetone using the following procedure:

1. Add the following to the 500 mL graduated cylinder labeled “chl only”
   a. 450 mL acetone
   b. 50 mL DI water

2. Once the DI water is added to the graduated cylinder the volume in the graduated cylinder will read slightly less than 500 mL due to density differences between acetone and water.

3. 5 drops 1N NaHCO$_3$ (sodium bicarbonate)
   a. Add 1 drop of 1N NaHCO$_3$ per 100 mL of solution.
   b. Since 500 mL of 90% acetone was prepared, 5 drops of 1N NaHCO$_3$ are added to the final solution.

4. Preparation instructions for 1N NaHCO$_3$ are in Section 5.3.2.2.

5. Cover the graduated cylinder loosely with aluminum foil, place it onto a magnetic stirrer, turn the stirrer on and stir the contents of the graduated cylinder well.

6. Store the solution in the labeled acetone repipette bottle.
5.3.2.2 Preparation of 100 mL of 1N NaHCO₃ (sodium bicarbonate)

Preparation of reagents used in the analysis of chlorophyll-a should be completed in room 002. Prepare 100 mL of 1N NaHCO₃ using the following procedure:

1. Add 8.4 g of NaHCO₃ to a 250 mL non-acid washed glass beaker.
2. Fill the beaker with DI water until it reaches 100 mL.
3. Stir using the 1” magnetic stir bar until the NaHCO₃ has dissolved.
4. Pour the solution into the 125 mL brown glass bottled labeled 1N NaHCO₃. Add a label to the bottle with the data of preparation and the initials of the technician that prepared the solution. The solution will last indefinitely.

5.3.3 Analysis of Extracted Chlorophyll-a Samples

1. Gather the needed supplies.
   a. Extracted chlorophyll-a samples in the aluminum foil covered white racks located in the refrigerator in room 019.
   b. Filter remover (6" piece aluminum wire, with bent tip) – room 019A
   c. Liquid chlorophyll-a standards and calibration blank - refrigerator in room 019.
   d. Solid secondary standard - box in drawer under fluorometer.
   e. Repipette containing 90% acetone for dilution - fume hood in chlorophyll closet.
   f. 1 mL Brinkmann pipette, with blue tip - borrowed daily from room 018.
2. Allow at least 30 minutes for extracted samples and standards to reach room temperature prior to analysis. Fluorometric readings are temperature dependent.
3. Remember to keep the lights off during analysis and preparation. The door to the chlorophyll closet may be left open if there is no one in room 019 and the lights are off. This is recommended to provide ventilation.
4. While the samples are warming to room temperature, shake the first sample vigorously. Remove the cap and using the filter remover, remove the filter. The acetone soaked filter should be placed on a Kimwipe, and left in the fume hood until completely dried. This takes only a few minutes. The old dry filters are then thrown away.
5. Replace the cap on the vial, and wipe outside of vial with a Kimwipe (removes fingerprints/moisture which interfere with readings).
6. Place the tube back in the rack.
7. Repeat Steps 4 through 6 for the remaining samples on the rack.
8. By the time Steps 4 through 6 have been completed for all the samples, the samples should have reached room temperature.
9. Check the calibration of the instrument using both the primary liquid standards as well as the solid standard. Refer to Section 5.2.4.2 for a description of the procedure.
10. Load the first sample vial into the fluorometer:
   a. Open the fluorometer sample holder lid.
   b. Insert cuvette holder if necessary (metal prong to the left, clear prong to the right). Check to make sure that the holder is properly seated.
   c. Wipe off any fingerprints on the vial.
   d. Hold the sample vial by its cap and gently place it into the fluorometer sample holder.
   e. Close sample holder lid.
11. Immediately press <*>.

   This initiates the following sequence: a 7 second delay for signal stabilization (DLY on display), a 12 second averaging period (AVG on display), then a 5 second display of readout (END on display.)
12. Record the reading on the data sheet. If the fluorometer reads “over” the sample must be diluted and re-analyzed using the procedure found in Section 5.3.3.1 or 5.3.3.2.
13. Analyze all the samples & blanks on the rack and then repeat to obtain a second (replicate) value.
   a. If the method blank or filter blank returns a value greater than 0.03 µg/L chlorophyll-a as read on the fluorometer then this should be noted on the project data sheet.
   b. Refer to Section 5.2.2 for corrective action necessary if the blanks are greater than 0.03 µg/L chlorophyll-a as read on the fluorometer.
14. Once all the samples on the rack have been analyzed a second time, remove the cuvette holder and analyze the solid secondary standard. This value should be less than or equal to 15%D from the know value. Refer to Section 5.2.5 for information on procedure to follow if this value is greater than 15%D.
15. Repeat steps 10 – 14 for the remaining racks.

Clean-up
1. Pour acetone extract from the vials into the waste acetone bottle located in the fume hood in the fluorometer room. Make sure to recap the bottle when done (see Section 4.0 for the waste disposal procedure.
2. Leave the test tubes in racks in the fume hood until they are to be washed. See Section 5.3.4 for information on the vial washing procedure.
3. Clean off the work surface.
4. Return the liquid primary standards and standard blank to the aluminum foil box and place them back into the refrigerator.
5. Make a copy of the completed data sheets. Put the original data sheet in the chlorophyll-a file in room 002. Place the copy of the data sheet in the “Chl to be entered" file in the in-basket next to the computer in room 002.
6. Fill out the chlorophyll log book in room 019A.
5.3.3.1 Procedure for Diluting Off-Scale Samples

If the fluorometer reads “over” the affected sample must be diluted and re-analyzed using the procedure below:

1. Obtain chlorophyll-a test tubes, caps and test tube rack from the cabinet above the fluorometer and the 1.0 mL pipette from room 018.
2. Mix the over range sample by inverting the sample vial several times.
3. Set the over range sample in a rack with a clean empty vial in front of the existing sample.
4. Pipette 1.0 mL of the over range sample into the clean vial.
5. Add 5.0 mL of 90% acetone to the diluted sample using the acetone repipette.
   a. Be sure to check the calibration of the repipettor as described in Section 5.3.2 Step 6.
6. Cap the vial and shake it.
7. Indicate on the data sheet which samples have been diluted and how.
   a. Record sample dilutions on the data sheet in the following manner: 1.0 mL sample + 5.0 mL acetone.
8. Read the samples on the fluorometer as described in Section 5.3.3.
9. If the sample is still over range repeat the dilution procedure using 1 mL of the diluted sample and 5.0 mL of 90% acetone following steps 2 – 8.

5.3.3.2 Alternate Procedure for off-scale samples

Use this procedure if it is known that a large number of samples (ie: greater than 10 samples) will be off-scale due to high chlorophyll-a concentrations.

1. Recalibrate the fluorometer. Calibration instructions are found in Section 5.2.4.
   a. During the recalibration when asked for the maximum range, press 9 to change and enter 1000. This will double the linear range of the fluorometer (which means you will be able to read concentrations twice as high).
   b. Note on the project data sheet that the instrument was calibrated at the higher level.
2. Run the samples as usual (refer to Section 5.3.3).
3. At the end of the run either re-calibrate using the 500 maximum range or leave a note on fluorometer indicating the new setting.
5.3.4 Washing Chlorophyll-a Vials

Although the vials are disposable, they are washed to allow for re-use using the following procedure:

1. Allow acetone to evaporate from vials in the fume hood.
2. Soak vials and caps in soapy water. *Do not acid soak.*
3. Use a brush to gently scrub the vials (vials break easily).
4. Rinse vials with tap water, then 3 times with DI water.
5. Store vials inverted in a test tube rack to dry.
6. Dry caps inverted on a paper towel.

6.0 CALCULATIONS

The equation below is used to determine the final chlorophyll-a concentration of a single undiluted sample. All data are reported to one decimal place. Values less than 0.2 µg/L chlorophyll-a are reported as <0.2 µg/L chlorophyll-a.

\[
\text{Chlorophyll-a (µg/L)} = \frac{(\text{Fo})(\text{Fs}) \times \text{Extraction volume (mL)} \times \text{High Solid Calibrant (Initial)}}{\text{Filtered volume (mL)} \times \text{High Solid Calibrant (Analysis day)}}
\]

\(\text{Fo}\) = Sample reading from the fluorometer (for an undiluted sample).
\(\text{Fs}\) = Concentration of the liquid standard divided by the mean fluorometric reading. Since the fluorometer reading is set to the value of the liquid standard, \(\text{Fs} = 1\) by definition.

**Extraction volume** = Volume (mL) of 90% acetone used to extract chlorophyll-a from frozen filters, assumed to be 5 mL

**Filtered Volume** = Volume (mL) of sample water filtered through the filter, variable but usually 50 mL, entered on spreadsheet for each sample.

**High Solid Calibrant (Initial)** = Average fluorometric reading of the *high solid secondary standard on day of instrument calibration* (this information is found on the Chlorophyll Calibration Record data sheet).

**High Solid Calibrant (Analysis day)** = Average fluorometric reading of the *high solid secondary standard on day that samples are analyzed*.

If the sample was diluted prior to being analyzed on the fluorometer, then \(\text{Fo}\) must be calculated using the equation below. \(\text{Fo}\) is then used in the equation above to determine the final chlorophyll-a concentration in the sample. If the sample was not diluted then the \(\text{Fo}\) value reported on the chlorophyll-a data sheet can be used directly in the equation above.

\[
\text{Fo} = \frac{\text{Value recorded (µg/L)} \times \text{Sample volume used for dilution (mL)} + \text{acetone volume used for dilution (mL)}}{\text{Sample volume used for dilution (mL)}}
\]

Note: The sample volume used for dilution is typically 1 mL, and the acetone volume used for dilution is typically 5 mL.
7.0 REFERENCES


### Chlorophyll-a Daily Standards Record

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Chlorophyll-a Calibration Record

Calibration Data Sheet for liquid standards

filename:c://aawwexcel/labproc/chlorophyll/chl standards log.xls, 8/3/04 ltg

Standards are purchased from Turner Designs

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Shaded cell is the value "High Solid Calibrant (initial)" used in the calculation of chlorophyll-a

Range setting: _____

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### Data sheets for storage of chlorophyll-a samples

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<td>Smith &amp; Sayles Res.</td>
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## Chlorophyll-a Analysis Data Sheet

### Name of rack: __________________________

### Set up date: __________________________

### Sample diluted: __________

### How diluted: __________

### Analysis Date: __________________________

### Analysis Technician: __________________________

### Linear Range (set at calibration)

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<td>Fo1 / Fo2</td>
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<table>
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<th>31</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>Volume Filtered</td>
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<td>Fo1 / Fo2</td>
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</table>

### Comments:

Updated 7/25/03

#### File name:

C:/www/excel_pro/chlorophyll/chlorophyll data sheet.xls
# Standard Operating Procedure 013

**URIWW-SOP-8**

**Chloride Analysis**

**Date:** 6/09  
**Revision:** 4  
**Author:** Linda Green

## University of Rhode Island Watershed Watch

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1.0 PURPOSE AND DESCRIPTION

The purpose of this method is to determine the concentration of chloride in freshwater or Individual Sewage Disposal System (ISDS) samples. This method is not appropriate for estuarine or marine samples. Samples are filtered and then analyzed on an autoanalyzer using a colorimetric technique to determine chloride concentration. This method is appropriate for undiluted samples ranging from less than 5 to 50 mg/L Cl⁻ and samples diluted to return values in this range.

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

General laboratory good housekeeping procedures should be practiced as outlined in Standard Operating Procedure (SOP) 001 – General Laboratory Safety.

ISDS samples should be treated as a potential biological hazard. All personnel handling ISDS samples should wear a laboratory coat, gloves and goggles.

Several chemicals are utilized in this procedure. Two of the chemicals have minimal hazards: sodium chloride (NaCl) and the Brij-35% surfactant solution. Sodium chloride may cause eye irritation on contact. The Brij-35% solution is not listed for a specific hazard. General safe handling practices should be used when working with both these chemicals.

The following chemicals should be used in the laboratory hood only. Technicians working with these chemicals must wear laboratory goggles, a laboratory coat and gloves. The fume funnel above the autoanalyzer must be turned on before using the mercuric thiocyanide reagent.

Nitric Acid (HNO₃) is corrosive and can cause severe burns to exposed body parts.

Methanol (CH₃OH) is a flammable liquid that should be kept away from all open flames. Exposure of the skin and eyes may cause irritation. Methanol should be used in the hood as inhalation of the vapors may cause irritation and damage to the nervous system.

Mercuric thiocyanide is extremely toxic, it may be fatal if swallowed. It is harmful if inhaled or absorbed through the skin and causes irritation and burns to skin, eyes and respiratory tract. Extreme caution should be exercised when using this chemical.

Ferric nitrate nonahydrate (Fe(NO₃)₃·9H₂O) is an oxidizer that can cause severe burns to eyes and skin. Inhalation of material may cause respiratory irritation.
Further information regarding chemicals utilized in this procedure is found in the laboratory Material Safety Data Sheet (MSDS) binder found in the laboratory where the chemical of interest is stored. General laboratory information regarding safe handling of chemicals is located in SOP 001a – University Safety and Waste Handling.

Waste Reagents
Waste reagents must be collected for later disposal by URI Safety and Health.

2.2 Technician Training/Qualifications

General training in laboratory technique and use of the Astoria-Pacific Model 303a Astoria® Analyzer (autoanalyzer) must be completed prior to analyzing samples using this method. Technician training will be provided by Linda Green, Laboratory Project Manager – Nutrients. No Technician is allowed to use the autoanalyzer without the permission of Linda Green.

3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astoria-Pacific Model 303a Astoria® Analyzer (autoanalyzer)</td>
<td>Equipped with a 480 nm filter for analysis of chloride</td>
<td></td>
</tr>
<tr>
<td>Personal computer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigerator</td>
<td>Set at 4 °C</td>
<td></td>
</tr>
<tr>
<td>Ultrapure water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drying oven</td>
<td>Capable of weighing to 0.0001 g</td>
<td></td>
</tr>
<tr>
<td>Balance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 mL HDPE plastic bottles</td>
<td>Sample storage</td>
<td></td>
</tr>
<tr>
<td>Brinkman pipettes and tips</td>
<td>0-100 µl and 100-1000 µl</td>
<td></td>
</tr>
<tr>
<td>10 – 200/250 mL Class A volumetric flasks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 to 4 – 1L Class A volumetric flasks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitric acid (HNO₃)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol (CH₃OH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mercuric Thiocyanide (Hg(SCN)₂)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferric nitrate nonahydrate (Fe(NO₃)₃·9H₂O)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brij-35% solution surfactant</td>
<td>Available from Astoria-Pacific</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External standards</td>
<td>Obtained from AccuStandard or other outside vendor</td>
<td></td>
</tr>
<tr>
<td>Millipore AP40 glass fiber filters (GFF) for TCLP</td>
<td>Gelman Type A/E</td>
<td>Millipore #AP4002500</td>
</tr>
</tbody>
</table>

Chloride Analysis SOP 013
filename S:\WW\awwword\LABPROC\all QAPPs\LABQAPPs\QAPP Rev5 -0609\SOPs\SOP 013 chloride.doc
<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter forceps</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filter housings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 mL Syringe</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory. Temporary replacement of the autoanalyzer may be made through arrangement with Astoria-Pacific. Other equipment may be obtained through arrangement with other scientists in the department maintaining similar equipment.

### 4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (Ambient and ISDS)</td>
<td>120 mL acid washed plastic bottle</td>
<td>Filtered and then frozen</td>
<td>10 mL</td>
<td>1 year</td>
</tr>
</tbody>
</table>

**Disposal**

Samples may be disposed of only after final quality assurance checks are completed and the data found to be acceptable using criteria found in Section 5.2 of this SOP. Samples may be disposed of by rinsing down the drain with water. ISDS samples are considered a potential biological hazard, a laboratory coat, gloves and goggles should be worn when disposing of these samples.

### 5.0 METHOD DESCRIPTION

#### 5.1 Scheduling Considerations

Preparation of the stock standard, if necessary, must be completed at least two days prior to sample analysis. Check to be sure enough color reagent is available. If necessary prepare more reagent using instructions found in the Astoria-Pacific manual.

Remove samples to be analyzed from the freezer and place in the refrigerator to thaw at least 1 day prior to planned analysis.

#### 5.2 Quality Assurance/Quality Control

##### 5.2.1 Method Detection Limit

The Method Detection Limit (MDL) for this assay is determined annually and has been 0.3 mg/L chloride (Cl⁻) or less. The Limit of Quantitation or Reporting Limit (RL) for this assay is 3 mg/L Cl⁻ or lower. The upper limit of reporting is 50 mg/L Cl⁻ without dilution and is set by the upper standard. Results are reported to the nearest whole number. Updated MDL and RL values are calculated on an annual basis, check with the Laboratory Manager for the most up-to-date values.
5.2.2 Method Blanks

Method blanks consist of Ultrapure water treated as a sample. A method blank is analyzed after every 15 samples. This is approximately 6% of the samples analyzed. Method blanks must not be greater than 2 mg/L Cl⁻.

Corrective Action
If any method blank is found to be greater than 2 mg/L Cl⁻ then the method blank is re-analyzed. If the method blank is still greater than 2 mg/L Cl⁻ then the instrument is re-calibrated and any samples analyzed between the accepted method blank and the unacceptable method blank are re-analyzed.

5.2.3 Sample Replication

Sample replication is completed in two ways. The autoanalyzer analyzes two aliquots from every sample cup for Cl⁻. The relative percent deviation (%RPD) between the values obtained from the same sample cup must not be greater than 15%.

If the samples being analyzed originated from an ISDS project then two aliquots of the same sample are poured into two separate autoanalyzer cups for 100% of the samples. The %RPD for values obtained for the same sample in two separate cups is 20%.

If the samples being analyzed originated from an ambient water site then 10% (1 sample in 10) are analyzed in duplicate cups. The %RPD must not be greater than 20% for these samples.

%RPD is calculated as follows:

\[ \%\text{RPD} = \left| \frac{\text{Result of Replicate 1 (µg/L)} - \text{Result of Replicate 2 (µg/L)}}{\text{Average of Result of Replicate 1 (µg/L) and Result of Replicate 2 (µg/L)}} \right| \times 100 \]

Corrective Action
If the %RPD is greater than 15% for an analysis from the same sample cup or greater than 20% when comparing sample values between duplicates placed in different sample cups then the sample is re-analyzed. If the sample replicates are still not within 15%RPD and 20%RPD and the instrument check standards are within the acceptable Quality Assurance/Quality Control guidelines then the deviation is noted on the project data sheet and the run continued.

5.2.4 Calibration

Instrument standardization is completed by determining the absorbance of a series of known working standards. The autoanalyzer software performs a standard linear regression analysis of the standard concentration versus the absorbance and returns a standard equation for a line \( y = mx + b \) and a graph. The autoanalyzer is standardized with a minimum of 6 working standards including a blank (see Section 5.3.1.2). An acceptable linear regression for a calibration sequence will have a coefficient of determination \( R^2 \) value of at least 0.990.

Corrective Action
If the \( R^2 \) value returned by the standardization procedure is less than 0.990 due to only 1 standard, then the standard may be removed and the calibration accepted. If the \( R^2 \) value is less than 0.990 due to more than 1 standard, then the autoanalyzer is re-calibrated. No
samples are analyzed until an acceptable $R^2$ value is recorded. Trouble shooting information is located in the autoanalyzer manual.

5.2.5 Calibration Check/Laboratory Control Standards

Environmental Protection Agency Water Pollution Proficiency Test Study
The laboratory participates in the Environmental Protection Agency Water Pollution Proficiency Test Study on a yearly basis. Unknown samples are purchased from an outside vendor and analyzed for chloride. URIWW results are sent to the vendor who provides a comparison to the actual value and a performance evaluation. Study results are maintained by the laboratory.

Daily Calibration Check
External standards are purchased from an outside vendor and are analyzed as samples during each analysis batch at a ratio of 2% of the samples run (1-2 standards per 90 samples). The percent difference ($\%D$) must not be greater than 20% for the external standards. External standards are prepared by diluting the purchased high concentration standard with UltraPure water to a value approximately at the middle of the range of the standards being used for calibration.

Check calibrants consisting of the middle standard from the standards used to calibrate the instrument are run at a ratio of 1 check standard per 15 samples, or approximately 7% of the samples analyzed. The $\%D$ must not be greater than 20% for the check standards.

Percent difference is calculated as follows:

$$\%D = \frac{\text{Reported value}}{\text{Known value}} \times 100$$

Corrective Action
If the $\%D$ is greater than 20% for either an external standard or check calibrant then the standard is re-analyzed. If the $\%D$ is still greater than 20% then the instrument is re-calibrated and the samples analyzed between the acceptable check calibrant or external standard and the unacceptable check calibrant or external standard are re-analyzed.

5.2.6 Matrix Spikes

Matrix spikes are used to verify that the chemical and physical characteristics of the samples being analyzed are not sufficiently different from the standards used in calibration to cause the analyte of interest to respond differently in the sample when compared to the calibrant.

There are two main ways to prepare matrix spikes. The more commonly used method is to pour a water sample into a volumetric flask and add a small amount of a highly concentrated standard. The amount of standard added allows the final solution to be approximately double the original concentration of the sample. The second method, used by URIWW involves measuring a known volume of water sample into an autoanalyzer cup, adding a known volume of a high calibrant or standard to the cup and calculating the expected final concentration. In both cases, using the Astoria-Analyzer system the spike concentration is calculated and entered in the CC/QC area. The unspiked sample is run, immediately followed by the spiked sample, and the autoanalyzer system calculates the spike recovery, which should be between 80 – 120%. Sample spikes are run on 30% of samples or one sample in 30.
The percent recovery is calculated as follows:

\[
\% \text{ recovery} = \frac{\text{SR-UR}[V3/(V1+V3)]}{(V1 \times C1)/(V3+V1)}
\]

Where:
- C1 = concentration of spiking solution
- V3 = volume of unspiked sample (=1900 ul)
- V1 = volume of spike solution (=100 ul)
- SR = spiked result
- UR = unspiked result

The matrix spike procedure utilized by URIWW is based on 1/1992 Astoria Pacific Technical Bulletin Appendix which states that “If higher dilutions of the sample matrix by the spike solution have been demonstrated not to change the matrix significantly, a rapid procedure of spiking directly into the sample cup can be used … an appropriate volume and concentration of spike solution is accurately pipetted to into the cup then mixed and analyzed.” This approach is also based on personal communication with Allen Clement, Astoria-Pacific International Technical Support, June 2006.

**Corrective Action**

If the % recovery for a spiked sample is not between 80 and 120% the sample and associated sample matrix spike will be re-analyzed. If the value is still not within the acceptable range but all other spiked samples are within QA/QC limits and calibration checks are also acceptable then the sample data will be flagged, but no further action will be taken. If more than one spiked sample is not within acceptable percent recovery then additional samples will be spiked to determine if the sample matrix is causing interference. Appropriate corrective action will be determined in consultation with the laboratory manager.

### 5.3 Analysis Method

#### 5.3.1 Preparation

At least two days prior to analysis, check to be sure enough color reagent is available. If the reagent must be prepared refer to the Astoria-Pacific manual for instructions.

Preparation of standards takes two days to complete, therefore check to determine if it is necessary to prepare new stock standard prior to the expected sample analysis date.

Move samples to be analyzed from the freezer to the refrigerator at least a day before analysis, to allow them time to thaw.

This method utilizes several types of water. Ultrapure water refers to highly purified water which is prepared by further purifying DI water using the URIWW Aries-Vaptronics system. SOP 002 – Laboratory Water provides information regarding the different types of laboratory water available and the methods to obtain each type of water.

**Glassware preparation for preparation of standards**

All glassware used to prepare standards must be soapy water washed, DI water rinsed, 10% HCl soaked overnight and then soaked in Ultrapure water overnight. Never use glassware that has just come from acid soaking. It must equilibrate in DI water first.
5.3.1.1 Preparation of 1000 ppm NaCl Stock Solution

1. Dry approximately 5 g of sodium chloride (NaCl) at 140 °C (284 °F) in the drying oven for 4 hours.
   a. After it has been dried place it into a desiccator immediately. It should be stored in the desiccator until use.
   b. Note: Na = 22.99 g Na/mole NaCl
      Cl = 35.45 g Cl/mole NaCl
      NaCl = 58.44 g/mole
      Ratio of NaCl/Cl = 58.44/35.45 = 1.648
      1.648 g NaCl is equal to 1.0 g Cl.

2. Check the calibration of the balance using the 1 g weight. Use a balance that weighs to at least 0.0001 g.
   a. The calibration weights are located in the drawer under the balance. Never touch calibration weights with anything but forceps. The oils on fingers may change the weight of the standard.
   b. Values returned by the balance should be within 10% of the actual calibration weight value. If a deviation of greater than 10% is noted then the balance will be serviced and re-calibrated. The balance should not be used in this procedure.

3. Refer to the table below to prepare varying amounts of the stock standard. Dilute to the final volume using Ultrapure water. This solution requires no special preservative and will last indefinitely.

<table>
<thead>
<tr>
<th>Mass of dried NaCl (g)</th>
<th>Final Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.648 g</td>
<td>1000</td>
</tr>
<tr>
<td>0.824 g</td>
<td>500</td>
</tr>
<tr>
<td>0.412g</td>
<td>250</td>
</tr>
</tbody>
</table>

5.3.1.2 Preparation of 100 mL of Working Standards

1. Check the calibration of the balance using the 1 g weight. Use a balance that weighs to at least 0.0001 g.
   a. The calibration weights are located in the drawer under the balance. Never touch calibration weights with anything but forceps. The oils on fingers may change the weight of the standard.
   b. Values returned by the balance should be within 10% of the actual calibration weight value. If a deviation of greater than 10% is noted then the balance will be serviced and re-calibrated. The balance should not be used in this procedure.
2. Calibrate the pipette. The nominal volume on the pipettes is not always accurate. Adjust pipette as needed and recheck the calibration with each change in pipette delivery volume.

   a. Pipettes are calibrated by setting the pipette at the desired volume and the pipetting this amount of Ultrapure water onto a weighting dish on the calibrated balance. The density of water at room temperature is essentially 1, therefore the weight of the delivered Ultrapure water in mg equals the volume delivered in µl.

   b. If the volume delivered is not the desired volume, adjust the pipette and repeat the check of volume until it is correct. Recheck the pipette with each change in pipette delivery volume.

3. Fill the 100 mL volumetric flasks part way with Ultrapure water. Use appropriate sized and calibrated micropipette to add stock solution to the volumetric flask, according to chart below.

   **Preparation of Working Standards**

   Note: All information contained in this table is for the preparation of working standards in 100 mL volumetric flasks.

<table>
<thead>
<tr>
<th>Desired Concentration (mg/L or ppm)</th>
<th>Volume of 1000 mg/L stock to add to 100 mL volumetric flask</th>
<th>Dilution Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µl</td>
<td>mL</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>1000</td>
<td>1.0</td>
</tr>
<tr>
<td>15</td>
<td>1500</td>
<td>1.5</td>
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<td>20</td>
<td>2000</td>
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<td>25</td>
<td>2500</td>
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<td>30</td>
<td>3000</td>
<td>3.0</td>
</tr>
<tr>
<td>40</td>
<td>4000</td>
<td>4.0</td>
</tr>
<tr>
<td>50</td>
<td>5000</td>
<td>5.0</td>
</tr>
</tbody>
</table>

4. Bring the volumetric flask to volume with Ultrapure water.

5. Cover the volumetric flask with parafilm and mix by inverting the flask.

6. Allow the standard to sit at least 1/2 hr. before using.

7. Store working standards in the 4 °C refrigerator.

5.3.2 Procedure – Day of Analysis

   5.3.2.1 Sample Filtration

Samples may arrive in the laboratory as filtered or unfiltered samples. Unfiltered samples must be filtered immediately using Millipore AP40 GFF for TCLP filters. After samples are filtered they must be placed in the freezer until they are analyzed. The procedure for filtering samples is described below:

1. Locate previously cleaned bottle(s) that will be used to hold the filtered water. Label the bottle with the sample location, sample depth, date of sample collection and the text “filtered”.
2. Prepare a filter housing by placing one glass fiber filter, gridded side down, onto the metal screen on the bottom of the filter assembly.

3. Always use the tweezers to handle filters.

4. Place the black rubber gasket on top of the filter, and screw the filter holder back together.

5. Shake the unfiltered sample bottle well.

6. Use a syringe to draw up approximately 20 mL of sample into the syringe. Use this water to rinse the syringe and then discard the water.

7. Take apart the syringe by pulling the plunger all the way out.

8. Attach the filter holder to the syringe.

9. Mix the sample bottle again and obtain the sample to be filtered by pouring water from the bottle into the syringe (with the filter holder facing down).
   a. Pour approximately 50 mL of water from the sample bottle into the syringe.
   b. At least 50 mL of water must be left in the sample bottle as this remaining water will be used for other assays.
   c. The exact volume filtered is not important unless the filter is to be utilized for chlorophyll-a analysis (see SOP 012 – Chlorophyll-a Analysis, Welschmeyer Method).

10. Attach the syringe plunger, and slowly push the water through the filter housing with even pressure catching the filtered water in the bottle labeled “filtered”.

11. If the filter becomes clogged, remove the filter and start the procedure over with another aliquot of sample.

12. After filtering the water take the filter holder off the syringe and unscrew the two halves. Discard the filter unless it is to be used for chlorophyll-a analysis.

13. Take apart the syringe.

14. Rinse all apparatus with DI water. Place upside down on a paper towel to dry.

5.3.2.2 Matrix Spikes

Matrix spikes are completed on 30% of samples. Use the procedure outlined below to prepare matrix spikes:

1. Use a matrix spike solution that is 500 ppm Cl⁻, instructions for making the solution are below.

2. Check and confirm the calibration of the following pipettes and volumes on the Mettler 0.5 mg balance using Ultrapure water:
   a. 100 µl (yellow tip Brinkmann)
   b. 900 µl (black Eppendorf, blue tip)
   c. 1000 µl (blue tip Brinkmann)

3. Select a water sample to spike that is known to have detectable results and is not at or below the limit of detection. Ideally it should be at the low end of mid-range. Remember that Cl⁻ is on-scale up to 40-50 ppm.
4. First fill a cup with the unspiked sample. Put on sample rack.
5. Next pipette 1900 µl of the sample into another 2 ml sample cup.
6. Add 100 µl of the 500 ppm spike solution to the 1900 µl of sample (instructions for making spike solution are below). This will give you a solution that is spiked with 25 ppm Cl\(^-\). In other words with 100% recovery your spiked samples will be 25 ppm Cl\(^-\) higher than the unspiked sample. (See below for more details.)
7. Mix using pipette. Put the spiked sample on the rack right after the unspiked sample.
8. (Optional) Set up a replicate spiked cup.
9. To use the autoanalyzer matrix spike automatic calculation, edit the chloride method. This doesn’t have to be repeated each time, once you have set it up.
   a. Go to edit for your chloride method
   b. Go to Channel Properties, CC/QC tab
   c. Enter SPK1 for spike number
   d. Enter 25 for spike concentration
10. The matrix spike result will be printed on the sample run report, right after the spiked sample.
11. Calculate results as an alternative to the AP software method.
   a. See 06 AMM 1st tri may 03.xls for the formula in excel to cut and paste, remembering to use appropriate cells for the calculation.
   b. Calculation is also below:

\[
\% \text{ recovery} = \frac{\{\text{SR}-\text{UR}\} \times (V3+V1)}{\{V1 \times C1\} \times (V3+V1)}
\]

Excel formula:
\[
= \frac{\{\text{SR}(V3+V1) \} - (\text{UR} \times V3)}{(V1 \times C1)} \times 100
\]

Where:
- \(C1\) = concentration of spiking solution
- \(V3\) = volume of unspiked sample (=1900 µl)
- \(V1\) = volume of spike solution (=100 µl)
- \(SR\) = spiked result
- \(UR\) = unspiked result

**Matrix spike solution for Chloride**

These instruction allow for the creation of 100 ml of matrix spike solution that is 500 ppm Cl\(^-\)

1. Fill a 100 ml volumetric flask about ½ full with Ultrapure water
2. Pipette in 50 ml of chloride stock solution (it is 1,000 ppm Cl\(^-\))
   a. \(df=2\) (100 ml / 50 ml)
   b. final Cl\(^-\) concentration is 1000/2 = 500 ppm Cl\(^-\)
3. Fill volumetric flask to the line with MQ water, shake repeatedly to mix

**To spike your water sample (also above)**

1. Pipette 1000 µl of the water sample into a 2 ml autoanalyzer sample cup
2. Pipette 100 µl of the chloride spike solution into the water sample in the sample cup
3. Pipette 900 µl water sample (more of the same one) into sample cup
4. Mix by using the pipette used in (3) to withdraw and push back in mixed sample/spike solution in sample cup. Repeat 3-4 times.
5. **Remember to place the matching unspiked sample right before the spiked sample in the sample tray!**

### 5.3.2.3 Sample Analysis

Detailed instructions regarding operation of the Astoria-Pacific automated spectrometer (autoanalyzer) will not be repeated here. This information is located in the autoanalyzer operation manuals. A summary of the analytical procedure is found below:

1. Allow all filtered samples, standards and blanks to warm to room temperature. This takes place while the autoanalyzer is being prepared.
2. Obtain reagents needed for the analysis. The chloride analysis only utilizes a color reagent. This reagent should be allowed to warm to room temperature prior to use. The color reagent contains mercuric thiocyanide, a highly toxic chemical. The fume funnel above the autoanalyzer must be on before this reagent (color reagent) is used.
   a. Lab personnel must wear gloves, lab coat and eye protection when using this reagent. Waste reagents must be collected for later disposal by URI Safety and Health.
   b. Further information regarding this reagent is located in the Laboratory MSDS binder. Further information regarding health and safety is located in SOP 001 – General Laboratory Safety and SOP 001a – University Safety and Waste Handling Document.
3. A summary of Autoanalyzer Operation is provided below:
   a. A calibrated volume of sample is introduced into the autoanalyzer by the sampler.
   b. The sample is delivered to the analytical cartridge by a peristaltic pump. It is then combined with reagents and air bubbles in a continuously moving stream.
   c. As the sample moves through the cartridge it passes through mixing coils and a heating bath, as specified by test conditions.
   d. A color is produced by the specific analyte in the sample. The intensity of the color is determined by the amount of analyte present.
   e. This colored stream then flows to the photometer where the color intensity is measured and converted to an electronic signal. The electronic signal is sent to a computer where the Astoria-Pacific FASPaC II® software program processes the signal to create a standard curve and numerical results for the standards and samples as well as a real-time recorder tracing of peaks.
4. Each autoanalyzer rack has 90 places for samples, blanks and QC samples. A sample run can have up to three racks (270 samples).
   a. Standards are set up on their own rack. A set of standards is run at the beginning of each run. A separate set of standards is run at the end, and are averaged with those run at the beginning.
b. A check calibrant and blank are run after every 15 samples and are used to monitor the run (see Section 5.2 for further information).

c. Each sample and standard are recorded on a sample log sheet as they are poured into analytical sample cups. 1 sample out of 15 is analyzed in duplicate (two separate analyzer sample cups).

d. The autoanalyzer is set to analyze each sample cup twice.

5. The autoanalyzer is connected to a personal computer. The Astoria-Pacific FASPac II® program analyzes the data reported by the autoanalyzer. The program performs a linear regression on the standards and determines sample concentrations.

   a. A graph of concentration vs. absorbance of standards is displayed and can be manipulated to remove obvious outliers.

   b. The standard curve is typically rejected if $R^2$ is less than 0.95 unless this is due to 1 standard in the suite. (see Section 5.2 for further information)

   c. During the run the real time peak tracing are monitored to check for in-range heights, proper shapes and any irregularities, so that samples can be re-analyzed, space permitting during the current run, or saved for re-analysis in a later run.

   d. At the end of a run each peak is checked to make sure the marker is in the proper spot and any irregularities are accounted for (analytical blips, empty sample cups, etc). Once each peak in a run has been reviewed, the results of the run are exported and saved as Excel files, and printed.

6. After the computer results are printed, they are again compared to the peak tracing, particularly to take note of carryover of high to low peaks and correct any keyboarding errors.

7. QC results are compared to previous results as well as "true values". If the results of the run meet the QA/QC criteria set forth in this SOP then the results of the run are deemed acceptable.

8. After the printout has been approved the data are entered into appropriate Excel spreadsheet files, where it is subsequently re-checked for data entry errors.

9. After the data is approved samples may be disposed of in accordance with Section 4.0.

10. Sample bottles are cleaned by acid washing following SOP 003 – General Labware Cleaning Procedure.

6.0 CALCULATIONS

Chloride concentrations are calculated in the Astoria Pacific FASPac II program from the average of standard curves. Data are reported to the nearest whole number. Values less than the reporting limit are reported as $<$ (numerical value of the) RL mg/L Cl$^-$. 
7.0 REFERENCES

Method referenced: Chloride (4500-Cl⁻) and Automated Ferricyanide Method (4500-Cl⁻ E).


8.0 DOCUMENTATION

Data consisting of the sample results, peaks and calibration curves are exported from the computer running the autoanalyzer into Excel data files. The excel files are then printed and saved electronically. Data are reported to the nearest whole number.
### Sample Log Sheet

<table>
<thead>
<tr>
<th>Pos#</th>
<th>Contents &amp; Date</th>
<th>DF</th>
<th>Pos#</th>
<th>Contents &amp; Date</th>
<th>DF</th>
<th>Pos#</th>
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</tbody>
</table>

Check Calibrant # & concentration (CC# _____) = ______ ug/l

---

**File/Run Name:** Analyst:

**Method Name:** Source of Standards:

**Date:** Conc. Range of Standards:

**Analysis of/in:** % light: ref ch1 ch2

**File Name:** c:\awwexcel\labproc\RFArelated\Astoria Analyzer sample cup log
# Standard Operating Procedure 014
(Prior number URIWW-SOP-3)

## Ammonia Analysis

**University of Rhode Island Watershed Watch**

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1.0 PURPOSE AND DESCRIPTION

This method determines the concentration of ammonia in an ambient (river, pond, lake), marine or Individual Sewage Disposal System (ISDS) samples. Samples are filtered and then analyzed on an autoanalyzer using a colorimetric technique to determine ammonia concentration. Results are reported as µg of ammonia (NH₃) as Nitrogen (N) per Liter. This method is appropriate for undiluted samples ranging from less than 40 to 2000 µg/L NH₃-N and samples diluted to return values in this range.

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

General laboratory good housekeeping procedures should be practiced as outlined in Standard Operating Procedure (SOP) 001 – General Laboratory Safety.

ISDS samples should be treated as a potential biological hazard. All personnel handling ISDS samples should wear a laboratory coat, gloves and goggles.

Several chemicals are utilized in this procedure that should be treated with extreme caution. Technicians working with these chemicals must wear laboratory goggles, a laboratory coat and gloves. The fume funnel above the autoanalyzer must be turned on before using the phenol reagent or the sodium nitroferricyanide reagent. The following chemicals should be used only in the laboratory funnel ventilation or laboratory hood.

Liquid phenol (carbolic acid) (C₆H₅OH) – Phenol is corrosive and toxic. Phenol may cause irritation or burns to skin, eyes, respiratory and digestive tract if exposed. Phenol is readily absorbed through the skin and may be fatal if inhaled, absorbed through the skin or swallowed. Phenol exposure may cause liver and kidney damage and central nervous system depression. Phenol is a mutagen, hygroscopic (absorbs moisture from the air) and light sensitive. Liquid phenol and phenol vapors are combustible.

Sodium nitroferricyanide (sodium nitroprusside dihydrate), (Na₄Fe(CN)₆(NO)·2H₂O) – Sodium nitroferricyanide is toxic. It may cause eye, skin and respiratory tract irritation on contact. It is harmful if swallowed, inhaled, or absorbed through the skin. Contact with acids liberates hydrogen cyanide, an extremely toxic gas.

The following chemicals should be treated with caution. Ammonia sulfate ((NH₄)₂SO₄), sodium citrate dihydrate (Na₃C₆H₅O₇·2H₂O) and sodium potassium tartrate (NaKC₄H₄O₆·4H₂O) may cause eye, skin and respiratory tract irritation upon exposure. Sodium hydroxide (NaOH) and sodium hypochlorite (bleach) (NaOHCl) are corrosive and may cause burns to exposed body
parts and eyes. Sulfuric Acid (H₂SO₄) is corrosive and may burn exposed body parts and eyes. The Brij-35% surfactant solution is not listed for a specific hazard. General safe handling practices should be used when working with this chemical.

Further information regarding chemicals utilized in this procedure is found in the laboratory Material Safety Data Sheet (MSDS) binder found in the laboratory where the chemical of interest is stored. General laboratory information regarding safe handling of chemicals is located in SOP 001a – University Safety and Waste Handling.

Waste Reagents
Waste reagents must be collected for later disposal by URI Safety and Health.

2.2 Technician Training/Qualifications

General training in laboratory technique and use of the Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer (autoanalyzer) must be completed prior to analyzing samples using this method. Technician training will be provided by Linda Green, Laboratory Project Manager – Nutrients. No Technician is allowed to use the autoanalyzer without the permission of Linda Green.

3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astoria®-Analyzer Model 303A</td>
<td>Equipped with a 630 nm filter for analysis of ammonia-nitrogen</td>
<td></td>
</tr>
<tr>
<td>Personal computer</td>
<td></td>
<td></td>
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<tr>
<td>Refrigerator</td>
<td>Set at 4 °C</td>
<td></td>
</tr>
<tr>
<td>Balance</td>
<td>Capable of weighing to 0.0001 g</td>
<td></td>
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<tr>
<td>Source of Ultrapure water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brinkman adjustable pipettes</td>
<td>0-100 µL and 100-1000 µL</td>
<td></td>
</tr>
<tr>
<td>10 200-250 mL Class A volumetric flasks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-4 1 L Class A volumetric flasks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plastic bottles</td>
<td>Sample storage 120 mL HDPE plastic bottles</td>
<td></td>
</tr>
<tr>
<td>Ammonia sulfate ((NH₄)₂SO₄)</td>
<td>Primary Standard</td>
<td></td>
</tr>
<tr>
<td>Brij-35% solution surfactant</td>
<td></td>
<td>Alpkem Inc.</td>
</tr>
<tr>
<td>Liquid phenol (carbolic acid) (C₆H₅OH)</td>
<td></td>
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<tr>
<td>Sodium citrate dihydrate (Na₃C₆H₅O₇·2H₂O)</td>
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<tr>
<td>Sodium hydroxide (NaOH)</td>
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<tr>
<td>Sodium hypochlorite (bleach) (NaOHCl)</td>
<td></td>
<td>Purchased locally (CVS or supermarket)</td>
</tr>
</tbody>
</table>


Required Material | Notes | Re-order information
--- | --- | ---
Sodium nitroferricyanide (sodium nitroprusside dehydrate) (Na₂Fe(CN)_5(NO)·2H₂O) |  | 
Sodium potassium tartrate (NaK₃C⁴H₄O₆·4H₂O) |  | 
Sulfuric acid (H₂SO₄) |  | 
Sodium chloride (NaCl) | For preparation of Artificial Seawater needed to analyze marine samples | 
Magnesium sulfate (MgSO₄) | For preparation of Artificial Seawater needed to analyze marine samples | 
Sodium bicarbonate (NaHCO₃) | For preparation of Artificial Seawater needed to analyze marine samples | 

Spare Equipment
Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory. Temporary replacement of the autoanalyzer may be made through arrangement with Astoria-Pacific. Other equipment may be obtained through arrangement with scientists in the Natural Resources Science department maintaining similar equipment.

4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>120 mL acid washed plastic bottle</td>
<td>Filtered and frozen</td>
<td>20 mL</td>
<td>12 months</td>
</tr>
</tbody>
</table>

Disposal
Samples may be disposed of only after final quality assurance checks are completed and the data found to be acceptable using criteria found in Section 5.2 of this SOP. Samples may be disposed of by rinsing down the drain with water. ISDS samples are considered a potential biological hazard, a laboratory coat, gloves and goggles must be worn when disposing of these samples.

5.0 METHOD DESCRIPTION

5.1 Scheduling Considerations

The amount of primary ammonia standard previously prepared should be checked at least two days prior to the date of expected sample analysis. If necessary, prepare the primary standard as discussed in Section 5.3.1.1. Approximately 100 mL of the primary standard is necessary to prepare the working standards.

Samples to be analyzed should be removed from the freezer and placed in the refrigerator to thaw at least a day before the date of expected sample analysis.
The available volume of each reagent utilized in this analysis should also be assessed at least two days prior to the date of expected sample analysis. Four reagents are needed for the NH$_3$ analysis: the sodium phenate solution, sodium hypochlorite solution, EDTA reagent and sodium nitroprusside solution. Additionally, Artificial Seawater is used as a rinse, carrier and to prepare standards when analyzing marine samples. If necessary, prepare more reagent using instructions found in the Astoria-Pacific manual.

### 5.2 Quality Assurance/Quality Control

#### 5.2.1 Method Detection Limit

The Method Detection Limit (MDL) is set at 3.0 µg/L NH$_3$-N. The Limit of Quantitation or Reporting Limit (RL) is set at 30 µg/L NH$_3$-N. The maximum concentration reported without dilution is 2000 µg/L NH$_3$-N for ISDS samples and 1000 µg/L NH$_3$-N for ambient and marine samples, based on the highest calibration standard. Values reported for NH$_3$-N are reported to the nearest 10 (i.e.: 152 µg/L NH$_3$-N is reported as 150 µg/L NH$_3$-N and 66 µg/L NH$_3$-N is reported as 70 µg/L NH$_3$-N). Updated MDL and RL values are calculated on an annual basis, check with the Laboratory Manager for the most up-to-date values.

#### 5.2.2 Method Blanks

Method blanks consist of Ultrapure water treated as a sample for all samples except marine samples. Method blanks for marine samples consist of Artificial Seawater treated as a sample. A method blank is analyzed after every 15 samples; this corresponds to 6% of the samples analyzed. Method blanks must not be greater than 30 µg/L NH$_3$-N.

**Corrective Action**

If any method blank is found to be greater than 30 µg/L NH$_3$-N then the method blank is re-analyzed. If the method blank is still greater than the acceptable value then the instrument is recalibrated and any samples analyzed between the accepted method blank and the unacceptable method blank are re-analyzed.

#### 5.2.3 Sample Replication

Sample replication is completed in two ways. The autoanalyzer analyzes two aliquots from every sample cup for NH$_3$. The relative percent deviation (%RPD) between the values obtained from the same sample cup must not be greater than 15%.

If the samples being analyzed originated from an ISDS project then two aliquots of the same sample are poured into two separate autoanalyzer cups for 100% of the samples. The %RPD for values obtained for the same sample in two separate cups is 20%.

If the samples being analyzed originated from an ambient water or marine site then 10% (1 sample in 10) are analyzed in duplicate cups. The %RPD must not be greater than 20% for these samples.

%RPD is calculated as follows:

\[
%RPD = \left| \frac{\text{Result of Replicate 1 (µg/L)} - \text{Result of Replicate 2 (µg/L)}}{\text{Average of Result of Replicate 1 (µg/L) and Result of Replicate 2 (µg/L)}} \right| \times 100
\]
Corrective Action
If the %RPD is greater than 15% for an analysis from the same sample cup or greater than 20% when comparing sample values between duplicates placed in different sample cups then the sample is re-analyzed. If the sample replicates are still not within 15%RPD and 20%RPD and the instrument check standards are within the acceptable Quality Assurance/Quality Control guidelines then the deviation is noted on the project data sheet and the run continued.

5.2.4 Calibration

Instrument standardization is completed by determining the absorbance of a series of known working standards. The autoanalyzer software performs a standard linear regression analysis of the standard concentration versus absorbance and returns a standard equation for a line \( y = mx + b \) and a graph. The autoanalyzer is standardized with a minimum of 4 working standards and a blank (see Section 5.3.1.2). An acceptable linear regression for a calibration sequence will have a coefficient of determination \( (R^2) \) value of at least 0.990.

Corrective Action
If the \( R^2 \) value returned by the standardization procedure is less than 0.990 due to only 1 standard, then the standard may be removed and the calibration accepted. If the \( R^2 \) value is less than 0.990 due to more than 1 standard, then the autoanalyzer is re-calibrated. No samples are analyzed until an acceptable \( R^2 \) value is recorded. Trouble shooting information is located in the autoanalyzer manual.

5.2.5 Calibration Check/Laboratory Control Standard

Environmental Protection Agency Water Pollution Proficiency Test Study
The laboratory participates in the Environmental Protection Agency Water Pollution Proficiency Test Study on a yearly basis. Unknown samples are purchased from an outside vendor and analyzed for NH₃. URIWW results are sent to the vendor who provides a comparison to the actual value and a performance evaluation. Study results are maintained by the laboratory.

Daily Calibration Check
External standards are purchased from an outside vendor and are analyzed as samples during each analysis batch at a ratio of 2% of the samples run (1-2 standards per 90 samples). The percent difference (%D) must not be greater than 20% for the external standards. External standards are prepared by diluting the purchased high concentration standard with either UltraPure water (all samples except marine samples) or Artificial Seawater (for marine samples) to a value approximately at the middle of the range of the standards being used for calibration.

Check calibrants consisting of the middle standard from the standards used to calibrate the instrument are run at a ratio of 1 check calibrant per 15 samples, or approximately 7% of the samples analyzed. The %D must not be greater than 20% for the check calibrant.

Percent difference is calculated as follows:

\[
\%D = \frac{\text{Reported value} \times 100}{\text{Known value}}
\]
Corrective Action
If the %D is greater than 20% for either an external standard or check calibrant then the standard is re-analyzed. If the %D is still greater than 20% then the instrument is re-calibrated and the samples analyzed between the acceptable check calibrant or external standard and the unacceptable check calibrant or external standard are re-analyzed.

5.2.6 Matrix Spikes

Matrix spikes are used to verify that the chemical and physical characteristics of the samples being analyzed are not sufficiently different from the standards used in calibration to cause the analyte of interest to respond differently in the sample when compared to the calibrant.

There are two main ways to prepare matrix spikes. The more commonly used method is to pour a water sample into a volumetric flask and add a small amount of a highly concentrated standard. The amount of standard added allows the final solution to be approximately double the original concentration of the sample. The second method, used by URIWW involves measuring a known volume of water sample into an autoanalyzer cup, adding a known volume of a high calibrant or standard to the cup and calculating the expected final concentration. In both cases, using the Astoria-Analyzer system the spike concentration is calculated and entered in the CC/QC area. The unspiked sample is run, immediately followed by the spiked sample, and the autoanalyzer system calculates the spike recovery, which should be between 80 – 120%. Sample spikes are run on 30% of samples or one sample in 30.

The percent recovery is calculated as follows:

\[
\text{% recovery} = \frac{\{\text{SR-UR}\} \{\text{V3}/(\text{V1+V3})\}}{\{(\text{V1} \times \text{C1})/(\text{V3+V1})\}}
\]

Where:
- C1 = concentration of spiking solution
- V3 = volume of unspiked sample (=1900 ul)
- V1 = volume of spike solution (=100 ul)
- SR = spiked result
- UR = unspiked result

The matrix spike procedure utilized by URIWW is based on 1/1992 Astoria Pacific Technical Bulletin Appendix which states that “If higher dilutions of the sample matrix by the spike solution have been demonstrated not to change the matrix significantly, a rapid procedure of spiking directly into the sample cup can be used … an appropriate volume and concentration of spike solution is accurately pipetted to into the cup then mixed and analyzed.” This approach is also based on personal communication with Allen Clement, Astoria-Pacific International Technical Support, June 2006.

Corrective Action
If the % recovery for a spiked sample is not between 80 and 120% the sample and associated sample matrix spike will be re-analyzed. If the value is still not within the acceptable range but all other spiked samples are within QA/QC limits and calibration checks are also acceptable then the sample data will be flagged, but no further action will be taken. If more than one spiked sample is not within acceptable percent recovery then additional samples will be spiked to determine if the sample matrix is causing interference. Appropriate corrective action will be determined in consultation with the laboratory manager.
5.3 Analysis Method

5.3.1 Preparation

At least 2 days before analysis, the amount of each reagent needed for the analysis should be checked. Four reagents are needed for the NH₃ analysis: the sodium phenate solution, sodium hypochlorite solution, EDTA reagent and sodium nitroprusside solution. If necessary, prepare more reagent using instructions found in the Astoria-Pacific manual. Additionally, if the samples are from a marine system, Artificial Seawater will need to be prepared, instructions are located in the Astoria Pacific manual “Saline Water Automated Analysis of Sea, Estuarine, and Brackish Water”.

Preparation of the primary standard takes two days to complete, therefore check to determine if it is necessary to prepare new primary standard prior to the expected sample analysis date. The primary standard can be stored for up to 6 months. Working standards are replaced after 2 months.

Samples to be analyzed should be removed from the freezer and placed in the refrigerator to thaw at least a day before the date of expected sample analysis.

This method utilizes several types of water. Ultrapure water refers to highly purified water which is prepared by further purifying deionized (DI) water using the URIWW Aries-Vaponics system. SOP 002 – Laboratory Water provides information regarding the different types of laboratory water available and the method to obtain water.

Glassware preparation for preparation of standards
All glassware utilized for standard preparation must be soapy water washed using non-phosphorus containing detergent, DI water rinsed, 10% HCl soaked overnight and then soaked in Ultrapure water overnight.

Never use glassware that has just come from acid soaking; it must equilibrate in Ultrapure water first.

5.3.1.1 Preparation of 100 mg/L NH₃-N Primary Ammonia Standard

1. Store standard grade ammonium sulfate ((NH₄)₂SO₄) in the desiccator.
   a. The formula weight of (NH₄)₂SO₄ is 132.14 g/ mole
   b. There are 2 moles of N per 1 mole of (NH₄)₂SO₄ for a total nitrogen mass of 28.02 g N per 1 mole (NH₄)₂SO₄.
2. The 100 mg/L (ppm) primary NH₃ standard is prepared using the following formula:
   
   $$100 \text{ mg/L primary NH}_3 \text{ standard} = 0.4716 \text{ g (NH}_4\text{)}_2\text{SO}_4 \text{ per liter}$$

   $$100 \text{ ppm N} \times \text{formula weight (NH}_4\text{)}_2\text{SO}_4 = 0.1 \text{ g/L} \times 132.14 \text{ g} = 0.4716 \text{ g (NH}_4\text{)}_2\text{SO}_4 / \text{L}$$

   Weight of N in (NH₄)₂SO₄ = 28.02 g
3. Check the calibration of the balance using the 1 g weight. Use a balance that weighs to at least 0.0001 g.
   a. The calibration weights are located in the drawer under the balance. Never touch the calibration weights with anything but forceps. The oils on the fingers may change the weight of the standard.
b. Values returned by the balance should be within 10% of the actual calibration weight value. If a deviation of greater than 10% is noted then the balance will be serviced and re-calibrated. If the balance is found to be outside of the acceptable calibration range then it will not be used in this procedure.

4. To prepare varying amounts of the primary standard refer to the table below. Dilute to the final volume using Ultrapure water.

a. Note: 100 mg/L NH$_3$-N = 100 µg/mL NH$_3$-N = 100 ppm NH$_3$-N

<table>
<thead>
<tr>
<th>Mass of (NH$_4$)$_2$SO$_4$ to be added to flask (g)</th>
<th>Final Volume (mL)</th>
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</thead>
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<td>0.4716</td>
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</tr>
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<td>0.2358</td>
<td>500</td>
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<td>0.0943</td>
<td>100</td>
</tr>
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</table>

5. Cover the volumetric flask with parafilm and mix by inverting the flask at least 30 times.

6. Place a label on the flask indicating the contents of the flask, date of preparation and the initials of the technician preparing the standard.

7. The primary standard is stable for 6 months after which it should be discarded. It is stored in the refrigerator in the volumetric flask it was prepared in.

8. The primary standard must be allowed to sit for 24 hours prior to use.

### 5.3.1.2 Preparation of Working Standards

1. Check the calibration of the balance using the procedure outlined in Section 5.3.1.1.

2. Calibrate the adjustable pipette. The nominal volume on the pipette is not always accurate. Adjust the pipette as needed and recheck the calibration with each change in pipette delivery volume.

   a. Pipettes are calibrated by setting the pipette at the desired volume and pipetting this amount of Ultrapure water onto a weighting dish on a calibrated balance.

   b. The density of water at room temperature is essentially 1 g/mL, therefore the weight of the delivered volume of Ultrapure water in g equals the volume delivered in mL. If the volume delivered is not the desired volume, adjust the pipette and repeat the check of volume until it is correct. Recheck the pipette with each change in pipette delivery volume.

3. Fill the 100 or 200 mL volumetric flasks to be used to prepare working standards part way with Ultrapure water or Artificial Seawater. Artificial seawater is used when making standards for analysis of marine samples; Ultrapure water is used for all other sample types. Use an appropriately sized and calibrated adjustable pipette to add stock solution to the volumetric flask, according to chart below.
a. Generally, the following standards are prepared for ISDS samples: 0, 250, 500, 1000, 1500 and 2000 µg/L NH₃-N.

b. Generally, the following standards are prepared for ambient and marine samples: 0, 25, 50, 125, 250, and 500 µg/L NH₃-N.

## Preparation of Working Standards

<table>
<thead>
<tr>
<th>Desired Concentration (µg/L NH₃-N)</th>
<th>Volume of primary NH₃ standard to add to 100 mL volumetric flask (µL)</th>
<th>Volume of primary NH₃ standard to add to 200 mL volumetric flask (µL)</th>
<th>Dilution Factor</th>
</tr>
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<tbody>
<tr>
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<tr>
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</tr>
<tr>
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<td>1500</td>
<td>3000 µL, (3 mL)</td>
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</tr>
<tr>
<td>2000</td>
<td>2000</td>
<td>4000 µL, (4 mL)</td>
<td>50</td>
</tr>
</tbody>
</table>

4. Bring the volumetric flask to volume with Ultrapure or Artificial Seawater, as appropriate.
5. Cover the volumetric flask with parafilm and mix by inverting the flask at least 30 times.
6. Place a label on the flask indicating the contents of the flask, date of preparation and the initials of the technician preparing the standard.
7. Allow the standard to sit at least 1/2 hr. before using.
8. Store working standards in the 4 °C refrigerator.
9. Working standards are replaced after 1 week.

### 5.3.2 Procedure – Day of Analysis

#### 5.3.2.1 Sample Filtration

Samples may arrive in the laboratory as filtered or unfiltered samples. Unfiltered samples must be filtered immediately using Millipore AP40 GFF for TCLP filters. After samples are filtered they must be placed in the freezer until they are analyzed. The procedure for filtering samples is described below:

1. Locate previously cleaned plastic bottle(s) that will be used to hold the filtered water. Label the bottle with the sample location, sample depth, date of sample collection and the text “filtered”.
2. Prepare a filter housing by placing one glass fiber filter, gridded side down, onto the metal screen on the bottom of the filter assembly.
3. Always use the tweezers to handle filters.
4. Place the black rubber gasket on top of the filter, and screw the filter holder back together.
5. Shake the unfiltered sample bottle well.

6. Use a syringe to draw up approximately 20 mL of sample into the syringe. Use this water to rinse the syringe and then discard the water.

7. Take apart the syringe by pulling the plunger all the way out.

8. Attach the filter holder to the syringe.

9. Mix the sample bottle again and obtain the sample to be filtered by pouring water from the bottle into the syringe (with the filter holder facing down).
   a. Pour approximately 50 mL of water from the sample bottle into the syringe.
   b. At least 50 mL of water must be left in the sample bottle as this remaining water will be used for other assays.
   c. The exact volume filtered is not important unless the filter is to be utilized for chlorophyll-a analysis (see SOP 012 – Chlorophyll-a Analysis, Welschmeyer Method).

10. Attach the syringe plunger, and slowly push the water through the filter housing with even pressure catching the filtered water in the bottle labeled “filtered”.

11. If the filter becomes clogged, remove the filter and start the procedure over with another aliquot of sample.

12. After filtering the water take the filter holder off the syringe and unscrew the two halves. Discard the filter unless it is to be used for chlorophyll-a analysis.

13. Take apart the syringe.

14. Rinse all apparatus with DI water. Place upside down on a paper towel to dry.

---

5.3.2.2 Matrix Spikes

Matrix spikes are completed on 30% of samples. Use the procedure outlined below to prepare matrix spikes:

1. Use a matrix spike solution that is 10,000 ppb NH$_3$-N, instructions for making the solution are below.

2. Check and confirm the calibration of the following pipettes and volumes on the Mettler 0.5 mg balance using Ultrapure water:
   a. 100 µl (yellow tip Brinkmann)
   b. 900 µl (black Eppendorf, blue tip)
   c. 1000 µl (blue tip Brinkmann)

3. Select a water sample to spike that is known to have detectable results and is not at or below the limit of detection. Ideally it should be at the low end of mid-range.

4. First fill a cup with the unspiked sample. Put on sample rack.

5. Next pipette 1900 µl of the sample into another 2 ml sample cup.

6. Add 100 µl of the 10,000 ppb spike solution to the 1900 µl of sample (instructions for making spike solution are below). This will give you a solution that is spiked with 500 ppb NH$_3$-N. In other words with 100% recovery your spiked samples will be 500 ppb NH$_3$-N higher than the unspiked sample. (See below for more details.)
7. Mix using pipette. Put the spiked sample on the rack right after the unspiked sample.

8. (Optional) Set up a replicate spiked cup.

9. To use the autoanalyzer matrix spike automatic calculation, edit the ammonia method. This doesn't have to be repeated each time, once you have set it up.
   a. Go to edit for your ammonia method
   b. Go to Channel Properties, CC/QC tab
   c. Enter SPK1 for spike number
   d. Enter 500 for spike concentration

10. The matrix spike result will be printed on the sample run report, right after the spiked sample.

11. Calculate results as an alternative to the AP software method.
   a. See 06 AMM 1st tri may 03.xls for the formula in excel to cut and paste, remembering to use appropriate cells for the calculation.
   b. Calculation is also below:

% recovery = \[ \frac{\{SR-UR\}(V3/(V1+V3))}{[(V1 x C1)/(V3+V1)]} \]

Excel formula:
\[ = \frac{\{SR(V3+V1)\} - (UR x V3)}{(V1 x C1)} \times 100 \]
\[ = 100 \times \left\{ \frac{SR(2000) - UR(1900)}{100C1} \right\} \]

Where:
C1 = concentration of spiking solution
V3 = volume of unspiked sample (=1900 ul)
V1 = volume of spike solution (=100 ul)
SR = spiked result
UR = unspiked result

Matrix spike solution for Ammonia

These instructions allow for the creation of 100 ml of matrix spike solution that is 10,000 ppm NH₃-N.

1. Fill a 100 ml volumetric flask about ½ full with Ultrapure water
2. Pipette in 10 ml of ammonia stock solution (it is 100 ppm NH₃-N)
   a. df = 10 = 100 ml / 10 ml
   b. final NH₃-N concentration is 100 ppm/10 = 10 ppm NH₃-N = 10,000 ppb NH₃-N
3. Fill volumetric flask to the line with MQ water, shake repeatedly to mix

To spike your water sample (also above)

1. Pipette 1000 µl of the water sample into a 2 ml autoanalyzer sample cup
2. Pipette 100 µl of the ammonia spike solution into the water sample in the sample cup
3. Pipette 900 µl water sample (more of the same one) into sample cup
4. Mix by using the pipette used in (3) to withdraw and push back in mixed sample/spike solution in sample cup. Repeat 3-4 times.
5. Remember to place the matching unspiked sample right before the spiked sample in the sample tray!
5.3.2.3  Sample Analysis

Detailed instructions regarding operation of the Astoria-Pacific automated spectrometer (autoanalyzer) will not be repeated here. This information is located in the autoanalyzer operation manuals. A summary of the analytical procedure is found below:

1. Allow all filtered samples, standards and blanks to warm to room temperature. This takes place while the autoanalyzer is being prepared.

2. Obtain reagents needed for the analysis. The NH$_3$ assay utilizes four reagents: the sodium phenate solution, sodium hypochlorite solution, EDTA reagent and sodium nitroprusside solution. If analyzing marine samples, Artificial Seawater will be needed as well. All reagents should be allowed to warm to room temperature prior to use. Remember that this procedure involves the use of 2 highly toxic reagents, phenol and nitroferricyanide. The fume funnel above the autoanalyzer must be on before using these reagents.
   a. Lab personnel must wear gloves, lab coat and eye protection when handling reagents. Waste reagents must be collected for later disposal by URI Safety and Health.
   b. Further information regarding reagents is located in the Laboratory MSDS binder. Further information regarding health and safety is located in SOP 001 – General Laboratory Safety and SOP 001a – University Safety and Waste Handling Document.

3. A summary of Autoanalyzer Operation is provided below:
   a. A calibrated volume of sample is introduced into the autoanalyzer by the sampler.
   b. The sample is delivered to the analytical cartridge by a peristaltic pump. It is then combined with reagents and air bubbles in a continuously moving stream.
   c. As the sample moves through the cartridge it passes through mixing coils and a heating bath, as specified by test conditions.
   d. A color is produced by the specific analyte in the sample, blue for the NH$_3$ assay. The intensity of the color is determined by the amount of analyte present.
   e. This colored stream then flows to the photometer where the color intensity is measured and converted to an electronic signal. The electronic signal is sent to a computer where the Astoria-Pacific FASPac II® software program processes the signal to create a standard curve and numerical results for the standards and samples as well as a real-time recorder tracing of peaks.

4. Each autoanalyzer rack has 90 places for samples, blanks and QC samples. A sample run can have up to three racks (270 samples).
   a. Standards are set up on their own rack. A set of standards is run at the beginning of each run. A separate set of standards is run at the end, and are averaged with those run at the beginning.
   b. A check calibrant and blank are run after every 15 samples and are used to monitor the run (see Section 5.2 for further information).
c. Each sample and standard is recorded on a sample log sheet as they are poured into analytical sample cups.

d. 1 sample out of 10 is analyzed in duplicate for ambient and marine samples and every sample is analyzed in duplicate for ISDS samples (two separate analyzer sample cups).

e. The autoanalyzer is set to analyze each sample cup twice.

5. The autoanalyzer is connected to a personal computer. The Astoria-Pacific FASPac II® program analyzes the data reported by the autoanalyzer. The program performs a linear regression on the standards and determines sample concentrations.

a. A graph of concentration verses absorbance of standards is displayed and can be manipulated to remove obvious outliers.

b. The standard curve is rejected if $R^2$ is less than 0.95 unless this is due to 1 standard in the suite. (see Section 5.2 for further information.)

c. During the run the real time peak tracing are monitored to check for in-range heights, proper shapes and any irregularities, so that samples can be re-analyzed, space permitting during the current run, or saved for re-analysis in a later run.

d. At the end of a run each peak is checked to make sure the marker is in the proper spot and any irregularities are accounted for (analytical blips, empty sample cups, etc). Once each peak in a run has been reviewed, the results of the run are exported and saved as Excel files, and printed.

6. After the computer results are printed, they are again compared to the peak tracing, particularly to take note of carryover of high to low peaks and correct any keyboarding errors.

7. QC results are compared to previous results as well as "true values". If the results of the run meet the QA/QC criteria set forth in this SOP then the results of the run are deemed acceptable.

8. After the printout has been approved, the data are entered into appropriate Excel spreadsheet files, where they are subsequently re-checked for data entry errors.

9. After the data are approved samples may be disposed of in accordance with Section 4.0.

10. Sample bottles are cleaned by acid washing following SOP 003 – General Labware Cleaning Procedure

6.0 CALCULATIONS

Ammonia is calculated in the Astoria Pacific FASPac II program from the average of standard curves. Data are reported to the nearest 10 for NH$_3$-N. Values less than the reporting limit are reported as < (numerical value of the) RL µg/L NH$_3$-N.
7.0 REFERENCES


8.0 DOCUMENTATION

Data consisting of the sample results, peaks and calibration curves are exported from the computer running the autoanalyzer into Excel data files. The excel files are then printed and saved electronically.
## Sample Log Sheet

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<td></td>
<td></td>
<td>44</td>
<td></td>
<td></td>
<td>59</td>
<td></td>
<td></td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td></td>
<td>45</td>
<td></td>
<td></td>
<td>60</td>
<td></td>
<td></td>
<td>75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Check Calibrant # & concentration(CC# ___) = ______ ug/l
Orthophosphate & Nitrate + Nitrite Analysis

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1.0 PURPOSE AND DESCRIPTION

This method is for the simultaneous colorimetric analysis of dissolved reactive phosphorus (DRP) and nitrate + nitrite (NO$_3$+NO$_2$) in filtered ambient (river, pond, lake), marine or Individual Sewage Disposal System (ISDS) samples through analysis on an autoanalyzer. The method may also be utilized for the analysis of just DRP or NO$_3$+NO$_2$. If marine samples are being analyzed, they are run with ambient samples; artificial seawater is not used as a carrier nor for preparation of standards and blanks. NO$_3$+NO$_2$ results for marine samples run in this manner will be accurate, but PO$_4$ results will not be accurate, and are therefore not reported. The method outlined here is the same as the method for the determination of total phosphorus and total nitrogen with the following exceptions:

- Water samples are filtered
- Water samples are not digested prior to analysis

DRP is a measure of orthophosphate (PO$_4$) as well as a small amount of condensed phosphate (pyro-, tripoly, etc. phosphate) that is hydrolyzed by the procedure. Since the amount of condensed phosphate hydrolyzed is generally considered low, the terms dissolved reactive phosphorus and orthophosphate are often interchanged. In this SOP the term orthophosphate or PO$_4$ will be used to represent the DRP analysis. Results are reported as µg of orthophosphate as Phosphorus (PO$_4$-P) per liter.

The NO$_3$+NO$_2$ analysis is run simultaneously with that of the PO$_4$ analysis. The NO$_3$+NO$_2$ method determines the total concentration of NO$_3$ plus NO$_2$ in a sample. Generally, the concentration of NO$_2$ in a sample is low. During the analytical procedure NO$_3^-$ is converted to NO$_2^-$ which then reacts with method reagents to form a color, the intensity of which is related to concentration. Therefore, the only way to determine NO$_3$ solely is to first determine NO$_2$ and subtract this from the NO$_3$ + NO$_2$ value. Results are reported as µg of Nitrate + Nitrite as Nitrogen (NO$_3$/NO$_2$-N) per liter.

This method is appropriate for undiluted samples ranging from less than 30 to 2000 µg/L NO$_3$/NO$_2$-N and less than 4 to 200 µg/L PO$_4$-P as well as samples diluted to return values in this range.
2.0 HEATH AND SAFETY CONSIDERATIONS

2.1 Hazards

General laboratory good housekeeping procedures should be practiced as outlined in Standard Operating Procedure (SOP) 001 – General Laboratory Safety.

ISDS samples should be treated as a potential biological hazard. All personnel handling ISDS samples should wear a laboratory coat, gloves and goggles.

Several chemicals are utilized in this procedure. Note that ammonium hydroxide, chloroform, sulfuric acid and phosphoric acid must be used in the hood. Specific hazards of each chemical are listed under the notes section of the required materials table (Section 3.0) of this SOP. When using any chemical general safety procedures should be followed and technicians must wear goggles, gloves and a laboratory coat.

Further information regarding chemicals utilized in this procedure is found in the laboratory Material Safety Data Sheet (MSDS) binder found in the laboratory where the chemical of interest is stored. General laboratory information regarding safe handling of chemicals is located in SOP 001a – University Safety and Waste Handling.

Waste Reagents
Waste reagents must be collected for later disposal by URI Safety and Health.

2.2 Technician Training/Qualifications

General training in laboratory technique and use of the Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer (autoanalyzer) must be completed prior to analyzing samples using this method. Technician training will be provided by Linda Green, Laboratory Project Manager – Nutrients. No Technician is allowed to use the autoanalyzer without the permission of Linda Green.

3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>105 °C drying oven</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astoria®-Analyzer Model 303A</td>
<td>Equipped with an 880 nm filter and 540 nm filter for analysis of</td>
<td></td>
</tr>
<tr>
<td>Segmented Continuous Flow Autoanalyzer (autoanalyzer)</td>
<td>PO₄ and NO₃+NO₂, respectively.</td>
<td></td>
</tr>
<tr>
<td>Personal computer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigerator</td>
<td>Set to 4 °C</td>
<td></td>
</tr>
<tr>
<td>Analytical balance</td>
<td>Capable of weighing to 0.0001 g</td>
<td></td>
</tr>
<tr>
<td>Ultrapure water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Brinkman pipettes</td>
<td>1-100 µL and 100-1000 µL</td>
<td></td>
</tr>
<tr>
<td>600-1000 mL acid-washed beaker, reserved for P use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required Material</td>
<td>Notes</td>
<td>Re-order information</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Cover glass, or Al foil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metal tray</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squeeze bottle containing Ultrapure water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-15 200-250 mL volumetric flasks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-4 1000 mL Class A volumetric flasks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 mL plastic HDPE bottles, acid washed</td>
<td>Sample storage</td>
<td></td>
</tr>
<tr>
<td><strong>Phosphorus reagents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium phosphate monobasic (KH₂PO₄)</td>
<td>Hygroscopic (absorbs moisture from the air). May cause eye, skin, respiratory and digestive tract irritation.</td>
<td></td>
</tr>
<tr>
<td>Antimony potassium tartrate trihydrate (C₈H₄K₂O₁₂Sb₂·3H₂O)</td>
<td>May cause eye, skin, respiratory and digestive irritation upon contact. Harmful if swallowed. May cause liver, kidney and heart damage.</td>
<td></td>
</tr>
<tr>
<td>Ammonium molybdate tetrahydrate ((NH₄)₆Mo₇O₂₄·4H₂O)</td>
<td>May cause eye, skin and respiratory tract irritation upon contact. May be harmful if swallowed.</td>
<td></td>
</tr>
<tr>
<td>L-Ascorbic acid (C₆H₈O₆)</td>
<td>Light sensitive. Air sensitive. May cause eye, skin and respiratory tract irritation. Strong reducing agent. Fire and explosion risk when in contact with oxidizing agents.</td>
<td></td>
</tr>
<tr>
<td>DowFax surfactant</td>
<td>May cause eye burns upon contact.</td>
<td>Available from Astoria-Pacific</td>
</tr>
<tr>
<td>Sulfuric acid, concentrated (H₂SO₄)</td>
<td>Will cause burns to exposed skin, eyes, respiratory and digestive tract.</td>
<td></td>
</tr>
<tr>
<td>Required Material</td>
<td>Notes</td>
<td>Re-order information</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Chloroform (CHCl₃)</td>
<td>May cause central nervous system depression. May cause cardiac disturbances. May cause cancer based on animal studies. This substance has caused adverse reproductive and fetal effects in animals. May be harmful if swallowed. Causes eye, skin and respiratory tract irritation. Light sensitive.</td>
<td></td>
</tr>
<tr>
<td><strong>Nitrogen Reagents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium nitrate (KNO₃)</td>
<td>Strong oxidizer. Contact with other materials may cause a fire. May cause respiratory tract, eye and skin irritation. May cause methemoglobinemia (negatively impacts hemoglobin). May cause kidney damage. Hygroscopic (absorbs moisture from the air). May be harmful if swallowed.</td>
<td></td>
</tr>
<tr>
<td>Ammonium chloride (NH₄Cl)</td>
<td>May cause skin, respiratory and digestive tract irritation. May be harmful if swallowed. Causes eye irritation. Hygroscopic (absorbs moisture from the air).</td>
<td></td>
</tr>
<tr>
<td>Disodium EDTA (Disodium ethylenediamine tetraacetic acid dihydrate) (C₁₀H₁₄N₂Na₂O₈·2H₂O)</td>
<td>Causes eye, skin and respiratory tract irritation. May be harmful if swallowed.</td>
<td></td>
</tr>
<tr>
<td>Ammonium hydroxide (NH₄OH)</td>
<td>Causes eye, skin, digestive and respiratory tract burns.</td>
<td></td>
</tr>
<tr>
<td>Cupric sulfate pentahydrate (CuSO₄·5H₂O)</td>
<td>Harmful if swallowed. Causes irritation and may burn the digestive tract, respiratory tract, eye and skin. Hygroscopic (absorbs moisture from the air). Possible sensitizer.</td>
<td></td>
</tr>
<tr>
<td>Sulfanilimide (C₆H₆N₂O₂S)</td>
<td>May cause reproductive and fetal effects. May cause eye, skin and respiratory tract irritation. May be harmful if swallowed or inhaled.</td>
<td></td>
</tr>
<tr>
<td>N-1-Napthylethylenediamine (NED) (N-(1-Naphthyl)ethylenediamine dihydrochloride) (C₁₀H₁₇NHCH₂CH₂NH₂ · HCl)</td>
<td>Will cause eye, skin and respiratory system irritation.</td>
<td></td>
</tr>
</tbody>
</table>
### Required Material

<table>
<thead>
<tr>
<th>Material</th>
<th>Notes</th>
<th>Re-order Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>2HCl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brij-35% solution surfactant</td>
<td></td>
<td>Available from Alpkem</td>
</tr>
<tr>
<td>Phosphoric acid (H₃PO₄)</td>
<td>Hydroscopic (absorbs moisture from the air). Will cause burns of exposed skin, eyes, respiratory and digestive tract.</td>
<td></td>
</tr>
</tbody>
</table>

### Spare Equipment

Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory. Temporary replacement of the autoanalyzer may be made through arrangement with Astoria-Pacific. Other equipment may be obtained through arrangement with other scientists in the Natural Resources Science department maintaining similar equipment.

### 4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>120 mL acid washed plastic bottles</td>
<td>Filtered and then frozen</td>
<td>20 mL</td>
<td>12 months</td>
</tr>
</tbody>
</table>

### Disposal

Samples may be disposed of only after final quality assurance checks are completed and the data found to be acceptable using criteria found in Section 5.2 of this SOP. Samples may be disposed of by rinsing down the drain with water. ISDS samples are considered a potential biological hazard, a laboratory coat, gloves and goggles should be worn when disposing of these samples.

### 5.0 METHOD DESCRIPTION

#### 5.1 Scheduling Considerations

The amount of primary and intermediate standard previously prepared should be checked at least two days prior to the date of expected sample analysis. If necessary, prepare the standards as discussed in Section 5.3.1. Approximately 100 mL of the primary standard is necessary to prepare the intermediate standard. Approximately 100 mL of the intermediate standard is necessary to prepare the working standards.

Samples to be analyzed should be removed from the freezer and placed in the refrigerator to thaw at least a day before the date of expected sample analysis.

The available volume of each reagent utilized in this analysis should be assessed. Two reagents are needed for the NO₃+NO₂ analysis: the ammonium chloride buffer and the color reagent. One reagent is necessary for the PO₄ analysis, this mixed reagent is prepared daily by addition of several other reagents: potassium antimonyl tartrate solution, ammonium molybdate solution, asorbic acid solution and sulfuric acid solution. The asorbic acid solution is only stable for 1 week. If necessary prepare more reagent using instructions found in the Astoria-Pacific manual.

#### 5.2 Quality Assurance/Quality Control
5.2.1 Method Detection Limit

The Method Detection Limit (MDL) is set at 0.45 µg/L PO₄-P for the orthophosphate assay and 2.3 µg/L NO₃/NO₂-N for the nitrate + nitrite assay. The Limit of Quantitation or Reporting Limit (RL) is set at 4.5 µg/L PO₄-P for the orthophosphate assay and 23 µg/L NO₃/NO₂-N for the nitrate + nitrite assay.

The upper RL or the maximum concentration reported by the orthophosphate assay without dilution is 200 µg/L PO₄-P for ambient and marine samples and 2000 µg/L PO₄-P for ISDS samples. The maximum concentration reported by the nitrate + nitrite assay without dilution is 2000 µg/L NO₃/NO₂-N for all samples. The upper RL is based on the maximum standard used to calibrate the autoanalyzer. Values reported for PO₄-P are reported to the nearest whole number, those reported for NO₃/NO₂-N are reported to the nearest 10’s (ie: 126 µg/L NO₃/NO₂-N is reported as 130 µg/L NO₃/NO₂-N and 44.3 µg/L PO₄-P is reported as 44 µg/L PO₄-P).

Updated MDL and RL values are calculated on an annual basis, check with the Laboratory Manager for the most up-to-date values.

5.2.2 Method Blanks

Method blanks consist of Ultrapure water treated as a sample. A method blank is analyzed after every 6 samples; this corresponds to 6% of the samples analyzed. Method blanks must not be greater than 2 µg/L PO₄-P or 20 µg/L NO₃/NO₂-N.

Corrective Action
If any method blank is found to be greater than 2 µg/L PO₄-P or 20 µg/L NO₃/NO₂-N then the method blank is re-analyzed. If the method blank is still greater than the acceptable value then the instrument is re-calibrated and any samples analyzed between the accepted method blank and the unacceptable method blank are re-analyzed.

5.2.3 Sample Replication

Sample replication is completed in two ways. The autoanalyzer analyzes two aliquots from every sample cup for both PO₄ and NO₃/NO₂. The relative percent deviation (%RPD) between the values obtained from the same sample cup must not be greater than 15%.

If the samples being analyzed originated from an ISDS project then two aliquots of the same sample are poured into two separate autoanalyzer cups for 100% of the samples. The %RPD for values obtained for the same sample in two separate cups is 20%.

If the samples being analyzed originated from an ambient water or marine site then 10% (1 sample in 10) are analyzed in duplicate cups. The %RPD must not be greater than 20% for these samples.

%RPD is calculated as follows:

\[
\%\text{RPD} = \left| \frac{\text{Result of Replicate 1 (µg/L)} - \text{Result of Replicate 2 (µg/L)}}{\text{Average of Result of Replicate 1 (µg/L) and Result of Replicate 2 (µg/L)}} \times 100
\]

Corrective Action
If the %RPD is greater than 15% for an analysis from the same sample cup or greater than 20% when comparing sample values between duplicates placed in different sample cups then the sample is re-analyzed. If the sample replicates are still not within 15%RPD and 20%RPD and the instrument check standards are within the acceptable Quality Assurance/Quality Control guidelines then the deviation is noted on the project data sheet and the run continued.

5.2.4 Calibration

Instrument standardization is completed by determining the absorbance of a series of known working standards. The autoanalyzer software performs a standard linear regression analysis of the standard concentration versus absorbance and returns a standard equation for a line ($y = mx + b$) and a graph. The autoanalyzer is standardized with a minimum of 5 working standards and a blank (see Section 5.3.1.3 and 5.3.1.5). An acceptable linear regression for a calibration sequence will have a coefficient of determination ($R^2$) value of at least 0.990.

Corrective Action

If the $R^2$ value returned by the standardization procedure is less than 0.990 due to only 1 standard, then the standard may be removed and the calibration accepted. If the $R^2$ value is less than 0.990 due to more than 1 standard, then the autoanalyzer is re-calibrated. No samples are analyzed until an acceptable $R^2$ value is recorded. Trouble shooting information is located in the autoanalyzer manual.

5.2.5 Calibration Check/Laboratory Control Standard

Environmental Protection Agency Water Pollution Proficiency Test Study

The laboratory participates in the Environmental Protection Agency Water Pollution Proficiency Test Study on a yearly basis. Unknown samples are purchased from an outside vendor and analyzed for PO$_4$ and NO$_3$+NO$_2$. The results of the analysis are compared to the actual value and a performance evaluation provided. Study results are maintained by the laboratory.

Daily Calibration Check

External standards are purchased from an outside vendor and are analyzed as samples during each analysis batch at a ratio of 2% of the samples run (1-2 standards per 90 samples). The percent difference (%D) must not be greater than 20% for the external standards. External standards are prepared by diluting the purchased high concentration standard with UltraPure water to a value approximately at the middle of the range of the standards being used for calibration.

Check calibrants consisting of the middle standard from the standards used to calibrate the instrument are run at a ratio of 1 check calibrant per 15 samples, or approximately 7% of the samples analyzed. The %D must not be greater than 20% for the check calibrant.

Percent difference is calculated as follows:

\[
\%D = \frac{\text{Reported value} \times 100}{\text{Known value}}
\]

Corrective Action

If the %D is greater than 20% for either an external standard or check calibrant then the standard is re-analyzed. If the %D is still greater than 20% then the instrument is re-calibrated.
and the samples analyzed between the acceptable check calibrant or external standard and the unacceptable check calibrant or external standard are re-analyzed.

5.2.6 Matrix Spikes

Matrix spikes are used to verify that the chemical and physical characteristics of the samples being analyzed are not sufficiently different from the standards used in calibration to cause the analyte of interest to respond differently in the sample when compared to the calibrant.

There are two main ways to prepare matrix spikes. The more commonly used method is to pour a water sample into a volumetric flask and add a small amount of a highly concentrated standard. The amount of standard added allows the final solution to be approximately double the original concentration of the sample. The second method, used by URIWW involves measuring a known volume of water sample into an autoanalyzer cup, adding a known volume of a high calibrant or standard to the cup and calculating the expected final concentration. In both cases, using the Astoria-Analyzer system the spike concentration is calculated and entered in the CC/QC area. The unspiked sample is run, immediately followed by the spiked sample, and the autoanalyzer system calculates the spike recovery, which should be between 80 – 120%. Sample spikes are run on 30% of samples or one sample in 30.

The percent recovery is calculated as follows:

\[
\% \text{ recovery} = \frac{SR-UR[V3/(V1+V3)]}{[(V1 \times C1)/(V3+V1)]}
\]

Where:
- \(C1\) = concentration of spiking solution
- \(V3\) = volume of unspiked sample (=1900 ul)
- \(V1\) = volume of spike solution (=100 ul)
- \(SR\) = spiked result
- \(UR\) = unspiked result

The matrix spike procedure utilized by URIWW is based on 1/1992 Astoria Pacific Technical Bulletin Appendix which states that “If higher dilutions of the sample matrix by the spike solution have been demonstrated not to change the matrix significantly, a rapid procedure of spiking directly into the sample cup can be used … an appropriate volume and concentration of spike solution is accurately pipetted to into the cup then mixed and analyzed.” This approach is also based on personal communication with Allen Clement, Astoria-Pacific International Technical Support, June 2006.

**Corrective Action**

If the % recovery for a spiked sample is not between 80 and 120% the sample and associated sample matrix spike will be re-analyzed. If the value is still not within the acceptable range but all other spiked samples are within QA/QC limits and calibration checks are also acceptable then the sample data will be flagged, but no further action will be taken. If more than one spiked sample is not within acceptable percent recovery then additional samples will be spiked to determine if the sample matrix is causing interference. Appropriate corrective action will be determined in consultation with the laboratory manager.

5.3 Analysis Method

5.3.1 Preparation
At least 2 days before analysis the amount of each reagent needed for the analysis should be checked. Two reagents are needed for the NO₃+NO₂ analysis: the ammonium chloride buffer and the color reagent. One reagent is necessary for the PO₄ analysis, this reagent is a mixture of several other reagents: potassium antimonyl tartrate solution, ammonium molybdate solution, ascorbic acid solution and sulfuric acid solution. The ascorbic acid solution is only stable for 1 week. If necessary, prepare more reagent using instructions found in the Astoria-Pacific manual.

Preparation of the primary standard takes two days to complete, therefore check to determine if it is necessary to prepare new primary standard prior to the expected sample analysis date. Primary and intermediate standards can be stored for up to 6 months. Working standards are replaced after 2 months.

Samples to be analyzed should be removed from the freezer and placed in the refrigerator to thaw at least a day before the date of expected sample analysis.

This method utilizes several types of water. Ultrapure water refers to highly purified water which is prepared by further purifying deionized (DI) water using the URIWW Aries-Vaponics system. SOP 002 – Laboratory Water provides information regarding the different types of laboratory water available and the method to obtain water.

Glassware preparation for preparation of standards
All glassware utilized for standard preparation must be soapy water washed using non-phosphorus containing detergent. DI water rinsed, 10% HCl soaked overnight and then soaked in Ultrapure water overnight.

Never use glassware that has just come from acid soaking; it must equilibrate in Ultrapure water first.

All phosphorus glassware should be dedicated to the phosphorus assay and not used for any other analyses.

5.3.1.1 Preparation of Primary Orthophosphate Standard (100 ppm)

1. The standards utilized for the PO₄ and NO₃+NO₂ analyses are the same as those used for SOP 016 – Total Phosphorus and Nitrogen Analysis.

2. Dry approximately 0.75 g of potassium phosphate (KH₂PO₄) in the drying oven (set at 105 °C) for 1 hour. After drying the chemical remove it from the oven and store it in a desiccator.
   a. Formula weight of KH₂PO₄ = 136.1 g / mole
   b. There is 1 mole of P per 1 mole of KH₂PO₄ for a total phosphorus mass of 30.97 g P / mole KH₂PO₄.

3. Check the calibration of the balance using the 1 g weight. Use a balance that weighs to at least 0.0001 g.
   a. The calibration weights are located in the drawer under the balance. Never touch the calibration weights with anything but forceps. The oils on the fingers may change the weight of the standard.
   b. Values returned by the balance should be within 10% of the actual calibration weight value. If a deviation of greater than 10% is noted then the balance will be
serviced and re-calibrated. If the balance is found to be outside of the acceptable calibration range then it will not be used in this procedure.

4. The 100 ppm primary PO₄ standard is prepared using the following formula:

\[
100 \text{ ppm P} \times \text{formula weight (KH₂PO₄)} = 0.1 \text{ g/L} \times 136.1 \text{ g} = 0.4394 \text{ g KH₂PO₄/L}
\]

Weight of P in KH₂PO₄ = 30.97 g

5. Therefore, to prepare 1 L of 100 mg/L primary PO₄ standard, place 0.4394 g of KH₂PO₄ into a 1 L class A volumetric flask.
   a. To prepare varying amounts of the primary standard refer to the table below.
   b. Dilute to the final volume using Ultrapure water.
   c. Note: This solution is 100 mg PO₄-P per L = 100 µg PO₄-P per mL = 100 ppm.

<table>
<thead>
<tr>
<th>Mass of (NH₄)₂PO₄ to be added to flask (g)</th>
<th>Final Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4394</td>
<td>1000</td>
</tr>
<tr>
<td>0.2127</td>
<td>500</td>
</tr>
<tr>
<td>0.1099</td>
<td>250</td>
</tr>
</tbody>
</table>

6. Dilute to volume using Ultrapure water.
7. To preserve the standard add 2 mL of chloroform per L using a glass pipette.
8. Place a piece of parafilm over the top of the volumetric flask and mix by inverting the flask at least 30 times.
9. Place a label on the flask indicating the contents of the flask, date of preparation and the initials of the technician preparing the standard.
10. The primary standard is stable for 6 months after which it should be discarded. It is stored in the refrigerator in the volumetric flask it was prepared in.
11. The primary standard must be allowed to sit for 24 hours prior to use.

5.3.1.2 Preparation of Intermediate Orthophosphate Standard (10 ppm)

1. Using a 100 mL class A volumetric pipette add 100 mL of the primary orthophosphate standard (100 ppm) to a 1 L class A volumetric flask.
   a. Do not place the volumetric pipette directly into the bottle containing the primary standard, place a little more than 100 mL of the primary standard into a small weigh dish or beaker and pipette from this container.
   b. Dispose of any unused primary standard by rinsing down the drain.
   c. Do not put the unused primary standard back into the primary standard storage bottle.
2. Use Ultrapure water to fill the volumetric flask to volume. This solution is 10 ppm (µg/mL or mg/L) or 10,000 ppb PO₄-P.
3. Place a label on the flask indicating the contents of the flask, date of preparation and the initials of the technician preparing the standard.

4. The intermediate standard is stable for 6 months after which it should be discarded. It is stored in the 4 °C refrigerator in the volumetric flask it was prepared in.

5.3.1.3 Preparation of Orthophosphate Working Standards

1. Note that working orthophosphate and nitrate/nitrite standards are made together in the same volumetric flasks, although the procedures are listed separately.

2. Check the calibration of the balance using the procedure outlined in Section 5.3.1.1.

3. Calibrate the adjustable pipette. The nominal volume on the pipette is not always accurate. Adjust the pipette as needed and recheck the calibration with each change in pipette delivery volume.
   a. Pipettes are calibrated by setting the pipette at the desired volume and pipetting this amount of Ultrapure water onto a weighting dish on a calibrated balance.
   b. The density of water at room temperature is essentially 1 g/mL, therefore the weight of the delivered volume of Ultrapure water in g equals the volume delivered in mL.
   c. If the volume delivered is not the desired volume, adjust the pipette and repeat the check of volume until it is correct.
   d. Recheck the pipette with each change in pipette delivery volume.

4. Fill the appropriately sized class A volumetric flasks to be used to prepare working standards part way with Ultrapure water. Use an appropriately sized and calibrated adjustable pipette to add intermediate stock solution to the volumetric flask, according to chart below.

5. Bring the volumetric flask to volume with Ultrapure water.

6. Cover each flask with parafilm and mix by inverting the flask at least 30 times.

7. Allow the standard to sit at least 1/2 hour before using.

8. Store working standards in the 4 °C refrigerator. Working standards are replaced every month.

<table>
<thead>
<tr>
<th>Ambient Water (lakes, ponds, streams) &amp; Marine</th>
<th>ISDS</th>
<th>Final Concentration of Standard (µg PO₄-P/L)</th>
<th>Ambient Water &amp; Marine (200 mL volumetric flasks)</th>
<th>ISDS Samples (100 mL volumetric flasks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards to prepare:</td>
<td></td>
<td>Volume of intermediate orthophosphate standard to use (µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X X</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>X</td>
<td>2.5</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>5</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>10</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>15</td>
<td>300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. The standards utilized for the PO₄ and NO₃+NO₂ analyses are the same as those used for SOP 016 – Total Phosphorus and Nitrogen Analysis.

2. Dry the approximate amount of primary standard grade potassium nitrate (KNO₃) needed for standard preparation in a drying oven set at 105 °C for 24 hours (refer to the table in Step 3 below). Once the material has dried store it in a desiccator.

3. Using a calibrated balance (see Section 5.3.1.1 for balance calibration procedure) weigh out the amount of KNO₃ needed to prepare the desired volume of primary nitrate/nitrite standard. This solution is 100 mg NO₃-N per L, 100 µg or 100 ppm.

Weight of KNO₃ needed for primary NO₃ standard based on volume prepared:

<table>
<thead>
<tr>
<th>KNO₃ (g)</th>
<th>Volume of Primary Standard Prepared (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7218</td>
<td>1000</td>
</tr>
<tr>
<td>0.3609</td>
<td>500</td>
</tr>
<tr>
<td>0.1805</td>
<td>250</td>
</tr>
<tr>
<td>0.0722</td>
<td>100</td>
</tr>
</tbody>
</table>

4. Dilute to volume using Ultrapure water.
5. Add 2 mL of chloroform per L of standard using a glass pipette.
6. Place a piece of parafilm over the top of the volumetric flask and mix by inverting the flask at least 30 times.
7. Place a label on the flask indicating the contents of the flask, date of preparation and the initials of the technician preparing the standard.
8. The primary standard is stable for 6 months after which it should be discarded. It is stored in the 4 °C refrigerator in the volumetric flask it was prepared in.
9. The primary standard must be allowed to sit for 24 hours prior to use.

5.3.1.5 Preparation of Working Nitrate/Nitrite Standards
1. The preparation of working nitrate/nitrite standards requires no intermediate stock solution.

2. Note that working orthophosphate and nitrate/nitrite standards are made together in the same volumetric flasks, although the procedures are listed separately.

3. Calibrate the balance (see Section 5.3.1.1 for balance calibration procedure).

4. Calibrate the adjustable pipette (see Section 5.3.1.3).

5. Fill the appropriate sized class A volumetric flasks to be used to prepare working standards part way with Ultrapure water. Use an appropriately sized and calibrated adjustable pipette to add stock standard each volumetric flask, according to chart below.

6. Brine volumetric flasks to volume with Ultrapure water.

7. Cover each flask with parafilm and mix by inverting each flask at least 30 times.

8. Allow the standards to sit at least 1/2 hour before using.

9. Store working standards in the 4 °C refrigerator in the volumetric flasks they were prepared in. Working standards are replaced every month.

### Preparation of Nitrate/Nitrite Working Standards

<table>
<thead>
<tr>
<th>Ambient Water (lakes, ponds, streams) &amp; Marine</th>
<th>ISDS</th>
<th>Final Concentration of Standard (µg NO₃-N/L)</th>
<th>Ambient Water &amp; Marine (200 mL volumetric flasks)</th>
<th>ISDS Samples (100 mL volumetric flasks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards to prepare:</td>
<td></td>
<td>Volume of nitrate/nitrite primary standard to use (µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>25</td>
<td>50</td>
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<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>75</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
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<td>200</td>
<td></td>
</tr>
<tr>
<td>X</td>
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<td>500</td>
<td>250</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>500</td>
<td>1000 (1.0 mL)</td>
<td>500</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>1000</td>
<td>2000 (2.0 mL)</td>
<td>1000 (1.0 mL)</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>1500</td>
<td>3000 (3.0 mL)</td>
<td>1500 (1.5 mL)</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>2000</td>
<td>4000 (4.0 mL)</td>
<td>2000 (2.0 mL)</td>
</tr>
</tbody>
</table>

### 5.3.2 Procedure – Day of Analysis

#### 5.3.2.1 Column Regeneration

The cadmium column used to reduce NO₃ to NO₂ must be regenerated after every 4 autoanalyzer runs. The method utilized for column regeneration is located in the Astoria-Pacific manual.

#### 5.3.2.2 Sample Filtration

Samples may arrive in the laboratory as filtered or unfiltered samples. Unfiltered samples must be filtered immediately using Millipore AP40 GFF for TCLP filters. After samples are filtered
they must be placed in the freezer until they are analyzed. The procedure for filtering samples is described below:

1. Locate previously cleaned bottle(s) that will be used to hold the filtered water. Label the bottle with the sample location, sample depth, date of sample collection and the text “filtered”.

2. Prepare a filter housing by placing one glass fiber filter, gridded side down, onto the metal screen on the bottom of the filter assembly.

3. Always use the tweezers to handle filters.

4. Place the black rubber gasket on top of the filter, and screw the filter holder back together.

5. Shake the unfiltered sample bottle well.

6. Use a syringe to draw up approximately 20 mL of sample into the syringe. Use this water to rinse the syringe and then discard the water.

7. Take apart the syringe by pulling the plunger all the way out.

8. Attach the filter holder to the syringe.

9. Mix the sample bottle again and obtain the sample to be filtered by pouring water from the bottle into the syringe (with the filter holder facing down).
   a. Pour approximately 50 mL of water from the sample bottle into the syringe.
   b. At least 50 mL of water must be left in the sample bottle as this remaining water will be used for other assays.
   c. The exact volume filtered is not important unless the filter is to be utilized for chlorophyll-a analysis (see SOP 012 – Chlorophyll-a Analysis, Welschmeyer Method).

10. Attach the syringe plunger, and slowly push the water through the filter housing with even pressure catching the filtered water in the bottle labeled “filtered”.

11. If the filter becomes clogged, remove the filter and start the procedure over with another aliquot of sample.

12. After filtering the water take the filter holder off the syringe and unscrew the two halves. Discard the filter unless it is to be used for chlorophyll-a analysis.

13. Take apart the syringe.

14. Rinse all apparatus with DI water. Place upside down on a paper towel to dry.

5.3.2.3 Matrix Spikes

Matrix spikes are completed on 30% of samples. Use the procedure outlined below to prepare matrix spikes:

1. Use a matrix spike solution that is 1000 ppb P and 10,000 ppb N03-N, instructions for making the solution are below.

2. Check and confirm the calibration of the following pipettes and volumes on the Mettler 0.5 mg balance using Ultrapure water:
   a. 100 µl (yellow tip Brinkmann)
b. 900 µl (black Eppendorf, blue tip)  
c. 1000 µl (blue tip Brinkmann)

3. Select a water sample to spike that is known to have detectable results and is not at or below the limit of detection. Ideally it should be at the low end of mid-range.

4. First fill a cup with the unspiked sample. Put on sample rack.

5. Next pipette 1900 µl of the sample into another 2 ml sample cup.

6. Add 100 µl of the mixed (1000 ppb P and 10,000 ppb NO₃-N) spike solution to the 1900 µl of sample (instructions for making spike solution are below) This will give you a solution that is spiked with 50 ppb P and 500 ppb NO₃-N. In other words with 100% recovery your spiked samples will be 50 ppb P and 500 ppb NO₃-N higher than the unspiked sample. (See below for more details.)

7. Mix using pipette. Put the spiked sample on the rack right after the unspiked sample.

8. (Optional) Set up a replicate spiked cup.

9. To use the autoanalyzer matrix spike automatic calculation, edit your nitrogen method and then edit your phosphorus method. This doesn’t have to be repeated each time, once you have set it up.
   a. Go to edit for your nitrogen method
   b. Go to Channel Properties, CC/QC tab
   c. Enter SPK1 for spike number
   d. Enter 500 for spike concentration

10. For phosphorus you have to set up a different spike number (2)
   a. Go to edit for your phosphorus method
   b. Go to Channel Properties, CC/QC tab
   c. Enter SPK2 for the spike number
   d. Enter 50 for the spike concentration

11. The matrix spike result will be printed on the sample run report, right after the spiked sample.

12. Calculate results as an alternative to the AP software method.
   a. See 06 AMM 1st tri may 03.xls for the formula in excel to cut and paste, remembering to use appropriate cells for the calculation.
   b. Calculation is also below:

   % recovery = \([\frac{{SR-UR(V3/(V1+V3))}}{{(V1 \times C1)/(V3+V1)}}]\)

   Excel formula:
   \[= \frac{{([SR(V3+V1)] - (UR \times V3))}}{V1 \times C1} \times 100\]
   \[= 100 \times \frac{{([SR(2000) - UR(1900)])}}{(100)C1}\]

   Where:
   C1 = concentration of spiking solution  
   V3 = volume of unspiked sample (=1900 µl)  
   V1 = volume of spike solution (=100 µl)  
   SR = spiked result  
   UR = unspiked result

**Mixed matrix spike solution for Nitrogen and Phosphorus**
These instruction allow for the creation of 100 ml of matrix spike solution that is 1,000 ppb P and 10,000 ppb NO₃-N

1. Fill a 100 ml volumetric flask about ½ full with Ultrapure water
2. Pipette in 10 ml of intermediate P stock solution (it is 10 ppm P)
   a. df=10 (100 ml / 10 ml)
   b. final P concentration is 10 ppm/10 = 1 ppm, = 1000 ppb P
3. Pipette in 10 ml of N stock solution (it is 100 ppm N)
   a. df= 10 (100 ml / 10 ml)
   b. final N concentration is 100/10 = 10 ppm, = 10000 ppb N
4. Fill volumetric flask to the line with MQ water, shake repeatedly to mix

   **To spike your water sample (also above)**

1. Pipette 1000 µl of the water sample into a 2 ml autoanalyzer sample cup
2. Pipette 100 µl of the mixed Nitrogen/Phosphorus spike solution into the water sample in the sample cup
3. Pipette 900 µl water sample (more of the same one) into sample cup
4. Mix by using the pipette used in (3) to withdraw and push back in mixed sample/spike solution in sample cup. Repeat 3-4 times.
5. **Remember to place the matching unspiked sample right before the spiked sample in the sample tray!**

   **5.3.2.4 Sample Analysis**

Detailed instructions regarding operation of the Astoria-Pacific automated spectrometer (autoanalyzer) will not be repeated here. This information is located in the autoanalyzer operation manuals. A summary of the analytical procedure is found below:

1. Allow all filtered samples, standards and blanks to warm to room temperature. This takes place while the autoanalyzer is being prepared.
2. Obtain reagents needed for the analysis. The PO₄ assay only utilizes one reagent, the mixed reagent. The mixed reagent must be prepared daily, preparation instructions are located in the Astoria-Pacific manual. The NO₃+NO₂ analysis utilizes two reagents: ammonium chloride buffer and color reagent. All reagents should be allowed to warm to room temperature prior to use.
   a. **Lab personnel must wear gloves, lab coat and eye protection when handling reagents. Waste reagents must be collected for later disposal by URI Safety and Health.**
   b. Further information regarding reagents is located in the Laboratory MSDS binder. Further information regarding health and safety is located in SOP 001 – General Laboratory Safety and SOP 001a – University Safety and Waste Handling Document.
3. A summary of Autoanalyzer Operation is provided below:
   a. A calibrated volume of sample is introduced into the autoanalyzer by the sampler.
b. The sample is delivered to the analytical cartridge by a peristaltic pump. It is then combined with reagents and air bubbles in a continuously moving stream.

c. As the sample moves through the cartridge it passes through mixing coils and a heating bath, as specified by test conditions.

d. A color is produced by the specific analyte in the sample, blue for the PO₄ assay and pink for the NO₃+NO₂ assay. The intensity of the color is determined by the amount of analyte present.

e. This colored stream then flows to the photometer where the color intensity is measured and converted to an electronic signal. The electronic signal is sent to a computer where the Astoria-Pacific FASPac II® software program processes the signal to create a standard curve and numerical results for the standards and samples as well as a real-time recorder tracing of peaks.

4. Each autoanalyzer rack has 90 places for samples, blanks and QC samples. A sample run can have up to three racks (270 samples).

   a. Standards are set up on their own rack. A set of standards is run at the beginning of each run. A separate set of standards is run at the end, and are averaged with those run at the beginning.

   b. A check calibrant is run after every 15 samples and a blank is run after every 15 samples to monitor the run (see Section 5.2 for further information).

   c. Each sample and standard is recorded on a sample log sheet as they are poured into analytical sample cups.

   d. 1 sample out of 10 is analyzed in duplicate for ambient and marine samples and every sample is analyzed in duplicate for ISDS samples (two separate analyzer sample cups).

   e. The autoanalyzer is set to analyze each sample cup twice.

   f. 1 sample out of 30 is a matrix spike.

5. The autoanalyzer is connected to a personal computer. The Astoria-Pacific FASPac II® program analyzes the data reported by the autoanalyzer. The program performs a linear regression on the standards and determines sample concentrations.

   a. A graph of concentration verses absorbance of standards is displayed and can be manipulated to remove obvious outliers.

   b. The standard curve is rejected if R² is less than 0.95 unless this is due to 1 standard in the suite (see Section 5.2 for further information).

   c. During the run the real time peak tracing are monitored to check for in-range heights, proper shapes and any irregularities, so that samples can be re-analyzed, space permitting during the current run, or saved for re-analysis in a later run.

   d. At the end of a run each peak is checked to make sure the marker is in the proper spot and any irregularities are accounted for (analytical blips, empty sample cups, etc). Once each peak in a run has been reviewed, the results of the run are exported and saved as Excel files, and printed.
6. After the computer results are printed, they are again compared to the peak tracing. Particularly take note of carryover of high to low peaks and correct any keyboarding errors.

7. QC results are compared to previous results as well as "true values". If the results of the run meet the QA/QC criteria set forth in this SOP then the results of the run are deemed acceptable.

8. After the printout has been approved, the data are entered into appropriate Excel spreadsheet files, where they are subsequently re-checked for data entry errors.

9. After the data are approved samples may be disposed of in accordance with Section 4.0.

10. Sample bottles are cleaned by acid washing following SOP 003 – General Labware Cleaning Procedure

6.0 CALCULATIONS

Orthophosphate and nitrate/nitrite concentrations are calculated in the Astoria Pacific FASpac II program from the average of standard curves analyzed at the beginning and end of the sample run. Data are reported to the nearest whole number for PO₄-P and the nearest 10’s for NO₃/NO₂-N. Values less than the reporting limit are reported as < (numerical value of the) RL µg/L PO₄-P or µg/L NO₃/NO₂-N.

If marine samples were analyzed, only the NO₃/NO₂-N are reported, PO₄-P values are discarded as they are inaccurate when run in the manner described in this SOP (namely, using UltraPure water as a carrier).

7.0 REFERENCES


Method referenced: Automated Cadmium Reduction (4500-NO₃ – F), Automated Ascorbic Acid Reduction Method (4500-P-F)


8.0 DOCUMENTATION

Data consisting of the sample results, peaks and calibration curves are exported from the computer running the autoanalyzer into Excel data files. The Excel files are then printed and saved electronically.
| Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF |
|------|----------------|----|------|----------------|----|------|----------------|----|------|----------------|----|------|----------------|----|------|----------------|----|------|----------------|----|------|----------------|----|------|----------------|----|
| 1    |                |    | 16   |                |    | 31   |                |    | 46   |                |    | 61   |                |    | 76   |                |    |
| 2    |                |    | 17   |                |    | 32   |                |    | 47   |                |    | 62   |                |    | 77   |                |    |
| 3    |                |    | 18   |                |    | 33   |                |    | 48   |                |    | 63   |                |    | 78   |                |    |
| 4    |                |    | 19   |                |    | 34   |                |    | 49   |                |    | 64   |                |    | 79   |                |    |
| 5    |                |    | 20   |                |    | 35   |                |    | 50   |                |    | 65   |                |    | 80   |                |    |
| 6    |                |    | 21   |                |    | 36   |                |    | 51   |                |    | 66   |                |    | 81   |                |    |
| 7    |                |    | 22   |                |    | 37   |                |    | 52   |                |    | 67   |                |    | 82   |                |    |
| 8    |                |    | 23   |                |    | 38   |                |    | 53   |                |    | 68   |                |    | 83   |                |    |
| 9    |                |    | 24   |                |    | 39   |                |    | 54   |                |    | 69   |                |    | 84   |                |    |
| 10   |                |    | 25   |                |    | 40   |                |    | 55   |                |    | 70   |                |    | 85   |                |    |
| 11   |                |    | 26   |                |    | 41   |                |    | 56   |                |    | 71   |                |    | 86   |                |    |
| 12   |                |    | 27   |                |    | 42   |                |    | 57   |                |    | 72   |                |    | 87   |                |    |
| 13   |                |    | 28   |                |    | 43   |                |    | 58   |                |    | 73   |                |    | 88   |                |    |
| 14   |                |    | 29   |                |    | 44   |                |    | 59   |                |    | 74   |                |    | 89   |                |    |
| 15   |                |    | 30   |                |    | 45   |                |    | 60   |                |    | 75   |                |    | 90   |                |    |

Sample Log Sheet

SR1  SYNC (High std)  SR6  C3=  SR11  C8=  File/run name:  Analyst:
SR2  CO (carryover-blank)  SR7  C4=  SR12  C9=  Method name:  Source of Standards:
SR3  W (wash)  SR8  C5=  SR13  C10=  Date:  Conc. Range of Standards:
SR4  C1=  SR9  C6=  SR14  C11=  Analysis of/in:  °light: ref _____ ch1 ____ ch2 _____
SR5  C2=  SR10  C7=  SR15  C12=  SYNC abs: ch 1 ________ ch2 _____

Check Calibrant # & concentration(CC# _____) = ______ ug/l

filename c:\awwexcel\labproc\RFArelated\Astoria Analyzer sample cup log
Standard Operating Procedure 016
(Prior number URIWW-SOP-1A)
Total Phosphorus and Nitrogen Analysis
University of Rhode Island Watershed Watch

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1.0 PURPOSE AND DESCRIPTION

This method is for the simultaneous analysis of total phosphorus and total nitrogen in ambient (river, pond, lake), marine or Individual Sewage Disposal System (ISDS) samples. This procedure follows much of the procedure outlined in Standard Operating Procedure (SOP) 015 – Orthophosphate and Nitrate + Nitrite analysis. The main differences are that the procedure is performed on unfiltered samples and the unfiltered samples are digested prior to analysis. The digestion step converts all forms of nitrogen and phosphorus to nitrate (NO$_3$) and orthophosphate (PO$_4$), respectively. Once samples have been digested the analysis is that for NO$_3$+NO$_2$ and PO$_4$ as discussed in SOP 15. Samples are analyzed on an autoanalyzer using a colorimetric technique to determine PO$_4$ and NO$_3$+NO$_2$ concentration. Results are returned as µg of phosphorus (P) per liter and µg of Nitrogen (N) per liter. This method is appropriate for undiluted samples ranging from less than 4 to 200 µg/L P and less than 40 to 2000 µg/L N as well as samples diluted to return values in this range.

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

General laboratory good housekeeping procedures should be practiced as outlined in SOP 001 – General Laboratory Safety.

ISDS samples should be treated as a potential biological hazard. All personnel handling ISDS samples should wear a laboratory coat, gloves and goggles.

Several chemicals are utilized in this procedure. Note that ammonium hydroxide, chloroform, sulfuric acid and phosphoric acid must be used in the hood. Specific hazards of each chemical are listed under the notes section of the required materials table (Section 3.0) of this SOP. When using any chemical general safety procedures should be followed and technicians shall wear goggles, gloves and a laboratory coat.

Further information regarding chemicals utilized in this procedure is found in the laboratory Material Safety Data Sheet (MSDS) binder found in the laboratory where the chemical of interest is stored. General laboratory information regarding safe handling of chemicals is located in SOP 001a – University Safety and Waste Handling.
**Waste Reagents**
All hazardous waste will be collected in appropriate containers, labeled with a URI hazardous waste label and held in the laboratory Hazardous Waste Accumulation Area for pickup and proper disposal by URI Safety and Risk Management.

**2.2 Technician Training/Qualifications**

General training in laboratory technique and use of the Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer (autoanalyzer) must be completed prior to analyzing samples using this method. Technician training will be provided by Linda Green, Laboratory Project Manager – Nutrients. No Technician is allowed to use the autoanalyzer without the permission of Linda Green.

**3.0 REQUIRED MATERIALS**

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drying oven</td>
<td>Set at 105 °C</td>
<td></td>
</tr>
<tr>
<td>Water bath</td>
<td>Capable of reaching 100 °C</td>
<td></td>
</tr>
<tr>
<td>Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer (autoanalyzer)</td>
<td>Equipped with a 880 nm filter and 540 nm filter for analysis of orthophosphate and nitrate/nitrite, respectively.</td>
<td></td>
</tr>
<tr>
<td>Personal computer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigerator</td>
<td>Capable of maintaining 4 °C</td>
<td></td>
</tr>
<tr>
<td>Analytical balance</td>
<td>Capable of weighing to 0.0001 g</td>
<td></td>
</tr>
<tr>
<td>Ultrapure water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desiccator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heated magnetic stirrer and stir bars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pipette dispenser, preferably electronic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>600 - 1000 mL acid-washed beakers</td>
<td>Reserved for phosphorus chemistry use</td>
<td></td>
</tr>
<tr>
<td>Cover glass, or Al foil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squeeze bottle containing Ultrapure water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-15 200 mL volumetric flasks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-4 500 &amp; 1000 mL class A volumetric flasks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vials (for phosphorus digestion)</td>
<td>Description: Borosilicate glass, Type 1, class B, 45 mL (or 11 drams) capacity, 28 mm O.D. X 108 mm high, thread 24-400</td>
<td>Fisher Catalog #03-339-5D 144 vials/case</td>
</tr>
<tr>
<td>Caps for vial</td>
<td>24 mm I.D, 24-400 thread, polypropylene</td>
<td>Fisher Catalog #02-923-14B Nalgene #5150-0240 12 vials/pack</td>
</tr>
<tr>
<td>20 mL volumetric pipettes</td>
<td>class B accuracy +/- 0.06 mL Calibrated “to deliver”</td>
<td>Fisher Catalog #13-650N 1 pipette/pack</td>
</tr>
</tbody>
</table>
### Required Material

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue coated-wire rack</td>
<td>60-72 vial capacity. Rack is cataloged as a “Whirl-pak Bag Rack”. It is found in the “bag” section of the Fisher catalog, not in the rack section.</td>
<td>Fisher Catalog #01-812-5G NASCO # B1048, with a capacity of 12, 710 mL samples (NASCO #B1020) Sold singly</td>
</tr>
<tr>
<td>White plastic racks</td>
<td>24 vial capacity, this type of rack can be placed into the oven (to dry vials) and will not float in the water bath.</td>
<td>Fisher Catalog #14-809D</td>
</tr>
<tr>
<td>Repipette low profile dispenser (for dispensing digesting reagent)</td>
<td>This repipette variety can withstand most reagents, has two openings and has a capacity for dispensing up to 10 mL.</td>
<td>Fisher Catalog #13-687-35</td>
</tr>
<tr>
<td>100-250 mL brown glass bottles</td>
<td>Qorpak, with TFE-lined closures</td>
<td>Fisher Catalog 240 mL bottles #03-320-8D 24 bottles/case 120 mL bottles #03-320-8C 24 bottles/case</td>
</tr>
</tbody>
</table>

### Chemical Reagents for Processing Samples

<table>
<thead>
<tr>
<th>Chemical Reagent</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Persulfate (K₂S₂O₈)</td>
<td>Strong oxidizer. Contact with other material may cause a fire. Causes digestive and respiratory tract irritation. May cause allergic respiratory or skin reaction. May be harmful if swallowed. May cause severe eye, skin and respiratory tract irritation with possible burns. Used as the sample digesting reagent.</td>
<td>Fisher Catalog #P282-500, 500 g, certified, ACS, low nitrogen (&lt;0.001%)</td>
</tr>
<tr>
<td>Boric Acid powder (H₃BO₃)</td>
<td>Used for sample digestion reagent. May cause respiratory tract, eye and skin irritation. Causes digestive tract irritation. May cause central nervous system effects. May cause adverse reproductive effects.</td>
<td>Fisher Catalog #A74-500, certified ACS, 500 g, Phosphate &lt;= 0.001%</td>
</tr>
<tr>
<td>Sodium Hydroxide pellets (NaOH)</td>
<td>Causes eye and skin burns. Hygroscopic (absorbs moisture from the air). Causes digestive and respiratory tract burns. Used for sample digestion reagent.</td>
<td>Fisher Catalog #S318-500, certified ACS, 500 g Phosphate compounds &lt;= 0.001%, Nitrogen compounds &lt;= 0.001%</td>
</tr>
<tr>
<td>Potassium Phosphate Monobasic (KH₂PO₄), also known as potassium dihydrogen phosphate</td>
<td>Hygroscopic (absorbs moisture from the air). May cause eye, skin, respiratory and digestive tract irritation.</td>
<td>Fisher Catalog #P382-500, primary standard, crystalline, 500 g</td>
</tr>
<tr>
<td>Required Material</td>
<td>Notes</td>
<td>Re-order information</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Potassium Nitrate (KNO₃)</td>
<td>Strong oxidizer. Contact with other material may cause a fire. May cause respiratory tract, eye and skin irritation. May cause methemoglobinemia. May cause kidney damage. Hygroscopic (absorbs moisture from the air). May be harmful if swallowed.</td>
<td>Fisher Catalog #P383-100, primary standard</td>
</tr>
</tbody>
</table>

**Reagents for Autoanalyzer. Orthophosphate Analysis**

<table>
<thead>
<tr>
<th>Chloroform (CHCl₃)</th>
<th>May cause central nervous system depression. May cause cardiac disturbances. May cause cancer based on animal studies. This substance has caused adverse reproductive and fetal effects in animals. May be harmful if swallowed. Causes eye, skin and respiratory tract irritation. Light sensitive.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimony Potassium Tartrate Trihydrate (C₈H₄K₂O₁₂Sb₂·3H₂O)</td>
<td>May cause eye, skin, respiratory and digestive irritation upon contact. Harmful if swallowed. May cause liver, kidney and heart damage.</td>
<td>Fisher Catalog #A 867-500</td>
</tr>
<tr>
<td>Ammonium Molybdate Tetrahydrate ((NH₄)₆Mo₇O₂₄·4H₂O)</td>
<td>May cause eye, skin and respiratory tract irritation upon contact. May be harmful if swallowed.</td>
<td>Fisher Catalog #A674-500</td>
</tr>
<tr>
<td>L-Ascorbic Acid (C₆H₈O₆)</td>
<td>Light sensitive. Air sensitive. May cause eye, skin, and respiratory tract irritation. Strong reducing agent. Fire and explosion risk in contact with oxidizing agents.</td>
<td>Fisher Catalog #A61-100</td>
</tr>
<tr>
<td>DowFax Surfactant for P analysis</td>
<td>May cause eye burns upon contact.</td>
<td>Available from Astoria-Pacific</td>
</tr>
<tr>
<td>Sulfuric Acid, concentrated (H₂SO₄)</td>
<td>Will cause burns of exposed skin, eyes, respiratory and digestive tract.</td>
<td>Fisher Catalog #A300-212</td>
</tr>
</tbody>
</table>

**Nitrate/Nitrite Reagents**

| Ammonium Chloride (NH₄Cl) | May cause skin, respiratory and digestive tract irritation. May be harmful if swallowed. Causes eye irritation. Hygroscopic (absorbs moisture from the air). | |
### Required Material

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disodium EDTA (Disodium Ethylenediamine Tetraacetic Acid Dihydrate) ( (\text{C}<em>{10}\text{H}</em>{14}\text{N}<em>{2}\text{Na}</em>{2}\text{O}<em>{8}\cdot2\text{H}</em>{2}\text{O}) )</td>
<td>Causes eye, skin and respiratory tract irritation. May be harmful if swallowed.</td>
<td></td>
</tr>
<tr>
<td>Ammonium Hydroxide ( (\text{NH}_{4}\text{OH}) )</td>
<td>Causes eye, skin, digestive and respiratory tract burns.</td>
<td></td>
</tr>
<tr>
<td>Cupric Sulfate Pentahydrate ( (\text{CuSO}<em>{4} \cdot 5\text{H}</em>{2}\text{O}) )</td>
<td>Harmful if swallowed. Causes digestive and respiratory tract, eye and skin irritation with possible burns. Hygroscopic (absorbs moisture from the air). Possible sensitizer.</td>
<td>Sigma Catalog #c7631</td>
</tr>
<tr>
<td>Sulfanilimide ( (\text{C}<em>{6}\text{H}</em>{6}\text{N}<em>{2}\text{O}</em>{2}\text{S}) )</td>
<td>May cause reproductive and fetal effects. May cause eye, skin, and respiratory tract irritation. May be harmful if swallowed or inhaled.</td>
<td>Fisher Catalog #04525-100</td>
</tr>
<tr>
<td>N-1-Napthylethylenediamine (NED) ( (N-(1-\text{Naphthyl})\text{ethylenediamine dihydrochloride}) \ (\text{C}<em>{10}\text{H}</em>{7}\text{NHCH}<em>{2}\text{CH}</em>{2}\text{NH}_{2} \cdot 2\text{HCl}) )</td>
<td>Will cause eye, skin and respiratory system irritation.</td>
<td>Sigma Catalog #N-9125</td>
</tr>
<tr>
<td>Brij-35% solution surfactant</td>
<td></td>
<td>(Available from Alpkem)</td>
</tr>
<tr>
<td>Glycine ( (\text{NH}<em>{2}\text{CH}</em>{2}\text{COOH}) )</td>
<td>May be absorbed through intact skin. May cause respiratory tract, eye and skin irritation.</td>
<td>Fisher Catalog #G46-500 (500 gram bottle- smallest amount available).</td>
</tr>
<tr>
<td>Phosphoric Acid ( (\text{H}<em>{3}\text{PO}</em>{4}) )</td>
<td>Hygroscopic (absorbs moisture from the air). Will cause burns of exposed skin, eyes, respiratory and digestive tract.</td>
<td></td>
</tr>
</tbody>
</table>

### Spare Equipment

Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory. Temporary replacement of the autoanalyzer may be made through arrangement with Astoria-Pacific. Other equipment may be obtained through arrangement with other scientists in the Natural Resources Science department maintaining similar equipment.

### 4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>125 or 250 mL acid washed brown glass bottles</td>
<td>4 °C</td>
<td>100 mL</td>
<td>14 days at 4 °C 3 months once pipetted into vials</td>
</tr>
</tbody>
</table>

### Disposal
Field samples and digested samples may be disposed of only after final quality assurance checks are completed and the data found to be acceptable using criteria found in Section 5.2 of this SOP. Samples may be disposed of by rinsing down the drain with water. ISDS samples are considered a potential biological hazard, a laboratory coat, gloves and goggles should be worn when disposing of these samples.

5.0 METHOD DESCRIPTION

5.1 Scheduling Considerations

The amount of primary and intermediate standard previously prepared should be checked at least two days prior to the date of expected sample analysis. If necessary, prepare the primary standards as discussed in Section 5.3.2. Approximately 100 mL of the primary standard is necessary to prepare the intermediate standard. Approximately 100 mL of the intermediate standard is necessary to prepare the working standards.

The available volume of each reagent utilized in this analysis should be assessed. Two reagents are needed for the NO₃/NO₂ analysis: the ammonium chloride buffer and the color reagent. One reagent is necessary for the PO₄ analysis, this mixed reagent is prepared daily by addition of several other reagents: potassium antimonyl tartrate solution, ammonium molybdate solution, ascorbic acid solution and sulfuric acid solution. The ascorbic acid solution is only stable for 1 week. If necessary prepare more reagent using instructions found in the Astoria-Pacific manual.

Preparation of vials to be used in the digestion of samples must occur at least 3 days prior to a sample event. Direction regarding the preparation of the vials is in Section 5.3.1.1 of this SOP.

5.2 Quality Assurance/Quality Control

5.2.1 Method Detection Limit

The Method Detection Limit (MDL) is set at 0.3 µg/L P for the total phosphorus assay and 3.2 µg/L N for the total nitrogen assay. The Limit of Quantitation or Reporting Limit (RL) is set at 3 µg/L P for the total phosphorus assay and 30 µg/L N for the total nitrogen assay. Updated MDL and RL values are calculated on an annual basis, check with the Laboratory Manager for the most up-to-date values.

The upper RL or the maximum concentration reported by the total phosphorus assay without dilution of the digested sample is 200 µg/L P for ambient and marine samples and 2000 µg/L P for ISDS samples. The maximum concentration reported by the total nitrogen assay without dilution of the digested sample is 2000 µg/L N for all samples. The upper RL is based on the maximum standard used to calibrate the autoanalyzer. Values reported for total phosphorus are reported to the nearest whole number, those reported for total nitrogen are reported to the nearest 10’s (ie: 46 µg/L N = 50 µg/L N and 13.6 µg/L P = 14 µg/L P).

5.2.2 Method (Digestion) Blanks

Method (digestion) blanks consist of 20 ml Ultrapure water digested as a sample and then analyzed as a sample. A minimum of four (4) digestion blanks are prepared per sample run. A method (digestion) blank is analyzed after every 15 samples; this corresponds to 6% of the samples analyzed. Method (digestion) blanks must not be greater than 3 µg/L P and 20 µg/L N.
Corrective Action
If any method (digestion) blank is found to be greater than 3 µg/L P or 20 µg/L N then this will be noted on the project data sheet. Since these blanks are digested it is not possible to re-prepare the blanks. If all the digestion blanks are outside of accepted quality assurance/quality control guidelines then the run will be considered contaminated and the data marked accordingly. If all the digestion blanks are outside of acceptable guidelines, but are very close to one another in value then it is possible to subtract this value from the final sample results, especially if the “0” standard used in calibration is also elevated. This will be determined by discussion with Linda Green – Laboratory Manager.

5.2.3 Sample Replication

Sample replication is completed in three ways. The autoanalyzer analyzes two aliquots from every sample cup for both total phosphorus and total nitrogen. The relative percent deviation (%RPD) between the values obtained from the same sample cup must not be greater than 15%.

The digested sample is poured into two separate autoanalyzer cups for 10% of the samples. The %RPD for values obtained for the same sample in two separate cups is 20%.

Replicate digestions of the same sample are completed on 20% of the ambient and marine samples. The %RPD for value obtained for two separate digestions of the same sample is 25%.

Triplicate digestions of the same sample are completed on 100% of the ISDS samples. The %RPD for value obtained for two separate digestions of the same sample is 25%.

%RPD is calculated as follows:

\[
\%RPD = \left| \frac{\text{Result of Replicate 1 (µg/L)} - \text{Result of Replicate 2 (µg/L)}}{\text{Average of Result of Replicate 1 (µg/L) and Result of Replicate 2 (µg/L)}} \right| \times 100
\]

Corrective Action
If the %RPD is greater than 15% for an analysis from the same sample cup or greater than 20% when comparing sample values between duplicates placed in different sample cups then the sample is re-analyzed. If the sample replicates are still not within 15%RPD and 20%RPD and the instrument check standards are within the acceptable Quality Assurance/Quality Control guidelines then the deviation is noted on the project data sheet and the run continued.
### 5.2.4 Calibration

Instrument standardization is completed by determining the absorbance of a series of known digested working standards. The autoanalyzer software performs a standard linear regression analysis of the standard concentration versus absorbance and returns a standard equation for a line \( y = mx + b \) and a graph. The autoanalyzer is standardized with a minimum of 5 digested working standards and a blank (see Section 5.3.2.3 and 5.3.2.5). An acceptable linear regression for a calibration sequence will have a coefficient of determination \( (R^2) \) value of at least 0.990.

Two (2) vials containing each standard are digested as samples if greater than 60 samples are being prepared for analysis. If less than 60 samples are being prepared then only 1 vial containing each standard are digested. The vials containing standard are placed randomly throughout the sample vials during the digestion step. After digestion 1 vial for each standard concentration is selected to be used as the standard to calibrate the autoanalyzer. Otherwise, no random selection of the standards to be run is necessary, as only one vial per standard was prepared. Three vials containing 20 mL of Ultrapure water digested as samples are prepared as method blanks or the zero standard (see Section 5.2.2.2). After digestion, 1 vial is selected as the zero standard the other two vials are used as method blanks and dilution liquid for off scale samples.

**Corrective Action**

If the \( R^2 \) value returned by the standardization procedure is less than 0.990 due to only 1 standard, then the standard may be removed and the calibration accepted. If the \( R^2 \) value is less than 0.990 due to more than 1 standard, then the autoanalyzer is re-calibrated. No samples are analyzed until an acceptable \( R^2 \) value is recorded. Trouble shooting information is located in the autoanalyzer manual.

### 5.2.5 Calibration Check/Laboratory Control Standard

**Environmental Protection Agency Water Pollution Proficiency Test Study**

The laboratory participates in the Environmental Protection Agency Water Pollution Proficiency Test Study on a yearly basis. Unknown samples are purchased from an outside vendor and analyzed for total phosphorus and total nitrogen. URIWW results are sent to the vendor who provides a comparison to the actual value and a performance evaluation. Study results are maintained by the laboratory.

**Daily Calibration Check**

External standards are purchased from AccuStandard or another outside vendor and are digested and analyzed as samples during each analysis batch at a ratio of 2 standards per run (150 vials). The percent difference \( (%D) \) must not be greater than 20% for the external standards.

Check calibrants consisting of the middle standard from the digested standards used to calibrate the instrument are run at a ratio of 1 check calibrant per 15 samples, or approximately 6% of the samples analyzed. The %D must not be greater than 20% for the check calibrant.

Percent difference is calculated as follows:

\[
%D = \frac{\text{Reported value} \times 100}{\text{Known value}}
\]
Corrective Action
If the %D is greater than 20% for either an external standard or check calibrant then the standard is re-analyzed. If the %D is still greater than 20% then the instrument is re-calibrated and the samples analyzed between the acceptable check calibrant or external standard and the unacceptable check calibrant or external standard are re-analyzed.

External Glycine Standard
Glycine is used as an external standard for only the total nitrogen analysis of ISDS samples. The glycine standard is prepared at a concentration of 100 ppm N, which is much higher than the typical concentration found in ambient or marine samples. This standard is analyzed to assess the completeness of digestion in the higher N concentration ISDS samples. Generally, ambient and marine samples have a total nitrogen concentration of less than 1 ppm N.

Three (3) vials containing the external standard are prepared per ISDS sample run (see Section 5.2.5.1 for preparation information). The %D must be less than or equal to 10%. Calculation of %D is discussed in the section above

Corrective Action
If the %D is greater than 10% for the external glycine standard then the standard is re-analyzed. If the %D is still greater than 10% then the deviation is noted on the project data sheet. If the %D for all the external glycine standards analyzed in a run is greater than 20% then the results of the external standards, digestion blanks and samples will be analyzed to determine if it will be necessary to re-digest samples or reject data due to quality assurance/quality control deviations. Final determination as to the course of action rests with Linda Green – Laboratory Manager.

5.2.5.1 Preparation of 100 ppm Glycine Total Nitrogen External Standard (ISDS samples only)

1. The formula weight of glycine (NH₂CH₂COOH) is 75.07 g glycine / mole.
   a. Glycine is 18.7% nitrogen.
   b. 1 mol glycine / 1 mole N = 75 g (glycine) / 14 g (N) = 5.36

2. To prepare a solution of 100 mg/L (ppm) of N which is equivalent to 0.1 g N/L use the following equation:

   \[
   100 \text{ mg/L N} = \frac{0.1 \text{ g N}}{L} \times \frac{75 \text{ g glycine}}{14 \text{ g N}} = 0.536 \text{ g glycine / L}
   \]

   Therefore, 0.536 grams of glycine per liter of solution are needed to prepare a 100 ppm N solution.

3. Dry approximately 0.75 g of glycine in the drying oven set at 105 °C for at least 3 hours.

4. After drying, store the glycine in a desiccator.

5. Rinse a 1 L volumetric flask and fill it partly with Ultrapure water.
6. Check the calibration of the balance using the procedure found in Section 5.3.2.1, Step 3.

7. Weigh 0.5360 g of glycine on a disposable weighing tray using the analytical balance.

8. Use a squirt bottle to transfer the glycine to the volumetric flask.

9. Dilute to volume with Ultrapure water.

10. Cap the flask and mix thoroughly.

11. Place a label on the flask indicating the contents of the flask, date of preparation and the initials of the technician preparing the standard.

12. Let the standard sit at least 1/2 hour before using.

13. Store the standard in the refrigerator in the volumetric flask it was prepared in.

14. The standard should be discarded after 3 months.

**5.2.5.2 Use of the Glycine Standard**

1. Bring the volumetric flask containing the glycine standard to room temperature.

2. Calibrate the adjustable pipette using the procedure outlined in Section 5.3.2.3, Step 3. The nominal volume on the pipette is not always accurate. Adjust the pipette as needed and recheck the calibration with each change in pipette delivery volume.

3. Pipette 1 mL of the standard into each of 3 total nitrogen/total phosphorus vials.

4. Add 19 mL of Ultrapure water to each of the 3 vials.

5. Add 5 mL of digesting reagent to each of the 3 vials.

6. Cap each vial and place them randomly in a rack with the other samples to be analyzed.

**Analytical Notes:**

1. Percent recovery was 95-100% (%D less than or equal to 5%) when the 100 ppm glycine was less than 3 months old. It declined to 90-95% when the glycine standard was 3-6 months old.

2. On 12/00 the above procedure was performed using 2 mL glycine and 18 mL water. Percent recovery was <=85%, suggesting that that high concentration of nitrogen exhausts the digesting capacity of the reagent.

**5.2.6 Matrix Spikes**

Matrix spikes are used to verify that the chemical and physical characteristics of the samples being analyzed are not sufficiently different from the standards used in calibration to cause the analyte of interest to respond differently in the sample when compared to the calibrant.

Matrix spikes are added to the undigested sample. A small amount of highly concentrated standard is added directly to an aliquot of sample before digestion. The amount of standard added allows the final solution to be approximately double the original concentration of the sample. The unspiked sample is run, immediately followed by the spiked sample, and the autoanalyzer system calculates the spike recovery, which should be between 80 – 120%. Sample spikes are run on 30% of samples or one sample in 30.
The percent recovery is calculated as follows:

\[
\% \text{ recovery} = \frac{\text{SR-UR}[V_3/(V_1+V_3)]}{(V_1 \times C_1)/(V_3+V_1)}
\]

Where:
- \( C_1 \) = concentration of spiking solution
- \( V_3 \) = volume of unspiked sample (= 19 ml)
- \( V_1 \) = volume of spike solution (= 1 ml)
- \( \text{SR} \) = spiked result
- \( \text{UR} \) = unspiked result

**Corrective Action**

If the % recovery for a spiked sample is not between 80 and 120% the sample and associated sample matrix spike will be re-analyzed. If the value is still not within the acceptable range but all other spiked samples are within QA/QC limits and calibration checks are also acceptable then the sample data will be flagged, but no further action will be taken. If more than one spiked sample is not within acceptable percent recovery then additional samples will be spiked to determine if the sample matrix is causing interference. Appropriate corrective action will be determined in consultation with the laboratory manager.

### 5.3 Analysis Method

#### 5.3.1 Preparation – At Least 3 Days Prior to Field Sample Collection

1. **Prepare Digestion Vials**
   1. Obtain digestion vials
   2. Empty contents of vials, if they have been previously used.
   3. Rinse the vial caps with Ultrapure water 3-5 times. Set caps to dry, inverted on paper towels.
   4. Place previously used vials in a tub of (preferably) hot tap water. Use the tub labeled “label soak only”. The labels of the samples are easier to remove after the vials have soaked.
   5. While vials are soaking begin to remove labels from vials.
   6. Once sample labels have been removed, place vials in another tub filled with hot tap water and non-phosphate detergent. Use the tub labeled “soapy soak only”. Do not use the same tub for label removal and soapy soak. The labels are high in phosphorus and will contaminate the vials.
   7. Scrub out the vials using a brush and the soapy water.
   8. Rinse the vials with DI water 3-5 times.
   9. Acid wash vials (not caps) by placing vials in a 10% hydrochloric acid tub in fume hood in room 002. Let vials soak overnight.
   10. Empty out acid from vials back into tub, rinse 3 times with Ultrapure water inside and out.
   11. Fill vials with Ultrapure water and let sit overnight with the vial caps on.
12. If the vials cannot be pre-digested the next day leave them filled with MQ water, cap and store, labeled "ready for pre-digestion."

13. Pre-digest vials by adding 5 mL digesting reagent to each vial (Preparation of digestion reagent Section 5.3.4.2). Using the re-pipette labeled "old digesting reagent".
   a. Cap the vials tightly.
   b. Vials do not have to be pre-digested immediately and can be stored with digesting reagent in them. Just remember to label them as to their contents.

14. Place capped vials in a rack and in a water bath. If needed add DI water to the water bath to approximately 1/3 of vial height.

15. Put the cover on the water bath. It will not reach 100 °C if the cover is off.

16. Turn on water bath heat at switch and bring to boiling. This takes 45-60 minutes. Boil for 15 minutes. Turn off the water bath.

17. Let the vials cool to room temperature in the water bath overnight.

18. Remove cooled vials and empty out digesting reagent.

19. Rinse vials and caps 3-5 times with Ultrapure water.

20. Fill the vials completely with Ultrapure water and cap them. Allow vials to sit at least 24 hours prior to use. If vials are not used immediately they are left filled with Ultrapure water until the day they are to be used and labeled “ready for use”. Vials for clean lakes samples and for ISDS samples are stored in separate areas of room 002.

5.3.2 Preparation – At least 2 days before field samples collected

The amount of each reagent needed for the analysis should be checked. Two reagents are needed for the total nitrogen analysis: the ammonium chloride buffer and the color reagent. One reagent is necessary for the total phosphorus analysis; this reagent is a mixture of several other reagents: potassium antimonyl tartrate solution, ammonium molybdate solution, asorbic acid solution and sulfuric acid solution. The asorbic acid solution is only stable for 1 week. If necessary, prepare more reagent using instructions found in the Astoria-Pacific manual.

Preparation of the primary standard takes two days to complete, therefore check to determine if it is necessary to prepare new primary standard prior to the expected sample analysis date. Primary and intermediate standards can be stored for up to 6 months. Working standards are replaced after 2 months.

This method utilizes several types of water. Ultrapure water refers to highly purified water which is prepared by further purifying deionized (DI) water using the Aries-Vaptronics system. SOP 002 – Laboratory Water provides information regarding the different types of laboratory water available and the method to obtain water.

Glassware preparation for preparation of standards
All glassware utilized for standard preparation must be soapy water washed using non-phosphorus containing detergent, DI water rinsed, 10% HCl soaked overnight and then soaked in Ultrapure water overnight.

Never use glassware that has just come from acid soaking; it must equilibrate in Ultrapure water first.
All phosphorus glassware should be dedicated to the phosphorus assay and not used for any other analyses.

5.3.2.1 Preparation of Primary Orthophosphate Standard (100 ppm)

1. The standards utilized for the TN/TP analyses are the same as those used for SOP 015 – Orthophosphate and Nitrate + Nitrite Analysis.

2. Dry approximately 0.75 g of potassium phosphate (KH$_2$PO$_4$) in the drying oven (set at 105 °C) for 1 hour. After drying the chemical remove it from the oven and store it in a desiccator.
   a. Formula weight of KH$_2$PO$_4$ = 136.1 g / mole
   b. There is 1 mole of P per 1 mole of KH$_2$PO$_4$ for a total phosphorus mass of 30.97 g P / mole KH$_2$PO$_4$.

3. Check the calibration of the balance using the 1 g weight. Use a balance that weighs to at least 0.0001 g.
   a. The calibration weights are located in the drawer under the balance. Never touch the calibration weights with anything but forceps. The oils on the fingers may change the weight of the standard.
   b. Values returned by the balance should be within 10% of the actual calibration weight value. If a deviation of greater than 10% is noted then the balance will be serviced and re-calibrated. If the balance is found to be outside of the acceptable calibration range then it will not be used in this procedure.

4. The 100 mg/L primary PO$_4$ standard is prepared using the following formula:

\[
100 \text{ ppm P} \times \text{formula weight (KH}_2\text{PO}_4) = 0.1 \text{ g/L} \times 136.1 \text{ g} = 0.4394 \text{ g KH}_2\text{PO}_4/\text{L}
\]

\[
\text{Weight of P in KH}_2\text{PO}_4 = 30.97 \text{ g}
\]

5. Therefore, to prepare 1 L of 100 mg/L primary PO$_4$ standard, place 0.4394 g of KH$_2$PO$_4$ into a 1 L class A volumetric flask. Or:
   a. To prepare varying amounts of the primary standard refer to the table below.
   b. Dilute to the final volume using Ultrapure water.
   c. Note: This solution is 100 mg PO$_4$-P per L = 100 µg PO$_4$-P per mL = 100 ppm.

<table>
<thead>
<tr>
<th>Mass of (NH$_4$)$_2$PO$_4$ to be added to flask (g)</th>
<th>Final Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4394</td>
<td>1000</td>
</tr>
<tr>
<td>0.2127</td>
<td>500</td>
</tr>
<tr>
<td>0.1099</td>
<td>250</td>
</tr>
</tbody>
</table>

6. Dilute to volume using Ultrapure water.
7. Add 2 mL of chloroform per L using a glass pipette to preserve the standard.

8. Place a piece of parafilm over the top of the volumetric flask and mix by inverting the flask at least 30 times.

9. Place a label on the flask indicating the contents of the flask, date of preparation and the initials of the technician preparing the standard.

10. The primary standard is stable for 6 months after which it should be discarded. It is stored in the refrigerator in the volumetric flask it was prepared in.

11. The primary standard must be allowed to sit for 24 hours prior to use.

5.3.2.2 Preparation of Intermediate Orthophosphate Standard (10 ppm)

1. Using a 100 mL class A volumetric pipette add 100 mL of the primary orthophosphate standard (100 ppm) to a 1 L class A volumetric flask.
   a. Do not place the volumetric pipette directly into the bottle containing the primary standard, place a little more than 100 mL of the primary standard into a small weigh dish or beaker and pipette from this container.
   b. Dispose of any unused primary standard by rinsing down the drain.
   c. Do not put the unused primary standard back into the primary standard storage bottle.

2. Use Ultrapure water to fill the volumetric flask to volume. This solution is 10 ppm (µg/mL or mg/L) or 10,000 ppb PO₄-P

3. Place a label on the flask indicating the contents of the flask, date of preparation and the initials of the technician preparing the standard.

4. The intermediate standard is stable for 6 months after which it should be discarded. It is stored in the 4 °C refrigerator in the volumetric flask it was prepared in.

5.3.2.3 Preparation of Orthophosphate Working Standards

1. Note that working orthophosphate and nitrate/nitrite standards are made together in the same volumetric flasks, although the procedures are listed separately.

2. Check the calibration of the balance using the procedure outlined in Section 5.3.2.1.

3. Calibrate the adjustable pipette. The nominal volume on the pipette is not always accurate. Adjust the pipette as needed and recheck the calibration with each change in pipette delivery volume.
   a. Pipettes are calibrated by setting the pipette at the desired volume and pipetting this amount of Ultrapure water onto a weighting dish on a calibrated balance. The density of water at room temperature is essentially 1 g/mL; therefore the weight of the delivered volume of Ultrapure water in g equals the volume delivered in mL.
   b. If the volume delivered is not the desired volume, adjust the pipette and repeat the check of volume until it is correct.
   c. Recheck the pipette with each change in pipette delivery volume.

4. Fill the appropriately sized class A volumetric flasks to be used to prepare working standards part way with Ultrapure water. Use an appropriately sized and calibrated
adjustable pipette to add intermediate stock solution to the volumetric flask, according to chart on the next page.

5. Bring the volumetric flask to volume with Ultrapure water.
6. Cover each flask with parafilm and mix by inverting the flask at least 30 times.
7. Allow the standard to sit at least a 1/2 hour before using.
8. Store working standards in the 4 °C refrigerator. Working standards are replaced every month.

**Preparation of Orthophosphate Working Standards:**

<table>
<thead>
<tr>
<th>Ambient Water (lakes, ponds, streams)</th>
<th>ISDS</th>
<th>Final Concentration of Standard (µg PO₄-P/L)</th>
<th>Ambient Water (200 mL volumetric flasks)</th>
<th>ISDS Samples (100 mL volumetric flasks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards to prepare:</td>
<td></td>
<td>Volume of intermediate orthophosphate standard to use (µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>10</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>15</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>20</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>25</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>50</td>
<td>1000 (1.0 mL)</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>100</td>
<td>2000 (2.0 mL)</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>150</td>
<td>3000 (3.0 mL)</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>200</td>
<td>4000 (4.0 mL)</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>250</td>
<td>2500 (2.5 mL)</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>500</td>
<td>5000 (5.0 mL)</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>1000</td>
<td>10,000 (10.0 mL)</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>1500</td>
<td>15,000 (15.0 mL)</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>2000</td>
<td>20,000 (20.0 mL)</td>
<td></td>
</tr>
</tbody>
</table>

**5.3.2.4 Preparation of Primary Nitrate/Nitrite Standard (100 ppm)**

1. The standards utilized for the PO₄ and NO₃+NO₂ analyses are the same as those used for SOP 016 – Total Phosphorus and Nitrogen Analysis.
2. Dry the approximate amount of primary standard grade potassium nitrate (KNO₃) needed for standard preparation in a drying oven set at 105 °C for 24 hours (refer to the table in Step 3 below). Once the material has dried store it in a desiccator.
3. Using a calibrated balance (see Section 5.3.2.1 for balance calibration procedure) weigh out the amount of KNO₃ needed to prepare the desired volume of primary nitrate/nitrite standard. This solution is 100 mg NO₃-N per L, 100 µg NO₃-N per mL, or 100 ppm.
Weight of KNO₃ needed for primary NO₃ standard based on volume prepared:

<table>
<thead>
<tr>
<th>KNO₃ (g)</th>
<th>Volume of Primary Standard Prepared (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7218</td>
<td>1000</td>
</tr>
<tr>
<td>0.3609</td>
<td>500</td>
</tr>
<tr>
<td>0.1805</td>
<td>250</td>
</tr>
<tr>
<td>0.0722</td>
<td>100</td>
</tr>
</tbody>
</table>

4. Dilute to volume using Ultrapure water.
5. Add 2 mL of chloroform per L of standard using a glass pipette.
6. Place a piece of parafilm over the top of the volumetric flask and mix by inverting the flask at least 30 times.
7. Place a label on the flask indicating the contents of the flask, date of preparation and the initials of the technician preparing the standard.
8. The primary standard is stable for 6 months after which it should be discarded. It is stored in the 4 °C refrigerator in the volumetric flask it was prepared in.
9. The primary standard must be allowed to sit for 24 hours prior to use.

5.3.2.5 Preparation of Working Nitrate/Nitrite Standards

1. The preparation of working nitrate/nitrite standards requires no intermediate stock solution.
2. Note that working orthophosphate and nitrate/nitrite standards are made together in the same volumetric flasks, although the procedures are listed separately.
3. Calibrate the balance (see Section 5.3.2.1 for balance calibration procedure).
4. Calibrate the adjustable pipette (see Section 5.3.2.3).
5. Fill the appropriate sized class A volumetric flasks to be used to prepare working standards part way with Ultrapure water. Use an appropriately sized and calibrated adjustable pipette to add stock standard each volumetric flask, according to chart below.
6. Bring volumetric flasks to volume with Ultrapure water.
7. Cover each flask with parafilm and mix by inverting each flask at least 30 times.
8. Allow the standards to sit at least 1/2 hour before using.
9. Store working standards in the 4 °C refrigerator in the volumetric flasks they were prepared in. Working standards are replaced every month.
Preparation of Nitrate/Nitrite Working Standards

<table>
<thead>
<tr>
<th>Ambient Water (lakes, ponds, streams)</th>
<th>Final Concentration of Standard (µg NO3-N/L)</th>
<th>ISDS Samples (200 mL volumetric flasks)</th>
<th>ISDS Samples (100 mL volumetric flasks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards to prepare:</td>
<td>Volume of intermediate nitrate/nitrite standard to use (µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X X 0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X 50</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X 75</td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X 100</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X 200</td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X X 250</td>
<td>500</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>X X 500</td>
<td>1000 (1.0 mL)</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>X X 1000</td>
<td>2000 (2.0 mL)</td>
<td>1000 (1.0 mL)</td>
<td></td>
</tr>
<tr>
<td>X X 1500</td>
<td>3000 (3.0 mL)</td>
<td>1500 (1.5 mL)</td>
<td></td>
</tr>
<tr>
<td>X X 2000</td>
<td>4000 (4.0 mL)</td>
<td>2000 (2.0 mL)</td>
<td></td>
</tr>
</tbody>
</table>

5.3.3 Procedure – Day of Sample Collection

5.3.3.1 Sample Preparation

1. Prepare the Clean Vials
   a. Use vials that have been cleaned and pre-digested as described in Section 5.3.1.1. These vials should have been previously filled with Ultrapure water and capped.
2. Pour out Ultrapure water and rinse vials and caps 3 times with Ultrapure water. After rinsing shake out as much excess water as possible.
3. Stand vials upright in rack.
4. Put rack in 105 °C drying oven until vials are dry (about 15-20 minutes). Do not put caps in the oven. Stack caps face down on a clean paper towel.
5. Prepare labels for samples and standards.
6. Remove samples and standards from refrigerator, bring to room temperature.
7. Just prior to filling vials shake the samples/standards well.
8. Use a pipette to transfer an aliquot of the unfiltered sample into a clean and labeled vial.
   a. Ambient water sites (rives, lakes, ponds): pipette 20 mL of unfiltered sample into each vial.
      i. Prepare a replicate every 10 samples.
   b. ISDS sites: Pipette 1.0 mL of unfiltered sample and 19.0 mL Ultrapure water into each vial.
      i. ISDS samples are digested in triplicate (3 vials are digested per ISDS sample)
   c. All site: Complete a matrix spike every 30 samples. Instructions are below in Section 5.3.3.2.
9. Rinse pipette well (with Ultrapure water) between samples or switch tips if using pipette with disposable tips.
10. Prepare standards by pipetting 20 mL of each standard into a clean and labeled vial. Method blanks and standards should be randomly interspersed among the samples after the standards and blanks are prepared.
   a. If greater than 60 samples are being prepared, prepare 2 vials containing each standard.
   b. If 60 or less samples are being prepared only pipette 1 vial for each standard.
   c. Prepare the zero standard/method blank by pipetting 20 mL of Ultrapure water into each of 3 vials.
11. Prepare external standards and the glycine standard according to Section 5.2.5.
12. Cap vials.
13. Samples may be stored up to 3 months in the refrigerator until the digesting reagent is added and the samples digested.

5.3.3.2 Matrix Spikes

Matrix spikes are completed on 30% of samples. Use the procedure outlined below to prepare matrix spikes:

1. Use a matrix spike solution that is 1000 ppb P and 10,000 ppb N\textsubscript{O\textsubscript{3}}-N, instructions for making the solution are below.
2. Check and confirm the calibration of the following pipettes and volumes on the Mettler 0.5 mg balance using Ultrapure water:
   a. 1000 µl (1 mL) (blue tip Brinkmann)
3. Select a water sample to spike that is known to have detectable results and is not at or below the limit of detection. Ideally it should be at the low end of mid-range.
4. Pipette 19 mL of sample into a clean vial.
5. Add 1 mL of the mixed (1000 ppb P and 10,000 ppb NO\textsubscript{3}-N) spike solution to the 19 mL of sample (instructions for making spike solution are below). This will give you a solution that is spiked with 50 ppb P and 500 ppb NO\textsubscript{3}-N. In other words with 100% recovery your spiked samples will be 50 ppb P and 500 ppb NO\textsubscript{3}-N higher than the unspiked sample. (See below for more details.)
6. Cap the vial and mix by inverting the sample vial. Put the spiked sample on the rack right after the unspiked sample.
7. (Optional) Set up a replicate spiked cup.
8. When you run the spiked sample on the autoanalyzer use the matrix spike automatic calculation, edit your nitrogen method and then edit your phosphorus method. This doesn’t have to be repeated each time, once you have set it up.
   a. Go to edit for your nitrogen method
   b. Go to Channel Properties, CC/QC tab
   c. Enter SPK1 for spike number
   d. Enter 500 for spike concentration
9. For phosphorus you have to set up a different spike number (2)
   a. Go to edit for your phosphorus method
   b. Go to Channel Properties, CC/QC tab
c. Enter SPK2 for the spike number
d. Enter 50 for the spike concentration

10. The matrix spike result will be printed on the sample run report, right after the spiked sample.

11. Calculate results as an alternative to the AP software method.
   a. See 06 AMM 1st tri may 03.xls for the formula in excel to cut and paste, remembering to use appropriate cells for the calculation.
   b. Calculation is also below:

   \[
   \text{% recovery} = \frac{\text{SR-UR} (V_3/(V_1+V_3))}{(V_1 \times C_1)/(V_3+V_1)}
   \]

   Excel formula:
   \[
   = \frac{\text{[SR(V_3+V_1)] - (UR \times V_3)}}{(V_1 \times C_1)} \times 100 = 100 \times \frac{\text{[SR(2000) - UR(1900)]}}{\text{[(100)C1]}}
   \]

   Where:
   C1 = concentration of spiking solution
   V3 = volume of unspiked sample (= 19 mL)
   V1 = volume of spike solution (=1 mL)
   SR = spiked result
   UR = unspiked result

### Mixed matrix spike solution for Nitrogen and Phosphorus

These instructions allow for the creation of 100 ml of matrix spike solution that is 1,000 ppb P and 10,000 ppb NO₃-N

1. Fill a 100 ml volumetric flask about ½ full with Ultrapure water
2. Pipette in 10 ml of intermediate P stock solution (it is 10 ppm P)
   a. df=10 (100 ml / 10 ml)
   b. final P concentration is 10 ppm/10 = 1 ppm, = 1000 ppb P
3. Pipette in 10 ml of N stock solution (it is 100 ppm N)
   a. df= 10 (100 ml / 10 ml)
   b. final N concentration is 100/10 = 10 ppm, = 10000 ppb N
4. Fill volumetric flask to the line with MQ water, shake repeatedly to mix

To spike your water sample (also above)

1. Pipette 19 mL of the unfiltered water sample into a TN/TP sample vial.
2. Pipette 1 mL of the mixed Nitrogen/Phosphorus spike solution into the water sample in the sample vial.
3. Cap the sample vial and mix by inverting the vial.
4. Remember to place the matching unspiked sample right before the spiked sample in the sample tray!
5.3.4 Procedure – Day of Sample Digestion

5.3.4.1 Digestion Procedure

1. Obtain racks of standards and samples from the refrigerator.
2. Prepare the digestion solution. The digestion solution is prepared daily as described below in Section 5.3.4.2.
3. Pump the repipette containing digesting reagent several times to make sure that there are no air bubbles in the delivery tube.
4. Check calibration to make sure that repipette is dispensing desired volume by dispensing "one shot" into a tared beaker on a balance: 5 mL = 5 grams. Adjust repipette if necessary. Remember to check the calibration of the balance using the procedure outlined in Section 5.3.2.1.
5. Remove the caps from the vials and dispense 5.0 mL of the digesting reagent into each vial. 5.0 mL of digesting reagent is used for all samples and standards.
6. Cap each vial tightly and shake vigorously. Place in rack.
7. Once the digestion solution has been added to each vial put the rack of vials into room temperature water bath. Add DI water to water bath to approximately a 1/4 inch below the level of liquid in the sample vials.
8. Put the lid on the water bath and switch it on. It will take 45 – 60 minutes to reach boiling temperature.
9. Boil gently for 15 minutes. The thermometer in the water bath must reach and maintain a temperature of at least 100 °C for 15 minutes; record the temperature on the water bath temperature log sheet.
10. Turn off water bath and allow the vials to cool to room temperature (overnight) in the water bath. Keep the cover on the water bath.
11. The next day, remove the racks of vials from the water bath.
12. Store racks in refrigerator until day of analysis. Samples should be analyzed within 48 hours of the time of digestion. If the samples are being analyzed the same day they are removed from the water bath do not refrigerate the samples prior to analysis.

5.3.4.2 Preparation of Potassium Persulfate Digesting Reagent

1. The potassium persulfate digesting reagent should be made fresh on the day of use.
2. Use a beaker with a magnetic stir bar that has been specifically reserved for total phosphorus digestions. These beakers are stored in a labeled glass cabinet in room 002.
3. Determine the amount of potassium persulfate digesting reagent needed. Each sample requires 5.0 mL digesting reagent.
4. Weigh the dry reagents (potassium persulfate and boric acid) into the beaker referring to the table below for reagent and volume information.
Digested Reagent for Total Phosphorus and Total Nitrogen

<table>
<thead>
<tr>
<th>Final Volume of Potassium Persulfate (mL)</th>
<th>Beaker Size for Reagent Preparation (mL)</th>
<th>Potassium Persulfate (g)</th>
<th>Boric Acid (g)</th>
<th>1N NaOH solution (mL)</th>
<th>Ultrapure Water (mL)</th>
<th>Approximate number of vials reagent volume will fill</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>1000</td>
<td>50</td>
<td>30</td>
<td>350</td>
<td>900</td>
<td>190</td>
</tr>
<tr>
<td>500</td>
<td>500</td>
<td>25</td>
<td>15</td>
<td>175</td>
<td>450</td>
<td>90</td>
</tr>
<tr>
<td>250</td>
<td>500</td>
<td>12.50</td>
<td>7.50</td>
<td>87.50</td>
<td>200</td>
<td>45</td>
</tr>
<tr>
<td>200</td>
<td>500</td>
<td>10</td>
<td>5</td>
<td>70</td>
<td>150</td>
<td>35</td>
</tr>
</tbody>
</table>

5. Add the sodium hydroxide solution.
6. Using Ultrapure water, dilute to the appropriate volume according to the chart above.
7. Cover beaker with aluminum foil.
8. Place beaker onto a heating magnetic stirrer.
9. Stir with low heat until all crystals are dissolved. This takes 5-10 minutes.
10. When crystals are dissolved, pour solution into appropriate sized volumetric flask specifically reserved for “TP only.”
11. Let digesting reagent cool.
12. Once reagent has cooled add Ultrapure water to the final volume.
13. Transfer to an amber Repipette, labeled “new digesting reagent.” Label with current date.
14. Any digesting reagent in the repipette from a prior digestion can be poured into the "old TP digest" repipette and used for "pre-digesting" vials.

5.3.5 Procedure – Day of Analysis

5.3.5.1 Column Regeneration

The cadmium column used to reduce NO₃ to NO₂ must be regenerated after every 4 autoanalyzer runs. The method utilized for column regeneration is located in the Astoria-Pacific manual.

5.3.5.2 Sample Analysis

Detailed instructions regarding operation of the Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyer will not be repeated here. This information is located in the autoanalyzer operation manuals. A summary of the analytical procedure is found below:

1. Allow all digested samples, standards and blanks to warm to room temperature. This takes place while the autoanalyser is being prepared.
2. Obtain reagents needed for the analysis. The PO₄ assay only utilizes one reagent, the mixed reagent. The mixed reagent must be prepared daily, preparation instructions are located in the Astoria-Pacific manual. The NO₃+NO₂ analysis utilizes two reagents:
ammonium chloride buffer and color reagent. All reagents should be allowed to warm to room temperature prior to use.

a. Lab personnel must wear gloves, lab coat and eye protection when handling reagents. Waste reagents must be collected for later disposal by URI Safety and Health.

b. Further information regarding reagents is located in the Laboratory MSDS binder. Further information regarding health and safety is located in SOP 001 – General Laboratory Safety and SOP 001a – University Safety and Waste Handling Document.

3. A summary of Autoanalyzer Operation is provided below:

a. A calibrated volume of sample is introduced into the autoanalyzer by the sampler.

b. The sample is delivered to the analytical cartridge by a peristaltic pump. It is then combined with reagents and air bubbles in a continuously moving stream.

c. As the sample moves through the cartridge it passes through mixing coils and a heating bath, as specified by test conditions.

d. A color is produced by the specific analyte in the sample, blue for the PO₄ assay and pink for the NO₃+NO₂ assay. The intensity of the color is determined by the amount of analyte present.

e. This colored stream then flows to the photometer where the color intensity is measured and converted to an electronic signal. The electronic signal is sent to a computer where the Astoria-Pacific FASPac II® software program processes the signal to create a standard curve and numerical results for the standards and samples as well as a real-time recorder tracing of peaks.

4. Each autoanalyzer rack has 90 places for samples, blanks and QC samples. A sample run can have up to three racks (270 samples).

a. Standards are set up on their own rack. A set of standards is run at the beginning of each run. A separate set of standards is run at the end, and are averaged with those run at the beginning, using standards from a different vial that those used for the first set of standards.

b. See Section 5.2 for information regarding the frequency of blanks and standards.

c. Each sample and standard is recorded on a sample log sheet as they are poured into analytical sample cups.

d. 1 sample out of 10 is analyzed in duplicate (two separate analyzer sample cups).

e. The autoanalyzer is set to analyze each sample cup twice.

f. 1 sample out of 30 is a matrix spike.

5. The autoanalyzer is connected to a personal computer. The Astoria-Pacific FASPac II® program analyzes the data reported by the autoanalyzer. The program performs a linear regression on the standards and determines sample concentrations.

a. A graph of concentration verses absorbance of standards is displayed and can be manipulated to remove obvious outliers.
b. The standard curve is rejected if $R^2$ is less than 0.95 unless this is due to 1 standard in the suite. (see Section 5.2 for further information)

c. During the run the real time peak tracing are monitored to check for in-range heights, proper shapes and any irregularities, so that samples can be re-analyzed, space permitting during the current run, or saved for re-analysis in a later run.

d. At the end of a run each peak is checked to make sure the marker is in the proper spot and any irregularities are accounted for (analytical blips, empty sample cups, etc). Once each peak in a run has been reviewed, the results of the run are exported and saved as Excel files, and printed.

6. After the computer results are printed, they are again compared to the peak tracing. Particularly take note of carryover of high to low peaks and correct any keyboarding errors.

7. QC results are compared to previous results as well as "true values". If the results of the run meet the QA/QC criteria set forth in this SOP then the results of the run are deemed acceptable.

8. After the printout has been approved, the data are entered into appropriate Excel spreadsheet files, where they are subsequently re-checked for data entry errors.

9. After the data are approved samples may be disposed of in accordance with Section 4.0.

10. Sample bottles are cleaned by acid washing following SOP 003 – General Labware Cleaning Procedure. Digestion vials are cleaned as specified in Section 5.3.1.1 of this SOP.

6.0 CALCULATIONS

Total nitrogen and total phosphorus concentrations are calculated in the Astoria Pacific FASPac II program from the standard curve analyzed at the beginning and end of the sample run. Data are reported to the nearest 10’s for total nitrogen and the nearest whole number for total phosphorus. Values less than the reporting limit are reported as $<(\text{numerical value of the}) \, \text{RL} \, \mu g/L \, \text{TP or TN}$.

7.0 REFERENCES


     Method referenced: Persulfate Method (with automated cadmium reduction) (Section 4500-N$_{org.}$ D).

     Method referenced: Automated Ascorbic Acid Reduction Method (Section 4500-P F. Phosphorus).


8.0 DOCUMENTATION

Data consisting of the sample results, peaks and calibration curves are exported from the computer running the autoanalyzer into Excel data files. The Excel files are then printed and saved electronically.
### Sample Log Sheet

<table>
<thead>
<tr>
<th>Pos#</th>
<th>Contents &amp; Date</th>
<th>DF</th>
<th>Pos#</th>
<th>Contents &amp; Date</th>
<th>DF</th>
<th>Pos#</th>
<th>Contents &amp; Date</th>
<th>DF</th>
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<th>DF</th>
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<th>Contents &amp; Date</th>
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<td>75</td>
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</tr>
</tbody>
</table>

Check Calibrant # & concentration(CC# _____) = ______ ug/l

Total Phosphorus and Nitrogen Analysis SOP 016
S:\WW\awwword\LABPROC\all QAPPs\LABQAPPs\QAPP RevS -0609\SOPs\SOP 016 TN&TP-MOD4.doc
1.0 PURPOSE AND DESCRIPTION .................................................................................................................. 1
2.0 HEALTH AND SAFETY CONSIDERATIONS ......................................................................................... 1
  2.1 HAZARDS ........................................................................................................................................ 1
  2.2 TECHNICIAN TRAINING/QUALIFICATIONS ....................................................................................... 1
3.0 REQUIRED MATERIALS ....................................................................................................................... 2
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6.0 CALCULATIONS .................................................................................................................................... 4
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Appendix A : Instructions for Operation of the Refractometer
1.0 PURPOSE AND DESCRIPTION

The purpose of this method is to determine the salinity of marine and estuarine samples. This method is not appropriate for determining the salt content of freshwater samples. Unfiltered samples are analyzed using a LaMotte Salinity Titration Kit and checked using a refractometer. This method is appropriate for undiluted samples ranging from 0.4 to 40 ppt.

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

General laboratory good housekeeping procedures should be practiced as outlined in Standard Operating Procedure (SOP) 001 – General Laboratory Safety.

Potassium chromate and silver nitrate are utilized in this procedure. Potassium chromate will cause eye, respiratory tract and skin irritation upon contact. It is a carcinogen and may cause liver and kidney damage. Silver nitrate may cause eye, skin, digestive and respiratory tract irritation with possible burns upon exposure. Ingestion may cause methemoglobinemia (lack of oxygen in the blood) and kidney damage. Use these chemicals in a well ventilated area wearing a laboratory coat, gloves and safety goggles. General safe handling practices should be used when working with all chemicals.

Further information regarding chemicals utilized in this procedure is found in the laboratory Material Safety Data Sheet (MSDS) binder found in the laboratory where the chemical of interest is stored. General laboratory information regarding safe handling of chemicals is located in SOP 001a – University Safety and Waste Handling.

Waste Reagents

Titrated samples and waste reagents may be rinsed down the drain with water.

2.2 Technician Training/Qualifications

General training in laboratory technique must be completed prior to analyzing samples using this method. Technician training will be provided by Linda Green, Laboratory Project Manager – Nutrients or Elizabeth Herron – Laboratory Project Manager - Microbiology.
3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>LaMotte Salinity Titration Kit (Model 7459-01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A366ATC Hand Held Salinity Refractometer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory. Several replacement test kits are available in the URIWW Laboratory.

4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (Marine and Estuarine)</td>
<td>60-125 mL acid washed brown glass bottle</td>
<td>None</td>
<td>30 mL</td>
<td>1 year</td>
</tr>
</tbody>
</table>

Disposal
Samples may be disposed of only after final quality assurance checks are completed and the data found to be acceptable using criteria found in Section 5.2 of this SOP. Samples may be disposed of by rinsing down the drain with water.

5.0 METHOD DESCRIPTION

5.1 Scheduling Considerations

There are no specific scheduling considerations for this method. Enough chemical should be available to complete the analysis.

5.2 Quality Assurance/Quality Control

5.2.1 Method Detection Limit

The Method Detection Limit (MDL) for this assay is 0.4 ppt salinity. The Limit of Quantitation or Reporting Limit (RL) for this assay is 0.4 ppt salinity. The upper limit of reporting is 40 ppt. Results are reported to the nearest tenth.

5.2.2 Method Blanks

No method blanks are completed in this assay.
5.2.3 Sample Replication

Sample replication is completed on 100% of samples. The difference between the replicates must be not greater than 2 ppt.

Corrective Action
If the difference between replicates is greater than 2 ppt then a third titration will be completed. If the sample replicates are still not within 2 ppt then the deviation is noted on the project data sheet.

5.2.4 Sample Comparison

At least 50% of the samples will be analyzed using a A366ATC Hand Held Salinity Refractometer or a similar unit. The operation instructions for this unit are attached in Appendix A. The refractometer unit may measure samples within the range of 0 – 100 ppt and is accurate to 1 ppt. The difference between the average of the values obtained using the test kit and the value obtained from the refractometer must not be greater than 2 ppt.

Corrective Action
If the difference between the salinity value obtained using the LaMotte Test Kit and the refractometer is greater than 2 ppt then the sample will be re-analyzed using both the test kit and the refractometer. If the difference is still greater than 2 ppt then the test kit titrant will be replaced and the sample reanalyzed. If the values are still greater than 2 ppt different then it will be assumed that the refractometer is in error and it will be repaired.

5.2.5 Calibration

Titrant is obtained from LaMotte, therefore no calibration is completed as the purchased titrant is of a known value.

5.3 Analysis Method

1. Safety goggles and gloves must be worn when completing this analysis. Care must be taken with this kit as the chemical reagents will permanently stain counter tops, tables and clothing as well as create long lasting stains on skin. Cover working surfaces with newspapers and wear gloves and protective clothing when using this kit.
2. Fill the demineralizer bottle with tap water. It is the big bottle with the brown-black crystals in it.
3. Recap, making sure that the spout is closed, and shake vigorously for 30 seconds.
4. Fill the titration vial (code 0648) to the 10 mL line with demineralized water.
5. Fill the 1 mL titrator syringe (code 0376) with the water sample. Wipe off the tip of the titrator syringe.
6. Dispense 0.5 mL of sample water into the titration vial containing the demineralized water.
7. Add 3 drops of the potassium chromate indicator solution (salinity reagent A). Cap the titration vial and swirl gently to mix. The solution will turn yellow.
8. Fill the 0 - 20 mL titrator syringe (code 0378) with the silver nitrate reagent (salinity reagent B).
9. Insert that titrator syringe into the hole in the cap of the titration vial.
10. Depress the plunger and add the silver nitrate dropwise. Swirl gently to mix the reagent with the water sample.
11. The end point of the reaction is when the color changes from yellow to cloudy pink-brown, sort of a grapefruit pink.
12. Read the titrator syringe where the tip of the plunger crosses the scale. Record the result. (Remember that the smallest divisions are 0.4 ppt.).
13. Repeat the test with another aliquot of the same sample.
14. Clean-up: Flush all unused reagents in the syringes and the solution in the titration vial down the drain with plenty of water. Take apart the syringes and rinse with tap water. Invert the syringe barrels to dry. Rinse the titration vial with tap water and invert to dry.

6.0 CALCULATIONS

Each division on the titrator is equal to 0.4 ppt. Therefore multiply the amount of titrant dispensed by 0.4 ppt to obtain the sample salinity. Data are reported to the nearest 10th of a unit.

7.0 REFERENCES

LaMotte Test Kit Instructions. www.lamotte.com

8.0 DOCUMENTATION

The results of each titration are recorded on the sample data sheet.
Green Hill and Ninigret Ponds  
SALINITY DATA  

*November 17, 2004 water collection*  
Please fill-in your initials, and the appropriate value.  
There should be something in each space.  
*Check sample log sheet to see if monitoring location has been received*

<table>
<thead>
<tr>
<th>Analysis Date</th>
<th>Tech's Initials</th>
<th>Monitoring Location</th>
<th>Refractometer Salinity</th>
<th>Kit Salinity Rep 1</th>
<th>Kit Salinity Rep 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GH-In Pond-mid-depth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GH-In Pond-deep</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>GH-Indigo Point</td>
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<td></td>
<td></td>
<td>GH-Sea Lea</td>
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<tr>
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<td></td>
<td>GH-Teal Road</td>
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<td>NP-Pond Street</td>
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<td>NP-Stumpy Point</td>
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<td>NP-Vigna's Dock</td>
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</tr>
</tbody>
</table>
Appendix A: Instructions for Operation of the Refractometer

A366ATC Operation Manual
Hand Held Salinity Refractometer with Automatic Temperature Compensation
(0-10%, and 1.000 to 1.070 specific gravity of Salt Water)

PARTS OF THE INSTRUMENT
1. eyepiece
2. zero adjustment screw
3. daylight plate
4. prism

SCALE CALIBRATION
The very first thing you should do before applying the actual sample solution to the refractometer prism glass is to calibrate the instrument to assure precision accuracy. It is done by performing the following:

Using a drop of distilled water, apply it to the prism glass as shown below. Close the cover gently, look through the viewfinder (eyepiece) and check to see that 0% (or 1,000). The boundary line between the colored and white fields intersects the scale anywhere but exactly 0% (1,000). Adjust the boundary line back to 0% (1,000) by turning the zero adjusting screw.

PRECAUTION
- Since the refractometer is an optical instrument, do not drop it or handle it roughly.
- Since the prism has a relatively soft surface, be careful not to scratch it.
- After each use, clean the prism surface and daylight plate with a soft cloth soaked in water and wipe off with a dry cloth.
- Do not hold the prism directly in a water stream of a water pipe, etc.
- If the prism surface is smeared with oil or similar liquids, it will repel the sample and obstruct the measurement. Wipe off the oil smear or contaminant with weakened detergent or suitable solvent.

HOW TO USE THE RefRACTOMETER
1. Open the daylight plate and apply one or two drops of a sample solution onto the prism surface.
2. Close the daylight plate gently. Then, the sample solution spreads into a thin film in between the daylight plate and the prism. Make sure the prism is completely covered and there are no air bubbles.
3. Hold the refractometer with the daylight plate facing upward, direct it toward the light and observe the liquid of view through the eyepiece. If the field of view is not clear, focus the image by turning the potion closest to your eye.
4. The upper field of view appears blue and the lower field appears white. Read the scale where the boundary line of the blue and white fields cross the scale. The scale reads in both parts per thousand and specific gravity of salt water.
### Conversion Table

<table>
<thead>
<tr>
<th>Refractometer Reading</th>
<th>NaCl% by Weight</th>
<th>MgCl2% by Weight</th>
<th>MgSO4% by Weight</th>
<th>K2SO4% by Weight</th>
<th>CaCO3% by Weight</th>
<th>Sucrose (lbs)</th>
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</thead>
<tbody>
<tr>
<td>0 PPT</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>10 PPT</td>
<td>1.0</td>
<td>0.7</td>
<td>0.9</td>
<td>1.4</td>
<td>0.8</td>
<td>1.3</td>
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<tr>
<td>20 PPT</td>
<td>2.1</td>
<td>1.4</td>
<td>1.8</td>
<td>2.9</td>
<td>1.5</td>
<td>2.5</td>
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<td>3.1</td>
<td>2.1</td>
<td>2.7</td>
<td>4.3</td>
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<td>5.8</td>
<td>3.0</td>
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<tr>
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<td>4.5</td>
<td>7.3</td>
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<td>4.2</td>
<td>5.4</td>
<td>8.8</td>
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<td>5.0</td>
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<td>6.6</td>
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<td>10.5</td>
<td>7.2</td>
<td>9.1</td>
<td>15.0</td>
<td>7.6</td>
<td>12.3</td>
</tr>
</tbody>
</table>

### Specifications

**Salinity Range:** 0 - 100 Parts per Thousand (PPT) and 1.000 - 1.070 specific gravity of saltwater

**Min. divisions:** 1 PPT and 0.001 specific gravity

**Accuracy:** 1 PPT and 0.001 specific gravity

**Dimensions:** 1.57" X 1.57" X 7.87" (4 X 4 X 20 cm)

**Weight:** 5.99 oz (170 g)
1.0  PURPOSE AND DESCRIPTION

The purpose of this method is to determine the number of enterococci bacteria within an ambient (lakes, ponds and rivers) or marine water sample. Marine samples must be diluted at least tenfold with sterile Ultrapure water. Samples are collected in sterile bottles, a measured volume aseptically transferred to a sterile IDEXX vessel, commercially prepared reagents are added and the solution poured into a Quanti-Tray or Quanti-Tray/2000 and sealed. The Quanti-Tray or Quanti-Tray/2000 is then incubated for 24 hours. The number of Quanti-Tray or Quanti-Tray/2000 cells that fluoresce under a UV light are used to determine the Most Probable Number (MPN) of bacteria per 100 mL.

This method is applicable to undiluted samples in the range of <1 to 2,419 enterococci/100 mL and samples diluted to return values in this range using the Quanti-Tray/2000. The method is applicable to undiluted samples in the range of <1 to 200 enterococci/100 mL and samples diluted to return values in this range using the Quanti-Tray.

2.0  HEALTH AND SAFETY CONSIDERATIONS

2.1  Hazards

Samples to be analyzed are a potential biological hazard. All personnel shall wear a lab coat, gloves and goggles when performing this procedure to protect them from accidental exposure.

Wastes in the form of used Quanti-Trays are also a potential biological hazard and will be disposed of following autoclaving. The procedure for sterilization of Quanti-Trays is found in Section 4.0 Sample Storage, Preservation and Disposal, of this SOP.

Wastes and materials pose a burn hazard immediately following autoclaving. Never remove materials from the autoclave without the proper insulated autoclave gloves. In addition, when opening the front door on the autoclave, steam will escape as soon as the door is cracked. Any exposed body parts near this steam could be burned. Specific information on autoclaving procedures can be found in the following SOPs: SOP 004 - General Autoclave Operation, SOP 005 - Bottle Autoclaving Procedure and SOP 006 - Waste Autoclaving Procedure.

General laboratory good housekeeping procedures should be practiced as outlined in Standard Operating Procedure (SOP) 001 - General Laboratory Safety. The only chemical utilized in this SOP is the Enterolert medium. The medium is not listed as hazardous but should be treated with care. Always wear protective clothing in the form of gloves, a laboratory coat and goggles when working with this chemical. Further information regarding this chemical may be found in
the laboratory Material Safety Data Sheet (MSDS) binder found in the laboratory where the chemical of interest is stored.

A UV light is utilized in this procedure, keep exposure to the UV light to a minimum. Use protective face shield or goggles and gloves when reading samples. General laboratory information regarding safe handling of chemicals is located in SOP 001a - University Safety and Waste Handling.

2.2 Technician Training/Qualifications

General training in laboratory technique, aseptic technique, and the proper use of the UV light and Quanti-Tray sealer must be completed prior to analyzing samples using this method. Technician training will be provided by Elizabeth Herron Project Manager – Microbiology

3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterolert Medium</td>
<td>Snap packs of sterile media vessels for 100 mL samples. Stable for 12 months at 2-30°C, away from light.</td>
<td>IDEXX Cat # WENT020 or WENT200</td>
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<tr>
<td>Quanti-Tray or Quanti-Tray/2000</td>
<td></td>
<td>IDEXX Cat # WQT100 (Quanti-Tray) or EQT-2K (Quanti-Tray/2000)</td>
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<td>IDEXX Quanti-Tray Sealer Model 2x</td>
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<td>IDEXX Cat # WQTS2X-115</td>
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<td>IDEXX Sterile Disposable Vessels</td>
<td>Marked at 100 mL, may have been resterilized and tested for sterility by URIWW</td>
<td>IDEXX Cat # WV120SBST-200</td>
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<tr>
<td>Quanti-Tray/2000 rubber insert</td>
<td></td>
<td>IDEXX Cat # WQTSRBR-2K</td>
</tr>
<tr>
<td>UV light and viewing box</td>
<td></td>
<td>UV light #WL160, Replacement lamp #WL161, UV cabinet #WCM10</td>
</tr>
<tr>
<td>Sterile Ultrapure water</td>
<td>4 L carboy used for Enterolert dilution</td>
<td></td>
</tr>
<tr>
<td>41 °C Incubator</td>
<td>Precision Scientific Model 4EG</td>
<td></td>
</tr>
<tr>
<td>Paper towels, safety goggles, lab gloves</td>
<td></td>
<td>UV goggles #WLG</td>
</tr>
<tr>
<td>Autoclave and maximum temperature thermometer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory and the Natural Resources Science department. Temporary replacement of large equipment such as the Quanti-Tray Sealer is available through arrangement with the manufacturer.
4.0 SAMPLE STORAGE, PRESERVATION AND DISPOSAL

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Volume</th>
<th>Holding Time</th>
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</thead>
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<tr>
<td>Water</td>
<td>Autoclavable 250 mL white plastic bottle</td>
<td>Kept at 4 °C in Sterile Bottles</td>
<td>100 mL</td>
<td>6 Hours</td>
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</table>

Disposal
Samples are archived for approximately 48 hours. Aqueous samples may only be disposed of after final quality assurance checks are completed and the data found to be acceptable using criteria found in Section 5.2 of this SOP. Aqueous samples are considered a potential biological hazard. Samples may be rinsed down the drain with running water. Personnel will wear gloves, eye protection and a laboratory coat when disposing of samples.

Disposal of used Quanti-Trays should be completed in accordance with SOP 006 - Waste Autoclaving Procedure.

Trays are not archived and may be disposed of immediately after counting and recording of data.

Cleaning and sterilization of sample and IDEXX mixing vessels

Sample bottles are cleaned in accordance with SOP 003 - General Labware Cleaning Procedure and SOP 005 - Bottle Autoclaving Procedure.

IDEXX mixing vessels are cleaned according to SOP 003 - General Labware Cleaning Procedure, then sterilized in the UV box for at least 5 minutes, with caps set inside up. Recapped sterilized mixing vessels are stored in the appropriate containers. Sterilization processing and sterility confirmation are recorded on datasheets stored in the Room 019 Maintenance notebook and provided in Section 8.0 Documentation, of this SOP.

5.0 METHOD DESCRIPTION

5.1 Scheduling Considerations

The information provided below is a basic outline of tasks that must be completed prior to the sampling day. Step-by-step instructions to complete each task are provided under Section 6.0.

At least 2 days prior to analysis, check that enough Enterolert media and sterile IDEXX mixing bottles are available for the anticipated number of samples to be analyzed. An operations check of the UV viewing box should also be completed at this time.

The day before a sampling event (24 hours) data sheets and a final check of equipment should be completed.
5.2 Quality Assurance/Quality Control

5.2.1 Method Detection Limit

The method detection limit is set by the product manufacturer at < 1 CFU/100 mL.

5.2.2 Method Blanks

Method blanks are determined by treating 100 mL of sterile Ultrapure water as a sample.

There will be 2 method blanks per run. The first blank will be prepared at the beginning of the sample run and the second at the end of the sample run. The method blank shall be less than 1 colony forming unit (CFU)/100 mL.

**Corrective Action**

If the method blank is equal or greater than 1 CFU/100 mL then the sample run will be considered contaminated and the samples reanalyzed. The field samples will be out of compliance with holding times if samples need to be reanalyzed. This will be noted on the data sheet.

5.2.3 Sample Replication

Sample replication is completed on 1 sample in 5; 20% of the collected field samples. Sample replication is completed by taking a second 100 mL (or 10 mL if an estuarine or marine sample) aliquot of the sample and treating it as a regular sample. Analysis results for replicate samples should be with 20% relative percent deviation (%RPD). %RPD is calculated as follows:

\[
\text{%RPD} = \left| \frac{\text{Result of Replicate 1 (CFU/100 mL)} - \text{Result of Replicate 2 (CFU/100 mL)}}{\text{Average of Result of Replicate 1 (CFU/100 mL) and Result of Replicate 2 (CFU/100 mL)}} \right| \times 100
\]

**Corrective Action**

If the %RPD is greater than 20% then the deviation is noted on the data sheet. The processing time to determine the sample value is greater than the holding time for the samples. Therefore, the samples will not be reanalyzed unless contamination is suspected as shown through a non-compliant method blank or the positive tray indicates that the method was compromised in another manner.

5.2.4 Calibration and Standards

5.2.4.1 Positive Trays

Calibration is completed in a qualitative way through an assessment of false negatives. A purchased enterococci bacterial sample is used to inoculate a sample of Ultrapure water. The standard is then treated as a sample. These samples are referred to as “positive trays”, 1 positive trays is prepared per sample batch. After incubation these trays must exhibit fluorescence.

**Corrective Action**

The inoculated trays (positive trays) must show fluorescence after incubation. If the trays do not show fluorescence then it is assumed that the run was in error and the batch of samples is
reanalyzed. The field samples will be out of compliance with holding times if samples need to be reanalyzed. This will be noted on the data sheet.

5.2.4.2 Calibration Check/Laboratory Control Standard

Environmental Protection Agency Water Pollution Proficiency Test Study
The laboratory participates in the Environmental Protection Agency Water Pollution Proficiency Test Study on a yearly basis. Unknown samples are purchased from an outside vendor and analyzed for enterococci. URIWW results are sent to the vendor who provides a comparison to the actual value and a performance evaluation. Study results are maintained by the laboratory.

Corrective Action
If study results return values outside acceptable limits then the laboratory will review the enterococci procedures and purchase another unknown to be analyzed for enterococci. If these data are also outside acceptable criteria then the laboratory will continue working with unknowns until the issue is resolved.

5.2.5 Incubators

The temperature of the 41 °C incubator is checked on a daily basis, when in use. Initial and final temperatures for incubations of samples are recorded on the project data reporting sheet (see Section 8.0). The acceptable temperature range for the 41 °C incubator is 41 +/- 0.5 °C.

Corrective Action
The incubator temperature must remain in the range specified above. If the incubator temperature is found to be outside the acceptable range, contact Elizabeth Herron and adjust the incubator temperature control. Professional maintenance of the incubator may be necessary if adjustment of the temperature control does not rectify the problem.

5.2.6 Sterility Check on IDEXX Mixing Bottles

IDEXX mixing bottles must be checked for sterility after cleaning and before being placed with the general stock of sterilized IDEXX mixing bottles. A bottle sterility check will be completed at a frequency of one sample bottle per sterilization run. Sterility checks will be completed by selecting one sterilized sample bottle at random after cleaning and sterilization of a batch of bottles has been completed. The IDEXX mixing bottle will then be filled with 100 mL of Ultrapure water capped and mixed. Once the water has been mixed it will be treated as a sample. All data will be recorded on the “Bacterial Sample Log & Worksheet: Bacteria Bottle Sterilization Confirmation” data sheet (see Section 8.0 Documentation).

Corrective Action
If the IDEXX mixing bottle blank returns a value greater than 0 CFUs then all the IDEXX mixing bottles sterilized in the run must be re-sterilized and the IDEXX mixing bottle sterility check completed again after the second sterilization process. No IDEXX mixing bottles will be placed into the general laboratory stock of sterilized mixing bottles until the sterility check returns a value of 0.
5.2.7 Sterility Check on Sample Bottles

Sample bottles after sterilization must be checked for sterility before being placed with the general stock of sterilized bottles. A bottle sterility check will be completed at a frequency of one sample bottle per sterilization run. Sterility checks will be completed by selecting one sterilized sample bottle at random after the sterilization run has been completed. The sample bottle will then be filled with Ultrapure water capped and mixed. Once the water has been mixed it will be filtered and treated as a sample. All data will be recorded on the “Bacterial Sample Log & Worksheet: Bacteria Bottle Sterilization Confirmation” data sheet (see Section 8.0 Documentation).

Corrective Action
If the bottle blank returns a value greater than 0 CFUs then all the sample bottles sterilized in the run must be re-sterilized and the bottle sterility check completed again after the second sterilization process. No sample bottles will be placed into the general laboratory stock of sterilized bottles until the sterility check returns a value of 0.

5.2.8 Germicidal Unit (UV Box)

The UV light box efficiency will be tested quarterly with a UV light meter. Each lamp will be tested individually. A lamp will be replaced if it emits less than 70% of its initial output. All efficiency checks will be recorded on the “Quarterly UV Germicidal Tube Record” data sheet (see Section 8.0 Documentation) and will be available for review.

Corrective Action
If a UV lamp is found to emit less than 70% of its initial output then it will be replaced. The date of replacement will be recorded on the efficiency check data sheet.

6.0 METHOD DESCRIPTION

Samples must be collected in sterilized bottles using proper sampling technique. The procedure for sterilizing sample bottles is located in SOP 005, Bottle Autoclaving Procedure. Samples should be analyzed immediately.

Prepare at least 1 blank consisting of 100 mL of sterile Ultrapure water at the beginning and end of the run. The blank should be treated as a sample.

For each sample repeat the following procedure:

1. Obtain a sterile IDEXX mixing vessel.
2. If the sample is from a freshwater source pour 100 mL of the sample into the vessel using the 100 mL mark on the bottle as a guide.
   a. If the sample is expected to return a value greater then the maximum acceptable value then dilution may be necessary, the procedure for diluting a sample is provided below. Alternatively, the Quanti-Tray/2000 may be utilized to obtain a greater maximum acceptable value, use previous data as a guide to determine appropriate measures.
   b. Complete a dilution by recording the sample volume on the project data sheet and use a pipette aide and sterile pipette to transfer the appropriate amount of sample to the sterile IDEXX mixing vessel.
c. Dilute to the 100 mL mark on the IDEXX mixing vessel using sterile Ultrapure water.

3. If the sample is from a saltwater source then you must dilute the sample before adding the reagent. The procedure for dilution is outlined below:
   a. Shake the sample vigorously (about 25 times in 7 seconds).
   b. Using the pipette-aide and a sterile 10 mL pipette, pipette 10 mL of sample into the sterile 100 mL vessel and then dilute to the 100 mL mark using sterile Ultrapure water.

4. Pour contents of one snap pack of Enterolert into the 100 mL vessel, cap and with a vigorous rotation of the wrist, mix until the medium is completely dissolved.

5. Record reagent lot number and expiration date on the data sheet.

6. With a permanent marker, record sample identification and volume on the front (plastic well side) of a Quanti-Tray or Quanti-Tray/2000.

7. Pour the sample into a Quanti-Tray or Quanti-Tray/2000 and seal it using the IDEXX Quanti-Tray Sealer.
   a. Instruction on how to use a Quanti-Tray or Quanti-Tray/2000 are attached below.
   b. Instructions on how to use the IDEXX Sealer are below:
      i. Figure showing IDEXX Sealer (www.idexx.com)
      ii. Turn the power switch on and the amber power light should illuminate. If the sealer is not operating properly contact Elizabeth Herron.
      iii. The sealer is warmed-up and ready to use when the green “Ready Light” comes on. It should take about 10 minutes. The sealer will not operate until the amber power light and green “Ready Light” are lit.
      iv. Put an empty Quanti-Tray or Quanti-Tray/2000 Rubber Insert on the Input Shelf with the large cutout facing away from the Sealer (as shown the figure above).
      v. Place a Quanti-Tray or Quanti-Tray/2000 filled with sample and reagent onto the Rubber Insert, making sure that the tray is properly seated in the Rubber Insert, and each well of the tray in its corresponding Rubber Insert hole.
vi. Slide the inset and tray into the sealer until the motor grabs the rubber insert and begins to pull it into the Sealer.

vii. The sealer will then seal the tray and push it partially out of the sealer. The process takes about 15 seconds.

viii. Remove the tray and insert from the Sealer.

ix. Check that the tray sealed properly; sample should be contained in each cell and not able to flow from cell to cell.

x. You can put the next tray into the sealer once the first tray has been fully drawn into the sealer.

xi. If there is a problem and the tray goes into the sealer improperly use the reverse button by pressing and holding it.

xii. Do not reverse the motor once the rubber insert is fully drawn into the input slot.

xiii. Turn off sealer when not in use.

xiv. Preventative maintenance information for the Sealer is attached in Section 8.0 of this SOP.

8. Record sample and processing information on the appropriate data worksheet.

9. Place the sealed tray into the 41 °C incubator foil side up for 24 hours. With multiple trays, stack no higher than 15 trays, and ensure that there is adequate airflow around the trays by not placing any trays on the floor of the incubator, or covering any one rack. The incubator should be set at 41 °C +/- 0.5°C.

10. After 24 hours remove the trays from the incubator and count the number of wells that exhibit fluorescence when placed under a UV light (6 watt, 365 nm). Use the UV viewing box.

11. The Most Probable Number is determined using the MPN table attached below.

12. Blanks should return a value of <1 CFU/mL. If the blanks are greater than this value the run is considered contaminated, re-run the procedure.
7.0 CALCULATIONS

Record the number of positive wells onto the sample data sheet. The number of positive large and small cells on the Quanti-Tray/2000 should be recorded in separate columns on the data sheet. Note that if you are running a sample in a Quanti-Tray, place a "—" in the column for "# pos. sm. cells" on the data sheet to indicate that you were using a Quanti-Tray and no small cells are available. If you are running a sample in a Quanti-Tray/2000 place a "0" in the column for "# pos. sm. cells" if none are positive, this will indicate that you were running the Quanti-Tray/2000 instead of the Quanti-Tray.

The numbers of positive wells as recorded on the sample data sheet are then input into the IDEXX data tables below to determine the Most Probably Number (MPN). You can use the data table directly if you did not dilute the sample. If you diluted the sample use the calculation below to determine the final MPN.

\[
\text{MPN} = \frac{\text{table value}}{100 \text{ mL}} \times 100 \text{ mL} \times \frac{\text{volume in mL of sample analyzed}}{100 \text{ mL}}
\]

Be sure to use the correct data tables; there are different tables for the Quanti-Tray and Quanti-Tray/2000 samples.

The final sample value should be less than 200 CFU/mL, if the Quanti-Tray has been used in the analysis and less than 2,419 CFU/mL, if the Quanti-Tray/2000 was used in the analysis. If the results are higher than acceptable, make a note on the data sheet and reanalyze the sample. The samples will be outside the holding time of 6 hours after the first analysis, be sure to note this on the data sheet.
## 51-Well Quanti-Tray MPN Table

<table>
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<tr>
<th>No. of wells giving positive reaction per 100 ml sample</th>
<th>Most Probable Number</th>
<th>95% Confidence Limits</th>
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<td>17</td>
<td>0.06</td>
<td>0.05</td>
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<tr>
<td>18</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>19</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>20</td>
<td>0.12</td>
<td>0.11</td>
</tr>
</tbody>
</table>

**IDEXX Quant-Trap / 2000 MPN Table**
8.0 REFERENCES


Method referenced: Heterotrophic Plate Count (9215).

### 8.0 DOCUMENTATION

Sample Data Sheet

<table>
<thead>
<tr>
<th>Monitoring Location</th>
<th>Sample/Set-Up Date</th>
<th>Setup tech</th>
<th>Dilution (mls)</th>
<th>Incubator temp. start</th>
<th>Incubator temp. end</th>
<th>Count tech</th>
<th># pos. lg wells</th>
<th># pos. sm wells</th>
<th>Table Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

**Notes:**
- If you are running a sample in a Quanti-Tray place a “—” in the column for “# pos. sm. cells” to indicate that you were using a Quanti-Tray and no small cells are available.
- If you are running a sample in a Quanti-Tray/2000 place a “0” in the column for “# pos. sm. cells” if none are positive, this will indicate that you were running the Quanti-Tray/2000 instead of the Quanti-Tray.
### Bacterial Sample Log & Worksheet: Bacteria Bottle Sterilization Confirmation

**mTEC Membrane Filtration Method**

**Analyst - set-up:**

**Incubator temp. start:**

**incubator temp. end:**

**Analyst - Counts:**

**Waterbath temp. start:**

**Waterbath temp. end:**

<table>
<thead>
<tr>
<th>Monitoring Location</th>
<th>Setup Date</th>
<th>Dil. (mls)</th>
<th>24 Hr Count</th>
<th>Total fecal (per 100ml)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch #4</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch #5</td>
<td></td>
<td>100</td>
<td></td>
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</tr>
</tbody>
</table>

**Enterolert IDEXX Method**

**Analyst - set-up:**

**Incubator temp. start:**

**Incubator temp. end:**

**Media Batch #:**

**Expiration:**

**Notes:** "-" = Quanti-tray used, no small wells

Final count determined by using the correct table

(51 well Quanti-Tray lg wells only, Quanti-Tray / 2000 with small wells)

<table>
<thead>
<tr>
<th>Enterococci</th>
<th>Setup Date</th>
<th>Dil. (mls)</th>
<th># lg pos. wells</th>
<th># sm pos. wells</th>
<th>Table Value</th>
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</thead>
<tbody>
<tr>
<td>Batch #4</td>
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<td></td>
<td>0</td>
<td>-</td>
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<td>0</td>
<td>-</td>
</tr>
<tr>
<td>IDEXX Blank</td>
<td></td>
<td>100</td>
<td></td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>URIWW Sterilized</td>
<td></td>
<td>100</td>
<td></td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
This form is intended to be used to maintain a record of QC requirements for sterilization of the IDEXX sample bottles. Once this sheet is full it should be replaced with a new blank sheet. The full sheet should be returned to the URIWW Micro Laboratory Binder in Room 019; blank sheets are also located in this binder. Any maintenance activity should also be recorded on this sheet. See Elizabeth with questions.
This form is intended to be used to maintain a quarterly record of the intensity of the germicidal UV labs in the sterilizing box in room 19. The intensity of EACH of the SIX tubes must be measured on a quarterly basis, with each tube measured four inches from each end, as well as in the center, with the average of those three readings used. Once this sheet is full it should be replaced with a new blank sheet. The full sheet should be returned to the URIWW Micro Laboratory Binder in Room 019; blank sheets are also located in this binder. Any maintenance activity should also be recorded on this sheet. See Elizabeth with questions.
Filling a Quanti-Tray or Quanti-Tray/2000 (source: www.idexx.com)

Quanti-Tray®

Introduction
IDXEX Quanti-Trays are designed to give quantitated bacterial counts of 100 ml samples using IDEXX Defined Substrate Technology® reagent products. Add the reagent/sample mixture to a Quanti-Tray, seal it in a Quanti-Tray Sealer and incubate per the reagent directions. Then count the number of positive wells and use the MPN table attached to determine the Most Probable Number (MPN).

Contents
This package contains 100 sterile, 51-well Quanti-Trays.

User Instructions

1. Use one hand to hold a Quanti-Tray upright with the well side facing the palm.
2. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends towards the palm.
3. Open the Quanti-Tray by pulling the foil tab away from the well side. Avoid touching the inside of the foil or tray.
4. Pour the reagent/sample mixture directly into the Quanti-Tray avoiding contact with the foil tab. Allow foam to settle.
5. Place the sample-filled Quanti-Tray onto the rubber tray carrier of the Quanti-Tray Sealer with the well side (plastic) of the Quanti-Tray facing down to fit into the carrier.
6. Seal according to Sealer instructions.
7. Incubate according to reagent directions.
8. Count positive wells and refer to the MPN table on the back of this instructions sheet to find the Most Probable Number (MPN).
9. Dispose of media in accordance with Good Laboratory Practices.

For Technical Assistance, visit www.idexx.com/water, or in the U.S. and Canada, call 1-800-321-0207 or 207-856-0496

IDEXX Laboratories, Inc. One IDEXX Drive, Westbrook, Maine 04092 USA

* Quanti-Tray and Defined Substrate Technology are trademarks or registered trademarks of IDEXX Laboratories, Inc. in the United States and other countries. US Patent Numbers: 4,825,789; 5,439,003; 5,519,092; Other patents pending.
Preventive Maintenance Instructions
Quanti-Tray* Sealer Model 2X

CAUTION: BURN HAZARD
- Cleaning should be performed by your trained personnel only
- Allow unit to cool at least 90 minutes before normal cleaning
- If hot machine must be opened, keep hands away from upper roller

Routine maintenance or 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. Please contact IDEXX Technical Service at 1-800-321-0207 or 1-207-856-0496 before proceeding if you have any questions.

www.idexx.com

IDEXX Laboratories, Inc.
One IDEXX Drive, Westbrook, ME 04092 USA
Tel.: 1-207-856-0496 or 1-800-321-0207
Fax: 1-207-856-0630

06-04457-01
Cleaning Procedure

The following picture outline is our recommended cleaning procedure:

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

2. Loosen hold-down screws, which secure the lower roller assembly to the bottom plate of the sealer.

3. Remove lower roller by lifting straight up and then out, to ensure roller clearance of locating pins on the bottom plate of the sealer.
   - Be careful not to touch the upper roller if it is hot.
4 Use mild detergent, diluted bleach or isopropyl alcohol to clean all accessible surfaces inside the sealer and the lower roller assembly.
   - Never use abrasive materials for cleaning.
   - Never use caustic cleaners.
   - Use alcohol only on cool sealer.
   - For stubborn deposits, allow soak time for cleaner to work.
   - Do not disassemble lower roller assembly.
Dry interior and roller assembly with paper towels or soft cloth.

5 Reinstall bottom roller assembly on locating pins and tighten hold-down screws.

6 Fasten access panel and reattach tray shelf.
   Sealer is now ready for use.
1.0 PURPOSE AND DESCRIPTION

The analytical balance located in Room 018 is capable to weighing to 4 decimal places. It is a delicate instrument and must be treated as such. Never place books or other documents on top of the balance, and clean up any chemical spills on or near the balance at once. The following procedure outlines how to check the calibration of the balance. This should be done on a daily basis. Actual calibration is completed by a trained service technician. All balance calibration checks should be recorded on the balance calibration data sheet (figure 1) located in the drawer below the balance.

1.1 Check Balance Level

Before each use, the balance level indicator should be checked. The level indicator is a small bubble that can be seen by looking down on the balance from above. It is in the back of the balance. The bubble should be centered within the printed circle. If it is not, adjust the feet of the balance until the bubble is within the circle. Check the box “Balance level?” on the data sheet after checking the balance.

1.2 Calibration Check

The calibration of the balance should be checked at least once a day, when in use. The calibration weights are in a box located in the drawer beneath the balance. Check the calibration by first making sure nothing is on the balance weigh pan, and then tare the balance. Once the balance is successfully tared use the tweezers to place the 50 mg certified weight onto the pan, and allow the balance to stabilize. NEVER HANDLE THE CERTIFIED WEIGHTS WITH YOUR FINGERS, THEY ARE PRECISELY CALIBRATED, AND EVEN THE OILS ON YOUR HANDS CAN AFFECT THE CALIBRATED WEIGHT.

Once the balance has stabilized, record the weight in the column “50 mg weight, Actual Reading” on the balance data sheet. The reading must be +/- 10% of the certified weight (50 mg). This is 0.0450 to 0.0550 mg. If the weight does not meet this criteria contact Linda Green or Elizabeth Herron, and do not use the balance.

After checking the balance with the 50 mg weight, complete the same procedure with the 20 g weight. Remember, don’t pick up the weight with your fingers; use the tweezers. Put the reading for the 20 g weight in the column “20 g weight, Actual Reading”. The reading must be +/- 1% of the certified weight (20 g). This is 20.2000 to 19.8000 g. If the weight does not meet this criteria contact Linda Green or Elizabeth Herron, and do not use the balance.

Make sure that the rest of the data sheet is filled out and the calibrated weights are put away. Do not take the calibration weights out of Room 019 without permission from Linda Green or Elizabeth Herron.
# URI Watershed Watch Laboratory
## Maintenance and Service Record - Mettler Toledo AB104 Balance

### calibration data sheet

<table>
<thead>
<tr>
<th>Date</th>
<th>Tech's Initials</th>
<th>Balance level?</th>
<th>50 mg weight Actual Reading</th>
<th>Expected Reading</th>
<th>20 g weight Actual Reading</th>
<th>Expected Reading</th>
<th>Actions taken (None, Serviced Instrument, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0450 to 0.0550 g</td>
<td></td>
<td>20.2000 to 19.8000 g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This form is intended to be used to maintain a record of calibration and maintenance of the Mettler Toledo balance. All maintenance activity should also be recorded on this sheet. Once this sheet is full it should be replaced with a new blank sheet. The full sheet should be returned to the URIWW Laboratory Maintenance Binder in Room 002; blank sheets are also located in this binder.

**Calibration and Maintenance Information:**

1. Each day the balance is used the level indicator should be checked. If the balance isn’t level it should be adjusted and the adjustment recorded on this data sheet.

2. The reading for the 50 mg calibration weight should be ±10% of the actual mass (0.0450 mg to 0.0550 mg).

   The reading for the 20 g calibration weight should be ±1% of the actual mass (20.2000 g to 19.8000 g).

   If the balance is outside these ranges contact L. Green or E. Herron; do not use the balance if it doesn’t calibrate correctly.
1.0 PURPOSE AND DESCRIPTION

The purpose of this method is to determine the number of live heterotrophic bacteria in water. This method is used by the University of Rhode Island Watershed Watch (URIWW) solely for the purpose of complying with the RI HEALTH rules and procedures for the certified analysis of fecal coliform samples. Sample results are not reported for any other purpose.

Samples of water from the laboratory’s Ultrapure water system are analyzed by mixing with media and then allowing the mixture to incubate. After the incubation the number of organisms present are counted and reported. The method presented here is modified from the IDEXX SimPlate for HPC Multi Dose method and correlates to the Pour Plate method using Total Plate Count Agar incubated at 35°C for 48 hours as described in Standard Methods for the Examination of Water and Wastewater, 19th ed.

2.0 HEALTH AND SAFETY CONSIDERATIONS

2.1 Hazards

General laboratory good housekeeping procedures should be practiced as outlined in Standard Operating Procedure (SOP) 001 - General Laboratory Safety. The only chemical utilized in this SOP is the media. The media is not listed as hazardous but should be treated with care. Always wear protective clothing in the form of gloves, a laboratory coat and goggles when working with this chemical. Further information regarding this chemical may be found in the laboratory Material Safety Data Sheet (MSDS) binder found in the laboratory where the chemical of interest is stored. A UV light is utilized in this procedure, keep exposure to the UV light to a minimum. Use protective face shield or goggles and gloves when reading samples. General laboratory information regarding safe handling of chemicals is located in SOP 001a - University Safety and Waste Handling.

2.2 Technician Training/Qualifications

General training in laboratory technique, aseptic technique and the proper use of the UV light must be completed prior to analyzing samples using this method. Technician training will be provided by Elizabeth Herron Project Manager – Microbiology.

3.0 REQUIRED MATERIALS

<table>
<thead>
<tr>
<th>Required Material</th>
<th>Notes</th>
<th>Re-order information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>Foil-packed sterile media vessels for 100 mL of media when hydrated</td>
<td>IDEXX Cat # WHPC-100</td>
</tr>
<tr>
<td>SimPlates</td>
<td>Comes with multi-dose kit</td>
<td></td>
</tr>
<tr>
<td>Required Material</td>
<td>Notes</td>
<td>Re-order information</td>
</tr>
<tr>
<td>-------------------</td>
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<td>----------------------</td>
</tr>
<tr>
<td>UV light and viewing box</td>
<td></td>
<td>UV light #WL160, Replacement lamp #WL161 UV cabinet #WCM10</td>
</tr>
<tr>
<td>Pipettes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 °C Incubator</td>
<td>Thermolyne Type 142300</td>
<td></td>
</tr>
<tr>
<td>Sterile Ultrapure water</td>
<td>4L carboy used for Enterolert dilution</td>
<td></td>
</tr>
<tr>
<td>Paper towels, safety goggles, lab gloves</td>
<td></td>
<td>UV goggles #WLG</td>
</tr>
</tbody>
</table>

Equipment is maintained by the University of Rhode Island Watershed Watch (URIWW) Laboratory and the Natural Resources Science department. Temporary replacement of large equipment such as incubators is available through arrangement with other scientists in the department maintaining similar equipment.

### 4.0 METHOD DESCRIPTION

1. Samples should be collected directly from the Ultrapure water system into a sterile bottle. Samples should be analyzed immediately.
2. Obtain a foil-packed sterile media vessel and dilute to the 100 mL mark with sterile Ultrapure water. Re-cap the vessel and shake to dissolve the media.
3. Setup 10 SimPlates. 8 of the plates will be used to test the Ultrapure water collected from the Ultrapure water system. The other two plates will be used as a blank at the beginning and end of the run. The blanks consist of 10 mL of rehydrated media.
4. Prepare the first blank. Pipette 10 mL rehydrated media onto the center of the SimPlate base.
5. Cover the SimPlate with the plate lid and gently swirl the plate to distribute the sample into the plate wells. Air bubbles do not interfere with the test. Label cover with a permanent marker.
6. Tip the plate 90° to 120° to drain excess sample into the absorbent pad on the plate.
7. Repeat the procedure for the Ultrapure water samples. (total of 8 plates)
8. Prepare the end blank. (10 mL of rehydrated media).
9. Invert the plates and place them into the 35 °C incubator for 48 hours. The incubator should be set at 35 °C +/- 0.5 °C.
10. Unused media can be refrigerated up to 5 days, if it is not used by then, it should be discarded by rinsing down the drain.
11. After 48 hours remove the plates from the incubator and count the number of wells that exhibit fluorescence when placed under a UV light (6 watt, 365 nm). Use the UV viewing box.
12. The Most Probable Number is determined using the MPN table attached below.
13. Blanks should return a value of <2 CFU/mL. If the blanks are greater then this value the run is considered contaminated, re-run the procedure.
5.0 CALCULATIONS

Input the number of positive wells into the table below to determine the MPN. Record the MPN on the data sheet. The MPN should be less than 500 CFU/mL, if the value is greater than 500 CFU/mL then re-run the test. If the results are still higher than 500 CFU/mL then begin to investigate any potential sources of contamination to the source water. Until the heterotrophic plate count returns a value less than 500 CFU/mL bacterial analyses should not be run.

<table>
<thead>
<tr>
<th># Positive Wells</th>
<th>MPN</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>lower</td>
</tr>
<tr>
<td>0</td>
<td>&lt;2</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>1</td>
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<table>
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<tr>
<th># Positive Wells</th>
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MPN is per ml of sample (pour-off is accounted for).
6.0 DOCUMENTATION

Bacterial Sample Log & Worksheet: Monthly HPC Analyses

IDEXX SimPlate for HPC Method

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7.0 REFERENCES

Method referenced: Heterotrophic Plate Count (9215).