# Quality Assurance Project Plan

# University of Rhode Island Watershed Watch Analytical Laboratory


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**Prepared for: U.S. Environmental Protection Agency N.E. Region 1**
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**Boston, Massachusetts 02114-2023**

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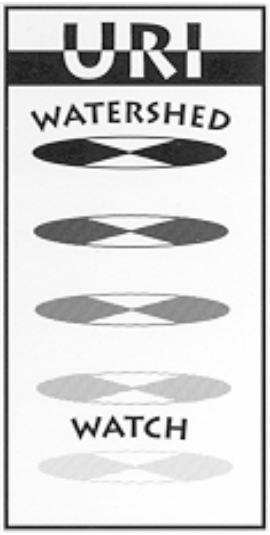
## University of Rhode Island
## Watershed Watch
## Analytical Laboratory

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Kingston, Rhode Island
2005

URI WATERSHED WATCH TECHNICAL REPORT NO. 4

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## List of Abbreviations

| Abbreviation | Definition |
|---|---|
| CA | Corrective Action |
| COC | Chain-of-Custody |
| %D | Percent Difference |
| DI | Deionized Water |
| DQIs | Data Quality Indicators |
| DO | Dissolved Oxygen |
| DQO | Data Quality Objectives |
| EPA-NE | Environmental Protection Agency – New England District (Region 1) |
| g | Gram |
| ISDS | Individual Sewage Disposal System |
| L | Liter |
| LCS | Laboratory Control Standard (standard analyzed as a sample) |
| MDL | Method Detection Limit |
| mL | Milli-liter |
| mg | Milli-gram |
| MSDS | Material Safety Data Sheet |
| NA | Not Applicable |
| QAPP | Quality Assurance Project Plan |
| QA/QC | Quality Assurance/Quality Control |
| $R^2$ | Coefficient of Determination |
| %RPD | Replicate Percent Difference |
| RL | Reporting Limit (Quantitation Limit) |

## List of Abbreviations (continued)

| Abbreviation | Definition |
|---|---|
| SOP | Standard Operating Procedure |
| SU | Standard Unit (pH units) |
| µg | Micro-gram |
| URIWW | University of Rhode Island Watershed Watch |

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## LIST OF FIGURES
## (Tables listed in Table of Contents)



## LIST OF APPENDICIES

## 1.0 PURPOSE AND DESCRIPTION

The University of Rhode Island Watershed Watch Program (URIWW) is a Cooperative Extension Water Quality Program in the Department of Natural Resources Science, College of the Environment and Life Sciences. The program is located in the Coastal Institute building on the URI Kingston campus. Begun in 1988, the URIWW program is a statewide volunteer monitoring program with over 300 volunteers. The program focuses on providing current information on the water quality of surface water resources throughout Rhode Island. It is a service provider to statewide and local decision-makers and is the sole source of long-term lake water quality data for Rhode Island. The URIWW laboratory provides analytical services to the Rhode Island Department of Environmental Management (RIDEM) and the Environmental Protection Agency, New England District (Region 1) (EPA-NE) as well as other URI researchers. It is a springboard for municipal board activities by volunteers, linked with all cooperative extension water quality activities. The program is intended to encourage communities and shoreline residents to understand the need to cooperatively manage and improve the water quality of all the water bodies within a watershed.

Information describing the URIWW program, program fact-sheets, water quality data as well as monitoring protocols are maintained at the following web-site: www.uri.edu/ce/wq. Basic information describing URIWW is also available in Appendix C.

The purpose of this Quality Assurance Project Plan (QAPP) is to provide guidance on the analytical procedures and quality assurance/quality control (QA/QC) tasks performed by the URIWW. The URIWW Laboratory provides analysis of samples for the following contaminants of concern: fecal coliforms, *Escherichia coli*, enterococci, salinity, total suspended solids, alkalinity, pH, biochemical oxygen demand (BOD), chlorophyll-a, chloride, ammonia, orthophosphate, nitrate + nitrite and total phosphorus and nitrogen. Assays are completed on ambient waters (rivers, lakes and streams), marine waters (ocean and estuaries) and Individual Sewage Disposal System (ISDS) samples.

This QAPP does not describe field collection or analysis procedures, that information is provided in other documents. A cross-reference between the information required by EPA-NE is provided in the table below. Note that information found in narrative format instead of in an EPA-NE table is listed as “in narrative”.

## Required Information Checklist

| EPA-NE Work-sheet number | Worksheet Title | Location In URIWW Laboratory QAPP |
|---|---|---|
| 1 | Title and approval | In narrative |
| 2 | Table of contents & document format | In narrative |
| 3 | Distribution list | In narrative |
| 4 | Project personnel sign-off sheet | All relevant personnel are included on the approval page |
| 5a | Organizational chart | Figure 1 |
| 5b | Communication pathway | Section 1.2 in narrative |
| 6 | Personnel responsibilities and qualification | Section 1.2 and 1.2.1 in narrative |
| 7 | Special personnel training requirements | Section 1.2.2 in narrative |
| 8a | Project scoping meeting attendance sheet, agenda | NA |
| 8b | Problem definition/site history & background | Section 1.0 in narrative |
| 9a | Project description | Section 1.0 in narrative |
| 9b | Contaminants of concern | Section 2.6.1 |
| 9c | Field & QC sample summary | NA |
| 10* | Project schedule timeline | Section 1.3 in narrative |
| 11a | Project quality objectives/decision statements | Section 2.0 in narrative |
| 11b | Measurement performance criteria table | Section 2.6.2 |
| 12a | Sampling design & rationale | NA |

| EPA-NE Work-sheet number | Worksheet Title | Location In URIWW Laboratory QAPP |
|---|---|---|
| 12b | Sampling locations, methods, SOP requirements table | NA |
| 13 | Project sampling SOP table | Appendix A |
| 14 | Field equipment calibration | NA |
| 15 | Field equipment maintenance | NA |
| 16 | Sampling handling, tracking, custody | Section 3.0 in narrative and Section 3.2 |
| 17 | Field method /SOP | NA |
| 18 | Field calibration | NA |
| 19 | Field maintenance | NA |
| 20 | Fixed lab. analytical , SOP reference table | Section 2.6.3 |
| 21 | Lab instrument maintenance & calibration table | Section 2.6.4 |
| 22a | Field sampling QC | NA |
| 22b | Field sampling QC continued | NA |
| 23a | Field analytical QC | NA |
| 23b | More field QC | NA |
| 24a | Lab analytical QC | Section 2.6.5 |
| 24b | More lab analytical QC | No multiple analytes |
| 25 | Non-direct measurement criteria | NA |
| 26 | Project documentation and records | Section 4.0 in narrative |
| 27a | Assessment and response | NA |
| 27b | Project assessment | NA |
| 27c | Project assessment plan | NA |

| EPA-NE Work-sheet number | Worksheet Title | Location In URIWW Laboratory QAPP |
|---|---|---|
| 28 | QA management reports | Section 4.0 in narrative |
| 29a | Data evaluation process | NA |
| 29b | Data validation summary | Section 5.0 in narrative |
| 29c | Data validation modifications | NA |
| 30 | Data usability assessment | NA |

**Notes:**
NA – Not applicable to this QAPP. This QAPP provides information regarding general laboratory protocols only. No project-specific information is contained in this general QAPP. No field sample collection or analysis information is provided in this QAPP and all data are generated in-house.

### 1.1 Quality Assurance Project Plan (QAPP) Objectives

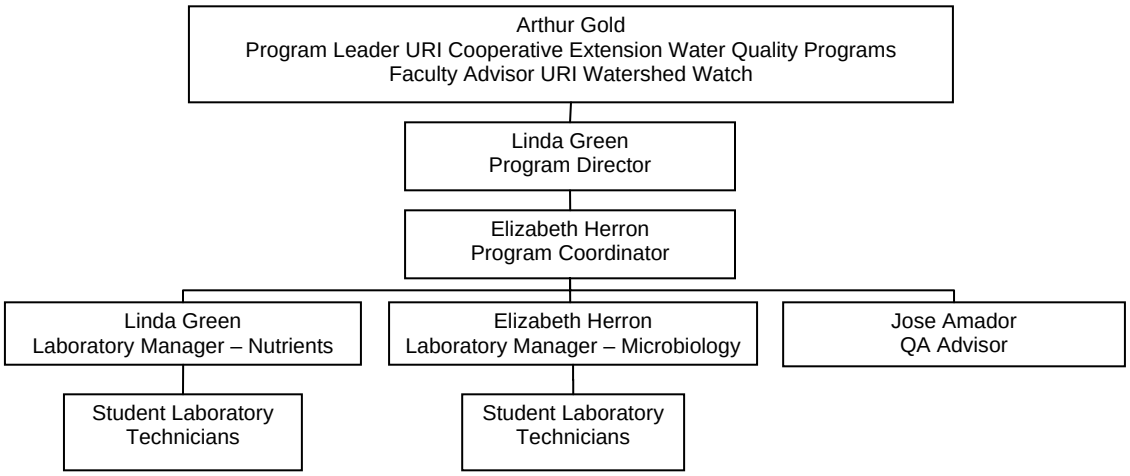
The objective of this QAPP is to present the organization, objectives and specific quality assurance/quality control (QA/QC) procedures associated with URIWW laboratory analysis protocols. Guidance on the analysis procedures for the following laboratory assays is provided in this document: fecal coliforms, *Escherichia coli*, enterococci, total suspended solids, alkalinity, pH, salinity, biochemical oxygen demand, chlorophyll-a, chloride, ammonia, orthophosphate, nitrate + nitrite and total phosphorus and nitrogen. Specific QA/QC criteria as well as documentation are outlined in individual Standard Operation Procedures (SOPs) located in Appendix A. This QAPP does not describe any field collection or analysis procedures that information is provided in other documents.

### 1.2 Organization and Communication

Dr. Arthur Gold is the Program Leader of the Cooperative Extension Water Quality Program and faculty advisor to the URIWW program. He will provide overall guidance related to projects performed by the URIWW program (Figure 1). Linda Green is the URIWW Program Director as well as the overall Laboratory Project Manager and Laboratory Manager for nutrient analyses. As such she is responsible for overall operation of the laboratory as well as the QA/QC of all non-microbiological related assays. Elizabeth Herron is the URIWW Program Coordinator and Laboratory Project Manager for microbiological analysis. She is responsible for the analysis and QA/QC of microbiological assays. Dr. Jose Amador will provide QA/QC guidance. Ms. Herron and Ms. Green are both responsible for the supervision of student laboratory technicians.

All changes to the QAPP or specific SOPs will completed only after review and acceptance by Ms. Green or Ms. Herron.

**Figure 1: University of Rhode Island Watershed Watch Laboratory Structure**




### 1.2.1 Personnel Qualifications

A brief description of the experience of principal laboratory personnel is described here. Resumes of key personnel are located in Appendix B. Dr. Arthur Gold is a watershed hydrologist and Professor in the Department of Natural Resources Science at URI. He has over 20 years of experience in the field of water resources; has published over 60 refereed journal articles and has served on numerous national and international committees dealing with water resources. Currently, Dr. Gold is the associate director of the URI Coastal Institute and is the program leader for the URI Cooperative Extension.

Linda Green has over 25 years of analytical laboratory related experience and has been the director of URIWW for over 18 years. She is the recipient of numerous awards and grants related to her work with the URIWW program and has authored numerous articles and technical publications. Ms. Green has hosted workshops on QA/QC in volunteer monitoring programs and is the sole volunteer monitoring representative on the National Water Quality Monitoring Council as well as a co-founding member of the Rhode Island Volunteer Monitoring Steering Committee.

Elizabeth Herron has over 15 years of experience in the field and is a former director of the North American Lake Management Society as well as a co-founding member of the Rhode Island Volunteer Monitoring Steering Committee. She has authored numerous articles and technical publications and has presented workshops and technical papers throughout the United States.

Dr. Jose Amador has 20 years of experience in the field of soil science, microbiology and nutrient chemistry. He has published over 40 peer reviewed articles. He is currently a Professor of microbial ecology and soil science at URI.

### 1.2.2 Training

Training of laboratory personnel is conducted by Linda Green and Elizabeth Herron. Laboratory training is provided on basic laboratory techniques as well as method-specific details. Training requirements for each assay are provided in analyte-specific SOPs, located in Appendix A. All laboratory assays are conducted by laboratory personnel; no volunteer monitors conduct any laboratory assays.

## 1.3 Schedule/Time-Line

This QAPP does not relate to a specific project, therefore no specific time-line or schedule is offered.

# 2.0 LABORATORY QUALITY OBJECTIVES AND MEASUREMENT PERFORMANCE CRITERIA

High quality data is the goal of all URIWW Laboratory analyses. Specific data quality objectives have been set on a method basis for method detection limits (MDL), precision, accuracy, comparability and completeness. Values specific to each of these objectives are located in analyte-specific SOPs located in Appendix A as well as below. Since this document is a general QAPP for laboratory assays only, there are no specific if/then statements linking laboratory criteria to project decisions.

## 2.1 Method Detection Limits (MDL) and Reporting Limit (RL)

The MDL is the analyte concentration where there is 99% confidence that the sample concentration is different than zero. Below the MDL it is uncertain if the concentration is not zero. The reporting limit (RL) is the value above which data have definable accuracy and precision. Each analyte of interest has a specific MDL and RL value. These values are located in the analyte-specific SOPs in Appendix A as well as worksheet 9b (see Section 2.6.1).

The analytical method MDL as reported in Section 2.6.1 for each assay is often different from the achievable laboratory MDL. Generally, the achievable laboratory MDL is higher than the analytical method MDL. This is often the case because the MDL listed for an analytical method is for the best case scenario. In this scenario, there are no other contaminants present in a sample that could cause interferences during sample analysis, the method blank would be extremely low and all equipment would function without error. Unfortunately, this is generally not the case. At the very low contaminant levels that the laboratory is able to analyze samples to it is easy to introduce some contamination from water or reagents. Therefore, the laboratory elevates the method MDL to a level that will account for these concerns.

## 2.2 Precision

Precision is an evaluation of the degree to which two or more measurements are in agreement as well as a measurement of random error. Precision will be assessed through the measurement of duplicate samples and subsequent calculation of the relative percent difference (%RPD) as described below.

$$\%RPD = \frac{|\text{Result of Replicate 1} - \text{Result of Replicate 2}|}{\text{Average of Result of Replicate 1 and Result of Replicate 2}} \times 100$$

Objectives for precision are located in the analyte specific SOPs (Appendix A) as well as worksheet 11b and 24a Section 2.6.2 and 2.6.5, respectively.

## 2.3 Accuracy

Accuracy is an evaluation of the degree to which a measured value and a known reference value or true value are in agreement. This is a measurement of systematic error and is often referred to as "bias". Laboratory accuracy is determined by the analysis of reference material and comparison of the resulting value to that of the accepted value. The difference between the accepted and reference value is the percent difference (%D). The %D is calculated as follows:

$$\%D = \frac{|\text{Known Value of Reference Material} - \text{Calculated Value of Reference Material}|}{\text{Known Value of Reference Material}} \times 100$$

Objectives for accuracy are located in the analyte specific SOPs (Appendix A) as well as worksheet 11b and 24a (Section 2.6.2 and 2.6.5, respectively).

Accuracy is determined during both routine sample analysis procedures as well as by yearly participation in the EPA Water Pollution Proficiency Test Study for the following assays: alkalinity, Biochemical oxygen demand, ammonia, chloride, nitrate + nitrite, orthophosphate, total nitrogen and phosphorus, pH and total suspended solids.

## 2.4 Comparability

All methods utilized by the URIWW Laboratory are based on methods found in *Standard Methods for the Examination of Water and Wastewater* published by the American Public Health Association, American Water Works Association and Water Environment Federation. Specific references for each method are found in the analyte specific SOPs (Appendix A).

## 2.5 Completeness

Completeness is a measure of the amount of valid data obtained from the laboratory methods compared to the amount that was expected to be obtained under normal conditions. Greater than 90% completeness of accepted field samples is expected. Completeness is calculated as follows:

$$\text{Completeness} = \frac{\text{Number of Valid Laboratory Measurements}}{\text{Number of Laboratory Measurements Planned}} \times 100$$

## 2.6 QA/QC Tables

Tables summarizing the QA/QC objectives for each analysis performed by the URIWW Laboratory are provided on the following pages. These tables specifically address the Data Quality Indicators (DQIs) or the procedures to be followed to provide assurance that an analytical procedure is returning valid results. Each DQI has a specific result that must be met before the data is considered acceptable. Information is also provided on the instruments utilized for each assay and the maintenance and calibration procedures that must be completed on each instrument. Analyte-specific tables provide information on the number of QA/QC samples to be prepared (blanks, replicates, etc.) and the expected result as well as the person(s) responsible for assessing any problems and determining the proper course of action, if necessary.

### 2.6.1 Contaminants of Concern and Other Target Analytes Table (Reference Limit and Evaluation Table) - Worksheet #9b

EPA-NE QAPP Worksheet #9b - Rev. 10/99

**Contaminants of Concern and Other Target Analytes Table (Reference Limit and Evaluation Table)**

| Analyte | CAS Number | Reporting Units | Project Action Limit (Units) (wet or dry weight) | Project Quantitation Limit (Units) (wet or dry weight) | Analytical Method | | Achievable Laboratory Limits | |
|---|---|---|---|---|---|---|---|---|
| | | | | | MDLs | Method RLs | MDLs | RLs |
| fecal coliforms – SOP 007 & 008 | | Colonies/100mL | NA – This is a generic QAPP for laboratory procedures | | 0 | | Variable: Dependent upon volume filtered | |
| *Escherichia coli* – SOP 007 | | Colonies/100mL | | | 0 | | Variable: Dependent upon volume filtered | |
| Enterococci – SOP 018 | | Colonies/100mL | | | 0 | | Variable: Dependent upon volume filtered | |
| Total Suspended Solids – SOP 009 | | mg/L TSS | | | Not Provided | | 1 | 1 |
| Alkalinity – SOP 010 | | mg/L $CaCO_3$ | | | Not Provided | | 0 | 0.1 |
| pH – SOP 010 | | Standard Unit (SU) | | | 1.0 | | 1.00 | 1.0 |
| Biochemical Oxygen Demand – SOP 011 | | mg/L BOD | | | Variable | | 2 | 2 |
| Salinity – SOP 017 | | ppt | | | 0.4 | | 0.4 | 0.4 |
| Chlorophyll a – SOP 012 | | µg/L chlorophyll-a | | | 0.1 | | 0.1 | 0.2 |
| Chloride – SOP 013 | 16887-00-6 | mg/L $Cl^-$ | | | 0.2 | | 2 | 5 |

EPA-NE QAPP Worksheet #9b - Rev. 10/99

## Contaminants of Concern and Other Target Analytes Table (Reference Limit and Evaluation Table)

| Analyte | CAS Number | Reporting Units | Project Action Limit (Units) (wet or dry weight) | Project Quantitation Limit (Units) (wet or dry weight) | Analytical Method | | Achievable Laboratory Limits | |
|---|---|---|---|---|---|---|---|---|
| | | | | | MDLs | Method RLs | MDLs | RLs |
| Ammonia – SOP 014 | 7664-41-7 | µg/L $NH_3$-N | | | 5 | | 20 | 40 |
| Orthophosphate – SOP 015 | | µg/L $PO_4$-P | | | 2 | | 2 | 4 |
| Nitrite + Nitrate – SOP 015 | | µg/L $NO_3/NO_2$-N | | | 10 | | 20 | 30 |
| Total Phosphorus – SOP 016 | | µg/L P | | | 2* | | 3 | 4 |
| Total Nitrogen – SOP 016 | | µg/L N | | | 10* | | 20 | 50 |

**Notes:**

*The MDLs for Total Phosphorus and Total Nitrogen were not provided by the method reference. Therefore, the method MDLs for orthophosphate and nitrate + nitrite were reported since the total phosphorus and nitrogen assays are based on the orthophosphate and nitrate + nitrite assays, respectively. After a sample is digested for total nitrogen and phosphorus the sample is analyzed as a nitrate + nitrite and orthophosphate sample (please refer to the analyte-specific SOP located in the URIWW Laboratory QAPP for more information).

### 2.6.2 Measurement Performance Criteria Table – Worksheet 11b

EPA-NE QAPP Worksheet #11b - Rev. 10/99

**Measurement Performance Criteria Table**

| Sampling Procedure | QC Sample and/or Activity Used to Assess Measurement Performance | Measurement Performance Criteria | Data Quality Indicators (DQIs) | Analytical Method/SOP |
|---|---|---|---|---|
| fecal coliform, *Escherichia coli,* enterococci | Method Blank | < 1 colony/100 mL | Bias | 007/008/018 |
| | Sample Replication | Not greater than 20%RPD | Precision | |
| | Inoculate a plate with a known positive plate (method for positive plates and QA check on new plates) | Positive growth | Bias/false negatives | |
| | Check incubator temperature | Temperature not greater than +/- 1 °C different then set temperature | Bias | |
| Total Suspended Solids | Method Blank | Not greater than 1 mg/L TSS | Bias | 009 |
| | Sample Replication | Not greater than 30%RPD | Precision | |
| | Check of balance calibration | Not greater than 10%D | Accuracy | |
| | EPA Water Pollution Proficiency Test Study (Analysis of Unknowns) | 2 standard deviation | Accuracy/ Comparability | |

EPA-NE QAPP Worksheet #11b - Rev. 10/99

## Measurement Performance Criteria Table

| Sampling Procedure | QC Sample and/or Activity Used to Assess Measurement Performance | Measurement Performance Criteria | Data Quality Indicators (DQIs) | Analytical Method/SOP |
|---|---|---|---|---|
| Alkalinity and pH | EPA Water Pollution Proficiency Test Study (Analysis of Unknowns) | 2 standard deviation | Accuracy/ Comparability | 010 |
| | Calibration | Electrode efficiency greater than 96% | Accuracy | |
| | Standards as Samples (Calibration check) | Change in standard not greater than 0.1 SU | Accuracy/ Precision | |
| Biochemical Oxygen Demand | Method Blank | Not greater than 2 mg/L BOD | Bias | 011 |
| | Sample Replication | Not greater than 20%RPD | Precision | |
| | EPA Water Pollution Proficiency Test Study (Analysis of Unknowns) | 2 standard deviation | Accuracy/ Comparability | |
| Salinity | Sample Replication | Not greater than 2 ppt different | Precision | 017 |
| | Sample Comparison | Not greater than 2 ppt different | Accuracy/ Comparability | |

EPA-NE QAPP Worksheet #11b - Rev. 10/99

## Measurement Performance Criteria Table

| Sampling Procedure | QC Sample and/or Activity Used to Assess Measurement Performance | Measurement Performance Criteria | Data Quality Indicators (DQIs) | Analytical Method/SOP |
|---|---|---|---|---|
| Chlorophyll-a | Method Blank | Not greater than 0.03 µg/L chlorophyll-a as read on the fluorometer | Bias | 012 |
| | Filter Blank | Not greater than 0.03 µg/L chlorophyll-a as read on the fluorometer | Bias | |
| | Sample Replication of Fluorometer reading | Not greater than 20%RPD | Precision | |
| | Calibration Check Using Liquid and Solid Standards | Not greater than 15%D | Accuracy | |
| | LCS (Calibration check using Solid Standard) | Not greater than 15%D | Accuracy/ Precision | |
| | Collection of field sample replicates | Not greater than 100% RPD | Precision | |

EPA-NE QAPP Worksheet #11b - Rev. 10/99

## Measurement Performance Criteria Table

| Sampling Procedure | QC Sample and/or Activity Used to Assess Measurement Performance | Measurement Performance Criteria | Data Quality Indicators (DQIs) | Analytical Method/SOP |
|---|---|---|---|---|
| Chloride | Method Blank | Not greater than 2 mg/L $Cl^-$ | Bias | 013 |
| | Sample Replication | Not greater than 15%RPD (Replicate from same sample cup)<br>Not greater than 20%RPD (Replicate from different sample cups) | Precision | |
| | Calibration | $R^2$ of calibration linear regression not less than 0.990 | Accuracy | |
| | EPA Water Pollution Proficiency Test Study (Analysis of Unknowns) | 2 standard deviation | Accuracy/<br>Comparability | |
| | Laboratory Control Samples (Purchased External Standards) | Not greater than 20%D | Accuracy/<br>Comparability | |
| | Standards as Samples (Calibration check) | Not greater than 20%D | Accuracy | |

EPA-NE QAPP Worksheet #11b - Rev. 10/99

## Measurement Performance Criteria Table

| Sampling Procedure | QC Sample and/or Activity Used to Assess Measurement Performance | Measurement Performance Criteria | Data Quality Indicators (DQIs) | Analytical Method/SOP |
|---|---|---|---|---|
| Ammonia | Method Blank | Not greater than 30 µg/L $NH_3$-N | Bias | 014 |
| | Sample Replication | Not greater than 15%RPD (Replicate from same sample cup)<br>Not greater than 20%RPD (Replicate from different sample cups) | Precision | |
| | Calibration | $R^2$ of calibration linear regression not less than 0.990 | Accuracy | |
| | EPA Water Pollution Proficiency Test Study (Analysis of Unknowns) | 2 standard deviation | Accuracy/<br>Comparability | |
| | Laboratory Control Samples (Purchased External Standards) | Not greater than 20%D | Accuracy/<br>Comparability | |
| | Standards as Samples (Calibration check) | Not greater than 20%D | Accuracy | |

EPA-NE QAPP Worksheet #11b - Rev. 10/99

## Measurement Performance Criteria Table

| Sampling Procedure | QC Sample and/or Activity Used to Assess Measurement Performance | Measurement Performance Criteria | Data Quality Indicators (DQIs) | Analytical Method/SOP |
|---|---|---|---|---|
| Orthophosphate & Nitrate + Nitrite | Method Blanks | Not greater than 2 µg/L $PO_4$-P and 20 µg/L $NO_3/NO_2$-N | Bias | 015 |
| | Sample Replication | Not greater than 15%RPD (Replicate from same sample cup)<br>Not greater than 20%RPD (Replicate from different sample cups) | Precision | |
| | Calibration | $R^2$ of calibration linear regression not less than 0.990 | Accuracy | |
| | EPA Water Pollution Proficiency Test Study (Analysis of Unknowns) | 2 standard deviation | Accuracy/<br>Comparability | |
| | Laboratory Control Samples (Purchased External Standards) | Not greater than 20%D | Accuracy/<br>Comparability | |
| | Standards as Samples (Calibration check) | Not greater than 20%D | Accuracy | |

EPA-NE QAPP Worksheet #11b - Rev. 10/99

## Measurement Performance Criteria Table

| Sampling Procedure | QC Sample and/or Activity Used to Assess Measurement Performance | Measurement Performance Criteria | Data Quality Indicators (DQIs) | Analytical Method/SOP |
|---|---|---|---|---|
| Total Phosphorus and Nitrogen Analysis | Method Blank | Not greater than 3 µg P/L and 5 µg N/L | Bias | 016 |
| | Digestion Blank | Not greater than 3 µg/L P and 10 µg/L N | Bias | |
| | Sample Replication | Not greater than 15%RPD (Replicate from same sample cup)<br>Not greater than 20%RPD (Replicate from different sample cups)<br>Not greater than 25%RPD (Replicate digestions) | Precision | |
| | Calibration | $R^2$ of calibration linear regression not less than 0.990 | Accuracy | |
| | EPA Water Pollution Proficiency Test Study (Analysis of Unknowns) | 2 standard deviation | Accuracy/ Comparability | |
| | Laboratory Control Samples (Purchased External Standards) | Not greater than 20%D | Accuracy/ Comparability | |

EPA-NE QAPP Worksheet #11b - Rev. 10/99

## Measurement Performance Criteria Table

| Sampling Procedure | QC Sample and/or Activity Used to Assess Measurement Performance | Measurement Performance Criteria | Data Quality Indicators (DQIs) | Analytical Method/SOP |
|---|---|---|---|---|
| Total Phosphorus and Nitrogen Analysis (continued) | Standards as Samples (Calibration check) | Not greater than 20%D | Accuracy | |
| | Glycine External Standard (ISDS sample runs only) | Not greater than 10%D | Accuracy | |

Note:
All QC Measurement Performance Criteria in this table are for assessment of analytical error only.

### 2.6.3 Fixed Laboratory Analytical Method/SOP Reference Table – Worksheet 20

**EPA-NE QAPP Worksheet #20 - Rev. 10/99**

**Fixed Laboratory Analytical Method/SOP Reference Table**

| Reference Number (SOP Number) | Title, Revision Date and/or Number | Analytical Parameter | Instrument |
|---|---|---|---|
| 007 | Ambient Waters Microbiological Procedure, Rev. 1: 11/04 | fecal coliforms and *Escherichia coli* | Incubator – 35 °C Precision incubator<br>Incubator – 44.5 °C Precision fecal coliform water bath<br>Autoclave |
| 008 | ISDS Microbiological Procedure, Rev. 1: 11/04 | fecal coliforms | Incubator – 35 °C Precision incubator<br>Incubator – 44.5 °C Precision fecal coliform water bath<br>Autoclave |
| 018 | Enterococci Analysis, Rev. 1: 11/04 | enterococci | Incubator - 44.5 °C Precision fecal coliform water bath<br>Autoclave |
| 009 | Total Suspended Solids Analysis, Rev. 1: 11/04 | Total suspended solids | Analytical Balance – Mettler Toledo AB 104<br>Drying Oven – Blue M Stabiltherm Mechanical Convection Oven |
| 010 | Alkalinity and pH Procedures, Rev. 1: 11/04 | Alkalinity and pH | pH Meter – Fisher Scientific Model AR20 |
| 011 | Biochemical Oxygen Demand Procedure, Rev. 1: 11/04 | Biochemical Oxygen Demand | Dissolved Oxygen (DO) meter – YSI Model 5000<br>Incubator – Fisher Low Temperature Incubator Model 307C |

**EPA-NE QAPP Worksheet #20 - Rev. 10/99**

## Fixed Laboratory Analytical Method/SOP Reference Table

| Reference Number (SOP Number) | Title, Revision Date and/or Number | Analytical Parameter | Instrument |
|---|---|---|---|
| 017 | Salinity Analysis, Rev. 1: 04/05 | Salinity | LaMotte Salinity Titration Kit (Model 7459-01)<br>A366ATC Hand Held Salinity Refractometer |
| 012 | Chlorophyll-a Analysis, Welschmeyer Method, Rev. 1: 11/04 | Chlorophyll-a | Fluorometer – Turner Designs Model TD-700 |
| 013 | Chloride Analysis, Rev. 1: 11/04 | Chloride ($Cl^-$) | Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer |
| 014 | Ammonia Analysis, Rev. 1: 11/04 | Ammonia ($NH_3$-N) | Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer |
| 015 | Orthophosphate and Nitrate + Nitrite Analysis, Rev. 1: 11/04 | Orthophosphate ($PO_4$-P) and Nitrate + Nitrite ($NO_3/NO_2$-N) | Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer |
| 016 | Total Phosphorus and Nitrogen Analysis, Rev. 1: 11/04 | Total phosphorus (P) and total nitrogen (N) | Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer |

Notes:
No SOP was modified for project work as this QAPP is for general laboratory procedures and not associated with a specific project.
All Fixed laboratory analytical methods are for definitive data
All fixed laboratory analytical methods are performed by URIWW laboratory
No analytical methods have Region 1 NESTS Method Codes.

### 2.6.4 Fixed Laboratory Instrument Maintenance and Calibration Table - Worksheet 21

**EPA-NE QAPP Worksheet #21 - Rev. 10/99**

**Fixed Laboratory Instrument Maintenance and Calibration Table**

| | | Maintenance, Testing and Inspection Activities | | | | | |
|---|---|---|---|---|---|---|---|
| **Activity** | **Instrument** | **Activity** | **Frequency** | **Acceptance Criteria** | **Corrective Action (CA)** | **Person Responsible for CA** | **SOP Reference** |
| fecal coliforms, *Escherichia coli* and enterococci | Incubator – 35 °C precision incubator | Check temperature | Each time used | 35 +/- 1 °C | Adjust temperature control | E. Herron | 007, 008, 018 & 004 |
| | Autoclave | Check temperature and pressure | Each time used | Must reach 121 °C and maintain for at least 15 minutes | Contact professional to provide maintenance service | E. Herron | |
| | Incubator – 44.5 °C precision fecal coliform bath | Check temperature | Each time used | 44.5 +/- 1 °C | Adjust temperature control | E. Herron | |

**EPA-NE QAPP Worksheet #21 - Rev. 10/99**

## Fixed Laboratory Instrument Maintenance and Calibration Table

| | | Maintenance, Testing and Inspection Activities | | | | | |
|---|---|---|---|---|---|---|---|
| **Activity** | **Instrument** | **Activity** | **Frequency** | **Acceptance Criteria** | **Corrective Action (CA)** | **Person Responsible for CA** | **SOP Reference** |
| Total Suspended Solids | Analytical Balance – Mettler Toledo AB 104 | Check calibration | Each day used | Not greater than 10%D (1 g +/- 0.1 g) | Contact professional to provide maintenance and calibration service | L. Green | 009 |
| | Drying Oven – Blue M Stabiltherm Mechanical Convection Oven | Check Temperature | Each time used | 105 +/- 5 °C | Adjust temperature | URIWW Staff | |
| Alkalinity and pH | pH Meter – Fisher Scientific Model AR20 | Calibrate | Each time used | Electrode Efficiency >96% | Replace standards then if calibration still a problem replace the electrode | URIWW Staff | 010 |
| | pH Probe – Fisher Scientific Model AR20 | Refill electrode with saturated KCl solution | Check before each use | KCl solution is within ¼ inch of top of electrode and filling hole is open | Re-fill electrode as needed | URIWW Staff | |

**EPA-NE QAPP Worksheet #21 - Rev. 10/99**

## Fixed Laboratory Instrument Maintenance and Calibration Table

| | | Maintenance, Testing and Inspection Activities | | | | | |
|---|---|---|---|---|---|---|---|
| **Activity** | **Instrument** | **Activity** | **Frequency** | **Acceptance Criteria** | **Corrective Action (CA)** | **Person Responsible for CA** | **SOP Reference** |
| Biochemical Oxygen Demand | Dissolved Oxygen (DO) meter – YSI Model 5000 | Check membrane | Each time used | No air bubbles under membrane and membrane completely intact | Replace membrane | URIWW Staff | 011 |
| | Dissolved Oxygen (DO) meter – YSI Model 5000 | Calibrate | Each time used | 99% Air Saturation | Replace membrane and re-calibrate. If still will not calibrate correctly use another meter. | URIWW Staff | |
| | Incubator – Fisher Low Temperature Incubator Model 307C | Check temperature | Each time used, and each day BOD bottles are in incubator | 20 +/- 1 °C over 5 day incubation period | Temperature fluctuation noted on project data sheet and incubator serviced | L. Green | |

**EPA-NE QAPP Worksheet #21 - Rev. 10/99**

## Fixed Laboratory Instrument Maintenance and Calibration Table

| | | Maintenance, Testing and Inspection Activities | | | | | |
|---|---|---|---|---|---|---|---|
| **Activity** | **Instrument** | **Activity** | **Frequency** | **Acceptance Criteria** | **Corrective Action (CA)** | **Person Responsible for CA** | **SOP Reference** |
| Salinity | LaMotte Salinity Titration Kit | Check that syringes are clean and undamaged | Each time used | Syringes are clean and undamaged | Replace syringes | URIWW Staff | 017 |
| | A366ATC Hand Held Salinity Refractometer | Check that prism is not damaged | Each time used | Prism is clean and not scratched or cracked | Send instrument to manufacturer for repair | L. Green | 017 |
| Chlorophyll-a | Fluorometer – Turner Designs Model TD-700 | Calibrate | Calibrate yearly, Check calibration daily | Daily – Not greater than 15%D | Re-calibrate and then replace light source if calibration continues to drift | URIWW Staff/ L. Green | 012 |

**EPA-NE QAPP Worksheet #21 - Rev. 10/99**

## Fixed Laboratory Instrument Maintenance and Calibration Table

| | | Maintenance, Testing and Inspection Activities | | | | | |
|---|---|---|---|---|---|---|---|
| **Activity** | **Instrument** | **Activity** | **Frequency** | **Acceptance Criteria** | **Corrective Action (CA)** | **Person Responsible for CA** | **SOP Reference** |
| Chloride, Ammonia, Orthophosphate and Nitrate + Nitrite, Total Phosphorus and Nitrogen | Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer | Calibrate | Each time used | $R^2$ of calibration linear regression not less than 0.95 | Re-calibrate | L. Green | 013, 014, 015, 016 |
| | | Check analytical tubing | Each time used | No cracks or clogs | Replace affected tubing | L. Green | |
| | | Check reagents flows | Each time used | No clogs in tubing causing pulsating flow | Replace affected tubing | L. Green | |
| | | Check light source voltage | Each time used | < 90V | Replace light source | L. Green | |
| | | Check baseline | Each time used | Should be smooth | Replace tubing/trouble shoot instrument using instruction manual | L. Green | |
| | | Check intersample bubble shape | Each time used | Bubble shape is uniform | Adjust tubing, flow or reagents | L. Green | |
| | | Check peak height and shape | Each time used | Check that peaks are not off scale | Dilute samples | L. Green | |

### 2.6.5 Fixed Laboratory Analytical QC Sample Table –Worksheet #24 a

**Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99**

**FECAL COLIFORMS, ESCHERICHIA COLI and ENTEROCOCCI**

| | | | |
|---|---|---|---|
| Medium/Matrix | Water | Analytical Method/ SOP Reference | SOP 007, 008 and 018 |
| Sampling SOP | NA | Laboratory Name | URIWW |
| Concentration Range (without dilution) | Ambient and ISDS samples:<br><1 – 80 colonies/100 mL (fecal coliforms and E. coli)<br>Ambient or marine samples:<br><2 – 80 colonies/100 mL (enterococci) | No. of Sample Locations | NA |

| Laboratory QC: | Frequency/ Number | Method/SOP QC Acceptance Limits | Corrective Action (CA) | Person(s) Responsible for CA | Data Quality Indicator (DQI) |
|---|---|---|---|---|---|
| Method Blank | 2/run or 2/100 plates, whichever is greater | Less than 1 colony/100 mL | Samples re-analyzed, data qualified as outside holding time | E. Herron | Bias |
| Laboratory Duplicate | 25% on non-diluted samples only | 20%RPD | Data qualified | E. Herron | Precision |
| | Diluted samples replication by comparison of samples at different dilutions | 20%RPD | Data qualified | E. Herron | Precision |
| Positive Plates | 2/run | Positive growth | Samples re-analyzed, data qualified as outside holding time | E. Herron | False Negatives |

Note:
No measurement performance criteria are provided in this table as this QAPP is for general laboratory procedures and not associated with a specific project.

**Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99**

## TOTAL SUSPENDED SOLIDS

| | | | |
|---|---|---|---|
| Medium/Matrix | Water | Analytical Method/ SOP Reference | SOP 009 |
| Sampling SOP | NA | Laboratory Name | URIWW |
| Concentration Range (without dilution) | Ambient and ISDS samples: <1 – 500 mg/L TSS | No. of Sample Locations | NA |

| Laboratory QC: | Frequency/ Number | Method/SOP QC Acceptance Limits | Corrective Action (CA) | Person(s) Responsible for CA | Data Quality Indicator (DQI) |
|---|---|---|---|---|---|
| Method Blank | 1/24 samples | Not greater than 1 mg/L TSS | Re-weigh, if still outside QC limits then data qualified | URIWW Staff | Bias |
| Laboratory Duplicate | 100% | Not greater than 30%RPD | Re-weigh samples then if still outside QC limits data is qualified | URIWW Staff | Precision |
| EPA Water Pollution Proficiency Test Study – Analysis of unknown | yearly | 2 standard deviation | Data reported to laboratory | NA | Accuracy/Comparability |
| Check Calibration of Balance | Daily, when in use | Not greater than 10%D | Service and re-calibrate | URIWW Staff | Accuracy |

Note:
No measurement performance criteria are provided in this table as this QAPP is for general laboratory procedures and not associated with a specific project.

**Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99**

## ALKALINITY AND pH

| | | | | |
|---|---|---|---|---|
| Medium/Matrix | Water | | Analytical Method/ SOP Reference | SOP 010 |
| Sampling SOP | NA | | Laboratory Name | URIWW |
| Concentration Range (without dilution) | pH ambient, marine and ISDS samples: 1 – 14 SU | Alkalinity<br>Ambient water : <0.1 – 30 mg/L $CaCO_3$<br>ISDS samples: <0.1 – 1000 mg/L $CaCO_3$ | No. of Sample Locations | NA |

| Laboratory QC: | Frequency/ Number | Method/SOP QC Acceptance Limits | Corrective Action (CA) | Person(s) Responsible for CA | Data Quality Indicator (DQI) |
|---|---|---|---|---|---|
| Calibrate pH meter | Each time used | Electrode Efficiency >96% | Replace standards then if calibration still a problem replace the electrode | URIWW Staff | Accuracy |
| pH: Standards as Samples (check of calibration using standards) | 1/15 samples | Change in standard not greater than 0.1 SU | Re-check values of calibrants (4 and 7 pH buffers), then recalibrate and re-analyze affected samples if necessary | URIWW Staff | Accuracy/Precision |
| EPA Water Pollution Proficiency Test Study – Analysis of unknown for pH and alkalinity | yearly | 2 standard deviation | Data reported to laboratory | NA | Accuracy/Comparability |

Note:
No measurement performance criteria are provided in this table as this QAPP is for general laboratory procedures and not associated with a specific project.

**Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99**

## BIOCHEMICAL OXYGEN DEMAND

| | | | |
|---|---|---|---|
| Medium/Matrix | Water | Analytical Method/ SOP Reference | SOP 011 |
| Sampling SOP | NA | Laboratory Name | URIWW |
| Concentration Range | Ambient samples (undiluted samples): <2 – 20 mg/L BOD<br>ISDS samples (diluted samples): <2 – 800 mg/L BOD | No. of Sample Locations | NA |

| Laboratory QC: | Frequency/ Number | Method/SOP QC Acceptance Limits | Corrective Action (CA) | Person(s) Responsible for CA | Data Quality Indicator (DQI) |
|---|---|---|---|---|---|
| Calibrate DO meter | Each time used | 99% Air Saturation | Replace membrane and re-calibrate. If still will not calibrate correctly use another meter. | URIWW Staff | Accuracy |
| Method Blank | 2/run | Not greater than 2 mg/L BOD | Data qualified | L. Green | Bias |
| Laboratory Duplicate | Each sample prepared at a minimum of 2 different dilutions. Acceptable dilutions compared as duplicates | Not greater than 20%RPD | Data qualified | L. Green | Precision |
| EPA Water Pollution Proficiency Test Study – Analysis of unknown | yearly | 2 standard deviation | Data reported to laboratory | NA | Accuracy/Comparability |

Note:
No measurement performance criteria are provided in this table as this QAPP is for general laboratory procedures and not associated with a specific project.

**Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99**

## SALINITY

| | | | |
|---|---|---|---|
| Medium/Matrix | Water | Analytical Method/ SOP Reference | SOP 017 |
| Sampling SOP | NA | Laboratory Name | URIWW |
| Concentration Level (undiluted samples) | Marine samples: 0.4 - 40 ppt | No. of Sample Locations | NA |

| Laboratory QC: | Frequency/ Number | Method/SOP QC Acceptance Limits | Corrective Action (CA) | Person(s) Responsible for CA | Data Quality Indicator (DQI) |
|---|---|---|---|---|---|
| Sample Replication | 100% | Not greater than 2 ppt different | Titrate a third aliquot of the sample. If still greater than 2 ppt different note deviation on project data sheet | URIWW Staff | Precision |
| Sample Comparison | 50% | Not greater than 2 ppt different | Re-analyze sample by test kit and refractometer. If difference still greater than 2 ppt then replace test kit and analyze another aliquot of sample. If still greater than 2 ppt different it will be assumed the refractometer is in error and it will be replaced. | URIWW Staff | Accuracy/ Comparability |

Note:
No measurement performance criteria are provided in this table as this QAPP is for general laboratory procedures and not associated with a specific project.

**Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99**

## CHLOROPHYLL-a

| | | | |
|---|---|---|---|
| Medium/Matrix | Water | Analytical Method/ SOP Reference | SOP 011 |
| Sampling SOP | NA | Laboratory Name | URIWW |
| Concentration Level (undiluted samples) | Ambient and marine samples: <0.2 – 100 µg/L chlorophyll-a | No. of Sample Locations | NA |

| Laboratory QC: | Frequency/ Number | Method/SOP QC Acceptance Limits | Corrective Action (CA) | Person(s) Responsible for CA | Data Quality Indicator (DQI) |
|---|---|---|---|---|---|
| Calibrate fluorometer | Calibrate yearly, Check calibration daily | Daily – Not greater than 15%D | Re-calibrate and then replace light source if calibration continues to drift | URIWW Staff/ L. Green | Accuracy |
| Method Blank | 1/rack (38 samples) | Not greater than 0.03 µg/L chlorophyll-a as read on the fluorometer | Re-analyze on fluorometer, then qualify samples associated with blank if necessary | URIWW Staff | Bias |
| Filter Blank | 1/rack (38 samples) | Not greater than 0.03 µg/L chlorophyll-a as read on the fluorometer | Re-analyze on fluorometer, then qualify samples associated with blank if necessary | URIWW Staff | Bias |
| Laboratory Duplicate (Replication of fluorometer reading) | 100% | Not greater than 20%RPD | Re-analyze on fluorometer, then qualify samples associated if still greater than QA objective | URIWW Staff | Precision |
| LCS (Check standard using solid standard) | 1/rack (38 samples) | Not greater than 15%D | Re-analyze on fluorometer, check value of primary standard, recalibrate if necessary, re-analyze associated samples | URIWW Staff | Accuracy |
| Collection of Field Sample Replicates | Each sample location | Not greater than 100%RPD | Deficiency will be noted on project data sheet | URIWW Staff | Precision |

Note:
No measurement performance criteria are provided in this table as this QAPP is for general laboratory procedures and not associated with a specific project.

## Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99
## CHLORIDE

| | | | |
|---|---|---|---|
| Medium/Matrix | Water | Analytical Method/ SOP Reference | SOP 013 |
| Sampling SOP | NA | Laboratory Name | URIWW |
| Concentration Range (without dilution) | Ambient and ISDS samples: 5 – 50 mg/L $Cl^-$ | No. of Sample Locations | NA |

| Laboratory QC: | Frequency/ Number | Method/SOP QC Acceptance Limits | Corrective Action (CA) | Person(s) Responsible for CA | Data Quality Indicator (DQI) |
|---|---|---|---|---|---|
| Calibrate | Each time used | $R^2$ of calibration linear regression not less than 0.990 | Re-calibrate | L. Green | Accuracy |
| Method Blank | 1/15 samples | Not greater than 2 mg/L $Cl^-$ | Re-analyze, then re-calibrate and re-analyze associated samples if necessary | L. Green | Bias |
| Laboratory Duplicate | Sample aliquot taken from same cup – 100%<br>ISDS samples poured into two separate cups – 100% samples<br>Ambient samples poured into two separate cups – 10% of samples | Not greater than 15%RPD<br>Not greater than 20%RPD<br>Not greater than 20%RPD | Re-analyze samples, if still greater than QC objective then note deviation on project data sheet | L. Green | Precision |
| LCS (Purchased External Standards) | 3/90 samples | Not greater than 20%D | Re-analyze standard, then recalibrate instrument and re-analyze associated samples if still greater than 20%D | L. Green | Accuracy/ Comparability |
| LCS (Calibrant) | 1/15 samples | Not greater than 20%D | Re-analyze standard, then recalibrate instrument and re-analyze associated samples if still greater than 20%D | L. Green | Accuracy |
| EPA Water Pollution Proficiency Test Study – Analysis of unknown | yearly | 2 standard deviation | Data reported to laboratory | NA | Accuracy/ Comparability |

Note:
No measurement performance criteria are provided in this table as this QAPP is for general laboratory procedures and not associated with a specific project.

## Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99
## AMMONIA

| | | | |
|---|---|---|---|
| Medium/Matrix | Water | Analytical Method/ SOP Reference | SOP 014 |
| Sampling SOP | NA | Laboratory Name | URIWW |
| Concentration range (without dilution) | Ambient and marine samples: <40 – 1000 µg/L $NH_3$-N<br>ISDS samples: <40 – 2000 µg/L $NH_3$-N | No. of Sample Locations | NA |

| Laboratory QC: | Frequency/ Number | Method/SOP QC Acceptance Limits | Corrective Action (CA) | Person(s) Responsible for CA | Data Quality Indicator (DQI) |
|---|---|---|---|---|---|
| Calibrate | Each time used | $R^2$ of calibration linear regression not less than 0.990 | Re-calibrate | L. Green | Accuracy |
| Method Blank | 1/10 samples | Not greater than 30 µg/L $NH_3$-N | Re-analyze, then re-calibrate and re-analyze associated samples if necessary | L. Green | Bias |
| Laboratory Duplicate | Sample aliquot taken from same cup – 100%<br>ISDS samples poured into two separate cups – 100% samples<br>Ambient & marine samples poured into two separate cups – 10% of samples | Not greater than 15%RPD<br>Not greater than 20%RPD<br>Not greater than 20%RPD | Re-analyze samples, if still greater than QC objective then note deviation on project data sheet | L. Green | Precision |
| LCS (Purchased External Standards) | 3/90 samples | Not greater than 20%D | Re-analyze standard, if still outside QC objective recalibrate instrument and re-analyze associated samples | L. Green | Accuracy/ Comparability |
| LCS (Calibrant) | 1/15 samples | Not greater than 20%D | Re-analyze standard, if still outside QC objective recalibrate instrument and re-analyze associated samples | L. Green | Accuracy |
| EPA Water Pollution Proficiency Test Study – Analysis of unknown | Yearly | 2 standard deviation | Data reported to laboratory | NA | Accuracy/ Comparability |

Note: No measurement performance criteria are provided in this table as this QAPP is for general laboratory procedures and not associated with a specific project.

## Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99
# ORTHOPHOSPHATE AND NITRATE + NITRITE

| | | | |
|---|---|---|---|
| Medium/Matrix | Water | Analytical Method/ SOP Reference | SOP 015 |
| Sampling SOP | NA | Laboratory Name | URIWW |
| Concentration range (without dilution) | Nitrate/Nitrite: Ambient, marine and ISDS samples: <30 – 2000 µg/L $NO_3/NO_2$-N<br>Orthophosphate: Ambient and marine samples: <4 – 200 µg/L $PO_4$-P<br>ISDS samples: <4 – 2000 µg/L $PO_4$-P | No. of Sample Locations | NA |

| Laboratory QC: | Frequency/ Number | Method/SOP QC Acceptance Limits | Corrective Action (CA) | Person(s) Responsible for CA | Data Quality Indicator (DQI) |
|---|---|---|---|---|---|
| Calibrate | Each time used | $R^2$ of calibration linear regression not less than 0.990 | Re-calibrate | L. Green | Accuracy |
| Method Blank | 1/10 samples | Not greater than 2 µg/L $PO_4$-P and 20 µg/L $NO_3/NO_2$-N | Re-analyze, then re-calibrate and re-analyze associated samples if necessary | L. Green | Bias |
| Laboratory Duplicate | Sample aliquot taken from same cup – 100% | Not greater than 15%RPD | Re-analyze samples, if still greater than QC objective then note deviation on project data sheet | L. Green | Precision |
| | ISDS samples poured into two separate cups – 100% samples | Not greater than 20%RPD | | | |
| | Ambient & marine samples poured into two separate cups – 10% of samples | Not greater than 20%RPD | | | |
| LCS (Purchased External Standards) | 3/90 samples | Not greater than 20%D | Re-analyze standard, if still outside QC objective recalibrate instrument and re-analyze associated samples | L. Green | Accuracy/ Comparability |
| LCS (Calibrant) | 1/15 samples | Not greater than 20%D | Re-analyze standard, if still outside QC objective recalibrate instrument and re-analyze associated samples | L. Green | Accuracy |
| EPA Water Pollution Proficiency Test Study – Analysis of unknown | yearly | 2 standard deviation | Data reported to laboratory | NA | Accuracy/ Comparability |

Note: No measurement performance criteria are provided in this table as this QAPP is for general laboratory procedures and not associated with a specific project.

## Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99
# TOTAL PHOSPHORUS AND NITROGEN

| | | | |
|---|---|---|---|
| Medium/Matrix | Water | Analytical Method/ SOP Reference | SOP 016 |
| Sampling SOP | NA | Laboratory Name | URIWW |
| Concentration range (without dilution) | Total N Ambient and ISDS samples: <30 – 2000 µg/L N<br>Total P Ambient samples: <4 – 200 µg/L P<br>ISDS samples: <4 – 2000 µg/L P | No. of Sample Locations | NA |

| **Laboratory QC:** | **Frequency/ Number** | **Method/SOP QC Acceptance Limits** | **Corrective Action (CA)** | **Person(s) Responsible for CA** | **Data Quality Indicator (DQI)** |
|---|---|---|---|---|---|
| Calibrate | Each time used | $R^2$ of calibration linear regression not less than 0.990 | Re-calibrate | L. Green | Accuracy |
| Method Blank | 1/10 samples | Not greater than 3 µg/L P and 5 µg/L N | Re-analyze, then re-calibrate and re-analyze associated samples if necessary | L. Green | Bias |
| Instrument Blank | 3/90 samples | Not greater than 10 µg/L N and 3 µg/L P | Re-analyze, if still outside QC criteria flag data | L. Green | Bias |
| LCS (Purchased External Standards) | 2/run (150 vials) | Not greater than 20%D | Re-analyze standard, then if still outside QC criteria recalibrate instrument and re-analyze associated samples | L. Green | Accuracy/ Comparability |

## Fixed Laboratory Analytical QC Sample Table – EPA NE QAPP Worksheet #24 a – Rev. 10/99
# TOTAL PHOSPHORUS AND NITROGEN (continued)

| Laboratory QC: | Frequency/ Number | Method/SOP QC Acceptance Limits | Corrective Action (CA) | Person(s) Responsible for CA | Data Quality Indicator (DQI) |
|---|---|---|---|---|---|
| Laboratory Duplicate | Sample aliquot taken from same cup – 100%<br><br>Sample poured into two separate cups – 10% of samples<br><br>Replicate digestions of sample 20% of marine and ambient samples<br><br>Triplicate digestions of 100% of ISDS samples | Not greater than 15%RPD<br><br>Not greater than 20%RPD<br><br>Not greater than 25%RPD<br><br>Not greater than 25%RPD | Re-analyze sample, if still outside QC criteria note deviation on project data sheet | L. Green | Precision |
| LCS (Calibrant) | 1/10 samples | Not greater than 20%D | Re-analyze standard, if still outside QC criteria recalibrate instrument and re-analyze associated samples | L. Green | Accuracy |
| Glycine External Total N Standard – ISDS samples only | 3/run | Not greater than 5%D | Re-analyze standard, if %D is greater than 20% then may be necessary to re-digest samples in the run. Otherwise, note deviation | L. Green | Accuracy |
| EPA Water Pollution Proficiency Test Study – Analysis of unknown | yearly | 2 standard deviation | Data reported to laboratory | NA | Accuracy/ Comparability |

Note: No measurement performance criteria are provided in this table as this QAPP is for general laboratory procedures and not associated with a specific project.

## 3.0 SAMPLE HANDLING, TRACKING AND CUSTODY REQUIREMENTS

A chain-of-custody (COC) form or sample log sheet will be completed for each set of samples by the person(s) responsible for collection and/or delivery of the samples to the laboratory. Sample log sheets are generally provided by the URIWW Laboratory. The COC form will include the following information:

1. Project Name
2. Project Location
3. Person(s) responsible for transporting samples
4. Date of sample collection
5. Sample identification name/number
6. Number and type of sample bottles

A technician will be responsible for checking that the samples listed on the sample log sheet correspond correctly with the samples received. A copy of the sample log sheet will be maintained in the project file.

### 3.1 Acceptance of Expendable Laboratory supplies

All expendable laboratory supplies such as test tubes, Petri dishes, chemicals and sample bottles will be inspected upon arrival by either Linda Green or Elizabeth Herron. Packages containing damaged material or packages that were open upon arrival will not be accepted. Chemicals will be marked with the date of acceptance as well as the date they are opened.

## 3.2 Sample Handling System – Worksheet 16

| EPA-NE QAPP Worksheet #16 - Rev. 10/99<br>**Sample Handling System** |
|---|
| **SAMPLE COLLECTION, PACKAGING AND SHIPMENT** |
| Sample Collection: Various persons<br>Sample Packing: Person(s) responsible for sample collection<br>Coordination of Shipment: Person(s) responsible for sample collection<br>Type of Shipment: Generally the person responsible for sample collection or their designee. |
| **SAMPLE RECEIPT AND ANALYSIS** |
| Responsible Organization: University of Rhode Island Watershed Watch Laboratory (URIWW)<br>Sample Receipt: URIWW Staff<br>Sample Custody and Storage: URIWW Staff<br>Sample Preparation: URIWW Staff<br>Sample Determinative Analysis: URIWW Staff |
| **SAMPLE ARCHIVAL** |
| Field Sample Storage (No. of days from sample collection): Dependent upon analysis – Refer to analyte-specific SOPs (Appendix A)<br>Sample Extract/Digestate Storage (No. of days from extraction/digestion): Dependent upon analysis – Refer to analyte-specific SOPs (Appendix A) |
| **SAMPLE DISPOSAL** |
| Responsible Organization and personnel: URIWW / URIWW Staff |

## 4.0 PROJECT DOCUMENTATION AND RECORDS

All sample log sheets will be retained by the laboratory in the project files. All hard copy sample data sheets and sample preparation worksheets as discussed in each analyte-specific SOP under Section 7.0 Documentation will also be retained in the project files. For assays that produce electronic files, the electronic file will be stored and a hard copy of the file contents will be produced. The hard copy will include a peak height tracing of each sample, a standard curve and a final data worksheet.

Project files are maintained in the main URIWW laboratory and Linda Green's office, in the URI Coastal Institute by Linda Green and Elizabeth Herron. Both locations are locked when staff are not present. Electronic data are stored on a password protected laboratory computer that is networked to several other password protected computers throughout the URIWW laboratories and offices. All laboratory data (electronic and hard copy) are retained for at least 10 years after project completion.

## 5.0 DATA VALIDATION

No general quality management reports are prepared. During the analysis of samples the technician completing sample analysis is responsible for recording any problems with meeting measurement performance criteria (Section 2.6.2) and/or instrument operational issues. Any failure of a sample to meet defined measurement performance criteria should be recorded and the data flagged for further review upon data entry and final data validation.

Data generated by each analysis is internally validated by either Ms. Green or Ms. Herron. The data validation process starts once the data has been produced and it is entered into Microsoft Excel files. After data has been entered into the appropriate file, URIWW staff completes an initial check to be sure all data was entered correctly. Then, Ms. Green or Ms. Herron check the data entered for errors and correct any. Outliers and inconsistencies are flagged for further review. If data collected by a volunteer monitor is flagged, then the monitor is contacted to check that the data sent to the laboratory were correct. Data are compared to value obtained for similar samples analyzed in the past. The decision to discard data will be made by either Ms. Green or Ms. Herron.

# Appendix A

**Standard Operation Procedures**
**List of SOPs**

| Description | SOP Number |
|---|---|
| General Laboratory Safety | 001 |
| University Safety and Waste Handling Document (located in the URIWW Laboratory, not included in this QAPP) | 001a |
| Laboratory Water | 002 |
| General Labware Cleaning Procedure | 003 |
| General Autoclave Operation | 004 |
| Bottle Autoclaving Procedure | 005 |
| Waste Autoclaving Procedure | 006 |
| Ambient Waters Microbiological Procedure | 007 |
| ISDS Microbiological Procedure | 008 |
| Total Suspended Solids Analysis | 009 |
| Alkalinity and pH Procedures | 010 |
| Biochemical Oxygen Demand Procedure | 011 |
| Chlorophyll-a Analysis, Welschmeyer Method | 012 |
| Chloride Analysis | 013 |
| Ammonia Analysis | 014 |
| Orthophosphate and Nitrate + Nitrite Analysis | 015 |
| Total Phosphorus and Nitrogen Analysis | 016 |
| Salinity Analysis | 017 |
| Enterococci Analysis | 018 |

# Appendix B

**Resumes for Key Laboratory Personnel**
**List of Resumes**

Arthur Gold, PhD

Linda Green, MS

Elizabeth Herron, MA

Jose Amador, PhD

# Appendix C

**Descriptive Information Regarding**
**University of Rhode Island Watershed Watch (URIWW)**

# Standard Operating Procedures

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

| Description | SOP Number |
|---|---|
| General Laboratory Safety | 001 |
| University Safety and Waste Handling Document | 001a |
| Laboratory Water | 002 |
| General Labware Cleaning Procedure | 003 |
| General Autoclave Operation | 004 |
| Bottle Autoclaving Procedure | 005 |
| Waste Autoclaving Procedure | 006 |
| Ambient Waters Microbiological Procedure | 007 |
| ISDS Microbiological Procedure | 008 |
| Total Suspended Solids Analysis | 009 |
| Alkalinity and pH Procedures | 010 |
| Biochemical Oxygen Demand Procedure | 011 |
| Chlorophyll-A Analysis, Welschmeyer Method | 012 |
| Chloride Analysis | 013 |
| Ammonia Analysis | 014 |
| Orthophosphate and Nitrate + Nitrite Analysis | 015 |
| Total Phosphorus and Nitrogen Analysis | 016 |
| Salinity Analysis | 017 |
| Enterococci Analysis | 018 |

# Standard Operating Procedure 001

# General Laboratory Safety

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

UNIVERSITY OF Rhode Island

**University of Rhode Island Watershed Watch**

## 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:
        i. Behind the sink in room 018.
        ii. On top of the refrigerator in room 002.
        iii. 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:
        i. Under the laboratory bench to the right of blue oven in room 018.
        ii. Next to the main sink in room 002.
        iii. 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 Patty Harrington (874-2495). 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 (ie 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.

# Standard Operating Procedure 002

# Laboratory Water

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

## 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.

# Standard Operating Procedure 003

## General Labware Cleaning Procedure

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

### 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 is soaked in a bath of dilute 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 it into labeled squeeze bottles.
2. Glassware is acid soaked, plasticware is not. 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 right 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. <u>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</u>. <u>Do not wash clean lakes sample bottles in ISDS tubs.</u>
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 Glassware Cleaning Procedure

### 3.2.1 Summary

The following is a general summary of the steps involved in cleaning glassware. Do not place any plasticware or plastic 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 tap water.
3. Wash in soapy water in the appropriate tub.
4. Rinse with tap water.
5. Soak glassware for at least 24 hours in tub of 10% hydrochloric acid.
6. Rinse with DI water.
7. Fill with DI or Ultrapure water and allow glassware 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 Glassware (Not Plasticware) Cleaning Procedure.

All glassware 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.

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 glassware. 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 glassware with tap water.
4. To remove labels fill a 5 gallon bucket, or the sink with (preferably) hot water and place glassware 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 glassware where the label was. Empty any water from the glassware.
6. Dump out and re-use the bucket or fill another bucket with tap water and a squirt of detergent. Add glassware and soak for at least a 1/2 hour. Use an appropriately sized brush to scrub bottles and glassware.
7. Rinse glassware 3 times with tap water, inside and out.
8. Put cleaned glassware into a glassware 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 glassware into the tub minimizing splashing. Remember the tub contains 10% hydrochloric acid. Make sure the glassware is completely covered by the acid solution. Label the tub with the date and time the glassware was placed into the tub. Soak the glassware for at least 24 hours.
10. After acid soaking, carefully remove glassware, emptying acid back into the bath. Put the glassware into a glassware carrying tub and bring it to a sink. Do not carry glassware dripping with acid to the sink.
11. Rinse each piece of glassware 3 times, inside and out, with DI water.
12. Fill each piece of glassware with DI water and allow it to soak for at least 24 hours, be sure to label the piece of glassware 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. 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 glassware cleaning procedure.
2. Follow steps 12 through 14 of the glassware cleaning procedure.

# Standard Operating Procedure 004

# General Autoclave Operation

**Date:** 11/04
**Revision:** 1
**Author:** Elizabeth Herron

**University of Rhode Island Watershed Watch**

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

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 at 250 °F / 121 °C with the thermostat knob. Reset the red “maximum pressure” indicator needle on the pressure gauge to zero.
7. 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).
8. 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.
9. 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.
10. When the timer reaches 0, the run is complete.
11. Turn the multi-purpose knob to EXH + DRY.
12. 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.

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.

# Standard Operating Procedure 005

# Bottle Autoclaving Procedure

UNIVERSITY OF Rhode Island

**University of Rhode Island Watershed Watch**

| | |
|---|---|
| **Date:** | **11/04** |
| **Revision:** | **1** |
| **Author:** | **Elizabeth Herron** |

## 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 value. 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. Close the door, and lock it by turning the handle clockwise. The door should be secure with a good seal, but only hand tight.
5. Set the temperature for 250 °F / 121 °C with the thermostat knob. Reset the red MAXIMUM PRESSURE indicator needle on the pressure gauge to zero.

6. 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.
7. When the timer reaches 0, the run is complete.
8. Turn the multi-purpose knob to EXH + DRY.
9. When the pressure gauge reads 0, turn the main switch to STOP, and the multi-purpose switch to 0.
10. Carefully open the door – steam will escape so do not put any body parts near the edge of the door while opening it.
11. Allow to cool or remove the load immediately. Be sure to use insulated gloves when handling the tray. It will be hot!
12. Unplug the unit if you are not running any more loads immediately.
13. Do not tighten bottle caps until the bottles are cooled to room temperature or they will warp.
14. 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).

# Standard Operating Procedure 006

# Waste Autoclaving Procedure

**Date:** 11/04
**Revision:** 1
**Author:** Elizabeth Herron

**University of Rhode Island Watershed Watch**

## 1.0 PURPOSE AND DESCRIPTION

This procedure is for autoclaving used media plates (mTec, mE, and EIA) using the autoclave in the basement of Woodward Hall.

## 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 bath 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 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 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.
2. Wearing gloves, remove the half filled bag of plates 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.
6. Set EXHAUST TIME for 5 minutes.
7. Select the UNWRAPPED setting (temperature should be set for 132 °C).
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. Once the wastes are cool enough to handle, bring the waste to the dumpster outside Woodward Hall 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!
12. Clean the autoclave tray with soap and hot water.
13. Place a new autoclave bag into the red waste container.

# Standard Operating Procedure 007 (Prior number URIWW-SOP-4A)

## Ambient Waters Microbiological Procedure

**Date:** 11/04
**Revision:** 1
**Author:** Elizabeth Herron

**University of Rhode Island Watershed Watch**

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# Standard Operating Procedure 007 (Prior number URIWW-SOP-4A)

# Ambient Waters Microbiological Procedure

**Date:** 11/04
**Revision:** 1
**Author:** Elizabeth Herron

**University of Rhode Island Watershed Watch**

## 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 ($KH_2PO_4$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), 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

| Required Material | Notes | Re-order information |
|---|---|---|
| **Coastal Institute in Kingston, room 081** | | |
| Autoclave | | |
| **University of Rhode Island (URI), Kingston Coastal Institute Watershed Watch (WW) Laboratory, room 002** | | |
| Autoclave safe white plastic bottles (125 – 500 mL) | | |
| 2 L autoclave safe flasks | | |
| Insulated autoclave gloves | | |
| Metal autoclave tray | | |
| Combination hot plate and magnetic stirrer | | |
| Magnetic stirring bar | | |
| Microbiology worksheets | Project data sheets are fond on the URIWW computer. See Section 8.0 Documentation | |
| Sodium Hydroxide (NaOH) | Caustic. Causes eye, skin, digestive and respiratory tract burns. Hygroscopic (absorbs moisture from the air). | |
| **Coastal Institute in Kingston, room 018** | | |
| Balance | Calibration weights in drawer beneath the balance | |
| **Coastal Institute in Kingston, room 019** | | |
| 35 °C Incubator | White Precision 5 $ft^3$ Incubator | Thomas catalog #6118L50 |
| 44.5 °C Water bath | Precision fecal coliform bath 66855 | Baxter catalog #W3182-2 |
| Petri dish rack (for water bath) | | Baxter catalog #W3182-11 |
| UV sterilization box | | |

| Required Material | Notes | Re-order information |
|---|---|---|
| Vacuum pump and manifold | | |
| Pipette-Aid filler/dispenser (electric) | | Fisher catalog # 13-681-15 |
| 2 - Glass 400 mL beakers marked URIWW | | |
| 4 - Side arm filter flasks | 500 mL or larger | |
| **Coastal Institute in Kingston, room 019, URIWW refrigerator** | | |
| mTEC media plates | less than a month old, stored media side up in a foil lined box in the refrigerator | |
| mTEC media mix | | Fisher catalog # DF0334-15-0 |
| Agar plate with E. coli | QC sample | American Type Culture Collection # 35922 |
| Tryptic soy broth (TSB) tubes | | |
| Agar mix | | Fisher catalog #BP1423-500 |
| Tryptic soy broth mix | | Fisher catalog #DF0370-17-3 |
| Stock $KH_2PO_4$ solution | Preparation instructions: Section 5.3.2.5 | |
| Stock $MgCl_2$ solution | Preparation instructions: Section 5.3.2.6 | |
| Urea substrate | Preparation instructions: Section 5.3.2.3 | |
| 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 |

| Required Material | Notes | Re-order information |
|---|---|---|
| Sterile grid membrane filters | | Fisher catalog # 09-719-1B or HAWG 047 S1 |
| **Coastal Institute in Kingston, room 019, Plastic drawers or boxes beneath the lab countertop** | | |
| Sterile plastic Petri dishes | | Fisher catalog # 08-757-19 |
| Sterile 1 mL pipettes | | Fisher catalog #13-678-25C |
| Sterile 10 mL pipettes | | Fisher catalog #13-678-25F |
| Sterile 25 mL pipettes | | Fisher catalog #13-676-29D |
| Pipette sterilizing pouches | | Fisher catalog # 01-812-53 |
| **Coastal Institute in Kingston, room 019, Drawer or shelf beneath the lab countertop** | | |
| Reusable test tubes | | Fisher catalog # 14-925J |
| Test tube rack | | Fisher catalog # 14-809-24 |
| Aluminum foil | | |
| **Coastal Institute in Kingston, room 019, In cabinet beneath the water bath** | | |
| Whirl-pak™ bags | | Fisher catalog # 01-812-5C |
| Clear biohazard autoclave bags | | Fisher catalog #01-826-5 |
| **Coastal Institute in Kingston, room 019, On shelf above the water bath** | | |
| Plastic carboy filled with phosphate buffer saline solution | Preparation instructions: Section 5.3.2.1 and 5.3.2.2 | |
| **Coastal Institute in Kingston, room 019, In the upper cabinet** | | |
| Urea | | Fisher catalog #DF0190-17-1 |
| Phenol red | | Sigma catalog #P2417 |
| Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) | | Fisher catalog #M33-500 |
| Potassium phosphate monobasic ($KH_2PO_4$) | | Fisher catalog #P382-500 |
| Sodium chloride (NaCl) | | Fisher catalog # S671-500 |
| 1 N Sodium Hydroxide (NaOH) | Causes eye, skin, digestive and respiratory tract burns. Caustic. Preparation instructions: 5.3.2.4. | |
| **Coastal Institute in Kingston, room 019, In the chemical cabinet** | | |
| 95% Ethanol in a 2 L glass bottle | | Available through the Agricultural |

| Required Material | Notes | Re-order information |
|---|---|---|
| | | Experiment Station office in Woodward Hall |

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Water | Autoclavable white plastic bottle<br>250 mL for freshwater samples<br>500 mL for estuarine and coastal samples | Kept at 4 °C in Sterile Bottles | 100 mL | 6 Hours |

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 with 20% relative percent deviation (%RPD). %RPD is calculated as follows:

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

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:

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

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 Calibration and Standards

#### 5.2.4.1 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

#### 5.2.4.2 Incubators

The temperature of the 35 °C and 44.5 °C incubators are 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 35 °C incubator is 35 +/- 1 °C. Acceptable temperature for the 44.5 °C incubator is 44.5 +/- 1 °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.

### 5.2.5 QA Check on New Plates

*E. coli* bacterium is the dominant fecal coliform bacteria found in ambient water. Therefore, a maintained plate of *E. coil* 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.

#### 5.2.5.1 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 $10^{-8}$ 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

| Volume of sample filtered (mL) | Dilution tube preparation | Volume placed on filter (mL) | Calculated Result (# of colonies divided by calculated result = colonies/100 mL) |
|---|---|---|---|
| 0.1 | 1 mL *E. coli* inoculated TSB sample into 9 mL of PBS | 1 | 1,000 or $10^{-3}$ |
| 0.01 | 1 mL $10^{3}$ sample into 9 mL of PBS | 1 | 10,000 or $10^{-4}$ |
| 0.001 | 1 mL $10^{4}$ sample into 9 mL of PBS | 1 | 100,000 or $10^{-5}$ |
| 0.0001 | 1 mL $10^{5}$ sample into 9 mL of PBS | 1 | 1,000,000 or $10^{-6}$ |
| 0.00001 | 1 mL $10^{6}$ sample into 9 mL of PBS | 1 | 10,000,000 or $10^{-7}$ |
| 0.000001 | 1 mL $10^{7}$ sample into 9 mL of PBS | 1 | 100,000,000 or $10^{-8}$ |

## 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

| | |
|---|---|
| Envirocide or Conflict | Magnetic stirring bar |
| Sterile Petri dishes | Magnetic stirring and heating plate |
| Autoclave | Metal tray |
| 2 - 2 L autoclave safe flasks | Aluminum foil |
| Insulated autoclave gloves | Sterile 25 mL pipette |
| Dehydrated mTEC media | Empty foil lined cardboard box |
| Deionized (DI) water | Electronic pipette-aid |

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 $H_2O$ 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 +/- 1 °C. The water bath should be approximately ¾ full of DI water, and set at 44.5 +/- 1 °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 $KH_2PO_4$ 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 $H_2O$
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 $KH_2PO_4$ 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 $H_2O$
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 it 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 $H_2O$
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 $KH_2PO_4$ solution

1. Add the following to a 1 L Erlenmeyer flask
   a. 34.0 g $KH_2PO_4$
   b. 500 mL DI $H_20$
2. Adjust to pH 7.2 $\pm$ 0.5 with 1*N* NaOH and dilute to 1 L with DI $H_20$
3. 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 $MgCl_2$ solution

Add 81.6 g $MgCl_2 \cdot 6H_2O$ to a 1 L Volumetric flask and dilute to 1 L with DI $H_20$.

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. 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).
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.

<u>Preparation of dilutions</u>

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 ºC 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 20 – 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. Using the Woodward Hall Autoclave only, autoclave the half full bags of plates in a metal tray for 20 minutes at 121 °C on the liquid cycle. Place the cooled bag into the dumpster (do not put in a trash can as the janitors will NOT dispose of it). See SOP 006 - Waste Autoclaving Procedure.

#### 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.

$$\frac{\text{fecal coliforms colonies}}{100\text{ mL}} = \frac{\text{number of yellow colonies counted}}{\text{volume in mL of sample filtered}} \times 100\text{ mL}$$

### 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.

$$\textit{E .coli} = \frac{\text{number of yellow colonies counted}}{\text{Volume in mL of sample filtered}} \times 100\text{ mL}$$

## 7.0 REFERENCES

APHA, AWWA & WEF. Standard Methods for the Examination of Water and Wastewater. 19th ed. Washington D.C: APHA, 1995.
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**

**Analyst - set-up:** **Analyst - Counts:**

**Incubator temp. start:** **Waterbath temp. start:**

**Incubator temp. end:** **Waterbath temp. end:**

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

### 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.

# Standard Operating Procedure 008 (Prior number URIWW-SOP-4B)

# ISDS Microbiological Procedure

**University of Rhode Island Watershed Watch**

**Date:** 11/04
**Revision:** 1
**Author:** Elizabeth Herron

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# Standard Operating Procedure 008 (Prior number URIWW-SOP-4B)

# ISDS Microbiological Procedure

**University of Rhode Island Watershed Watch**

**Date:** 11/04
**Revision:** 1
**Author:** Elizabeth Herron

## 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 ($KH_2PO_4$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), 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

| Required Material | Notes | Re-order information |
|---|---|---|
| **Coastal Institute in Kingston, room 081** | | |
| Autoclave | | |
| **University of Rhode Island (URI), Kingston Coastal Institute Watershed Watch (WW) Laboratory, room 002** | | |
| Autoclave safe white plastic sample bottles | 125 – 500 mL | |
| Autoclave safe flasks | 2 L | |
| Autoclave gloves (Insulated) | | |
| Metal autoclave tray | | |
| Combination hot plate and magnetic stirrer | | |
| Magnetic stirring bar | | |
| Sodium Hydroxide (NaOH) | Caustic. Causes eye, skin, digestive and respiratory tract burns. Hygroscopic (absorbs moisture from the air). | |
| **URI – WW Computer** | | |
| Microbiology worksheets | Project data sheets are found on the URIWW computer. See Section 8.0 Documentation | |
| **Coastal Institute in Kingston, room 018** | | |
| Balance | Calibration weights in drawer beneath the balance | |
| **Coastal Institute in Kingston, room 019** | | |
| 35° C Incubator | White Precision 5 $ft^3$ Incubator | Thomas catalog #6118L50 |
| 44.5° C Water bath | Precision Fecal coliform bath 66855 | Baxter catalog #W3182-2 |
| Petri dish rack for water bath | | Baxter catalog #W3182-11 |
| UV Sterilization box | | |
| Vacuum pump and manifold | | |
| Pipette-Aid filler/dispenser (electric) | | Fisher catalog # 13-681-15 |

| Required Material | Notes | Re-order information |
|---|---|---|
| 2 - Glass 400 mL beakers marked URIWW | | |
| 4 - Side arm filter flasks | 500 mL or larger | |
| Thermolyne Vortex Mixer | | Fisher catalog #12-814 |
| **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 | |
| mTEC media mix | | Fisher catalog # DF0334-15-0 |
| 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 $KH_2PO_4$ solution | Preparation instructions: Section 5.3.2.4 | |
| Stock $MgCl_2$ 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 |
| **Coastal Institute in Kingston, room 019, Plastic drawers or boxes beneath the lab countertop** | | |
| Sterile plastic Petri dishes | | Fisher catalog # 08-757-19 |

| Required Material | Notes | Re-order information |
|---|---|---|
| Sterile 1 mL pipettes | | Fisher catalog #13-678-25C |
| Sterile 10 mL pipettes | | Fisher catalog #13-678-25F |
| Sterile 25 mL pipettes | | Fisher catalog #13-676-29D |
| Pipette sterilizing pouches | | Fisher catalog # 01-812-53 |
| **Coastal Institute in Kingston, room 019, Drawer or shelf beneath the lab countertop** | | |
| Reusable test tubes | | Fisher catalog # 14-925J |
| Test tube rack | | Fisher catalog # 14-809-24 |
| Aluminum foil | | |
| **Coastal Institute in Kingston, room 019, In cabinet beneath the water bath** | | |
| Whirl-pak™ bags | | Fisher catalog # 01-812-5C |
| Clear biohazard autoclave bags | | Fisher catalog #01-826-5 |
| **Coastal Institute in Kingston, room 019, On shelf above the water bath** | | |
| Plastic carboy filled with phosphate buffer saline solution | Preparation instructions: Section 5.3.2.1 and 5.3.2.2 | |
| **Coastal Institute in Kingston, room 019, In the upper cabinet** | | |
| Phenol red | | Sigma catalog #P2417 |
| Sodium chloride | | Fisher catalog # S671-500 |
| Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) | | Fisher catalog # M33-500 |
| Potassium phosphate monobasic ($KH_2PO_4$) | | Fisher catalog # P382-500 |
| 1 N Sodium Hydroxide (NaOH) | Causes eye, skin, digestive and respiratory tract burns. Caustic. Preparation instructions: Section 5.3.2.3. | |
| **Coastal Institute in Kingston, room 019, In the chemical cabinet** | | |
| 95% Ethanol in a 2 L glass bottle | | Available through the Agricultural Experiment Station office in Woodward Hall |

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Water | Autoclavable 125 or 250 mL white plastic bottles. Sample bottle size is project dependent | Kept at 4 °C in sterile bottle | 100 mL | 6 Hours |

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:

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

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 Calibration and Standards

#### 5.2.4.1 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.

#### 5.2.4.2 Incubators

The temperature of the 35 °C and 44.5 °C incubators are checked on a daily basis, when in use. Initial and final temperatures for incubations of samples are recorded on the project data sheet (see Section 8.0). The acceptable temperature range for the 35 °C incubator is 35 +/- 1 °C. The acceptable temperature range for the 44.5 °C incubator is 44.5 +/- 1 °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.

### 5.2.5 QA Check on New Plates

*E. coli* bacterium is the dominant fecal coliform bacteria found in ambient water. Therefore, a maintained plate of *E. coil* 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 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 5.2.5.1 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.

#### 5.2.5.1 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 $10^{-8}$ 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

| | |
|---|---|
| Envirocide or Conflict | Magnetic stirring bar |
| Sterile Petri dishes | Magnetic stirring and heating plate |
| Autoclave | Metal tray |
| 2 - 2 L autoclavable flasks | Aluminum foil |
| Insulated autoclave gloves | Sterile 25 mL pipette |
| Dehydrated mTEC media | Empty foil lined cardboard box |
| Deionized (DI) water | Electronic pipette-aid |

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 $H_2O$ 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 +/- 1 °C. The water bath should be approximately ¾ full of DI water and set at 44.5 +/- 1 °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 $KH_2PO_4$ solution (preparation information in Section 5.3.2.4)
    b. 5 mL Stock $MgCl_2$ solution (preparation information in Section 5.3.2.5)
    c. 7 g NaCl
    d. 1 L DI $H_2O$
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 $KH_2PO_4$ solution (preparation information in Section 5.3.2.4)
    b. 20 mL Stock $MgCl_2$ solution (preparation information in Section 5.3.2.5)
    c. 28 g NaCl
    d. 4 L DI $H_2O$
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 it 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 $KH_2PO_4$ solution

1. Add the following to a 1 L Erlenmeyer flask
    a. 34.0 g $KH_2PO_4$
    b. 500 mL DI $H_20$
2. Adjust to pH 7.2 ± 0.5 with 1*N* NaOH and dilute to 1 L with DI $H_20$
3. 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 $MgCl_2 \cdot 6H_2O$ to a 1 L Volumetric flask and dilute to 1 L with DI $H_20$.

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. 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).
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 20 - 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. Using the Woodward Hall Autoclave only, autoclave the half full bags of plates in a metal tray for 20 minutes at 121°C on the liquid cycle. Place the cooled bag into the dumpster (do not put in a trash can as the janitors will NOT dispose of it). See SOP 006 - Waste Autoclaving Procedure.

#### 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.

$$\frac{\text{Fecal coliforms colonies}}{100\text{ mL}} = \frac{\text{Count}}{\text{Yields}}$$

**Notes regarding dilutions:**

| Volume of ISDS sample filtered (mL) | Dilution tube preparation | Volume placed on filter (mL) | Calculated Result (# of colonies divided by …) |
|---|---|---|---|
| 100 | None | 100 | 1 |
| 10 | None | 10 | 0.1 |
| 1 | None | 1 | 0.01 |
| 0.1 | 1 mL ISDS sample into 9 mL of PBS | 1 | $10^{-3}$ |
| 0.01 | 1 mL $10^{3}$ sample into 9 mL of PBS | 1 | $10^{-4}$ |
| 0.001 | 1 mL $10^{4}$ sample into 9 mL of PBS | 1 | $10^{-5}$ |
| 0.0001 | 1 mL $10^{5}$ sample into 9 mL of PBS | 1 | $10^{-6}$ |

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

## 7.0 REFERENCES

APHA, AWWA & WEF. Standard Methods for the Examination of Water and Wastewater. 19th ed. Washington D.C: APHA, 1995.
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:** _____ 36 Tubes

**Community: Block Island** 69 Plates

**Analyst – set-up:** **Analyst – counts:**

**Incubator temp. start:** **Waterbath temp. start:**

**Incubator temp. end:** **Waterbath temp. end:**

| Site | Location | Setup Date | Dilution | Vol. Filtered | (Yields) | 24 Hr. Count | Count per 100 mL |
|---|---|---|---|---|---|---|---|
| BI - 3 Rea | STE – 1 | | **$10^{-2}$** | **1** | **$10^{-3}$** | | |
| BI - 3 Rea | STE – 1 | | **$10^{-3}$** | **1** | **$10^{-4}$** | | |
| BI - 3 Rea | STE – 1 | | **$10^{-4}$** | **1** | **$10^{-5}$** | | |
| | | | | | | | |
| BI - 3 Rea | RTE | | **1** | **1** | **$10^{-2}$** | | |
| BI - 3 Rea | RTE | | **$10^{-2}$** | **1** | **$10^{-3}$** | | |
| BI - 3 Rea | RTE | | **$10^{-3}$** | **1** | **$10^{-4}$** | | |
| | | | | | | | |
| BI - 3 Rea | AXE | | **1** | **10** | **$10^{-1}$** | | |
| BI - 3 Rea | AXE | | **1** | **1** | **$10^{-2}$** | | |
| BI - 3 Rea | AXE | | **$10^{-2}$** | **1** | **$10^{-3}$** | | |
| | | | | | | | |
| Lab Positive | | | | | | | |
| Lab Positive | | | | | | | |
| Lab Positive | | | | | | | |
| Field Blank | | | **1** | **100** | **1** | | |
| Start Blank | | | **1** | **100** | **1** | | |
| End Blank | | | **1** | **100** | **1** | | |

**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.

UNIVERSITY OF Rhode Island

# Standard Operating Procedure 009 (Prior number URIWW-SOP-009)

# Total Suspended Solids Analysis

**University of Rhode Island Watershed Watch**

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

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# Standard Operating Procedure 009 (Prior number URIWW-SOP-009)

# Total Suspended Solids Analysis

**University of Rhode Island Watershed Watch**

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

## 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

| Required Material | Description | Notes |
|---|---|---|
| Drying oven | Blue-M Stabiltherm mechanical convection horizontal airflow electric utility oven set at 105 °C | Property of URI Natural Resources Science Dept. |
| Balance | Mettler Toledo AB 104 Balance Capable of weighing to 0.5 mg | Property of URI Natural Resources Science Dept. |
| Electronic desiccator | Desiccator contains several shelves and an automatic electronic dryer to maintain 30-40% relative humidity. | Fisher Scientific #08-647-30, list price $625. |

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

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Water | 500 mL HDPE Bottle | Kept at 4 °C | 500 mL | 1 week |

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-ruse 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:

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

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.

### 5.3 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

| ISDS Sample ID | Volume to Filter (mL) | | Sample ID | Volume to Filter (mL) |
|---|---|---|---|---|
| STE-R | 10-100 | | RSFE | 200 mL |
| RTFE | 200 | | RTE | 100 mL |
| SFE | 200 | | BFE | 150-200 mL |
| STE-S | 25-100 | | | |
| 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 TSS Samples

1. Dry filters in the aluminum weight dishes on an aluminum pan in a drying oven set a 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” column on data sheet.

#### 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 = final weight (g) = weigh dish + filter containing dried residue (g)
B = initial weight (g) = 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 = final weight (mg) = weigh dish + filter containing dried residue (mg)
B= initial weight (mg) = 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

*APHA, AWWA, WEF. Standard Methods* for the Examination of Water and Wastewater. 19th ed. Washington D.C.: APHA, 1995.
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

**Green Hill and Ninigret Ponds**
**TOTAL SUSPENDED SOLIDS DATA SHEET**
**FULL SUMMER COLLECTION (August 18, 2004)**

**Analyst Initials**

**Initial weights:** _____
**Sample set-up:** _____
**final weights:** _____

**Date Samples Analyzed:** _____

**Shake vigorously before pouring each sample (including replicates)**

| Sample Date | Sample # | Location | Volume Filtered | Initial Weight | Final Weight | Notes |
|---|---|---|---|---|---|---|
| | | Start Blank | | | | |
| | | GH-In Pond | | | | |
| | | GH-In Pond REP | | | | |
| | | GH-Indigo Point | | | | |
| | | GH-Indigo Point REP | | | | |
| | | GH-Sea Lea | | | | |
| | | GH-Sea Lea REP | | | | |
| | | GH-Teal Road | | | | |
| | | GH-Teal Road REP | | | | |
| | | GH-Twin Peninsula | | | | |
| | | GH-Twin Peninsula REP | | | | |
| | | NP-Crawford Dock | | | | |
| | | NP-Crawford Dock REP | | | | |

# Standard Operating Procedure 010 (Prior number URIWW SOP 7A&7B)

# Alkalinity and pH Procedures

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

## TABLE OF CONTENTS

# Standard Operating Procedure 010 (Prior number URIWW SOP 7A&7B)

# Alkalinity and pH Procedures

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

## 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 $CaCO_3$.

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

| Required Material | Notes | Re-order Information |
|---|---|---|
| Fisher Model AR 20 laboratory pH meter | Capable of reading to 0.01 unit with automatic temperature compensation electrode | |
| Combination pH electrode | Recommended by Fisher Tech Support | Fisher #13-620-185 |
| Saturated potassium chloride (KCl) solution. | This solution is used as the electrode filling solution | Fisher #SP138-500. |
| Fisher certified color-coded pH buffers - pH 4,7 & 10 | Note the expiration data before using the buffers | pH 7 (yellow) #SB107-500<br>pH 4.0 (pink) #SB101-500<br>pH 10 (blue) #SB115-500. |
| Magnetic stirrer and magnetic stir bars | | |
| 10 mL micro-buret, 0.02 mL subdivisions | NIST traceable, with 3-way plug | Fisher # 17115F-10. |
| 2 L of 0.02 N $H_2SO_4$ (Sulfuric Acid) | Preparation instructions: Section 5.4.1.1 | |
| Tubing and stoppers to connect the bottle containing the acid titrant to the buret. | | |
| 250 mL graduated cylinders | | |
| 250 mL beakers | | |

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Ambient Water | 250-500 mL white HDPE or brown glass bottle | Kept at 4 °C | Measured directly in sample bottle | Analyze immediately |
| ISDS Sample | 125 white HDPE bottle | Kept at 4 °C | Measured directly in sample bottle | Analyze immediately |

### 4.2 pH and Alkalinity

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Ambient Water | 500 mL white HDPE bottle | Kept at 4 °C | 200 mL | 24 Hours |
| ISDS Sample | 125 mL white HDPE bottle | Kept at 4 °C | 35-40 mL | 24 Hours |

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 $H_2SO_4$ (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 preformed 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

No samples are replicated during pH or alkalinity determinations.

### 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.
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 purchased from Fisher Scientific. Buffers are stored in bulk in the cabinet above and to the right of the pH meters.
    a. The buffers that are used for each calibration are kept in small wide-mouth plastic bottles, labeled with the contents and the date of filling. These bottles each have a small magnetic stirrer in them.
    b. The buffers are replaced monthly or sooner if they become cloudy or have precipitate in them. Remember to re-date the bottle when replacing the buffer.
    c. Check the expiration date on the buffer bottle prior to re-filling the bottles used for calibration. If the expiration date has passed, dispose of the buffer by rinsing it down the sink and use a newer bottle of buffer.
3. Remove the 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 every 10-15 samples by placing the pH probe in the 4.02 and 7.00 pH buffer standards. The resulting observation is recorded on the data sheet. A change in either standard by less than +/- 0.1 SU is considered acceptable. A change greater than this amount necessitates corrective action.

Corrective Action

Standard buffer values of the buffer should not change more than +/- 0.1 SU. If there is a change greater than +/- 0.1 SU re-check the values of the 4 and 7 standard buffers. If the change is still greater then this value recalibrate the instrument and re-run the samples between the acceptable calibration check and the unacceptable calibration check.

## 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. Check the pH meter calibration every 10-15 samples by immersing the pH probe in the pH 4 and 7 buffers and recording the result on the data sheet. Values of the buffer should not change more than +/- 0.1 SU, refer to Section 5.2.5 of this SOP.
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.

## 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 ($Na_2CO_3$) by placing approximately 2 g of $Na_2CO_3$ 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. $Na_2CO_3$ gram formula weight (gfw) = 106 g
    b. $Na_2CO_3$ gram-equivalent weight (geqw) = 53 g
2. Prepare a $Na_2CO_3$ solution of approx. 0.025 N
    a. Place 1.33 g of $Na_2CO_3$ in a 1 L volumetric flask
    b. Dilute with Ultrapure water
    c. $N = \frac{\text{weight } Na_2CO_3 \text{ used}}{\text{geqw}} = \frac{1.33 \text{ g}}{53 \text{ g}} = 0.025 \text{ N}$
    d. Preparation of 500 mL of solution: 0.665 g $Na_2CO_3$ to 500 mL Ultrapure water
    e. Preparation of 250 mL of solution: 0.332 g $Na_2CO_3$ to 250 mL Ultrapure water
3. Prepare a sulfuric acid ($H_2SO_4$) solution of approximately 0.02 N
    Dilute 40 mL of 1 N $H_2SO_4$ to 2 L using Ultrapure water
4. Put the 0.02 N $H_2SO_4$ solution into the clear glass bottle located on the shelf in room 002, above the pH meters. Pace 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 top save all data.

### 5.4.2 Procedure - Analysis of pH and Alkalinity

1. Drain off ~20 mL $H_2SO_4$ 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 $H_2SO_4$ 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. Check and record the value of the standard buffers used in the calibration of the pH meter every 10-15 samples. The recorded change in each buffer value should not be greater than +/- 0.1 SU. Review Section 5.2.5 for further information on procedure and 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.

## 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

$$\text{Alkalinity (mg/L)} = \frac{[(2B - C) \times (N\ H_2SO_4) \times (50{,}000)]}{\text{Volume of water sample (mL)}}$$

Where:

B = volume to pH ~4.5

C = volume to pH ~4.2

N = normality of titrant ($H_2SO_4$) (approx. 0.02N)

The exact normality of the $H_2SO_4$ 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

Standard Methods for the Examination of Water and Wastewater, 1995 edition. Alkalinity Titration Method (2320B) and pH Value – Electrometric Method (4500-$H^+$ B)

Standard Methods 1985 for the Examination of Water and Wastewater, Method 403, pp 268-273.

## 8.0 DOCUMENTATIONS

Data sheet for analysis of pH and alkalinity

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

| Electrode Efficiency | Sample Date | Monitoring Location | Tech's Initials | vol. (mL) | Initial pH | mL acid to ~4.5 | mL acid to ~4.2 | N of acid |
|---|---|---|---|---|---|---|---|---|
| | | Almy Pond | | | | | | |
| | | Alton Pond | | | | | | |
| | | Arnold Pond | | | | | | |
| | | Barber Pond | | | | | | |
| | | Belleville P - Lower | | | | | | |
| | | Belleville P - upper | | | | | | |
| | | Blackamore Pond | | | | | | |
| | | Bowdish Reservoir | | | | | | |
| | | Carbuncle Pond | | | | | | |
| | | Carr Pond (NK) | | | | | | |
| | | Carr Pond (WG) | | | | | | |
| | | Chapman Pond | | | | | | |
| | | Fenner Pond | | | | | | |
| | | Flat River Reservoir | | | | | | |

Data sheet for analysis of pH only

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

| Electrode Efficiency | Sample Date | Monitoring Location | Tech's Initials | Initial pH |
|---|---|---|---|---|
| | | **Tributaries - no alkalinity only pH** | | |
| | | Asseconk Swamp | | |
| | | Barber - Mud Brook | | |
| | | Belleville @ RR Xing | | |
| | | Belleville @ Sluiceway | | |
| | | Carr Inlet (NK) | | |
| | | FRR @ Zeke's Bridge | | |
| | | FRR@ Isle of Capri | | |
| | | FRR @ Maple Valley | | |
| | | Geo @ Capron Pond | | |
| | | Geo @ Harris | | |
| | | Shick - a @ Rte 2 | | |
| | | Shick - b @ Miskiania | | |

Data sheet for ISDS samples

**Date:** _____

**Community:** **Block Island**

**Please fill-in your initials, and the appropriate value.**

**There should be something in each space.**

**Use only 35 - 40 mL of ISDS samples for pH and alkalinity analyses!**

| Electrode Efficiency | Site | Monitoring Location | Tech's Initials | vol. (mL) | Initial pH | mL acid to ~4.5 | mL acid to ~4.2 | **N of acid** |
|---|---|---|---|---|---|---|---|---|
| | BI - 3 Rea | STE –1 | | | | | | |
| | BI - 3 Rea | STE –2 | | | | | | |
| | BI - 3 Rea | RTE | | | | | | |
| | BI - 3 Rea | AXE | | | | | | |
| | BI - 4 One | STE | | | | | | |
| | BI - 4 One | RTE | | | | | | |
| | BI - 4 One | AXE | | | | | | |
| | BI - 8 Brn | STE | | | | | | |
| | BI - 8 Brn | AXE | | | | | | |
| | BI - 8 Brn | PE | | | | | | |
| | BI - 10 Ort | STE | | | | | | |
| | BI - 10 Ort | AXE | | | | | | |
| | BI - 11 Der | STE | | | | | | |
| | BI - 11 Der | AXE | | | | | | |
| | BI - 12 Fla | STE-S | | | | | | |
| | BI - 12 Fla | RTE | | | | | | |
| | BI - 12 Fla | AXE | | | | | | |
| | BI Field Blank | | | | | | | |

# Standard Operating Procedure 011 (Prior number URIWW-SOP-6)

# Biochemical Oxygen Demand (BOD) Procedure

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

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# Standard Operating Procedure 011 (Prior number URIWW-SOP-6)

# Biochemical Oxygen Demand (BOD) Procedure

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

## 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

| Required Material | Notes | Re-order information |
|---|---|---|
| Incubator set at 20 +/- 1 °C | Capable of maintaining +/- 0.02 °C | Fisher Low Temperature Incubator Model 307C<br>Fisher #11-679-25C<br>List price $3400 |
| DO meter | YSI 5000 | Fisher #13-298-21<br>List price $1300 |
| BOD DO probe | YSI 5010<br>Self-stirring with 3 1/4" paddle | Fisher #13-298-23<br>List price $525 |
| BOD bottles | Clear 300 mL flared neck with a ground glass stopper | Fisher #09-926<br>Case of 24 $190 |
| 20 L Carboy | HDPE with spigot in bottom for nutrient buffer water | Fisher #02-963-5C<br>List price $110 |
| Hach BOD nutrient buffer pillows | | Hach Chemical Company #14863-98<br>25 per box List price $30 |
| Aquarium bubbler | | Purchased from local pet store - $5 |
| Deionized (DI) water | | |
| Pipette filler | Preferably electric | Drummond #4-000-110,<br>Fisher #13-681-15<br>List price $180 |
| Glass pipettes | 1, 10 and 25 mL | |
| Graduated cylinders | 100 and 250 mL | |
| Squeeze bottle full of DI water | | |
| 2 beakers | 1 for clean water to rinse pipettes between use<br>1 for dirty rinse water. | |
| Paper towels, safety goggles, lab gloves | | |

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Water (Ambient and ISDS samples) | White 500 mL HDPE | Kept at 4 ºC | 500 mL | 24 hours max<br>6 hours ideal |

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
If the BOD for the method blank is greater than 2 mg/L BOD 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:

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

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 Calibration and Standards

#### 5.2.4.1 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.

#### 5.2.4.2 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.

#### 5.2.4.3 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. Turn on the DO meter to allow a 30 minute warm-up period.
2. Calibrate the DO meter using the method outlined in Section 5.2.4.3.
3. Allow the samples to warm to room temperature.
4. 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 6 and place the bottle into the incubating refrigerator.
5. 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) Fill the remaining volume in each BOD bottle with nutrient buffer water to 1-2 mm above the base of ground glass neck.

6. Determine initial DO of the blanks 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.

7. Prepare the next sample starting from Step 5.

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

9. 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.

10. Turn off the DO meter.

11. 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.
12. Field sample disposal is completed in accordance with Section 4.0.

### 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.

$$BOD\ (mg/l) = \frac{(D_1 - D_2)}{P}$$

$D_1$ = Initial DO of diluted sample (mg/L $O_2$)
$D_2$ = DO of diluted sample after 5 days incubation at 20 °C (mg/L $O_2$)
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

| Expected BOD (mg/L) | Sample Amount (mL) | Final Volume (mL) |
|---|---|---|
| 1 – 50 | 50, 100, 200 | 300 |
| 50 – 100 | 10, 25, 50 | 300 |
| 100 – 350 | 6, 8, 10 | 300 |
| 350 – 500 | 1, 3, 5 | 300 |
| >= 500 | 0.5, 1, 2 | 300 |

## 7.0 REFERENCES

APHA, AWWA, WEF. Standard Methods for the Examination of Water and Wastewater. 19th ed. Washington D.C.: APHA, 1995.
Method referenced: Biochemical Oxygen Demand (5210), 5-Day BOD Test (5210 B). #5210.

YSI Inc. YSI Model 5000/5100 Dissolved Oxygen, Temperature Operations Manual. Yellow Springs, OH: YSI 1998.

## 8.0 DOCUMENTATION

Example of project data sheet

**Set-up analyst:** **5-day analyst:**
**Initial DO meter:** **Final DO meter:**
**Into incubator date/time:** **from incub. Date/time:**
**Inside of Incubator temp.:** **Inside of Incubator temp.:**

| BOD Bottle | Site | Location | mL Sample | Initial DO | 5-Day DO | Comments |
|---|---|---|---|---|---|---|
| | **INITIAL** | **lab nutrient BLANK** | **300** | | | |
| | BI - 3 Rea | STE – 1 | **4** | | | |
| | BI - 3 Rea | STE – 1 | **6** | | | |
| | BI - 3 Rea | STE – 1 | **8** | | | |
| | BI - 3 Rea | AXE | **50** | | | |
| | BI - 3 Rea | AXE | **100** | | | |
| | BI - 10 Ort | AXE | **10** | | | |
| | BI - 10 Ort | AXE | **20** | | | |
| | BI - 10 Ort | AXE | **50** | | | |
| | BI - 11 Der | AXE | **50** | | | |
| | BI - 11 Der | AXE | **100** | | | |
| | BI - 12 Fla | STE –S | **5** | | | |
| | BI - 12 Fla | STE –S | **7** | | | |
| | ***FIELD*** | ***Water BLANK*** | ***200*** | | | |
| | **FINAL** | **Lab nutrient BLANK** | **300** | | | |

# Standard Operating Procedure 012 (Prior number URIWW-SOP-5)

# Chlorophyll-a Analysis – Welschmeyer Method

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

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# Standard Operating Procedure 012 (Prior number URIWW-SOP-5)

# Chlorophyll-a Analysis Procedure – Welschmeyer Method

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

## 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

| Required Material | Notes | Re-order information |
|---|---|---|
| Fluorometer | Turner Designs model TD-700. Excitation filter 436nm, emission filter 680 nm | Turner Designs, 845 W Maude St, Sunnyvale, CA 94086, 408-749-0998. |
| Freezer | Maintained at -80 °C | |
| Walk-in cold room or refrigerator | Maintained at 4 °C | |
| Balance | Capable of weighing to 0.1 g room 018 | |
| Repipet | Volume range to 20 mL, set to dispense 5 mL | Barnstead/Thermolyne |
| 90% Acetone | 90% acetone is found in the repipet labeled "90% acetone"<br><br>Preparation instructions for 90% acetone are found in Section 5.3.2.1. | Fume hood, room 019a |
| Magnetic stirrer and stir bar dedicated to 90% acetone preparation | Located on the laboratory bench in room 002. Stir bar is always left in graduated cylinder used to prepare the 90% acetone. | |
| Magnetic stirrer and 1” stir bar | Located on side laboratory bench, room 002 | |
| 13x100 mm disposable glass test tubes | Stored in room 019A | Fisher # 14-958-10C, case of 1000 |
| Vial caps | Stored in room 019A | Fisher “Tainer Tops” #02-706-28 |
| 40 place test tube racks | Holes should fit 13x100 mm test tubes | |
| Deionized (DI) water | | |
| 10 - 100 mL graduated cylinders | | |
| Dedicated 500 mL graduated cylinder | 500 mL graduated cylinder labeled "chl only" | Fume hood or center lab bench room 002 |
| 250 mL beaker | Located in the non-acid washed glassware cabinet | |
| 10-25 mL to contain (TC) graduated cylinder | | |
| Dropper pipette | Left in small flask next to bottle of sodium bicarbonate ($NaHCO_3$) | |
| Filters | | Millipore AP40 GFF for TCLP #AP4002500 |
| Filter Housings | | |

| Required Material | Notes | Re-order information |
|---|---|---|
| DI saturated solution of magnesium carbonate ($MgCO_3$) | Stored in a drawer by the center sink in room 002 | |
| Forceps | | |
| Primary chlorophyll-a standard | 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. | Turner Designs # 10-850 |
| Secondary chlorophyll-a standard | This standard is a dark grey rod and has an indefinite shelf life. It is stored in room 019A next to the fluorometer. | Turner Designs |
| Acetone | Located in the flammable storage cabinet in room 002 | Fisher Optima #A-929 |
| Sodium bicarbonate ($NaHCO_3$) | Located on the top shelf of the glass chemical cabinet. | Fisher, Certified ACS, #S-233 |
| 1N $NaHCO_3$ | Located in the cabinet to the left of BOD incubator room 002 or next to the fume hood.<br><br>Preparation Instructions in Section 5.3.2.2. | |
| Chlorophyll-a data sheets | Stored in a drawer in room 019A. | |
| Filter remover wire | 6" aluminum wire with bent tip located in room 019A. | |
| Kim-wipe tissues | | |
| Aluminum foil | | |

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.

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Glass Fiber Filter | Aluminum Foil | Frozen | NA | 6 months |
| Water | 500 mL brown glass or plastic bottle | 4 °C | 100 mL | 24 hours |

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 can not 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:

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

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 and Standards

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:

$$\%D = \frac{|\text{Known Value} - \text{Reported Value}|}{\text{Known Value}} \times 100$$

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”.
4. Press <ENT> on Home screen, press <1> for set-up, press <1> for Mode. Toggle to choose Multi-optional Mode.
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. <u>The filters must not be exposed to light</u>.
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. <u>Do not touch the filter with anything but the forceps!</u>
    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 $NaHCO_3$ (sodium bicarbonate)

Preparation of reagents used in the analysis of chlorophyll-a should be completed in room 002. Prepare 100 mL of 1N $NaHCO_3$ using the following procedure:

1. Add 8.4 g of $NaHCO_3$ 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 $NaHCO_3$ has dissolved.
4. Pour the solution into the 125 mL brown glass bottled labeled 1N $NaHCO_3$. 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.

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

Fo = Sample reading from the fluorometer (for an undiluted sample).

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, 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 Fo must be calculated using the equation below. 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 Fo value reported on the chlorophyll-a data sheet can be used directly in the equation above.

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

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

APHA, AWWA, WEF. Standard Methods for the Examination of Water and Wastewater. 19th ed. Washington D.C.: APHA, 1995.
Method referenced: Chlorophyll (10200 H).

Arar, A.J. and G.B. Collins. EPA Method 445.0 - *In Vitro* Determination of Chlorophyll *a* and Phaeophytin *a* in Marine and Freshwater Algae by Fluorescence. Cincinnati OH: US EPA National Exposure Research Laboratory, Office of Research and Development, 1997.

Turner Designs. Measuring Extracted Chlorophyll a Free from the Errors Associated with Chlorophyll b and Phaeopigments. Sunnyvale CA: Turner Designs.

Turner Designs. Operation Manual for Model TD-700. Sunnyvale CA: Turner Designs.

Welschmeyer, N.A. "Fluorometric Analysis of Chlorophyll a in the Presence of Chlorophyll b and Phaeophytins." *Limnology and Oceanography* 39 (1994):1985-1992.

## 8.0 DOCUMENTATION

Chlorophyll-a Daily Standards Record

| Chlorophyll-a Standards Records | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Date | Tech. | CHL Sheet # | 90% Acetone | Low Solid Stnd | High Solid Stnd | Low Liquid Stnd | High Liquid Stnd | Comments |
| | | | | | | | | |
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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**

**Date of standardization:** **by:**

**lot #:**

**low chl conc:** µg/l

**high chl conc:** µg/l

**Range setting: 500**

| Vial Contents | rep1 | rep2 | rep3 | Average |
|---|---|---|---|---|
| **90% acetone blank** | | | | |
| **Vial A (18.1ug/l)** | | | | |
| **Vial B (18.1ug/l)** | | | | |
| **Vial C (181 ug/l)** | | | | |
| **Vial D (181 ug/l)** | | | | |
| **low solid standard** | | | | |
| **high solid standard** | | | | |

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

**Range setting: _____**

| Vial Contents | rep1 | rep2 | rep3 | Average |
|---|---|---|---|---|
| **90% acetone blank** | | | | |
| **Vial A (18.1ug/l)** | | | | |
| **Vial B (18.1ug/l)** | | | | |
| **Vial C (181 ug/l)** | | | | |
| **Vial D (181 ug/l)** | | | | |
| **low solid standard** | | | | |
| **high solid standard** | | | | |

Data sheets for storage of chlorophyll-a samples

**Storage Location:**

| | Sign in | Sign Out | | Sign in | Sign Out |
|---|---|---|---|---|---|
| **Monitoring Location** | **Initials/Date** | **Initials/Date** | **Monitoring Location** | **Initials/Date** | **Initials/Date** |
| Almy Pond | | | Saugatucket Pond | | |
| Alton Pond | | | Round Pond | | |
| Arnold Pond | | | Sand Pond | | |
| Barber Pond | | | Saugatucket Pond | | |
| Belleville P - Lower | | | Saw Mill Pond | | |
| Belleville P - upper | | | Scott Pond | | |
| Blackamore Pond | | | Secret Lake | | |
| Bowdish Reservoir | | | Silver Lake | | |
| Breakheart Pond | | | Silver Spring Lake | | |
| Brickyard Pond | | | Simmon Mills Pond | | |
| Browning Mill Pond | | | Sisson P. (Coventry) | | |
| Carbuncle Pond | | | Sisson P. (Ports.) | | |
| Carr Pond (NK) | | | Slack's Reservoir | | |
| Carr Pond (WG) | | | Slater Pond | | |
| Chapman Pond | | | Slatersville Res. - U | | |
| Fenner Pond | | | Smith & Sayles Res. | | |

# Chlorophyll-a Analysis Data Sheet

**CHLOROPHYLL-A**
**SHEET # 2004 -**
**____**

Name of rack: _____

Set up date: _____

Set up Technician: _____ **samples diluted: _____**

Analysis Date: _____ **how diluted: _____**

Analysis Technician: _____

| | START | END |
|---|---|---|
| 90% Acetone Blank | | |
| Low Solid Standard | | |
| High Solid Standard | | |
| Low Liquid Standard | | |
| High Liquid Standard | | |

Linear Range (set at calibration) _____

**entire rack once (Fo1), then re-read (Fo2). Record both readings.**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample **Date** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| Sample **Location** | | | | | | | | | | |
| Volume Filtered | | | | | | | | | | |
| **Fo1 / Fo2** | / | / | / | / | / | / | / | / | / | / |
| Sample **Date** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** |
| Sample **Location** | | | | | | | | | | |
| Volume Filtered | | | | | | | | | | |
| **Fo1 / Fo2** | / | / | / | / | / | / | / | / | / | / |
| Sample **Date** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** | **29** | **30** |
| Sample **Location** | | | | | | | | | **Acetone** | **Filter** |
| Volume Filtered | | | | | | | | | **Blank** | **Blank** |
| **Fo1 / Fo2** | / | / | / | / | / | / | / | / | / | / |
| Sample **Date** | **31** | **32** | **33** | **34** | **35** | **36** | **37** | **38** | **39** | **40** |
| Sample **Location** | | | | | | | | | | |
| Volume Filtered | | | | | | | | | | |
| **Fo1 / Fo2** | / | / | / | / | / | / | / | / | / | / |

filename c:\awwexcel\lab proc\chlorophyll\chlorophyll data sheet.xls

**Comments:**

updated 7/25/03 ltg

UNIVERSITY OF
Rhode Island

# Standard Operating Procedure 013 (Prior number URIWW-SOP-8)

## Chloride Analysis

**University of Rhode Island Watershed Watch**

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**TABLE OF CONTENTS**

# Standard Operating Procedure 013 (Prior number URIWW-SOP-8)

# Chloride Analysis

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

## 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 ($HNO_3$) is corrosive and can cause severe burns to exposed body parts.

Methanol ($CH_3OH$) 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 ($Fe(NO_3)_3 \cdot 9H_2O$) 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

| Required Material | Notes | Re-order information |
|---|---|---|
| Astoria-Pacific Model 303a Astoria® Analyzer (autoanalyzer) | Equipped with a 480 nm filter for analysis of chloride | |
| Personal computer | | |
| Refrigerator | Set at 4 °C | |
| Ultrapure water | | |
| Drying oven | | |
| Balance | Capable of weighing to 0.0001 g | |
| 60-125 mL brown glass Bottles | Qorpak, with TFE-lined closures | Fisher catalog #03-320-8D, 240 mL, case of 24<br>#03-320-8C, 120 mL, case of 24 |
| Brinkman pipettes and tips | 0-100 µl and 100-1000 µl | |
| 10 – 200/250 mL Class A volumetric flasks | | |
| 2 to 4 – 1L Class A volumetric flasks | | |
| Nitric acid ($HNO_3$) | | Fisher catalog # A200-500 |
| Methanol ($CH_3OH$) | | Fisher catalog # A452-4 |
| Mercuric Thiocyanide ($Hg(SCN)_2$) | | Purchased from Astoria-Pacific in 0.6 g bottles |
| Ferric nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$) | | Purchased from Astoria-Pacific in 30 g bottles |
| Brij-35% solution surfactant | | Available from Astoria-Pacific |
| Sodium chloride (NaCl) | | Fisher catalog # S233-500 |

| Required Material | Notes | Re-order information |
|---|---|---|
| External standards | | Obtained from AccuStandard or other outside vendor |
| Millipore AP40 glass fiber filters (GFF) for TCLP | Gelman Type A/E | Millipore #AP4002500 |
| Filter forceps | | |
| Filter housings | | |
| 60 mL Syringe | | |

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Water (Ambient and ISDS) | 60-125 mL acid washed brown glass bottle | Filtered and then refrigerated at 4 °C | 10 mL | 1 year |

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.

### 5.2 Quality Assurance/Quality Control

#### 5.2.1 Method Detection Limit

The Method Detection Limit (MDL) for this assay is 2 mg/L chloride ($Cl^-$). The Limit of Quantitation or Reporting Limit (RL) for this assay is 5 mg/L $Cl^-$. 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.

### 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:

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

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 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 AccuStandard or another outside vendor and are analyzed as samples during each analysis batch at a ratio of 3% of the samples run (3 standards per 90 samples). The percent difference (%D) must not be greater than 20% for the external standards.

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.

### 5.3 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.

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.

| Mass of dried NaCl (g) | Final Volume (mL) |
|---|---|
| 1.648 g | 1000 |
| 0.824 g | 500 |
| 0.412g | 250 |

#### 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.

| Desired Concentration (mg/L or ppm) | Volume of 1000 mg/L stock to add to 100 mL volumetric flask | | Dilution Factor |
|---|---|---|---|
| | µl | mL | |
| 5 | 500 | 0.5 | 200 |
| 10 | 1000 | 1.0 | 100 |
| 15 | 1500 | 1.5 | 66.7 |
| 20 | 2000 | 2.0 | 50 |
| 25 | 2500 | 2.5 | 40 |
| 30 | 3000 | 3.0 | 33.3 |
| 40 | 4000 | 4.0 | 25 |
| 50 | 5000 | 5.0 | 20 |

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. 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 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. <u>The fume funnel above the autoanalyzer must be on before this reagent (color reagent) is used.</u>
    a. <u>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.</u>

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.

   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 standard curve analyzed at the beginning of the sample run. Data are reported to the nearest whole number. Values less than 5 mg/L $Cl^-$ are reported as <5 mg/L $Cl^-$.

## 7.0 REFERENCES

APHA, AWWA, WEF. Standard Methods for the Examination of Water and Wastewater. 19th ed. Washington D.C.: APHA, 1995.
Method referenced: Chloride (4500-$Cl^-$) and Automated Ferricyanide Method (4500-$Cl^-$ E).

Astoria-Pacific Inc. *Astoria Analyzer Operations Manual*. Clackamas, OR: Astoria-Pacific Inc, 2002.

U S. Environmental Protection Agency. EPA Method 365 Series, EPA 600/4-79, rev. March 1983. Cincinnati, OH: Environmental Monitoring and Support Laboratory, 2001.

## 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

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **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

| Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

UNIVERSITY OF
Rhode Island

# Standard Operating Procedure 014 (Prior number URIWW-SOP-3)

## Ammonia Analysis

**University of Rhode Island Watershed Watch**

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**TABLE OF CONTENTS**

# Standard Operating Procedure 014 (Prior number URIWW-SOP-3)

# Ammonia Analysis

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

## 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 ($NH_3$) as Nitrogen (N) per Liter. This method is appropriate for undiluted samples ranging from less than 40 to 2000 µg/L $NH_3$-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 nitroferriccyanide reagent. The following chemicals should be used only in the laboratory funnel ventilation or laboratory hood.

Liquid phenol (carbolic acid) ($C_6H_5OH$) – 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 nitroferriccyanide (sodium nitroprusside dihydrate), ($Na_2Fe(CN)_5(NO)\cdot 2H_2O$) – Sodium nitroferriccyanide 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 ($(NH_4)_2SO_4$), sodium citrate dihydrate ($Na_3C_6H_5O_7\cdot 2H_2O$) and sodium potassium tartrate ($NaKC_4H_4O_6.4H_2O$) 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 ($H_2SO_4$) 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

| Required Material | Notes | Re-order information |
|---|---|---|
| Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer (autoanalyzer) | Equipped with a 630 nm filter for analysis of ammonia-nitrogen | |
| Personal computer | | |
| Refrigerator | Set at 4 °C | |
| Balance | Capable of weighing to 0.0001 g | |
| Source of Ultrapure water | | |
| Brinkman adjustable pipettes | 0-100 µL and 100-1000 µL | |
| 10 200-250 mL Class A volumetric flasks | | |
| 2-4 1 L Class A volumetric flasks | | |
| Brown glass bottles | Qorpak bottles with TFE-lined closures | Fisher catalog<br>240 mL bottle, case of 24<br>#03-320-8D<br>120 mL bottle, case of 24<br>#03-320-8C |
| Ammonia sulfate ($(NH_4)_2SO_4$) | Primary Standard | Fisher catalog # A938 |
| Brij-35% solution surfactant | | Alpkem Inc. |
| Liquid phenol (carbolic acid) ($C_6H_5OH$) | | Fisher catalog #A9311-500 |
| Sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) | | Fisher catalog # S279-500 |
| Sodium hydroxide (NaOH) | | Fisher catalog #S318-500 |

| Required Material | Notes | Re-order information |
|---|---|---|
| Sodium hypochlorite (bleach) (NaOHCl) | | Purchased locally (CVS or supermarket) |
| Sodium nitroferriccyanide (sodium nitroprusside dihydrate), ($Na_2Fe(CN)_5(NO)\cdot 2H_2O$) | | Sigma catalog # S-0501 |
| Sodium potassium tartrate ($NaKC_4H_4O_6.4H_2O$) | | Baker catalog # 3262-01 |
| Sulfuric acid ($H_2SO_4$) | | Fisher catalog # A300SI-212 |

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Water | 60 – 125 mL acid washed brown glass bottle | 4 °C and filtered | 20 mL | 30 days |

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.

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. 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 20 µg/L $NH_3$-N. The Limit of Quantitation or Reporting Limit (RL) is set at 40 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 (ie: 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).

### 5.2.2 Method Blanks

Method blanks consist of Ultrapure water treated as a sample. A method blank is analyzed after every 10 samples; this corresponds to 10% 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 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 $NH_3$. 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:

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

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 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 $NH_3$. 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 analyzed as samples during each analysis batch at a ratio of 3% of the samples run (3 standards per 90 samples). The percent difference (%D) must not be greater than 20% for the external standards.

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:

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

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.

## 5.3 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 $NH_3$ 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.

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.

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 $NH_3$-N Primary Ammonia Standard

1. Store standard grade ammonium sulfate ($(NH_4)_2SO_4$) in the desiccator.
    a. The formula weight of $(NH_4)_2SO_4$ is 132.14 g/ mole
    b. There are 2 moles of N per 1 mole of $(NH_4)_2SO_4$ for a total nitrogen mass of 28.02 g N per 1 mole $(NH_4)_2SO_4$.
2. The 100 mg/L (ppm) primary $NH_3$ standard is prepared using the following formula:

    100 mg/L primary $NH_3$ standard = 0.4716 g $(NH_4)_2SO_4$ per liter

    $$\frac{100 \text{ ppm N}}{\text{Weight of N in } (NH_4)_2SO_4} \times \frac{\text{formula weight } (NH_4)_2SO_4}{28.02 \text{ g}} = \frac{0.1 \text{ g/L} \times 132.14 \text{ g}}{} = 0.4716 \text{ g } (NH_4)_2SO_4 \text{ /L}$$

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

| Mass of $(NH_4)_2SO_4$ to be added to flask (g) | Final Volume (mL) |
|---|---|
| 0.4716 | 1000 |
| 0.2358 | 500 |
| 0.1179 | 250 |
| 0. 0943 | 100 |

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. 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.
    b. Generally, the following standards are prepared for ambient and marine samples: 0, 50, 125, 250, 500 and 1000.

Preparation of Working Standards

| Desired Concentration (µg/L $NH_3$-N) | Volume of primary $NH_3$ standard to add to 100 mL volumetric flask (µL) | Volume of primary $NH_3$ standard to add to 200 mL volumetric flask | Dilution Factor |
|---|---|---|---|
| 0 | 0 | 0 | |
| 50 | 50 | 100 µL | 2000 |
| 125 | 125 | 250 µL | 800 |
| 250 | 250 | 500 µL | 400 |
| 500 | 500 | 1000 µL, (1 mL) | 200 |
| 1000 | 1000 | 2000 µL, (2 mL) | 100 |
| 1500 | 1500 | 3000 µL, (3 mL) | 66.67 |
| 2000 | 2000 | 4000 µL, (4 mL) | 50 |

4. Bring the volumetric flask to volume with Ultrapure water.
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 every 2 months.

### 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. 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 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. 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.

b. A check calibrant is run after every 15 samples and a blank is run after every 10 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.

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 standard curve analyzed at the beginning of the sample run. Data are reported to the nearest 10 for $NH_3$-N. Values less than 40 µg/L $NH_3$-N are reported as <40 µg/L $NH_3$-N.

## 7.0 REFERENCES

APHA, AWWA, WEF. *Standard Methods for the Examination of Water and Wastewater*. 19th ed. Washington D.C.: APHA, 1995

Method referenced: Nitrogen (Ammonia) (4500-NH3), Automated Phenate Method (4500-NH4 G).

Astoria-Pacific Inc. Astoria Analyzer Operations Manual. Clackamas, OR: Astoria-Pacific Inc. 2002.

U S. Environmental Protection Agency. EPA Method 365 Series, EPA 600/4-79. Cincinnati OH: US EPA Environmental Monitoring and Support Laboratory, rev. March 1983.

## 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

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **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

| Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

# Standard Operating Procedure 015 (Prior number URIWW-SOP-2)

# Orthophosphate & Nitrate + Nitrite Analysis

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

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# Standard Operating Procedure 015 (Prior number URIWW-SOP-2)

# Orthophosphate & Nitrate + Nitrite Analysis

**Date:** 11/04
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**Author:** Linda Green

**University of Rhode Island Watershed Watch**

## 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 $NO_3$+$NO_2$. 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 2000 µg/L $PO_4$-P 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 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

| Required Material | Notes | Re-order information |
|---|---|---|
| 105 °C drying oven | | |
| Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer (autoanalyzer) | Equipped with an 880 nm filter and 540 nm filter for analysis of $PO_4$ and $NO_3$+$NO_2$, respectively. | |
| Personal computer | | |
| Refrigerator | Set to 4 °C | |
| Analytical balance | Capable of weighing to 0.0001 g | |
| Ultrapure water | | |
| 2 Brinkman pipettes | 1-100 µL and 100-1000 µL | |
| 600-1000 mL acid-washed beaker, reserved for P use | | |
| Cover glass, or Al foil | | |
| Metal tray | | |
| Squeeze bottle containing Ultrapure water | | |
| 10-15 200-250 mL volumetric flasks | | |
| 2-4 1000 mL Class A volumetric flasks | | |
| 60-250 mL Brown glass bottles, Qorpak, with TFE-lined closures | | Fisher Catalog:<br>240 mL, case of 24<br>#03-320-8D<br>120 mL, case of 24<br>#03-320-8C |

| Required Material | Notes | Re-order information |
|---|---|---|
| **Phosphorus reagents** | | |
| Potassium phosphate monobasic ($KH_2PO_4$) | Hygroscopic (absorbs moisture from the air). May cause eye, skin, respiratory and digestive tract irritation. | Fisher Catalog #P382-500, primary standard, crystalline, 500 g |
| Antimony potassium tartrate trihydrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$) | May cause eye, skin, respiratory and digestive irritation upon contact. Harmful if swallowed. May cause liver, kidney and heart damage. | Fisher Catalog #A 867-500 |
| Ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) | May cause eye, skin and respiratory tract irritation upon contact. May be harmful if swallowed. | Fisher Catalog #A674-500 |
| L-Ascorbic acid ($C_6H_8O_6$) | 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. | Fisher Catalog #A61-100 |
| DowFax surfactant | May cause eye burns upon contact. | Available from Astoria-Pacific |
| Sulfuric acid, concentrated ($H_2SO_4$) | Will cause burns to exposed skin, eyes, respiratory and digestive tract. | Fisher Catalog #A300-212 |
| Chloroform ($CHCl_3$) | 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. | |
| **Nitrogen Reagents** | | |
| Potassium nitrate ($KNO_3$) | 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. | Fisher Catalog #P383-100, primary standard |

| Required Material | Notes | Re-order information |
|---|---|---|
| Ammonium chloride ($NH_4Cl$) | May cause skin, respiratory and digestive tract irritation. May be harmful if swallowed. Causes eye irritation. Hygroscopic (absorbs moisture from the air). | |
| Disodium EDTA (Disodium ethylenediamine tetraacetic acid dihydrate) ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$) | Causes eye, skin and respiratory tract irritation. May be harmful if swallowed. | |
| Ammonium hydroxide ($NH_4OH$) | Causes eye, skin, digestive and respiratory tract burns. | |
| Cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) | Harmful if swallowed. Causes irritation and may burn the digestive tract, respiratory tract, eye and skin. Hygroscopic (absorbs moisture from the air). Possible sensitizer. | Sigma Catalog #c7631 |
| Sulfanilimide ($C_6H_8N_2O_2S$) | May cause reproductive and fetal effects. May cause eye, skin and respiratory tract irritation. May be harmful if swallowed or inhaled. | Fisher Catalog #04525-100 |
| N-1-Napthylethylenediamine (NED) (*N*-(1-Naphthyl)ethylenediamine dihydrochloride) ($C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$) | Will cause eye, skin and respiratory system irritation. | Sigma Catalog #N-9125 |
| Brij-35% solution surfactant | | Available from Alpkem |
| Phosphoric acid ($H_3PO_4$) | Hydroscopic (absorbs moisture from the air). Will cause burns of exposed skin, eyes, respiratory and digestive tract. | |

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Water | 60 – 125 mL acid washed brown glass bottles | Filtration and storage at 4 °C | 20 mL | 30 days |

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.

The available volume of each reagent utilized in this analysis should be assessed. Two reagents are needed for the $NO_3$+$NO_2$ analysis: the ammonium chloride buffer and the color reagent. One reagent is necessary for the $PO_4$ 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 2 µg/L $PO_4$-P for the orthophosphate assay and 20 µg/L $NO_3/NO_2$-N for the nitrate + nitrite assay. The Limit of Quantitation or Reporting Limit (RL) is set at 4 µg/L $PO_4$-P for the orthophosphate assay and 30 µg/L $NO_3/NO_2$-N for the nitrate + nitrite assay.

The upper RL or the maximum concentration reported by the orthophosphate assay without dilution is 200 µg/L $PO_4$-P for ambient and marine samples and 2000 µg/L $PO_4$-P for ISDS samples. The maximum concentration reported by the nitrate + nitrite assay without dilution is 2000 µg/L $NO_3/NO_2$-N for all samples. The upper RL is based on the maximum standard used to calibrate the autoanalyzer. Values reported for $PO_4$-P are reported to the nearest whole number, those reported for $NO_3/NO_2$-N are reported to the nearest 10's (ie: 126 µg/L $NO_3/NO_2$-N is reported as 130 µg/L $NO_3/NO_2$-N and 44.3 µg/L $PO_4$-P is reported as 44 µg/L $PO_4$-P).

#### 5.2.2 Method Blanks

Method blanks consist of Ultrapure water treated as a sample. A method blank is analyzed after every 10 samples; this corresponds to 10% of the samples analyzed. Method blanks must not be greater than 2 µg/L $PO_4$-P or 20 µg/L $NO_3/NO_2$-N.

Corrective Action
If any method blank is found to be greater than 2 µg/L $PO_4$-P or 20 µg/L $NO_3/NO_2$-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 $PO_4$ and $NO_3/NO_2$. 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:

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

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 AccuStandard or another outside vendor and are analyzed as samples during each analysis batch at a ratio of 3% of the samples run (3 standards per 90 samples). The percent difference (%D) must not be greater than 20% for the external standards.

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:

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

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.

## 5.3 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 $NO_3$+$NO_2$ analysis: the ammonium chloride buffer and the color reagent. One reagent is necessary for the $PO_4$ 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 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 $PO_4$ and $NO_3$+$NO_2$ analyses are the same as those used for SOP 016 – Total Phosphorus and Nitrogen Analysis.
2. Dry approximately 0.75 g of potassium phosphate ($KH_2PO_4$) 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 $KH_2PO_4$ = 136.1 g / mole
    b. There is 1 mole of P per 1 mole of $KH_2PO_4$ for a total phosphorus mass of 30.97 g P / mole $KH_2PO_4$.
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 ppm primary $PO_4$ standard is prepared using the following formula:

$$\frac{\text{100 ppm P x formula weight } (KH_2PO_4)}{\text{Weight of P in } KH_2PO_4} = \frac{\text{0.1 g/L x 136.1 g}}{\text{30.97 g}} = 0.4394 \text{ g } KH_2PO_4/L$$

5. Therefore, to prepare 1 L of 100 mg/L primary $PO_4$ standard, place 0.4394 g of $KH_2PO_4$ 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 $PO_4$-P per L = 100 µg $PO_4$-P per mL = 100 ppm.

| **Mass of $(NH_4)_2PO_4$ to be added to flask (g)** | **Final Volume (mL)** |
|---|---|
| 0. 4394 | 1000 |
| 0.2127 | 500 |
| 0.1099 | 250 |

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 $PO_4$-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 2 months.

**Preparation of Orthophosphate Working Standards:**

| **Ambient Water (lakes, ponds, streams)** | **ISDS** | **Final Concentration of Standard (µg $PO_4$-P/L)** | **Ambient Water (200 mL volumetric flasks)** | **ISDS Samples (100 mL volumetric flasks)** |
|---|---|---|---|---|
| **Standards to prepare:** | | | **Volume of intermediate orthophosphate standard to use (µL)** | |
| X | X | 0 | 0 | 0 |
| X | | 5 | 100 | |
| X | | 10 | 200 | |
| X | | 15 | 300 | |
| X | | 20 | 400 | |
| X | | 25 | 500 | |
| X | | 50 | 1000 (1.0 mL) | |
| X | | 100 | 2000 (2.0 mL) | |
| X | | 150 | 3000 (3.0 mL) | |
| X | | 200 | 4000 (4.0 mL) | |
| | X | 250 | | 2500 (2.5 mL) |
| | X | 500 | | 5000 (5.0 mL) |
| | X | 1000 | | 10,000 (10.0 mL) |
| | X | 1500 | | 15,000 (15.0 mL) |
| | X | 2000 | | 20,000 (20.0 mL) |

#### 5.3.1.4 Preparation of Primary Nitrate/Nitrite Standard (100 ppm)

1. The standards utilized for the $PO_4$ and $NO_3$+$NO_2$ 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 ($KNO_3$) 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 $KNO_3$ needed to prepare the desired volume of primary nitrate/nitrite standard. This solution is 100 mg $NO_3$-N per L, 100 µg $NO_3$-N per mL, or 100 ppm.

   Weight of $KNO_3$ needed for primary $NO_3$ standard based on volume prepared:

| **$KNO_3$ (g)** | **Volume of Primary Standard Prepared (mL)** |
|---|---|
| 0.7218 | 1000 |
| 0.3609 | 500 |
| 0.1805 | 250 |
| 0. 0722 | 100 |

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. 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 2 months.

**Preparation of Nitrate/Nitrite Working Standards**

| Ambient Water (lakes, ponds, streams) | ISDS | Final Concentration of Standard (µg NO3-N/L) | Ambient Water (200 mL volumetric flasks) | ISDS Samples (100 mL volumetric flasks) |
|---|---|---|---|---|
| **Standards to prepare:** | | | **Volume of nitrate/nitrite primary standard to use (µL)** | |
| X | X | 0 | 0 | 0 |
| X | | 50 | 100 | |
| X | | 75 | 150 | |
| X | | 100 | 200 | |
| X | | 200 | 400 | |
| X | X | 250 | 500 | 250 |
| X | X | 500 | 1000 (1.0 mL) | 500 |
| X | X | 1000 | 2000 (2.0 mL) | 1000 (1.0 mL) |
| X | X | 1500 | 3000 (3.0 mL) | 1500 (1.5 mL) |
| X | X | 2000 | 4000 (4.0 mL) | 2000 (2.0 mL) |

### 5.3.2 Procedure – Day of Analysis

#### 5.3.2.1 Column Regeneration

The cadmium column used to reduce $NO_3$ to $NO_2$ 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. 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 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 $PO_4$ 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 $NO_3$+$NO_2$ 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 $PO_4$ assay and pink for the $NO_3$+$NO_2$ 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.

b. A check calibrant is run after every 10 samples and a blank is run after every 10 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.

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

## 6.0 CALCULATIONS

Orthophosphate and nitrate/nitrite concentrations are calculated in the Astoria Pacific FASPac II program from the standard curve analyzed at the beginning of the sample run. Data are reported to the nearest whole number for $PO_4$–P and the nearest 10's for $NO_3/NO_2$-N. Values less than 4 µg/L $PO_4$–P are reported as <4 µg/L $PO_4$–P and values less than 30 µg/L $NO_3/NO_2$-N are reported as <30 µg/L $NO_3/NO_2$-N.

## 7.0 REFERENCES

APHA, AWWA, WEF. *Standard Methods for the Examination of Water and Wastewater*. 19th ed. Washington D.C.: APHA, 1995

Method referenced: Automated Cadmium Reduction (4500-$NO_3$ – F), Automated Ascorbic Acid Reduction Method (4500-P-F)

Astoria-Pacific Inc. Astoria Analyzer Operations Manual. Clackamas, OR: Astoria-Pacific Inc. 2002.

U S. Environmental Protection Agency. EPA Method 365 Series, EPA 600/4-79. Cincinnati OH: US EPA Environmental Monitoring and Support Laboratory, rev. March 1983.

## 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

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **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

| Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

# Standard Operating Procedure 016 (Prior number URIWW-SOP-1A)

# Total Phosphorus and Nitrogen Analysis

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

## TABLE OF CONTENTS

# Standard Operating Procedure 016 (Prior number URIWW-SOP-1A)

# Total Phosphorus and Nitrogen Analysis

**Date:** 11/04
**Revision:** 1
**Author:** Linda Green

**University of Rhode Island Watershed Watch**

## 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 difference is that 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 2000 µg/L P and less than 50 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

| Required Material | Notes | Re-order information |
|---|---|---|
| Drying oven | Set at 105 °C | |
| Water bath | Capable of reaching 100 °C | |
| Astoria®-Analyzer Model 303A Segmented Continuous Flow Autoanalyzer (autoanalyzer) | Equipped with a 880 nm filter and 540 nm filter for analysis of orthophosphate and nitrate/nitrite, respectively. | |
| Personal computer | | |
| Refrigerator | Capable of maintaining 4 °C | |
| Analytical balance | Capable of weighing to 0.0001 g | |
| Ultrapure water | | |
| Desiccator | | |
| Heated magnetic stirrer and stir bars | | |
| Pipette dispenser, preferably electronic | | |
| 600 - 1000 mL acid-washed beakers | Reserved for phosphorus chemistry use | |
| Cover glass, or Al foil | | |
| Squeeze bottle containing Ultrapure water | | |
| 10-15 200 mL volumetric flasks | | |
| 2-4 500 & 1000 mL class A volumetric flasks | | |
| Vials (for phosphorus digestion) | Description: Borosilicate glass, Type 1, class B, 45 mL (or 11 drams) capacity, 28 mm O.D. X 108 mm high, thread 24-400 | Fisher Catalog #03-339-5D<br>144 vials/case |
| Caps for vial | 24 mm I.D, 24-400 thread, polypropylene | Fisher Catalog #02-923-14B<br>Nalgene #5150-0240<br>12 vials/pack |
| 20 mL volumetric pipettes | class B accuracy +/- 0.06 mL<br>Calibrated “to deliver” | Fisher Catalog #13-650N<br>1 pipette/pack |

| Required Material | Notes | Re-order information |
|---|---|---|
| Blue coated-wire rack | 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. | Fisher Catalog #01-812-5G NASCO # B1048, with a capacity of 12, 710 mL samples (NASCO #B1020) Sold singly |
| White plastic racks | 24 vial capacity, this type of rack can be placed into the oven (to dry vials) and will not float in the water bath. | Fisher Catalog #14-809D |
| Repipette low profile dispenser (for dispensing digesting reagent) | This repipette variety can withstand most reagents, has two openings and has a capacity for dispensing up to 10 mL. | Fisher Catalog #13-687-35 |
| 100-250 mL brown glass bottles | Qorpak, with TFE-lined closures | Fisher Catalog<br>240 mL bottles #03-320-8D<br>24 bottles/case<br>120 mL bottles #03-320-8C<br>24 bottles/case |
| **Chemical Reagents for Processing Samples** | | |
| Potassium Persulfate ($K_2S_2O_8$) | 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. | Fisher Catalog #P282-500, 500 g, certified, ACS, low nitrogen (<0.001%) |
| Boric Acid powder ($H_3BO_3$) | 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. | Fisher Catalog #A74-500, certified ACS, 500 g, Phosphate <= 0.001% |
| Sodium Hydroxide pellets (NaOH) | Causes eye and skin burns. Hygroscopic (absorbs moisture from the air). Causes digestive and respiratory tract burns. Used for sample digestion reagent. | Fisher Catalog #S318-500, certified ACS, 500 g Phosphate compounds <= 0.001%, Nitrogen compounds <= 0.001% |
| Potassium Phosphate Monobasic ($KH_2PO_4$), also known as potassium dihydrogen phosphate | Hygroscopic (absorbs moisture from the air). May cause eye, skin, respiratory and digestive tract irritation. | Fisher Catalog #P382-500, primary standard, crystalline, 500 g |

| Required Material | Notes | Re-order information |
|---|---|---|
| Potassium Nitrate ($KNO_3$) | 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. | Fisher Catalog #P383-100, primary standard |
| **Reagents for Autoanalyzer. Orthophosphate Analysis** | | |
| Chloroform ($CHCl_3$) | 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. | |
| Antimony Potassium Tartrate Trihydrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$) | May cause eye, skin, respiratory and digestive irritation upon contact. Harmful if swallowed. May cause liver, kidney and heart damage. | Fisher Catalog #A 867-500 |
| Ammonium Molybdate Tetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) | May cause eye, skin and respiratory tract irritation upon contact. May be harmful if swallowed. | Fisher Catalog #A674-500 |
| L-Ascorbic Acid ($C_6H_8O_6$) | Light sensitive. Air sensitive. May cause eye, skin, and respiratory tract irritation. Strong reducing agent. Fire and explosion risk in contact with oxidizing agents. | Fisher Catalog #A61-100 |
| DowFax Surfactant for P analysis | May cause eye burns upon contact. | Available from Astoria-Pacific |
| Sulfuric Acid, concentrated ($H_2SO_4$) | Will cause burns of exposed skin, eyes, respiratory and digestive tract. | Fisher Catalog #A300-212 |
| **Nitrate/Nitrite Reagents** | | |
| Ammonium Chloride ($NH_4Cl$) | May cause skin, respiratory and digestive tract irritation. May be harmful if swallowed. Causes eye irritation. Hygroscopic (absorbs moisture from the air). | |

| Required Material | Notes | Re-order information |
|---|---|---|
| Disodium EDTA (Disodium Ethylenediamine Tetraacetic Acid Dihydrate) ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$) | Causes eye, skin and respiratory tract irritation. May be harmful if swallowed. | |
| Ammonium Hydroxide ($NH_4OH$) | Causes eye, skin, digestive and respiratory tract burns. | |
| Cupric Sulfate Pentahydrate ($CuSO_4 \cdot 5H_2O$) | Harmful if swallowed. Causes digestive and respiratory tract, eye and skin irritation with possible burns. Hygroscopic (absorbs moisture from the air). Possible sensitizer. | Sigma Catalog #c7631 |
| Sulfanilimide ($C_6H_8N_2O_2S$) | May cause reproductive and fetal effects. May cause eye, skin, and respiratory tract irritation. May be harmful if swallowed or inhaled. | Fisher Catalog #04525-100 |
| N-1-Napthylethylenediamine (NED) (*N*-(1-Naphthyl)ethylenediamine dihydrochloride) ($C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$) | Will cause eye, skin and respiratory system irritation. | Sigma Catalog #N-9125 |
| Brij-35% solution surfactant | | (Available from Alpkem) |
| Glycine ($NH_2CH_2COOH$) | May be absorbed through intact skin. May cause respiratory tract, eye and skin irritation. | Fisher Catalog #G46-500 (500 gram bottle- smallest amount available). |
| Phosphoric Acid ($H_3PO_4$) | Hydroscopic (absorbs moisture from the air). Will cause burns of exposed skin, eyes, respiratory and digestive tract. | |

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Water | 125 or 250 mL acid washed brown glass bottles | 4 °C | 100 mL | 14 days at 4 °C<br>3 months once pipetted into vials |

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 $NO_3/NO_2$ analysis: the ammonium chloride buffer and the color reagent. One reagent is necessary for the $PO_4$ 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.

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 3 µg/L P for the total phosphorus assay and 20 µg/L N for the total nitrogen assay. The Limit of Quantitation or Reporting Limit (RL) is set at 4 µg/L P for the total phosphorus assay and 50 µg/L N for the total nitrogen assay.

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 Blanks

##### 5.2.2.1 Method Blanks

Method blanks consist of undigested Ultrapure water treated as a sample. A method blank is analyzed after every 10 samples; this corresponds to 10% of the samples analyzed. Method blanks must not be greater than 3 µg/L P and 5 µg/L N.

Corrective Action
If any method blank is found to be greater than 3 µg/L P or 5 µg/L 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.

#### 5.2.2.2 Digestion Blanks

Digestion blanks consist of 20 mL of Ultrapure water digested as a sample and then analyzed as a sample. Three (3) digestion blanks are prepared per sample run. Digestion blanks must not be greater than 10 µg/L N and 3 µg/L P

Corrective Action
If any digestion blank is found to be greater than 3 µg/L P or 10 µg/L N then this will be noted on the project data sheet. Since the digestion 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 may be 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:

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

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 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 10 samples, or approximately 10% of the samples analyzed. The %D must not be greater than 20% for the check calibrant.

Percent difference is calculated as follows:

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

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.

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 5%. Calculation of %D is discussed in the section above

Corrective Action
If the %D is greater than 5% for the external glycine standard then the standard is re-analyzed. If the %D is still greater than 5% 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 ($NH_2CH_2COOH$) 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:

$$100 \text{ mg/L N} = \frac{0.1 \text{ g N}}{\text{L}} \times \frac{75 \text{ g glycine}}{14 \text{ g N}} = 0.536 \text{ g glycine / L}$$

   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.

#### 5.2.6 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.

### 5.3 Analysis Method

#### 5.3.1 Preparation – At Least 3 Days Prior to Field Sample Collection

##### 5.3.1.1 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 a10% 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 ($KH_2PO_4$) 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 $KH_2PO_4$ = 136.1 g / mole
    b. There is 1 mole of P per 1 mole of $KH_2PO_4$ for a total phosphorus mass of 30.97 g P / mole $KH_2PO_4$.
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:

$$\frac{100 \text{ ppm P} \times \text{formula weight } (KH_2PO_4)}{\text{Weight of P in } KH_2PO_4} = \frac{0.1 \text{ g/L} \times 136.1 \text{ g}}{30.97 \text{ g}} = 0.4394 \text{ g } KH_2PO_4/\text{L}$$

5. Therefore, to prepare 1 L of 100 mg/L primary $PO_4$ standard, place 0.4394 g of $KH_2PO_4$ 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.

| **Mass of $(NH_4)_2PO_4$ to be added to flask (g)** | **Final Volume (mL)** |
|---|---|
| 0. 4394 | 1000 |
| 0.2127 | 500 |
| 0.1099 | 250 |

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 $PO_4$-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 2 months.

**Preparation of Orthophosphate Working Standards:**

| Ambient Water (lakes, ponds, streams) | ISDS | Final Concentration of Standard (µg $PO_4$-P/L) | Ambient Water (200 mL volumetric flasks) | ISDS Samples (100 mL volumetric flasks) |
|---|---|---|---|---|
| **Standards to prepare:** | | | **Volume of intermediate orthophosphate standard to use (µL)** | |
| X | X | 0 | 0 | 0 |
| X | | 5 | 100 | |
| X | | 10 | 200 | |
| X | | 15 | 300 | |
| X | | 20 | 400 | |
| X | | 25 | 500 | |
| X | | 50 | 1000 (1.0 mL) | |
| X | | 100 | 2000 (2.0 mL) | |
| X | | 150 | 3000 (3.0 mL) | |
| X | | 200 | 4000 (4.0 mL) | |
| | X | 250 | | 2500 (2.5 mL) |
| | X | 500 | | 5000 (5.0 mL) |
| | X | 1000 | | 10,000 (10.0 mL) |
| | X | 1500 | | 15,000 (15.0 mL) |
| | X | 2000 | | 20,000 (20.0 mL) |

#### 5.3.2.4 Preparation of Primary Nitrate/Nitrite Standard (100 ppm)

1. The standards utilized for the $PO_4$ and $NO_3$+$NO_2$ 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 ($KNO_3$) 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 $KNO_3$ needed to prepare the desired volume of primary nitrate/nitrite standard. This solution is 100 mg $NO_3$-N per L, 100 µg $NO_3$-N per mL, or 100 ppm.

   Weight of $KNO_3$ needed for primary $NO_3$ standard based on volume prepared:

| $KNO_3$ (g) | Volume of Primary Standard Prepared (mL) |
|---|---|
| 0.7218 | 1000 |
| 0.3609 | 500 |
| 0.1805 | 250 |
| 0. 0722 | 100 |

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 2 months.

**Preparation of Nitrate/Nitrite Working Standards**

| Ambient Water (lakes, ponds, streams) | ISDS | Final Concentration of Standard (µg NO3-N/L) | Ambient Water (200 mL volumetric flasks) | ISDS Samples (100 mL volumetric flasks) |
|---|---|---|---|---|
| **Standards to prepare:** | | | **Volume of intermediate nitrate/nitrite standard to use (µL)** | |
| X | X | 0 | 0 | 0 |
| X | | 50 | 100 | |
| X | | 75 | 150 | |
| X | | 100 | 200 | |
| X | | 200 | 400 | |
| X | X | 250 | 500 | 250 |
| X | X | 500 | 1000 (1.0 mL) | 500 |
| X | X | 1000 | 2000 (2.0 mL) | 1000 (1.0 mL) |
| X | X | 1500 | 3000 (3.0 mL) | 1500 (1.5 mL) |
| X | X | 2000 | 4000 (4.0 mL) | 2000 (2.0 mL) |

### 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 sample into a clean and labeled vial.
    a. Ambient water sites (rives, lakes, ponds): pipette 20 mL of sample into each vial

        Prepare a replicate every 10 samples.
    b. ISDS sites: Pipette 1.0 mL of sample and 19.0 mL Ultrapure water into each vial.

        ISDS samples are digested in triplicate (3 vials are digested per ISDS sample)
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.

### 5.3.4 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.
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.

Digesting Reagent for Total Phosphorus and Total Nitrogen

| | | Add to beaker | | | | |
|---|---|---|---|---|---|---|
| **Final Volume of Potassium Persulfate (mL)** | **Beaker Size for Reagent Preparation (mL)** | **Potassium Persulfate (g)** | **Boric Acid (g)** | **1N NaOH solution (mL)** | **Ultrapure Water (mL)** | **Approximate number of vials reagent volume will fill** |
| 1000 | 1000 | 50 | 30 | 350 | 900 | 190 |
| 500 | 500 | 25 | 15 | 175 | 450 | 90 |
| 250 | 500 | 12.50 | 7.50 | 87.50 | 200 | 45 |
| 200 | 500 | 10 | 5 | 70 | 150 | 35 |

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 $NO_3$ to $NO_2$ 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 autoanalyzer is being prepared.
2. Obtain reagents needed for the analysis. The $PO_4$ 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 $NO_3$+$NO_2$ 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 $PO_4$ assay and pink for the $NO_3$+$NO_2$ 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.

   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.

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 is calculated in the Astoria Pacific FASPac II program from the standard curve analyzed at the beginning 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 4 µg/L P are reported as <4 µg/L P and values less than 50 µg/L N are reported as <50 µg/L N.

## 7.0 REFERENCES

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## 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

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **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

| Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# | Contents & Date | DF | Pos# |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

# Standard Operating Procedure 017

# Salinity Analysis

**University of Rhode Island Watershed Watch**

**Date:** 04/05
**Revision:** 1
**Author:** Marie Evans Esten

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# Standard Operating Procedure 017

# Salinity Analysis

**University of Rhode Island Watershed Watch**

**Date:** 04/05
**Revision:** 1
**Author:** Marie Evans Esten

## 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

| Required Material | Notes | Re-order information |
|---|---|---|
| LaMotte Salinity Titration Kit (Model 7459-01) | | |
| A366ATC Hand Held Salinity Refractometer | | |

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Water (Marine and Estuarine) | 60-125 mL acid washed brown glass bottle | None | 30 mL | 1 year |

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 competed 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 $10^{th}$ 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.

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***

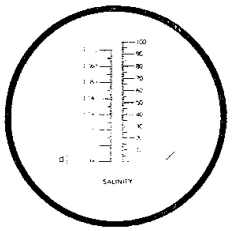
| Analysis Date | Tech's Initials | Monitoring Location | Refractometer Salinity | Kit Salinity Rep 1 | Kit Salinity Rep 2 |
|---|---|---|---|---|---|
| | | GH-In Pond-mid-depth | | | |
| | | GH-In Pond-deep | | | |
| | | GH-Indigo Point | | | |
| | | GH-Sea Lea | | | |
| | | GH-Teal Road | | | |
| | | GH-Twin Peninsula | | | |
| | | NP-Crawford Dock | | | |
| | | NP-Ft Neck Cove | | | |
| | | NP-Pond Street | | | |
| | | NP-Stumpy Point | | | |
| | | NP-Tockwotten Dock | | | |
| | | NP-Tom Cod Cove | | | |
| | | NP-Vigna's Dock | | | |
| | | | | | |

Appendix A : Instructions for Operation of the Refractometer

Rec'd 10/94

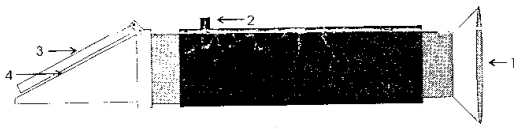
# A366ATC Operation Manual

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

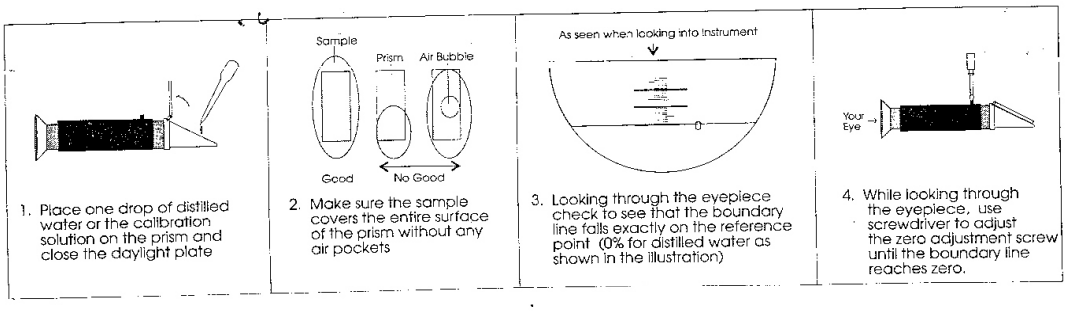
1. eyepiece
2. zero adjustment screw
3. daylight plate
4. prism




## 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). If 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.




1. Place one drop of distilled water or the calibration solution on the prism and close the daylight plate
2. Make sure the sample covers the entire surface of the prism without any air pockets
3. Looking through the eyepiece check to see that the boundary line falls exactly on the reference point (0% for distilled water as shown in the illustration)
4. While looking through the eyepiece, use screwdriver to adjust the zero adjustment screw until the boundary line reaches zero.

## 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 field of view through the eyepiece. If the field of view is not clear, focus the image by turning the portion 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**

| Refractometer Reading | NaCl % by weight | MgCl2 % by weight | MgSO4 % by Weight | K2SO4 % by weight | CaCl2 % by weight | Sucrose (Brix) |
|---|---|---|---|---|---|---|
| 0 PPT | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 10 PPT | 1.0 | 0.7 | 0.9 | 1.4 | 0.8 | 1.3 |
| 20 PPT | 2.1 | 1.4 | 1.8 | 2.9 | 1.5 | 2.5 |
| 30 PPT | 3.1 | 2.1 | 2.7 | 4.3 | 2.3 | 3.7 |
| 40 PPT | 4.1 | 2.8 | 3.6 | 5.8 | 3.0 | 4.9 |
| 50 PPT | 5.1 | 3.5 | 4.5 | 7.3 | 3.8 | 6.2 |
| 60 PPT | 6.2 | 4.2 | 5.4 | 8.8 | 4.5 | 7.4 |
| 70 PPT | 7.2 | 5.0 | 6.3 | 10.3 | 5.3 | 8.6 |
| 80 PPT | 8.3 | 5.7 | 7.2 | 11.8 | 6.0 | 9.8 |
| 90 PPT | 9.4 | 6.4 | 8.2 | 13.4 | 6.8 | 11.0 |
| 100 PPT | 10.5 | 7.2 | 9.1 | 15.0 | 7.6 | 12.3 |

**SPECIFICATIONS**

| | |
|---|---|
| SALINITY RANGE: | 0 - 100 Parts per Thousand (PPT) and 1.000 - 1.070 specific gravity of salt water |
| 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) |

# Standard Operating Procedure 018

# Enterococci Analysis

**Date:** 11/04
**Revision:** 1
**Author:** Elizabeth Herron

UNIVERSITY OF Rhode Island

**University of Rhode Island Watershed Watch**

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# Standard Operating Procedure 007 (Prior number URIWW-SOP-4A)

## Ambient Waters Microbiological Procedure

**Date:** 11/04
**Revision:** 1
**Author:** Elizabeth Herron

**University of Rhode Island Watershed Watch**

### 1.0 PURPOSE AND DESCRIPTION

The purpose of this method is to determine the concentration of enterococci bacteria within an ambient (lakes, ponds and rivers) or marine water samples. Samples are collected in sterile bottles, an aliquot of sample is filtered, and the resulting filter is placed into a media dish and incubated for 48 hours. After the 48 hour incubation the filter is placed into another media type and incubated for 20 – 30 minutes. After the final incubation the number of bacteria colonies are counted. This procedure is utilized for ambient and marine waters 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 60 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. Specific hazards of each chemical are listed under the notes section of the required materials table (Section 3.0) of this SOP. Be especially careful with the Nalidixic acid and the dehydrated mE media. Nalidixic acid causes photosensitivity and may cause allergic respiratory or skin reaction upon contact. The mE media is an irritant and potential carcinogen in its dried form so it must be handled under the fume hood with the mask on.

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 Sheets (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, 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

| Required Material | Notes | Re-order information |
|---|---|---|
| **Coastal Institute in Kingston, room 081** | | |
| Autoclave | | |
| **University of Rhode Island (URI), Kingston Coastal Institute Watershed Watch (WW) Laboratory, room 002** | | |
| Autoclave safe white plastic bottles (125 – 500 mL) | | |
| 2 L autoclave safe flasks | | |
| Insulated autoclave gloves | | |
| Metal autoclave tray | | |
| Combination hot plate and magnetic stirrer | | |
| Magnetic stirring bar | | |
| Sodium Hydroxide (NaOH) | Caustic. Causes eye, skin, digestive and respiratory tract burns. Hygroscopic (absorbs moisture from the air). | |
| Microbiology worksheets | Project data sheets are found on the URIWW computer. See Section 8.0 Documentation | |

| Required Material | Notes | Re-order information |
|---|---|---|
| **Coastal Institute in Kingston, room 018** | | |
| Balance | Calibration weights in drawer beneath the balance | |
| **Coastal Institute in Kingston, room 019** | | |
| Balance | Calibration weights are in drawer beneath the balance in room 018 | |
| 44.5 °C Incubator | Precision fecal coliform bath 66855 | Baxter catalog #W3182-2 |
| 41 °C Incubator | Thermolyne Type 142300 incubator | |
| UV sterilization box | | |
| Vacuum pump and manifold | | |
| Pipette-Aid filler/dispenser (electric) | | Fisher catalog # 13-681-15 |
| 2 - Glass 400 mL beakers marked URIWW | | |
| 4 - Side arm filter flasks | 500 mL or larger | |
| **Coastal Institute in Kingston, room 019, URIWW refrigerator** | | |
| mE agar plates | less than a month old, stored media side up in a labeled and foil lined box in the refrigerator | |
| mE agar mix | May cause skin, eye and respiratory tract irritation upon contact. Potential carcinogen. Use this chemical in the hood only with the mask. | Fisher catalog # DF0333-17-9, 500 g |
| 2,3,5 – Triphenyl tetrazolium chloride ($C_{19}H_{15}ClN_4$) | May cause skin, eye and respiratory tract irritation upon contact. May be harmful if swallowed. | Sigma #17779-10X10ML-F |
| Esculin Iron Agar (EIA) plates | Less than a month old, stored media side up in a labeled and dated foil lined box in the refrigerator | |
| EIA mix | | Fisher catalog #DF0488-15-4, 100 g |
| Stock $KH_2PO_4$ solution | Preparation instructions: Section 5.3.2.3 | |
| Stock $MgCl_2$ solution | Preparation instructions: Section 5.3.2.4 | |
| **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) | | |

| Required Material | Notes | Re-order information |
|---|---|---|
| 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 |
| **Coastal Institute in Kingston, room 019, Plastic drawers or boxes beneath the lab countertop** | | |
| Sterile plastic Petri dishes | | Fisher catalog # 08-757-19 |
| Sterile 1 mL pipettes | | Fisher catalog #13-678-25C |
| Sterile 10 mL pipettes | | Fisher catalog #13-678-25F |
| Sterile 25 mL pipettes | | Fisher catalog #13-676-29D |
| Pipette sterilizing pouches | | Fisher catalog # 01-812-53 |
| **Coastal Institute in Kingston, room 019, Drawer or shelf beneath the lab countertop** | | |
| Aluminum foil | | |
| **Coastal Institute in Kingston, room 019, In cabinet beneath the water bath** | | |
| Clear biohazard autoclave bags | | Fisher catalog #01-826-5 |
| **Coastal Institute in Kingston, room 019, On shelf above the water bath** | | |
| Plastic carboy filled with phosphate buffer saline solution | Preparation instructions: Section 5.3.2.1 and 5.3.2.2 | |
| **Coastal Institute in Kingston, room 019, In the upper cabinet** | | |
| Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) | 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. | Fisher catalog #M33-500 |
| Potassium phosphate monobasic ($KH_2PO_4$) | 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. | Fisher catalog #P382-500 |
| 10 N Sodium Hydroxide (NaOH) | Causes eye, skin, digestive and respiratory tract burns. Caustic. Preparation instructions: Section 5.3.1.1. | |
| Nalidixic acid ($C_{12}H_{12}N_2O_3$) | May cause irritation of the skin, eyes and respiratory tract upon contact. Causes photosensitivity and may cause allergic respiratory or skin reaction. Harmful if consumed. | Sigma N8878-5G |

| Required Material | Notes | Re-order information |
|---|---|---|
| Sodium chloride (NaCl) | 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. | Fisher catalog # S671-500 |
| **Coastal Institute in Kingston, room 019, In the chemical cabinet** | | |
| 95% Ethanol in a 2 L glass bottle | | Available through the Agricultural Experiment Station office in Woodward Hall |

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

| Matrix | Sample Container | Preservation | Volume | Holding Time |
|---|---|---|---|---|
| Water | Autoclavable white plastic bottle<br>250 mL for freshwater samples<br>500 mL for estuarine and coastal samples | Kept at 4 °C in Sterile Bottles | 100 mL | 6 Hours |

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 mE and EIA media 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 5.2.4.3 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 enterococci 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 filtered 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 50 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 with 20% relative percent deviation (%RPD). %RPD is calculated as follows:

$$\%\text{RPD} = \frac{\left|\text{Result of Replicate 1 (colonies/100 mL)} - \text{Result of Replicate 2 (colonies/100 mL)}\right|}{\text{Average of Result of Replicate 1 (colonies/100 mL) and Result of Relicate 2 (colonies/100 mL)}} \times 100$$

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:

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

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 Calibration and Standards

#### 5.2.4.1 Positive Plates

Calibration is completed in a qualitative way through an assessment of false negatives. A sample known to produce positive results for enterococci bacteria is filtered using the sample filtration procedure outlined in Section 5.3.4.3 and then treated as a sample. These plates are 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.

#### 5.2.4.2 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 +/- 1 °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.

#### 5.2.4.3 QA Check on New Plates

A QA check is completed on batches of new media plates. A sample known to produce positive results for enterococci bacteria is obtained and twelve aliquots are filtered using the sample filtration procedure (Section 5.3.4.3). Six filters are placed onto plates containing the new media and the remaining six onto plates containing the old media. The plates are then treated as samples. After incubation, the plates are visually inspected 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 approximately the same amount of 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 to be sure there is an adequate supply of mE and EIA 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 5.3.1.2 and 5.3.1.3 for the preparation of the mE and EIA plates, respectively.

##### 5.3.1.1 Preparation of 10 N NaOH

1. Obtain a 250 mL volumetric flask and fill it approximately ¾ full with ultrapure water.
2. Check the calibration of the balance in room 018 using the 1 g weight.
    a. The calibration weights are located in the drawer under the balance in room 018. 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. Contact Elizabeth Herron or Linda Green.
3. Weight out 100 g of NaOH.
4. Slowly add the NaOH to the volumetric flask while mixing.
5.

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.

6. Allow the flask to cool and add ultrapure water to bring the flask to volume once all the NaOH has dissolved.

#### 5.3.1.2 Preparation of mE Media and Plates

Materials

| | |
|---|---|
| Envirocide or Conflict | Magnetic stirring bar |
| Sterile Petri dishes | Magnetic stirring and heating plate |
| Autoclave | Metal tray |
| 2 - 2 L autoclave safe flasks | Aluminum foil |
| Insulated autoclave gloves | Sterile 25 mL pipette |
| Dehydrated mE media | Empty foil lined cardboard box |
| Deionized (DI) water | Electronic pipette-aid |
| Nalidixic acid | 10 N NaOH |
| 2,3,5-triphenyl-tetrazolium chloride | |

Procedure

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

500 mL of media is enough for approximately 85 - 100 plates

1. Check the water bath is approximately ¾ full of DI water, and set it at 44.5 +/- 1 °C. Turn the water bath on.
2. Check the calibration of the balance under the hood using the 1 g weight. (see Section 5.3.1.1 step 2 for a description of this procedure). Also note that since the balance is in the hood that the value returned by the balance may fluctuate due to the air flow.
3. Under the fume hood in room 019a weight out 35.6 g of dehydrated mE media into a 2 L Erlenmeyer flask. Weight out the mE media in the fume hood only, making sure to wear the mask.
4. Add 500 mL of DI $H_2O$ and a magnetic stirring bar.
5. Cover the flask loosely with aluminum foil and bring to room 002.
6. Heat and stir the re-hydrated mE media on the stirring/heating plate until the media is fully dissolved (to boiling if necessary).
7.

Once the media is dissolved place the Erlenmeyer onto a metal autoclave tray and autoclave the material 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.

8. While the mE media is autoclaving, sterilize the table top by wiping down the entire surface with the Envirocide or Conflict and allowing the surface to dry.
9. 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.
10. 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.
11. 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.
12. 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.
13. Place the flask with the boiling 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.
14. 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).
15. Mix 0.12 g of nalidixic acid into 2.5 mL of DI water and then add 0.1 mL of 10 N NaOH. This may be done in a small Erlenmeyer flask. Allow the mixture to dissolve and then add it to the cooled mE media.
16. Add 0.075 g of 2,3,5–triphenyl-tetrazolium chloride to the media and mix well.
17. 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 mE media from the flask.
18. Dispense 4 to 5 mL of mE media into each of the plates in the first row.
19. Repeat until all the media has been used.
20. 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.
21. QC check the plates against old plates using the procedure found in Section 5.2.4.3.
22. Plates may be stored in the refrigerator for up to a month.

### 5.3.1.3 Preparation of the EIA Media and Plates

| | |
|---|---|
| Envirocide or Conflict | Magnetic stirring bar |
| Sterile Petri dishes | Magnetic stirring and heating plate |
| Autoclave | Metal tray |
| 2 - 2 L autoclave safe flasks | Aluminum foil |
| Insulated autoclave gloves | Sterile 25 mL pipette |
| Dehydrated EIA media | Empty foil lined cardboard box |
| Deionized (DI) water | Electronic pipette-aid |

Procedure

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

500 mL of media is enough for approximately 85 - 100 plates

1. Check the water bath is approximately ¾ full of DI water, and set it at 44.5 +/- 1 °C. Turn the water bath on.
2. Check the calibration of the balance under the hood using the 1 g weight. (see Section 5.3.1.1 step 2 for a description of this procedure). Also note that since the balance is in the hood that the value returned by the balance may fluctuate due to the air flow.
3. Under the fume hood in room 019a weight out 8 g of dehydrated EIA media per 100 mL of desired final volume (500 mL media = 40 g EIA) into a 2 L Erlenmeyer flask.
4. Add the appropriate amount of DI $H_2O$ and a magnetic stirring bar.
5. Cover the flask loosely with aluminum foil and bring to room 002.
6. Heat and stir the re-hydrated EIA media on the stirring/heating plate until the media is fully dissolved (to boiling if necessary).
7. Once the media is dissolved place the Erlenmeyer onto a metal autoclave tray and autoclave the material 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.
8. While the EIA media is autoclaving, sterilize the table top by wiping down the entire surface with the Envirocide or Conflict and allowing the surface to dry.
9. 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.
10. 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.
11. 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.
12. 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.

13. Place the flask with the boiling 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.
14. 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).
15. 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 EIA media from the flask.
16. Dispense 4 to 5 mL of EIA media into each of the plates in the first row.
17. Repeat until all the media has been used.
18. 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.
19. QC check the plates against old plates using the procedure found in Section 5.2.4.3.
20. Plates may be stored 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 and 5.3.2.2.
3. Make sure the incubator is set to and holding the correct temperatures. The flask with the thermometer in the 41 °C incubator should be full of DI water, with the temperature reading 41 +/- 1 °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 $KH_2PO_4$ solution (preparation information in Section 5.3.2.3)
    b. 5 mL Stock $MgCl_2$ solution (preparation information in Section 5.3.2.4)
    c. 7 g NaCl
    d. 1 L DI $H_2O$
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 <u>using insulated autoclave gloves as the PBS will be very hot.</u>
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 $KH_2PO_4$ solution (preparation information in Section 5.3.2.3)
    b. 20 mL Stock $MgCl_2$ solution (preparation information in Section 5.3.2.4)
    c. 28 g NaCl
    d. 4 L DI $H_2O$
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 it 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 Stock $KH_2PO_4$ Solution

1. Add the following to a 1 L Erlenmeyer flask
    a. 34.0 g $KH_2PO_4$
    b. 500 mL DI $H_20$
2. Adjust to pH 7.2 $\pm$ 0.5 with 1 N NaOH and dilute to 1 L with DI $H_20$
3. Store excess solution in a labeled plastic bottle in the URIWW Refrigerator in room 002 of the Kingston Coastal Institute

#### 5.3.2.4 Preparation of Stock $MgCl_2$ Solution

Add 81.6 g $MgCl_2 \cdot 6H_2O$ to a 1 L Volumetric flask and dilute to 1 L with DI $H_20$.

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. 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).
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.

### 5.3.4 Procedure - Day of Sample Collection

#### 5.3.4.1 Initial Preparation

1. Take the appropriate number of mE 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 4 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 top (half that is not holding the media) of mE 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. It is important to keep the bottom of the Petri dish free of markings because the samples are read by looking at the bottom of the media dish.
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, 50 mL of ambient or marine 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 enterococci 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 or marine water samples that must be diluted are generally diluted to 1, 10 and 50 mL.
ii. Prepared dilutions should be filtered sequentially in a single filter funnel in the following order: 1 mL, 10 mL and 50 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 41 ºC incubator for 48 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.

#### 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 Transferring and Counting Filters

1. After approximately 48 hours allow a quantity of EIA plates equal to the number of mE plates incubating to come to room temperature.
2. After 48 hours transfer the membrane filters previously filtered to the EIA plates, transferring the appropriate plate lid.
3. Put the EIA plates into the 41 °C incubator for 20 – 30 minutes.
4. After 20 – 30 minutes remove the EIA plates from the incubator and organize the plates according to the data sheet for ease of data entry.
5. Count and record the number of pink to red colonies with black or reddish-brown precipitate on the underside of the membrane. 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 - 60 colonies for a diluted sample or 0 – 60 colonies for an undiluted sample. Higher or lower plate counts can be used if necessary, with the average of replicate plates used when possible.
    c. Record the number of colonies in the column marked EIA count.
6. Repeat for the remaining filters.

### 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. Using the Woodward Hall Autoclave only, autoclave the half full bags of plates in a metal tray for 20 min at 132 °C on the liquid cycle. Place the cooled bag into the dumpster (do not put in a trash can as the janitors will NOT dispose of it). See SOP 006 - Waste Autoclaving Procedure.

#### 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

Enteroccoi are reported in terms of the number of colonies per 100 mL. Enterococci counts of 0 are reported as <2 colonies/100 mL.

### 6.1 Calculation of Enterococci Results

Select the membrane filter with the number of colonies closest to the acceptable range (20 – 60 colonies) from the EIA 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 enterococci colonies per 100 mL to the nearest whole number. If the sample has not been diluted the acceptable range of colonies per filter is 0 – 60.

$$\frac{\text{enterococci colonies}}{100\ \text{mL}} = \frac{\text{number of colonies counted}}{\text{volume in mL of sample filtered}} \times 100\ \text{mL}$$

## 7.0 REFERENCES

APHA, AWWA & WEF. Standard Methods for the Examination of Water and Wastewater. 19th ed. Washington D.C: APHA, 1995.

Methods referenced: Microbiological Examination (9000), Fecal streptococcus and enterococcus groups (9230 A) and Membrane Filtration Technique for Members of the Enterococci (9230 C.)

## 8.0 DOCUMENTATION

Example Data Sheet

**Bacterial Sample Log & Worksheet: GH / NP COLLECTION (9/29/04)**
**mE Membrane Filtration Method**

**Analyst - set-up:** **Analyst - Counts:**
**Incubator temp. start:**
**incubator temp. end:**

| Monitoring Location | Setup Date | Dil. (mls) | EIA Count | Total Enteroccoci (per 100ml) |
|---|---|---|---|---|
| GH-In Pond | | | | |
| GH-Indigo Point | | | | |
| GH-Sea Lea | | | | |
| GH-Teal Road | | | | |
| GH-Twin Peninsula | | | | |
| NP-Crawford Dock | | | | |
| NP-Ft Neck Cove | | | | |
| NP-Pond Street | | | | |
| NP-Stumpy Point | | | | |
| NP-Tockwotten Dock | | | | |
| NP-Tom Cod Cove | | | | |
| NP-Vigna's Dock | | | | |
| | | | | |
| | | | | |
| | | | | |

**Understanding the URIWW Bacterial Data Sheet**

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

Setup Date: 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 50 mL are filtered, although if the bacteria levels are expected to be high smaller volumes may be filtered as well.

EIA Count: The number of pink to red colonies with black or reddish-brown precipitate on the underside of the membrane after the initial 48 hour incubation on the mE media and the 20 – 30 minute incubation of the EIA media. This number corresponds to the number of enterococci bacteria for the volume of water that was filtered.

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

Rhode Island Department of Health Beach Standards:
Freshwater beaches: Not to exceed a value of 61 enteroccoi/100 mL
Saltwater beaches: Not to exceed a value of 104 enterococci/100 mL
(Standards are based on the Maximum Probably Number (MPN) method which estimates the number of bacteria. This method is different then the method discussed in this SOP which provides an actual number of bacteria in a sample)