Buzzards Bay
Stormwater Collaborative

Quality Assurance Project Plan
Illicit Connection Detection and Stormwater Quality Monitoring
in the Buzzards Bay Watershed

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Buzzards Bay Stormwater Collaborative

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1.3 Distribution List

Organization	Contact
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Town of Dartmouth	Tim Barber, Director of Public Works 759 Russells Mills Rd. South Dartmouth, MA 02748 Tel: 508-999-0740 ext:2210 Email: tbarber@town.dartmouth.ma.us
Town of Fairhaven	Vincent Furtado, Department of Public Works Director 5 Arsene Street Fairhaven, MA 02719 Tel: 508-979-4030 Email: vfurtado@fairhaven-ma.gov
Town of Mattapoisett	Michael Lorenco, Town Administrator Mattapoisett Town Hall 16 Main Street Mattapoisett, MA 02739 Tel: 508-758-4100 Ext. 200 Email: mlorenco@mattapoisett.net
Town of Acushnet	Patrick Hannon, Conservation/Stormwater Agent Parting Ways Building 130 Main Street Acushnet, MA 02743 Tel: 508-998-0202 Email: phannon@acushnet.ma.us

Town of Bourne	Stephanie Fitch, Conservation Agent Bourne Town Hall 24 Perry Avenue - Room 201 Buzzards Bay, MA 02532-3441 Tel: 508-759-0600 Ext.1344 Email: sfitch@townofbourne.com
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Envirotech Laboratories Inc.	Ron Saari, President 8 Jan Sebastian Drive, Unit 12 Sandwich, MA 02563 Tel: 508-888-6460 Email: rsenviro@comcast.net
New Bedford Health Laboratory	Jane H. Wurm, Laboratory Director 1000 Rodney French Boulevard New Bedford, MA 02744 Tel: 508- 991-6199 Email: Jane.Wurm@NewBedford-ma.gov

1.4 Project Overview

The Buzzards Bay Stormwater Collaborative (BBSC) was established in 2015 with a grant from EPA's Healthy Communities Grant Program awarded to the Buzzards Bay Action Committee (BBAC), with additional financial and technical support from the Buzzards Bay National Estuary Program (NEP). The original BBSC consisted of five Buzzards Bay municipalities (Dartmouth, Fairhaven, Acushnet, Mattapoisett, and Wareham) working in partnership with the BBAC and the NEP to map stormwater networks and monitor water quality of both dry and wet weather discharges. The BBSC and its partners, with the assistance of the local departments of public works staff, began monitoring stormwater discharges within 100 feet of either a shellfish closed embayment and/or bacteria impaired waters (Massachusetts 2012 Integrated Waters List, Category 4a and 5). This monitoring program commenced under an EPA approved Quality Assurance Project Plan (QAPP) dated March 16, 2016, subsequently revised on December 12, 2017.

In 2019, staffing support for the BBSC field monitoring and mapping effort was transferred from the BBAC to the Massachusetts Maritime Academy (MMA) with funding from the Southeast New England Program for Coastal Watershed Restoration (SNEP). The program also expanded to include three additional towns (Westport, Marion and Bourne). During this period, financial support from Buzzards Bay municipalities increased appreciably. MMA staff and Cooperative (Co-Op) program students help and support local public works staff in a team-based approach for sampling stormwater discharges and mapping stormwater tasks. Between 2020 and 2022, the BBSC received funding through MassDEP's MS4 Municipal Assistance Grant Program. This funding was used to create and outfit a stormwater trailer to conduct illicit discharge detection and elimination (IDDE) investigations. IDDE investigations are a required component of EPA's National Pollutant Discharge Elimination System (NPDES) Municipal Separate Storm Sewer System (MS4) permit. The trailer is housed at MMA and the Stormwater Coordinator organizes the scheduling and site locations for the Co-Op students and Public Works staff.

Since the approval of the BBSC 2017 QAAP, the BBSC has evolved to include a new partnership with MMA and the inclusion of three new Buzzards Bay municipalities. This QAPP has been revised to reflect the organizational status and personnel changes within the BBSC and some minor changes to the monitoring protocols as defined in the 2017 QAPP.

1.5 Project Organization

The BBSC is jointly managed by the MMA and the NEP.

1. BBSC Administrative Manager - Financial (FAM)

   The MMA FAM is responsible for overseeing the financial administration of the BBSC. The FAM coordinates fiscal responsibility within the BBSC to ensure the funding obligations of the BBSC are being met. The FAM provides guidance in the development of new funding initiatives and coordinates educational curricula development for the Co-Op students.

2. BBSC Administrative (Technical) and Project Quality Assurance Officer (TA/QAO)

   The NEP Executive Director provides oversite to the technical components of the BBSC data collection program including analysis of the water quality data provided by the contracted laboratories.

3. Stormwater Coordinator (SWC)

   The MMA SWC manages all aspects of data collection and student field activities of the program. Duties include recruiting and then training MMA students in Global Positioning System (GPS) technology, proper water quality monitoring protocol and field verification of storm drain structures using Geographic Information System (GIS); tracks stormwater monitoring and illicit discharge investigation sites; provides planning, oversight and coordination with the field sampling/investigative crews (Municipal Public Works staff and/or Co-Op students); communicates with certified laboratories with regards to scheduling; provides training and oversite to MMA students' analysis of "in-house" (at MMA laboratory) field samples; ensures that the monitoring samples are properly documented, preserved, handled, and delivered to the appropriate laboratory;

maintains the BBSC IDDE investigative trailer and sampling kits.

4. NEP Technical Project Advisors and Quality Control Officers (TPA/QCO)
   The NEP Stormwater Specialists both serve as TPA/QCO to the BBSC. Each provides technical advice regarding sampling collection and monitoring sites, GIS issues, equipment use and replacement, ensures that procedures in the field and laboratory are performed in accordance with this QAPP, and keeps other leaders informed of project status in relation to the QAPP.

5. Municipal Public Work Directors (DPW Directors) and Field Sampling Teams (Field Teams)
   The Stormwater Coordinator consults with the DPW Directors to establish Field Teams. The Field Teams are assigned pre-determined sampling stations during wet- and dry-weather sampling events. Each team consists of the Stormwater Coordinator (or designee), 1-2 MMA Co-Op students and 1-2 staff from the municipal public works department. Responsibilities include assisting with pre-event planning, grab sample collection, collection of field measurements, accurate completion of field data sheets and equipment worksheets. If needed, team members might also deliver samples and field data sheets to the appropriate analytical laboratory. All field personnel involved in the project undergoes training prior to field sampling and a record of training are documented and filed in the BBSCs office. In addition, the Field Teams are responsible for collecting and documenting (GPS, photographs, or field notes) as appropriate.

Table 1. Project Organization

Function	Organization
BBSC Administrative Manager (Financial)	Kristin Osborne, MMA
BBSC Administrative Manager (Administrative)	Joseph Costa, NEP
Technical Advisors/QC Officers	Kevin Bartsch and Bernadette Taber, NEP
Stormwater Coordinator	Maura Flaherty MMA
Project QA Officer	Joseph Costa, NEP
EPA Project Officer	Alicia Grimaldi, U.S. EPA
EPA QA Officer	TBD, U.S. EPA
Nutrient, Phosphorus, TSS Laboratory	Ron Saari, Envirotech Laboratories Inc.
Biological Laboratories	Ron Saari, Envirotech Laboratories Inc. Jane Wurm, New Bedford Health Laboratory

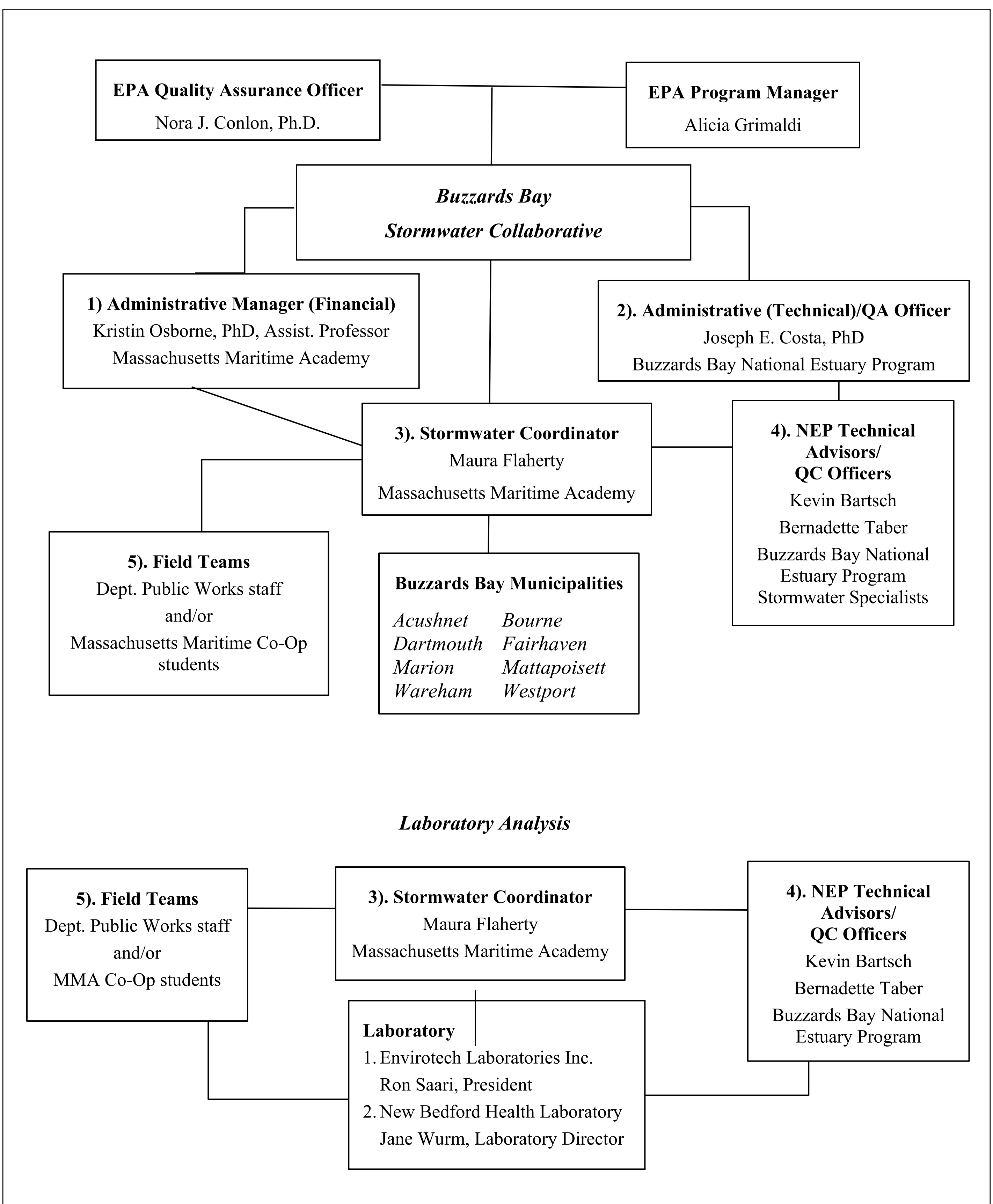

Figure 1 Organizational Chart

1.6 Problem Definition/Background

In Buzzards Bay, there are roughly 6,000 acres of shellfish growing areas indefinitely closed to shellfishing, another 6,000 acres closed after 0.2 inches or more of rain, and roughly 3,000 acres seasonally closed. These closures are due to bacterial contamination and are often largely the result of stormwater discharges. In addition, most of the Buzzards Bay municipalities contain an Urbanized Area (UA) as defined under EPA's MS4s permit. Monitoring and inspections in these areas must comply with the permit. The permit requires the municipalities to obtain an Authorization to Discharge for stormwater originating from UAs. To obtain a permit to discharge, each municipality must submit a Stormwater Management Plan that addresses the impact these discharges have on water quality, and it must include a mechanism in which to locate and eliminate illicit (non-stormwater) discharges. The elimination of illicit discharges to stormwater networks and the treatment of stormwater discharges conveying non-point sources of pollution will help reduce water quality impairments. These actions can only be taken if problem discharges can be identified and prioritized.

The identification of problem discharges and the development of solutions must begin with mapping stormwater networks and monitoring stormwater discharges for pollutants. In 2016, the BBAC, through its establishment of the BBSC began the process by obtaining funding through EPA's Healthy Communities Grant Program. This funding allowed Buzzards Bay municipalities within the BBSC to begin testing stormwater discharges in coastal waters that were closed to shellfishing. The closures were a result of high bacterial levels. In addition to bacteria, these discharges were also monitored for the presence of illicit discharges in accordance with EPA's New England Bacterial Source Tracking Protocol (Draft-January 2012).

Utilizing various funding sources, the BBSC has been able to expand the program and now includes a partnership with MMA. MMA, as an active partner in the BBSC, administers the financial component of the project, provides the facilities for training and equipment storage, employs the Stormwater Coordinator for the monitoring and mapping component and provides the Co-Op students for sampling and mapping tasks. The BBSC has also expanded the program to include three additional Buzzards Bay municipalities and expanded their monitoring initiative to encompass stormwater flows discharging from UAs as defined by EPA's MS4.

The QAPP for this monitoring program was originally approved in March 2016 under the BBAC administered Stormwater Collaborative and revised in December 2017. This current revision includes the new partnership with MMA and encompasses monitoring in the UAs of the eight BBSC municipalities (Westport, Dartmouth, Acushnet, Fairhaven, Mattapoisett, Marion, Wareham and Bourne). This QAPP includes protentional future stormwater monitoring (subject to as needed modification) by the BBSC, MMA, and NEP.

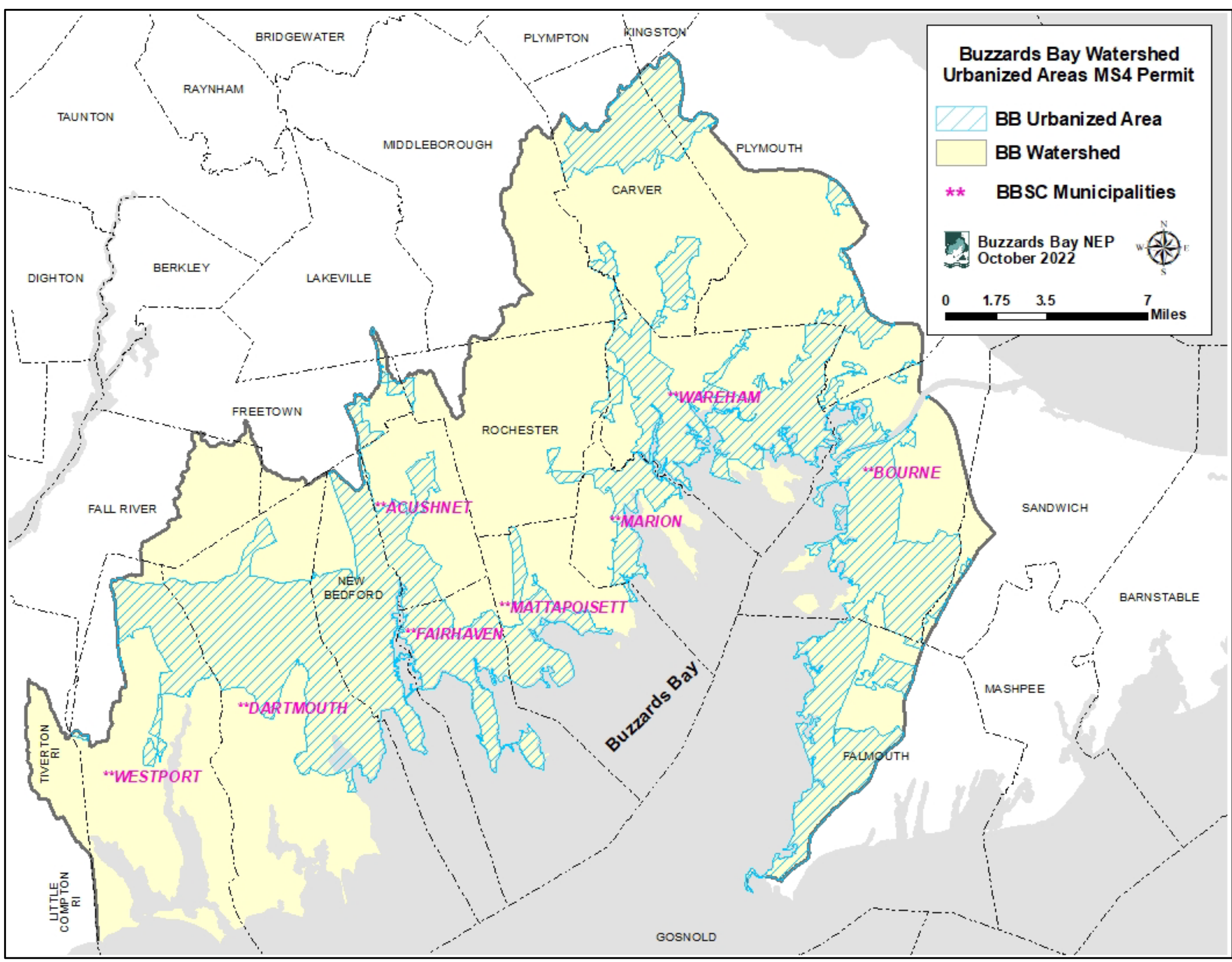

Figure 2. Buzzards Bay Watershed and Urbanized Areas

1.7 Project/Task Description and Schedule

For the purposes of this revised QAPP, the monitoring project scope has been defined as stormwater networks discharging into the waters within the Buzzards Bay watershed, including discharges to wetlands, saltwater, and freshwater, both within and outside of the MS4 UAs (Figure 2). As some of the collaborating towns encompass more than one drainage basin, monitoring may be expanded beyond the boundaries of the Buzzards Bay drainage basin upon the request of the municipality. Monitoring of discharges is on a priority basis, with the high priority discharges being located within defined UAs currently affecting impaired waters (Massachusetts 2018 Integrated Waters List, Category 4a and 5, Figure 3). In addition, each drainage pipe is investigated for potential illicit discharge connections utilizing equipment from the BBSC's stormwater trailer. Upon locating a potential illicit discharge, the investigative team documents the location of the suspected illicit discharge and collects a sample if the pipe is flowing. The individual municipality is notified of the potential illicit discharge and any monitoring results if available.

Since the immediate need of the Buzzards Bay communities is the upcoming monitoring requirements for their MS4 permit, this revised QAPP focuses on complying with those requirements and includes some additional monitoring parameters. This QAPP will also encompass future stormwater monitoring both within and outside the UAs and can be used for both screening purposes and to meet the needs of future permit compliance.

As stated previously, this monitoring program has been ongoing since 2017, encompassed under the original QAPP

approved in 2016 and then the revised and approved QAPP in December 2017. This current revision factors in the reorganization of the BBSC to include the partnership of the MMA and the subsequent staffing changes. Since the immediate need of the communities within the BBSC is the monitoring requirements for the MS4 permit, the QAPP (original and revisions) focused on complying with those requirements. As per MA MS4 protocol, each discharge is inspected during dry weather, (less than 0.1 inches of rainfall within the previous 24 hours). Information regarding the discharge's physical condition (size, shape, dimensions, material, physical condition etc.) is documented and the GPS location recorded (if not done previously). If the discharge is inaccessible or submerged, the next upstream structure (manhole or catchbasin) is inspected. Observations are also made as to the potential presence of an illicit discharge (olfactory and/or visual evidence of sewage). A grab sample is taken when a dry weather flow is present (see monitoring parameters below). If a dry weather flow is not present, but there is evidence of sewage at or near the discharge, the data collector informs the Stormwater Coordinator. The site is revisited as soon as possible during dry weather and a sample collected if a flow is observed.

For screening purposes, as a minimum, water quality samples collected during sampling events (dry or wet weather) are evaluated for all the parameters in the Field Test Kits plus the laboratory analysis for bacteria (Table 2). The bacteria analysis selected depends on the impairment of the receiving waters - Fecal coliform bacteria for saline waters and E. coli for freshwater. In addition, the screening also includes documentation of all the field observations. Additional samples are collected for laboratory analysis as required for permit compliance based upon the water quality of the receiving (impaired) or at the discretion of the NEP QA Officer or QC/Technical Program Advisor.

The number of samples collected in any given year is highly variable, depending on pipe flow characteristics, weather conditions (rain), availability of staff/Co-op students and laboratory availability to process samples. Since 2017, the lowest number of samples collected was 6 (2018 - partnership not established) to a high of 129 in 2021 Utilizing previous numbers of samples collected. the expected average for Collaborative monitoring within the upcoming years will probably range from 50 to 100 samples per year.

Table 2. Monitoring Parameters

- **Field Test Kits (Primary)**
  - Surfactants as detergents
  - Ammonia as Nitrogen
  - Conductivity/Salinity/Temperature
  - pH
  - Nitrates as Nitrogen
  - Chlorine (Hach-test strips or Pocket Colorimeter)
- **Field Observations (Primary)**
  - Color
  - Odor
  - Cloudiness (turbidity)
  - Sewage/Septic System waste products (toilet paper, sanitary products, etc.)
  - Flow characteristics
- **Laboratory Analysis (Primary)**
  - Enterococci and/or Fecal coliform bacteria - saline
  - *E. coli* bacteria - fresh water
- **Secondary Laboratory Analysis**
  - TN (Nitrates, Nitrites, Total Kjeldahl Nitrogen
  - Total Phosphorus
  - Total Suspended Solids
  - Turbidity
  - Oil & Grease (Hydrocarbons)s
  - Dissolved Oxygen
  - Biological Oxygen Demand
  - Metals
    - Aluminum
    - Arsenic
    - Cadmium
    - Chromium (total)
    - Copper
    - Lead

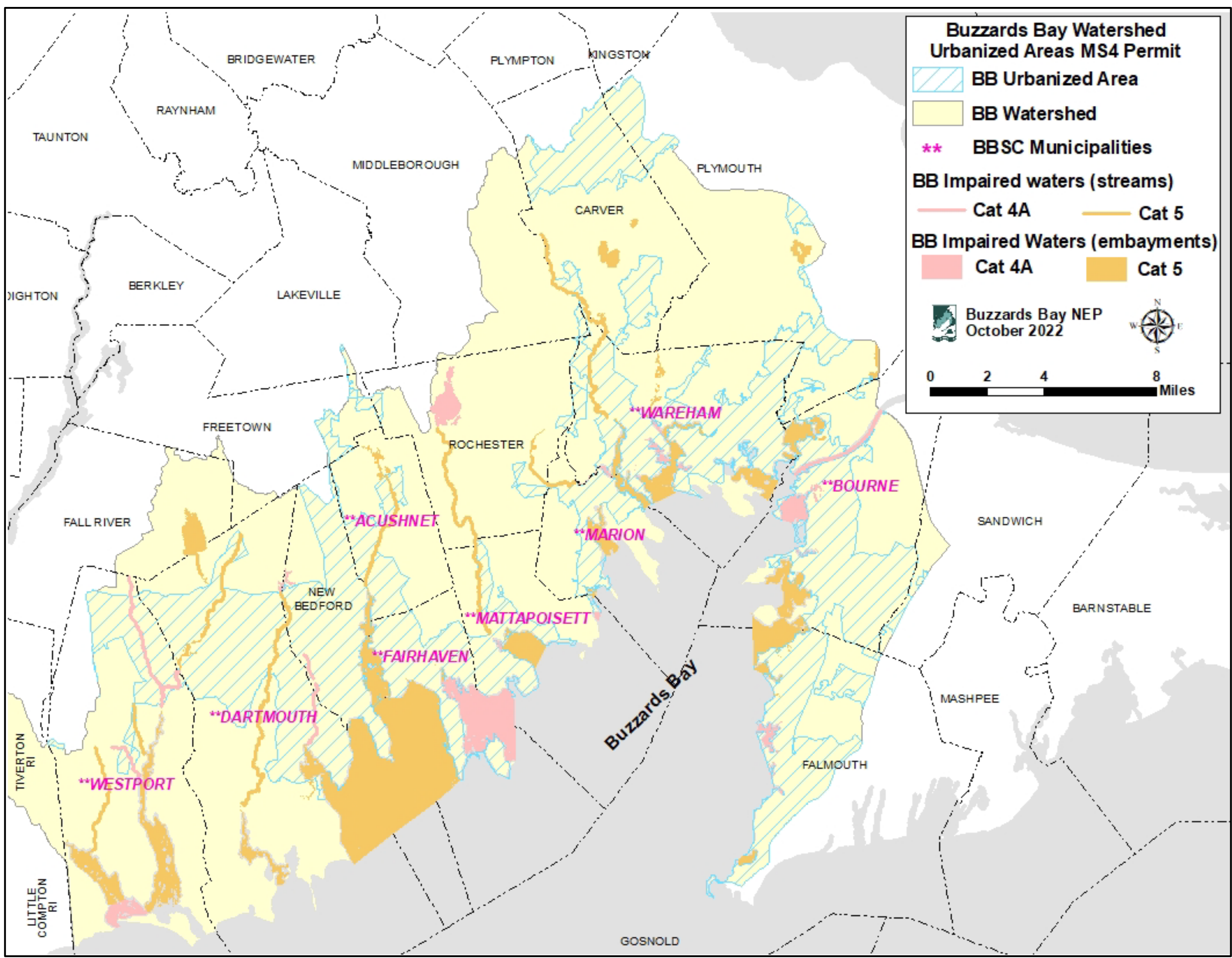

Figure 3. Buzzards Bay watershed and DEP impaired waters.

Project Scope and Schedule

Table 2 shows a summary of contaminants that are evaluated through this effort. Each round of sampling includes confirmation of sampling locations, tidal data, antecedent precipitation, and compilation of all field tests and field observations. Once the analytical data is received from the laboratories, the Stormwater Coordinator, QA Officer, and TPA reviews the data and make recommendations for future monitoring locations. Final decisions regarding locations are made in consultation with the municipal DPW Director or designee.

The assessment of outfalls and scope of work described above includes the following tasks, and according to the schedule in Table 3:

- Task 1: Gathering of catchment data
- Task 2: Initial ranking of discharges
- Task 3: Hiring of interns/training (or retraining) of staff
- Task 4: Implementation of Sampling Program
- Task 5: Reporting and Analysis

1.8 Data Quality Objectives

Monitoring data is collected by the BBSC Field Teams. The Field Teams consists of two trained individuals from the BBSC, MMA, NEP and/or municipal public works department.

The Field Teams meet the following water quality objectives:

- collect water quality data to characterize pollutants associated with stormwater runoff in predetermined areas as designated by the NEP and the individual municipalities.
- collect water quality data to identify illicit connections that may be primary contributors of pollutants to all waters in the Buzzards Bay watershed.

The methods and approaches utilized by the BBSC are aimed at meeting the required levels of precision, accuracy, field blank cleanliness, and detection limits. The minimum performance criteria for the bacteria sampling is given in Table 4, for field test sampling in Table 5 and 6, and for the optional future tests in **Error! Reference source not found.**, below.

1.9 Special Training Requirements/Certification

This sampling program consists of standard field sampling techniques, field analyses, laboratory analyses, and data validation techniques. All field personnel on the Field Teams are required to complete a brief training program to ensure all field sampling protocol is followed as specified in this QAPP, as described in Appendix C. The Stormwater Coordinator maintains a signup sheet to include the name of the trainee, date of training, and contact information.

1.10 Documentation and Records

Field data are recorded on standardized field data sheets (Appendix D) either on-site ("in the field") or at the MMA lab ("in house").

The selected analytical laboratories are certified by MassDEP to perform all analytical procedures that are required during the completion of this field sampling program. All laboratory personnel are to have had training in accordance with the procedures outlined in their QAPPs. A summary of the laboratory Standard Operating Procedures (SOPs) for the required analyses are provided in Appendix A.

The samples requiring laboratory analysis are picked up by the Stormwater Coordinator (or designee) and driven to the appropriate laboratory. Prior to releasing the samples, a member of the Field Team signs and dates the Chain of Custody (COC) -- either on the bottom of the field data sheets or a separate form. The Stormwater Coordinator (or designee) places the "in house" samples and laboratory samples into separate coolers and delivers the laboratory samples to the laboratory. Once the samples arrive at the laboratory, the Stormwater Coordinator signs the COC at either the bottom of the data sheet or one provided by the laboratory. The laboratory keeps the field data sheets and COC forms and makes copies for the Stormwater Coordinator. The Stormwater Coordinator brings the remaining "in-house" samples to the MMA lab for testing. The results of the "in house" testing are placed on the field data sheets. The field data sheets are then filed at the NEP office. The monitoring data is entered into a database, organized by municipal name, storm drain system, and then specific location (discharge points or structure) within each storm drain system.

Table 3. Project Schedule

	2017			2018												2027										
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
1 Gathering of catchment data																										
2 Initial Ranking		X	X	X	X																					
3 Hiring of Staff			X	X																						
4 Implementation of Monitoring Program																										
5 Reports and Analysis									X																	

Table 4. Laboratory Tests - Bacteria, New Bedford Health Laboratory and Envirotech Laboratories Inc.

Parameter	Sample Volume/ Container	Maximum Holding Time	Field Processing/ Preservation	Laboratory Precision	Method	Units	Method Detection Limits MDLs
Fecal Coliform	100 ml sterilize polyethylene	6 hours	Collect, label, store on blue ice	Logarithm range of duplicates, PT tests for bacterial analysis	Membrane Filtration, wastewater, SM9222D, 21th Edition 2005	cfu/100ml	10,100, or 1000 dilution
Enterococci	100 ml sterilize polyethylene	6 hours	Collect, label, store on blue ice	Logarithm range of duplicates, PT tests for bacterial analysis	EPA Office of Water, Method 1600, Membrane Filter Test EPA 821-R-97-004.	cfu/100ml	10,100, or 1000 dilution
E. coli	100 ml sterilize polyethylene	6 hours	Collect, label, store on blue ice	Logarithm range of duplicates, PT tests for bacterial analysis	Modified M TEC Agar for E coli (method 1603)	cfu/100ml	10,100, or 1000 dilution

Table 5. "In the field" Field Tests
(Sample analysis to be completed onsite)

Analyte(s) Measured	Equipment	Operating Range	Resolution	Accuracy
Ammonia	Hach Test Strips	0-6ppm	0.25ppm	+/- one half of a color block
Conductivity	HachPocket Pro, Multi 2	0 to 200 µS/cm or 2.00-19.9 mS/cm (auto-range)	0.01mS/0.1µS/1.0uS (range dependent)	±1.0%
Salinity	HachPocket Pro, Multi 2	0 to 10 ppt	0.01 ppt	±1%
Temperature	HachPocket Pro, Multi 2	0 to 50°C (32 to 122°F)	0.1°C	±0.5°C;
pH	HachPocket Pro, Multi 2	0.0-14.0	0.01	0.02
Chlorine	Hanna HI 762 Free Chlorine Meter (Ultra Low Range HI762)	0-500ppb	1ppb	±20 ppb ±4% of reading

Table 6. "In house" Field Tests

(Samples to be taken to the MMA lab for analysis)

Analyte(s) Measured	Equipment	Operating Range	Resolution	Accuracy	Holding time
Surfactants (detergents as MBAS)	CHEMetrics K-9400[1]	0-3 ppm	+ 1 color standard increment	+ 30% error at 0.25 ppm, 0.63 ppm, and 1.88 ppm	48 hours
Nitrates	LaMotte Nitrate-Nitrogen test kit (3615-01)	0.00 to 1.00 ppm	0.1ppm	0.1ppm	24 hours

[1]Organic sulfonates, sulfates, carboxylates, phosphates, and phenols as well as inorganic cyanates and thiocyanates may interfere. Sulfides may interfere negatively. Nitrate interferes positively; 10 ppm NO3-N may read as approximately 0.2 ppm. Isopropanol at up to 0.1% does not interfere. Chloride at up to 100 ppm does not interfere significantly. However, because higher chloride levels will interfere positively, this test kit is not recommended for the analysis of brine or seawater samples unless additional sample manipulation is performed.

Table 7. Other Laboratory Tests (Secondary tests based on need)

Parameter[1]	Max. Holding Time	Process &Storage	Method (Ref)	Units	Method Detection Limits (MDLS)
Nitrogen					
Nitrates	48 Hours	Filtered and stored in the dark at 4°C	EPA 300.0,	mg/L	0.01
Nitrites	48 Hours	Filtered and stored in the dark at 4°C	EPA 300.0,	mg/L	0.006
TKN	28 Days	Preserve with H2SO4 to pH <2, cool to 4° C	SM-4500 NH3 C	mg/L	0.6
Phosphorus	28 Days	120 ml plastic bottle, preserve with H_2SO_4 to a pH<2 cool to <6° C	SM4500-P-E, B	mg/L	0.005
Total Suspended Solids	7 Days	Analyze ASAP, Refrigerate or ice to 4°C .	SM 2540A.5	Mg/L	1.5
Turbidity	0 days	Analyze ASAP, Refrigerate or ice to 4°C	SM2130B	NTU	1.0
Oil and Grease	28 Days	500-1L narrow and wide-mouth glass bottles, preserve with HCl to a pH of <2, cool to <4ºC.	EPA 1664A, Ref: U.S. EPA-821-R-98-002, Feb 1999	mg/L	1.0
Dissolved Oxygen	ASAP	300 ml BOD bottle	SM-4500-O-G	Mg/L	1.0
Biological Oxygen Demand	ASAP	500ml plastic container	SM 5210 B	mg/L	1.0
Metals: Aluminum Arsenic Cadmium Tot Chromium Copper Lead	6 mo.	Filter through a 0.45 µm pore diameter membrane filter at the time of collection or as soon thereafter as practically possible. Use a portion of the filtered sample to rinse the filter flask, discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) nitric acid immediately following filtration to pH <2	EPA METHOD 200.7	mg/L	0.008 0.010 0.002 0.003 0.003 0.010

[1]Note: For samples collected and delivered to the laboratory within 6 hours, any specified preservatives are added by laboratory staff at the time of delivery

2.0 Data Generation and Acquisition

2.1 Sampling Process Design

The BBSC water quality monitoring program is designed to meet the project quality objectives discussed in Section 1.7 - Quality Objectives and Criteria. The initial monitoring program involves primarily dry weather observations and sampling (if discharge contains a dry weather flow) of priority outfall pipes and associated storm drain systems whose stormwater originates within an UA. Outfalls are prioritized based upon proximity to an impaired waterbody (the most recent Massachusetts Integrated Waters List, Category 4a and 5, Figure 3) and the potential for containing an illicit discharge. Based on EPA's MS4 permit requirements both wet and dry weather monitoring continues until all discharges within the UA have been evaluated. In addition, the BBSC utilizes this monitoring protocol to investigate, locate and then eliminate illicit discharges from within the storm drain network.

Sampling Description:

Stormwater water quality sampling is expected to be undertaken beginning in March of each year and continues as required under the MS4 permit. In general, water samples are collected from discharges flowing during dry weather, indicating the potential for an illicit discharge. Each discharge is monitored for the parameters listed under Field Test Kits in Table 2, along with Field Observations and Laboratory Analysis for bacteria. Under the current MS4 permit requirements, laboratory analysis is also required for discharges directly flowing into a water quality limited water (Category 4a waters) or water subject to an approved TMDL (Category 5 waters). Samples are taken from these direct discharges and analyzed for the impaired parameter listed under Category 4a or 5 as it relates to stormwater. These parameters are listed as secondary parameters in Table 2 as they are not monitored for every discharge. Appendix F lists the Impaired Waters within the Buzzards Bay watershed and the acceptable EPA methodologies for analysis.

Dry weather defined:

- Antecedent conditions where no more than 0.1inches of rainfall have occurred at least 48 hours prior to the sampling event;
- Not raining during sampling collection.

Wet weather defined:*

- Antecedent conditions where no more than 0.1inches of rainfall have occurred 72hours prior to the start of the rainfall event;
- During a rain event with a minimum rainfall of 0.25-inches and a minimum duration of 1 hour before the sample is collected.

Note: Due to the difficulty in obtaining wet weather samples (laboratory, DPW and field teams staffing constraints) along with inconsistent and unpredictable weather patterns, the SWS and/or the TPA may allow testing outside of the wet weather definition.

All samples are collected as close as possible to the discharge point and as close to low tide as feasible. Many of the pipe discharges in Buzzards Bay are partially or totally submerged, even during low tides. Tides are monitored using data provided by the National Oceanic and Atmospheric Administration (NOAA) at the NOAA Tide Predictions website.

Antecedent rain conditions and predicted rainfall are checked prior to an event by the Stormwater Coordinator. Precipitation forecasts are based on meteorological forecast models provided by the National Weather Service at the New Bedford Station. A final decision on whether samples will be collected is decided by the Stormwater Coordinator.

Prior to the first monitoring event, all the pre-selected discharge pipes/road cuts are assigned a unique site name (i.e., *Buzzards Bay Stormwater Atlas* FacilityID). The Stormwater Coordinator (or designee) conducts visual inspections of the sites, collecting GPS coordinates, photographs, and information regarding outfall pipe/road cut

(material, size, condition, etc.). During each monitoring event, the Field Team confirms the pipe/road cut location using an online GIS map (includes aerial map of discharge point, catch basins, flow direction, and street names) of each site and take additional photographs. Data is collected in accordance with Appendix B - Water Quality Grab Sample Collection Standard Operating Procedure and Appendix C – SOP: Field Water Quality Measurements. Environmental conditions (weather, antecedent rain, tide conditions, etc.) as indicated on the monitoring data sheet are filled out and flow characteristics noted along with any observations (odor, color, waste products).

Bacteria samples are collected first to minimize potential sediment contamination. Once the bacteria samples are collected, they are immediately placed in a cooler with blue ice. Separate bottles are then used to collect water samples for all "in the field" tests (pH, conductivity, temperature, salinity, chlorine and ammonia) and the "in house" (analysis to be done at MMA) tests (surfactants and nitrates). All "in house" samples are placed on blue ice. During each sampling event, up to two sequential duplicate samples are collected and analyzed by the appropriate laboratory identically to grab samples.

If the discharge pipe is submerged due to tidal conditions, a notation is made on the data sheet as to the depth of submersion. The Field Team then proceeds upgradient to the next available upstream manhole (or catchbasin if the storm drain system is catchbasin to catchbasin without any manholes). Under typical situations, this first manhole or catchbasin is the last of the stormwater entering the storm drain system and is representative of stormwater flow to the discharge pipe. At this first manhole, the Field Team take samples from every inlet pipe discharging under free-flow conditions into this first manhole (junction manhole). If the inlet pipe or pipes in the first manhole remains submerged, the Field Team notes the condition of the inlet pipe (submerged) and the outlet pipe (submerged or not) and collects the samples from the manhole sump. Any manhole with elevated salinity test results that does not appear to be tidally influenced (outlet pipe is free flowing) is investigated further for potential illicit discharges.

In addition to non-tidal salinity, all other parameters are evaluated by the Stormwater Coordinator and the NEP Quality Assurance Officer to determine the necessity of further investigation of individual storm drain systems for illicit discharges and/or pollution sources. At a minimum, efforts are made to evaluate storm drain systems whose previous samples were in a manhole sump (as opposed to free-flowing conditions). The water quality within the stagnant water of the sump may not be the best bacterial representation of the water quality within the storm drain system. In these circumstances, the Field Team rechecks the sampling site during the next sampling event, if the inlet(s) is still submerged, the Field Team proceeds to the next upstream manhole or catchbasin to collect samples/data.

Subsequently, if the water quality parameters indicate the potential presence of an illicit discharge and/or pollution source under free-flowing conditions, an immediate and more detailed search within the storm drain system is undertaken to determine the source of the problem.

2.2 Sampling Methods

Water quality sampling performed during this monitoring program includes the collection of grab samples, field measurements (both in the field and at the MMA lab), and quality control (QC) samples. Samples are collected and handled according to the SOPs listed in Table 8 and included in the associated Appendices of this QAPP.

Table 8. Project SOPs

Appendix	Project SOP
Appendix A	Laboratory SOPs and QAPPs
Appendix B	Water Quality Grab Sample Collection Standard Operating Procedure
Appendix C	Field Water Quality Measurements Standard Operating Procedure
Appendix D	Wet and Dry Weather Field Data Sampling Sheet
Appendix E	Data Collection Equipment and Equipment Calibration

Table 9. Water Quality Parameters and Indicators

Parameter	Indication of:
Surfactants (P)	Washing machine or sewer connection
Ammonia as Nitrogen (P)	Sewer connection
Temperature (P)	Impact to aquatic life
Conductivity (P)	Saline conditions, impacts on surfactant tests
Nitrates as Nitrogen (P/S)	Sewer connection
Bacteria (Enterococci, Fecal Coliform, *E. coli*) (P)	Presence of pathogens in fresh and saltwater
Phosphorus (S)	Washing machine or sewer connection, fertilizer
Total Kjeldahl Nitrogen (S)	Sewer connection, fertilizer
Hydrocarbons (Oil and Grease) (S)	Connection to high vehicle areas
Chlorine (P)	Connection to swimming pools
Total Suspended Solids (S)	Erosion, construction activities
Dissolved Oxygen (S)	Impact to aquatic life

(P)-Primary, (S)-Secondary

Note: all water quality parameters, except the secondary parameters, are tested for both the dry and wet-weather monitoring events. Chlorine is monitored using a Hannah Colorimeter or a Hach Pocket Colorimeter. Secondary parameters are analyzed by the laboratory when an outfall directly discharges into impaired water or at the discretion of the Stormwater Coordinator in consultation with the NEP Technical Advisors.

Pre-Sampling Activities

Preparation for sampling activities are the responsibility of the Technical Advisor and the Stormwater Coordinator. Responsibilities include assignment of site location identification, confirmation of discharge point location and safe access, collecting GPS coordinates, documenting point data (size, material, and condition), procurement, and field-testing of field equipment, training of Field Team members, weather tracking, review of SOPs, and laboratory coordination. Once in the field, the Stormwater Coordinator (or designee) establishes the sample staging areas, equipment distribution, confirmation of bottle labeling, and distribution of bottles and coolers provided by the subcontracted laboratories.

Sampling Procedure

All samples sent to the laboratory are grab samples collected in accordance with the laboratory SOPs found in Appendix A. Sample containers are sterile 100ml sample cups or clean, pre-preserved bottles supplied by the assigned analytical laboratory. The Field Team, on the appropriate monitoring field data sheets, records all data. Sample worksheets are provided in Appendix D-Wet and Dry Weather Field Data Sampling Sheet. Pre-labeled bottles are provided to each Field Team for each station and sampling round. Upon collection, sample bottles are handled in accordance with this QAPP, and the sample handling procedures provided by the assigned analytical laboratory. The chain of custody is signed and dated by the either the DPW Director (or designee) or the Stormwater Coordinator (or designee) before samples are transported to the appropriate laboratory and then again once the samples arrive at the laboratory. An example label is shown below, which shows the date and time the sample was taken, identification label, parameter being tested for, any pre-charged preservatives and the initials of the person who obtained the sample.

Field bottle sample identification labels (to be analyzed by the laboratories) consists of the Project ID (BBAC or NEP), Date and Time, Sample ID, Sample Type (wet or dry), Laboratory Analysis, Preservative (if any), and the collector's initials. The Sample ID numbering follows the point identification protocol as established in the NEP's Atlas of Stormwater Discharges in the Buzzards Bay Watershed (Stormwater Atlas).

Sample Identification Label

BBSC

Water Quality Sampling Program

Sample Date/Time: ______________________________

Sample ID: ______________________________________

Sample Type: ___________________________________

Laboratory Analysis: ______________________________

Preservative ________________

Collected By: ___________________________________

Laboratory Sample Identification - Field Code Designation

The laboratory identification protocol is as follows:

Project ID: BBSC Quality Sampling Program (BBAC-WQSP)

Sample ID:

Event Type: W (Wet Weather) or D (Dry Weather)

Sampling Type QG (Water Quality Grab Sample), FD (Field Duplicate Grab Sample) or FB (Field Blank)

"In the field" field measurements includes color, odor, and waste products observations in addition to, ammonia as nitrogen, pH, temperature, salinity, conductivity, and chlorine. "In house" (analysis in the MMA lab) includes

surfactants (detergents as MBAS) and nitrates. All data are collected in accordance with the procedures in Appendix C – SOP: Field Water Quality Measurements. Field measurements for conductivity, pH, and temperature (HachPocket Pro, Multi 2meter) are taken with a calibrated hand-held meter following the bacteria sampling and other laboratory samples.

Corrective Actions

Corrective action in the field may be required when conditions require a modification to this QAPP. Any member of the Field Team may identify a problem requiring corrective action. The Field Team, in consultation with the NEP Technical Advisor, TPA, or Stormwater Coordinator, implements the minor corrective actions and documents the action taken on the field on the data worksheets under the comments section. Major corrective actions including revision of field procedures, resampling, or retesting require approval by the Stormwater Coordinator and/or TPA prior to implementation. All major corrective actions are relayed to the individual Field Teams prior to the next sampling event to ensure consistency in the monitoring procedures. Corrective actions by the analytical laboratory are identified according to the laboratory Quality Assurance Plan. Any deviations from standard procedures, including corrective actions, which may jeopardize the integrity of the sample analysis, are communicated to the Stormwater Coordinator or NEP Technical Advisor and the TPA immediately and are reported in the final laboratory QA/QC report.

2.3 Sample Handling and Custody

Pre-Sampling Activities

All sample containers are sterile 100ml sample cups or clean, pre-preserved bottles supplied by the contracted laboratory in accordance with the applicable SOPs. Prior to the collection of samples for laboratory analysis, the sample containers are labeled using pre-printed, water-resistant labels. The labels include the sample ID, analysis requested, and preservative added and provides locations for collection date and time, and initials of the person who obtained the sample.

Chain of Custody (COC)

COC is located at the bottom of each data sheet (or on a separate from as requested by the laboratory) and is signed and dated by the Stormwater Coordinator (or designee) at the end of each sampling event. When transferring sample custody, the individuals relinquishing and receiving the samples signs and dates the COC. This COC documents the transfer of sample custody from the sampler to another person, to the laboratory, or to/from a secure storage area. The laboratory keeps the field data sheets and COC forms and makes copies for the Stormwater Coordinator.

Sample Handling

Laboratory samples are placed in coolers with sufficient blue ice to meet preservation and holding requirements. The COC form for the samples is placed in a waterproof plastic bag and accompanies the samples in the coolers. The Stormwater Coordinator or designee delivers the coolers.

2.4 Analytical Methods and Quality Control

- Monitoring parameters, analytical methodology, sample volumes, containers, sample processing, and storage for this project are listed in Table 4 through **Error! Reference source not found.**.
- Laboratory SOPs used for each analysis and associated QAs manuals are listed in Appendix A. Envirotech Laboratories Inc. and the New Bedford Health Department Laboratory conduct bacteria analysis, all other parameters are done through Envirotech Laboratories Inc.

Laboratory Analytical Methods/Quality Control

The analytical laboratory uses the procedures outlined in their QA Plan to ensure the reliability and validity of analytical results (Appendix A). Compliance with the laboratory QA plan is the responsibility of the laboratory QA

Officer. Laboratory reports are sent to the Stormwater Coordinator and the Technical Advisor within 28 days of the monitoring event and includes:

- Laboratory duplicates and/or blanks
- Matrix spikes and matrix spike duplicates (MS/MSDs)
- Laboratory Control Standard and Laboratory Control Standard Duplicates (LCS/LCSDs)

Specific information regarding the laboratory analysis of the water quality samples can be found in the laboratory SOPs in Appendix A. Specific criteria for the evaluation of laboratory precision and accuracy are provided in Table 5 and Section D1 – Data Review, Verification, and Validation. Any samples that do not meet the laboratory QC criteria are reanalyzed in the respective laboratory if sufficient sample volume is available.

2.5 "In the field" and "In House" Field Measurements/Quality Control

Field measurements are performed at either monitoring site locations ("In the field") or at the MMA lab ("In house") using portable field units, test kits, and test strips (Table 4). The instruction manuals for the usage of the portable meter and test strips are included in Appendix C. Specific procedures for quality control of the portable meter are discussed in Sections B6 – Instrument Testing, Inspection, and Maintenance and B7 – Instrument Calibration and Frequency.

Sample quality and testing reproducibility of the field parameters are assessed by field duplicates. The location of the field duplicate is determined before the sample round by the Stormwater Coordinator. Typically, at least one field duplicate is taken in each municipality during a sampling event. If more than 10 samples are taken per event (per municipality), then field duplicates are taken for every 10 samples. This duplicate sample is collected, handled, and analyzed/recorded for the same parameters as the original sample.

2.6 Instrument Testing, Inspection, and Maintenance

The laboratory performs routine preventative maintenance in accordance with their respective QA Plans and with manufacturer's specifications. The laboratory maintains factory-trained repair staff or maintains service contracts with applicable vendors.

The inspection, testing, and maintenance of all field equipment, instruments, and test kits are performed in accordance with the respective manufacturers' procedures. The Stormwater Coordinator (or designee) is responsible for ensuring calibration documentation is provided by the equipment supplier and is responsible for testing, inspection, and maintenance of all equipment prior to the first sampling event.

At the first sampling event, the Field Team visually inspects the field probes and performs a calibration check, as described in Section B7 – Instrument Calibration and Frequency. Results of that calibration check is noted on the field calibration sheets and Equipment Inspection, Testing, and Maintenance Sheets provided in Appendix E and any calibration errors are corrected at that time. The Stormwater Coordinator (or designee) is responsible for the proper maintenance and storage of the portable meters.

2.7 Instrument Calibration and Frequency

Laboratory Instruments

Calibration procedures and frequencies of all laboratory equipment is performed in accordance with the respective laboratory's QAPs, manufacturer's specifications, analytical SOPs, and written procedures approved by laboratory management. Records of calibration method and frequency are filed and maintained by the designated laboratory Quality Assurance Officers.

Field Instruments

Instruments and equipment used to conduct the field measurements are calibrated before each sampling event in accordance with manufacturer specifications. Any deviations in calibration which may affect final results are noted

on the equipment worksheets and corrective actions taken, as determined by the Stormwater Coordinator and/or TPA.

2.8 Inspection/Acceptance for Supplies and Consumables

All supplies to be used during the field sampling program are inspected (Table 10) by the Stormwater Coordinator or Field Team prior to the monitoring event to ensure that supplies are functioning properly, in satisfactory condition, and free of defects or contamination in accordance with the methods specified in Table 5. The Stormwater Coordinator and or Field Team keep extra supplies, stored and maintained in accordance with the respective manufacturers' or laboratory protocol.

Table 10. Supply Inspection Requirements and Acceptance Criteria

Supplies	Inspection Requirements
Sample Bottles	Visual inspection for cracks, breakage, cleanliness, and preservation solution (if needed)
Chemicals and Reagents (test strips)	Visual inspection for proper labeling, expiration dates, and appropriate grade
Water Quality Monitors (portable meters)	Calibration and operational check
Sampling equipment	Functional check and visual inspection for cracks, breakage, damage, cleanliness/contamination

2.9 Non-Direct Measurements

At a minimum, monitoring data is collected for dry weather events for each discharge point within the MS4 permit UA. Wet weather samples for the MS4 permit are collected as required for catchment investigations to located illicit discharges. The BBSC and the NEP utilizes this QAPP for future monitoring both within and outside the permitted UA. Rainfall is tracked using the NOAA weather station (www.erh.noaa.gov/box/dailystns.shtml) in New Bedford, Massachusetts. Whenever possible, the sampling collection coincides with low tide using tidal data from the NOAA website (NOAA Tide Predictions), with supplemental data from the local harbormaster's office.

2.10 Data Management

Laboratory analysis results and QA documentation is provided by the analytical laboratory to the Stormwater Coordinator and the TPA. Field measurements collected by the Field Team during sampling events is documented on the field data sheets (under Field Tests). The analytical laboratory keeps the original data sheets and provides copies to the Stormwater Coordinator (or designee).

The field and laboratory data are linked to the NEP's Stormwater Atlas and associated GIS database. The GIS database includes information regarding watershed size, impervious surface contribution, and land use characteristics associated with each discharge point. The GIS database also includes discharge pipe/road cut characteristics (pipe material, size, and condition) and other storm drain data collected in the field. Utilizing the GIS and monitoring data, the Stormwater Coordinator and the TPA continually reassess the monitoring program and making changes as needed. All water quality monitoring data and GIS data is available on the Buzzards Bay NEP website at stormwater.buzzardsbay.org.

3.0 Assessment and Oversight

3.1 Assessments and Response Actions

The Stormwater Coordinator is responsible for ensuring both laboratory and field activities are performed in accordance with the procedures established in this QAPP. Throughout the monitoring program, the Stormwater Coordinator works with the Field Teams directly, coordinating the day-to-day activities, ensuring on-site review of field activities including:

- Checking field data sheets, and field equipment operation and maintenance
- Checking/calibrating field equipment
- Checking status of sample collection, handling, and packaging procedures
- Ensuring Quality Assurance (QA) procedures are followed
- Ensuring Chain-of-Custody procedures are followed

Corrective actions in the field are implemented on a case-by-case basis. Minor response actions taken in the field to immediately correct a problem are conveyed to the Stormwater Coordinator (if unavailable then TPA) and documented on the data sheets. Major corrective actions require approval by the Stormwater Coordinator prior to implementation. Such actions may include revising procedures in the field, resampling, or retesting. All major corrective actions are relayed to the individual Field Teams prior to the next sampling event to ensure consistency in the monitoring procedures. Any corrective action undertaken by the laboratory are completed in accordance with the procedures outlined in their QA Plan. Any deviations from standard procedures, including corrective actions, which may jeopardize the integrity of the sample analysis, are communicated (verbally and in writing) to the Stormwater Coordinator and the Technical Advisor immediately and are reported in the final laboratory QA/QC report.

3.2 Reports

All field measurements collected by the Field Team during sampling events are recorded on the data sheets and provided to the Stormwater Coordinator upon completion of the field sampling event. The laboratory analysis data is sent to the Stormwater Coordinator within 28 days of the sampling event. The Stormwater Coordinator organizes and compiles all the data based on site location onto a spreadsheet.

The field and laboratory data are linked to the NEP's Stormwater Atlas and associated GIS database. The GIS database includes information regarding watershed size, impervious surface contribution and land use characteristics associated with each discharge point. The GIS database also includes discharge point characteristics (pipe material, size, and condition) and other storm drain data collected in the field. Once the final monitoring event is completed, the Stormwater Coordinator and the Technical Advisor assesses the monitoring and GIS data and provides a final report (including links to the NEP's Stormwater Atlas) to EPA and other interested parties. The report makes recommendations concerning future monitoring needs including potential expansion of the program into other municipalities.

4.0 Data Validation and Usability

4.1 Data Review, Verification, and Validation

As discussed in Section 2, the analytical laboratory uses the procedures outlined in their QA Plan to ensure the reliability and validity of analytical results (Appendix A). Laboratory reports are sent to the Stormwater Coordinator and the NEP QA Officer within 28 days of the monitoring event and includes:

- Laboratory duplicates and/or blanks
- Matrix spikes and matrix spike duplicates (MS/MSDs)
- Laboratory Control Standard and Laboratory Control Standard Duplicates (LCS/LCSDs).

Upon receipt of the laboratory reports, both the Stormwater Coordinator and the Technical Advisor reviews the laboratory and field test results to determine the general validity of sample results.

The accuracy of all the meters (parameters: pH, salinity, conductivity, and temperature) and other field parameters are assessed using QA procedures outlined in Section 2 and the accuracy/precision data as depicted in Table 5. Prior to each monitoring event, the Stormwater Coordinator selects a site (or sites) for collection of sequential field duplicates. Each field teams collects at least one field duplication for each monitoring event in each municipality. If more than 10 samples are taken per event (per municipality), then field duplicates are taken for every 10 samples. These duplicate samples are collected, handled, and analyzed/recorded for the same parameters as the original sample.

4.2 Verification and Validation Methods

General data evaluation includes review of holding times, laboratory duplicates, matrix spikes/matrix spike duplicates (MS/MSDs), laboratory control samples/laboratory control sample duplicates (LCS/LCSDs), sampling procedures, field blank results, equipment blank results as applicable, field duplicate results, and review of the analytical laboratory QA report. If the Field Team identifies the need for a corrective action, the NEP Technical Advisor are responsible for approving the implementation of the response action. Problems that are attributed to laboratory quality assurance issues are brought to the attention of the Stormwater Coordinator.

Appendix A. Laboratory SOPs and QAPPs

In the following pages are the QA Plan of Envirotech Laboratories Inc. of Sandwich, MA, and the City of New Bedford Health Department Laboratory SOP # 4.27 (2020).

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QUALITY ASSURANCE MANUAL

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Section 1 - Laboratory Facilities and Personnel

1.1 Ventilation

Central air conditioning is used to promote a well-ventilated environment which is free of dust, drafts and extreme temperature changes. Temperatures are maintained between 16 and 27ºC. Air conditioning is used to reduce contamination, permit more stable operation of incubators and decrease possible moisture problems with medias or analytical balances.

1.2 Organization of Laboratory Space

Entrance to the laboratory area is posted to minimize through traffic and visitors. Apart from the main laboratory area, a separate room, the chemical prep and incubation room, is used for the weighing and preparation of medias and reagents as well as various incubators and waterbaths for incubation purposes. The main laboratory area is divided into working sections which include a glassware preparation and storage area, a water purification and dishwashing area with safety shower and eyewash, and a laboratory work-bench area with analytical meters and filtration apparatus where samples are processed for analysis. The main laboratory area also has two fume hoods for working with hazardous or volatile materials. One fume hood is specific for working with acids and the other fume hood is reserved for use with organics. Sterilization is performed in the autoclave located in the Wastewater Treatment Laboratory.

1.3 Laboratory Bench Areas

A minimum of 2m linear bench space is provided per analyst in addition to areas specific for preparation and support activities. The laboratory bench tops provide a smooth, impervious surface which is chemically inert, corrosion resistant and has a minimum number of seams. The bench working surfaces are illuminated with even, glare-free lighting.

1.4 Walls and Floors

Walls are covered with a smooth finish that is readily cleaned and disinfected. Floors are coated with an impervious, washable surface which is textured to prevent slipping accidents.

1.5 Air Monitoring

Laboratory air quality is monitored on a monthly basis using Plate Count Agar in sterile dishes, set on lab bench surfaces (exposed) for a 15 minute period. Following 48 hours incubation at 35ºC, the number of colonies on the air density plates must not exceed 15 colonies/plate/15 minutes. Records of air quality monitoring are maintained in the lab Quality Assurance Manual.

1.6 Laboratory Cleanliness and Maintenance

Laboratory rooms are cleaned regularly (weekly). Routine cleaning includes washing of benches, shelves, floors and other areas prone to dust accumulation. Floors are wet mopped and treated with a disinfectant solution. Sweeping of floors and/or accumulation of clutter is not allowed. Benchtops are treated with a disinfectant solution before and after each use. Laboratory equipment is maintained with regular inspection and servicing as necessary. Procedures specific for maintenance of equipment are outlined in Standard Operating Procedures (SOPs) and records of repair and service are kept.

1.7 Laboratory Personnel

All bacteriological testing is performed by trained personnel, currently consisting of a lab director/supervisor/analyst (Jane Wurm). The Lab Director monitors and shares in defined bench-top work assignments to ensure that test procedures are precisely followed and that quality control measures are incorporated into the analyses. The Lab Director is responsible for ensuring the laboratory quality assurance program is followed. Job descriptions and training records for laboratory personnel are maintained on file, as a resume of pertinent experience, and are updated at least annually. Any additional training received between updates is documented in the personnel files and on the SOP sign-off sheet maintained in the Standard Operating Procedures Manual. Laboratory personnel are subject to annual performance evaluation reviews. Copies of performance evaluations are maintained on file with the training records. All Laboratory personnel participate in an annual Laboratory Ethics Training Program which is documented in the Laboratory Ethics Training Manual. Lab personnel are encouraged to take additional training for the advancement of skills and knowledge. The Lab Director is responsible for record keeping for laboratory services and archival of lab data. The Lab Director reviews all reports generated by support personnel for accuracy, and monitors record keeping procedures to ensure compliance with SOPs and appropriate regulatory guidelines.

1.8 Analytical Test Procedures

For the analysis of potable and non-potable waters, the analytes and methods employed are: Total Coliform by MF-SM9222B and E.coli by NA-mug -SM9222G, and for ambient and wastewaters the Fecal Coliform method employed is MF-SM9222D and the Enterococcus method used is EPA Modified Method 1600.

Section 2 - Laboratory Equipment and Instrumentation

2.1 Autoclaves

The autoclave is of sufficient size to prevent crowding of the interior. It is constructed to provide uniform sterilizing temperature of 121ºC within the chamber. The autoclave is equipped with digital readouts of pressure, temperature and time. Autoclave cycles must be completed within 45 minutes when a 15 minute sterilization cycle time is used, such as with sugar broths. Records are maintained in the Autoclave Logbook for each sterilization cycle and include items sterilized, date, start-up time, time temperature reached, time temperature off, time removed from autoclave, total time in autoclave, maximum registering thermometer temperature, heat sensitive tape usage and user initials. Sterilization effectiveness is monitored using spore ampules weekly (Standard Methods, 22nd Ed., 9020B section 4h) with results maintained in the Autoclave Logbook. The timing operation of the autoclave is checked against a stopwatch or accurate timepiece quarterly, with results recorded and maintained in the Autoclave Logbook. Periodic inspection is performed by qualified service representatives. Servicing is conducted whenever a deviation of sterilization time or effectiveness is observed and records of corrective action are maintained in the Autoclave Logbook.

2.2 Balances

Operation and routine maintenance is performed in accordance with manufacturers instructions. Balances and weights are serviced and checked annually by the Sealer of Weights and Measures. Balances are calibrated monthly with a set of S weights traceable to NIST and records of calibrations are maintained in the Balance Logbook. If balance calibrations vary from true values, adjustments are made to re-center the bubble and re-calibration is performed. Continued variance from true values will result in servicing, with records maintained in the Balance Logbook. In weighing less than a gram, an analytical balance with a sensitivity less than 1 mg at a 10 g load is used. For larger quantities, a top-loading balance with sensitivity of 0.1 g at a 150 g load is used. The accuracy of the analytical balance is checked each day it is to be used with

three reference weights and records are maintained in the balance logbook. The balance is wiped before and after each use with a soft brush. Spills are immediately cleaned-up with a damp towel. Weights are inspected with each use and are discarded if corrosion is observed. Weights are protected from manual contact, corrosion and laboratory atmosphere.

2.3 Colony Counters and Tallies

A standard colony counter, Quebec model, providing magnification and good visibility is used for heterotrophic plate counts. Grid plates are inspected for scratches. Internal mirror placement and cleanliness is maintained to ensure optimal illumination. Hand-held colony tallies are used and checked with each use to ensure accuracy. For 50mm plates, a Spencer dissection microscope with 10X magnification and fluorescent light source is used in counting colonies. Maintenance records are maintained in the Microscope Logbook.

2.4 pH Meter

An electronic pH meter (Orion Star A211) graduated in 0.01 pH units with temperature compensation and reference electrode is used. Commercial buffer solutions are used to standardize the meter daily when in use. Three buffers which bracket the pH of interest are standard practice. Also included in the calibration is a record of the slope and mV readings as a check of electrode accuracy. If the slope falls outside the range of 95% to 105%, the cause is investigated, corrective action is taken and documented in the pH meter Logbook. Records of pH meter calibrations are maintained in the pH Meter Logbook. Buffer solutions are dated when opened and are used prior to the manufacturer's expiration date. The lot numbers for each set of buffers in use is recorded at the end of the pH Meter Logbook. Buffer solutions are discarded after each use. Glass electrodes are stored in pH buffer solution when not in use. All samples and buffers are brought to room temperature prior to measurements. In between each sample or buffer, the electrodes are thoroughly rinsed with distilled water and wiped with a soft tissue. The maintenance of electrodes is performed as described in Standard Methods, 22nd Ed., 9020B,4c. Precision and accuracy checks are conducted at least daily when the pH meter is in use and results are recorded in the Laboratory Precision and Accuracy Logbook. When unacceptable results are obtained from precision or accuracy measurements, the cause is investigated, corrective action taken and recorded in the Precision and Accuracy Logbook.

2.5 Laboratory Refrigerators

The temperature of the laboratory refrigerators is checked and recorded twice daily. Each laboratory refrigerator is assigned a unique number and has its own temperature recording logbook. Refrigerators are cleaned monthly. All materials stored are identified and dated. The lab

refrigerators are used to cool and maintain samples at 4º +/- 2 ºC until tested and to store prepared media, reagents and control cultures. Refrigerator temperatures are measured using two thermometers with bulbs immersed in glycerin solution, in glass vials, on upper and lower shelves. If refrigerators show unacceptable temperature ranges, a service call is made to a repair technician and servicing is recorded in the Refrigerator Logbook.

2.6 Water Distillation and Deionization Systems

The laboratory water still (Barnstead MP-1) is fed from New Bedford city tap water. The still is drained and cleaned after each 3-5 days usage using a 10% HCl acid solution according to SOP #3.31 (1) and manufacturers instructions. Cleaning dates are recorded, with technician initials, in the system logbook. The distilled water produced is used to feed the laboratory water deionization system (Barnstead E-Pure). The E-Pure system combines pre-filtration, mixed-bed resins, activated carbon and aseptic final filtration with a 0.22 um pore membrane to produce a reagent grade microbiologically suitable water. The water deionization system is monitored daily when in use, by observing the resistivity reading displayed on the electronic meter and recording results in the system logbook. The reagent grade water produced is analyzed annually for trace metals (Cd, Cr, Cu, Pb, Zn and Ni). The E-Pure system cartridges are replaced at intervals recommended by the manufacturer or as indicated by analytical results. The reagent grade water is tested monthly for conductivity, and each time a new lot of media is prepared (must be < 2 uS/cm at 25ºC), residual chlorine (must be < 0.1 mg/L), pH and heterotrophic plate count (must contain < 500 cfu/mL). All reagent grade water quality control tests are performed in accordance with 310 CMR 42.08(5)(c)12 and Standard Methods for the Examination of Water and Wastewater, 22nd Ed., 2012 and pertinent laboratory SOPs. Records are maintained in the laboratory Water Systems Logbook. Any deviations from prescribed test parameter limits will result in investigation accordingly and corrective action with records maintained.

2.7 Waterbaths

Each laboratory waterbath is equipped with a gable cover and is identified with a unique number. Waterbaths have an adequate capacity for the workload. The level of water in the baths is kept above the level of liquid in incubating tubes. Waterbath temperatures are maintained at 44.5ºC +/- 0.2ºC. Temperatures are recorded twice daily with records maintained in the Waterbaths Logbook. Deviation from prescribed temperature maintenance is investigated and rectified by repair from an authorized service representative with records maintained. The thermometers used to monitor the temperatures of the waterbaths are graduated in 0.1ºC increments. Waterbath working thermometers are calibrated annually against the laboratory standards thermometer (traceable to NIST) at 44.5ºC and calibration records are maintained in the Thermometers Logbook.

2.8 Incubators

All laboratory incubators are identified with a unique number. Incubator temperatures are checked twice daily (morning and afternoon) on the shelf areas in use (top and bottom). Records are maintained in the logbooks specific for each incubator. Thermometers are immersed in water to the stem marking. Laboratory incubators are kept in areas where the temperature is maintained at 16 to 27ºC. The incubator working thermometers are calibrated annually against the laboratory standards thermometer (traceable to NIST) at the appropriate operational temperature, and calibration records are maintained in the Thermometers Logbook. Thermometers used in air incubators are graduated in 0.1ºC increments. Incubators are loaded so that there will be at least 2.54 cm of space between stacks of plates and from incubator walls. Humidity levels of incubators are maintained such that SPC agar plates do not lose more than 15% of agar weight after 48 +/- 2 hours of incubation at 35ºC. Agar weight loss is determined biannually and records are maintained in the laboratory Quality Assurance Manual. Deviation from prescribed temperatures or agar weight loss is investigated and remedied by corrective action or servicing if necessary, with records maintained.

2.9 Laboratory Fume Hoods

Hoods are located away from high traffic areas as a fire safety and hood velocity integrity measure. Fume hood face velocity should be at least 30.5 linear meters/minute and are checked annually with an airflow measurement device. No airflow restrictive devices shall be placed in the hood. The laboratory fume hoods are made of corrosion resistant materials.

2.10 Membrane Filtration Equipment

Before use, filtration units are assembled and checked for leaks. Units are discarded if inside surfaces are scratched or cracked. Filtration assemblies are washed and rinsed thoroughly after use, wrapped in non-toxic paper or foil, and sterilized by autoclaving. Membrane filter funnels are numbered and marked at appropriate graduations. Volume accuracy is determined by weight annually and documented in the Funnel Logbook. Tolerance must not exceed 2.5%.

2.11 Laboratory Thermometers

Accuracy of thermometers is checked annually against a NIST traceable thermometer. The NIST traceable thermometer is checked annually for accuracy at ice-point (distilled water ice). For incubators and waterbaths, thermometers with 0.1ºC increments are used. All thermometer incubation records are maintained in the laboratory Thermometers Logbook. Calibration corrections for each thermometer are marked on the thermometer identification tag, including

calibration date, ID number and calibration temperature. Working thermometers are appropriately immersed in stoppered glass tubes containing water, or in waterbaths above the stem marking. Metal field thermometers are calibrated against a NIST traceable working thermometer quarterly. The calibration check is documented in the Field Thermometer Logbook. A field thermometer which has greater than a 5° correction factor is discarded.

2.12 Pipettors

Eppendorf fixed and adjustable volume pipettors are used throughout the laboratory for dispensing of aqueous aliquots in the range of 10 uL to 1000 uL. The pipettors are assigned a unique ID number and are calibrated quarterly, using ten weighings each, on an analytical balance. Calibration results are recorded and maintained in the laboratory Pipettor Logbook. Pipet tips (Eppendorf) are placed into autoclavable trays and are sterilized prior to use.

2.13 Conductivity Meter

A YSI Model 30 Handheld SCT meter, SN 04F10421, is dedicated to the use of monitoring low level conductivity of the laboratory reagent grade water. The meter is calibrated monthly, using single-use, low level calibration standard. The conductivity probe is maintained with extraordinary cleanliness by thorough cleaning with distilled/deionized water and cleaning with a mild acid solution (per manufacturer instruction manual) as dictated by any deviation observed during calibration checks. If erroneous measurements are observed despite these measures, the meter is returned to the manufacturer for servicing. Records of calibration, maintenance and servicing are maintained in the laboratory Conductivity Meter Logbook.

2.14 Dissection Microscope

A Spencer Cycloptic Dissection Microscope with 10X-15X objective and Cyclospot Illuminator is used to count sheen colonies on m-Endo agar medium or enterococcus colonies on mEI medium. The microscope and cool light illuminator are maintained according to the Manufacturer Reference Manual (American Optical Co.) and serviced when necessary (ie; inability to focus) by an authorized factory representative. The microscope lenses are inspected for smears and dust particles with use and cleaned with lens cleaner and a lint free cloth. Maintenance and service records are maintained in the laboratory Microscope Logbook.

Section 3 - Laboratory Supplies

3.1 Glassware and Plasticware

Prior to each use, glassware is examined and items which are scratched, cracked or have chipped edges are discarded. Dilution bottles are made of borosilicate glass and are indelibly etched at the graduation level of 99 +/- 1 mL. Leakproof stopper closures are used with dilution bottles. Petri dishes are 12 mm deep and have bottoms of at least 80 mm inside diameter. Petri dishes for membrane filtration work have a 47 mm inside diameter. Petri dishes, whether sterilized glass or sterile plastic, are inspected for bubbles, scratches or cracks. Pipets are of glass or sterile plastic and are operated manually with a pipet aid. Pipets are marked to sharply contrast with pipetted solutions. As a general rule, for delivery of 1 mL volumes, pipets of no greater than 10 mL are used and for 0.1 mL volumes, pipets no greater than 1 mL are used. Manual use pipets have straight walls and tips are inspected for cracks to ensure accurate volume delivery. Disposable pipets and Eppendorf pipet tips are used one time only, placed in a disinfectant solution and are disposed of. Reusable glassware and plasticware is decontaminated if necessary and washed. In washing reusable sample containers, glassware and plasticware, a minimum of four fresh water rinses and a final rinse in distilled water is used to thoroughly rinse off all residual detergent. This is accomplished by running two complete wash and rinse cycles (the second cycle does not utilize detergent) in the lab dishwasher, with a final purified water rinse.
To ensure glassware cleanliness, the following quality control checks for clean glassware are made:

a) pH check - batches of clean glassware are spot checked for pH reaction by adding a few drops of 0.04% bromothymol blue (BTB) and observing the color reaction. BTB should be blue-green, in the neutral range. BTB indicator solution is purchased commercially or is prepared by adding 16 mL 0.01N NaOH to 0.1 g BTB and diluting to 250 mL with distilled deionized water. Records of pH checks are maintained in the laboratory Glassware Washing Logbook. If deviation from the prescribed pH range is observed, the cause is investigated by replacing reagents and reviewing the rinse procedure.

b) Check for Inhibitory Residues - the test is performed before using a new supply of detergent, to ensure the effectiveness of the rinsing procedure. The inhibitory residue test is performed according to Standard Methods for the Examination of Water and Wastewater, 22nd Ed., page 9-11, and laboratory SOP #4.11(1). Records of the Inhibitory Residue Test are maintained in the Glassware Washing Logbook. If inhibitory residue results, the cause is investigated by reviewing the detergent in use and the rinse procedure, with records of corrective action maintained.

After washing, glassware is inspected for excessive water beads and rewashed if necessary. All glassware and plasticware (polypropylene), including reusable sample containers, which have been exposed to bacteriological agents are autoclaved for 30 minutes prior to storage. The

sterility of reusable sample containers is determined with each autoclaved batch by addition of Tryptic Soy Broth to a sample container followed by incubation at 35°C for 24 and 48 hours. Results of sterility checks are recorded and maintained in the laboratory Autoclave Logbook. The sterility of irradiated disposable plasticware is determined with each new manufacturer lot. Laboratory disposables are also subjected to lot comparison/productivity tests with records maintained in the Laboratory Disposables Logbook. Lack of sterility will prompt immediate investigation into the sterilization procedures and autoclave maintenance, with servicing by an authorized service technician. Servicing and maintenance records are maintained.

3.2 Membrane Filter Equipment

Membrane filtration units are assembled and checked for leaks prior to use. The filtration units contain clearly marked graduations for measuring sample aliquots. Following use, the filtration units are washed and re-autoclaved prior to storage and reuse. When membrane filtration units are in use they are changed in between each series of samples or at a minimum of every 30 minutes. Filtration assemblies are autoclaved in non-toxic paper or aluminum foil for 30 minutes. Membrane filters are purchased through Thermo Fisher Scientific and come provided with certification for each new lot, which includes retention, pore size, flow rate, sterility, pH, percent recovery and limits for specific inorganic chemical extractables. The lot number of the filters and usage dates is recorded in the Membrane Filters Logbook. Volume accuracy of filtration units is checked annually and recorded in the Membrane Filtration Funnels Accuracy Logbook, with tolerance not to exceed 2.5%. The funnels are marked at the measured volume. Use of the membrane filters and apparatus is performed in accordance with laboratory SOP's. When a new lot of membrane filters is used, comparison tests of the lot in use is made against the new lot as described in Standard Methods for the Examination of Water and Wastewater, 22nd Ed., page 9-14.

3.3 Reagent Grade Water Quality

The laboratory reagent grade water supply is monitored for acceptability criteria as outlined in Standard Methods, 22nd Ed., Table 9020:II, page 9-12. The resistivity reading on the electronic display of the E-Pure deionization system is checked with each use to ensure a value of >16.7 megaohms/cm (per manufacturer instructions). Conductivity is monitored to ensure a value of <2 umhos/cm at 25°C. Records are maintained in the Conductivity/Salinity Logbook. The pH is checked with each pH meter calibration to be between 5.5 and 7.5 and recorded in the pH Meter Logbook. A heavy metals analysis (Cd, Cr, Cu, Ni, Pb and Zn) is performed annually and records are maintained in the Reagent Grade Lab Water Logbook. The metals analyzed must be < 0.05 mg/L. The total residual chlorine is determined monthly and records are maintained in the Reagent Grade Lab Water Logbook. The value obtained for the total residual chlorine must be

less than 0.1 mg/L. A heterotrophic plate count (HPC) is conducted monthly and records are maintained in the Reagent Grade Lab Water Logbook. HPC results must be <500 CFU/mL.

3.4 Laboratory Control Cultures

Laboratory control cultures are purchased commercially as inoculated swabs, or equivalent, bearing an ATCC reference number. The control cultures are revived and incubated on appropriate agar media slant tubes or broth tubes for 24 hours at 35°C. Control cultures are stored refrigerated and are aseptically transferred bimonthly to fresh agar slants or broth tubes. Each new set of control cultures is assigned a lot number based on the preparation date. Records for control cultures are maintained in the Control Cultures Logbook. For each new lot of prepared culture medium, the analytical procedures are tested with known positive and negative control cultures for the organism(s) under test. Control cultures used for each type of test are as follows:

Test	Positive Control	Negative Control
Total Coliforms	E. coli or Enterobacter	Staphylococcus
Fecal Coliforms	E. coli	Enterobacter
E. coli	E. coli	Enterobacter
Fecal Streptococci	Enterococcus faecalis	Staphylococcus or E. coli
Enterococci	Enterococcus faecalis	Staphylococcus or E. coli

Section 4 Media and Reagents

4.1 Reagents

Only reagents and chemicals of ACS grade are used for analyses of test samples. All reagents are prepared in distilled/deionized water and clean glassware, following pertinent laboratory SOP's. All chemicals and reagents are dated when received and when first opened for use. Reagents are made to volume using Class A volumetric glassware. Preparation details are recorded in the laboratory Media and Reagents Preparation Logbook. For storage, good-quality inert plastic or borosilicate glass bottles with tight fitting caps are used. Prepared reagents are labeled with name, concentration, date prepared, lot number, preparer initials, expiration date, storage conditions, and chemical hazard rating (if applicable) according to NFPA recommendations.

4.2 Culture Media

Commercially prepared media is used wherever possible for control of quality. Media is ordered in quantities to last no longer than 2 years. Upon receipt, media bottles are labeled with the date of receipt and the date when opened. The new media is logged into the Media Receipt Logbook with Manufacturer, lot number, amount received, date received, date opened and date discarded. Media that appears caked or exhibits signs of deterioration are discarded. The manufacturer's lot numbers are recorded on media preparation forms (maintained as raw data in the Media Preparation Logbook). Opened bottles of media are used within 2 years of opening. Culture medias are stored in a Fisher Scientific desiccator cabinet containing desiccant to protect dehydrated medias from humidity. Storage of unopened medias is no longer than the manufacturers expiration date. Newly purchased lots of media are compared against proven lots using recovery of pure culture isolates and/or natural samples.

a) Media Preparation - Medias are prepared in containers that are at least twice the volume of the media being prepared. Media are stirred while heating, either on a hot plate or in a waterbath. Prepared media is identified, dated, and assigned a lot number in the Media Preparation Logbook. Media lot numbers are recorded on raw data worksheets. All medias are prepared in distilled, deionized water which has been measured with pipets or graduated cylinders conforming to NIST and APHA standards. The pH of a portion of media from each lot is checked after sterilization and recorded in the Media Preparation Logbook. Minor adjustments in pH are made with 1M NaOH or HCl solution. The media is remade if large deviations in pH are observed (> 0.5 pH units). Prepared medias are examined for unusual color, darkening or precipitation and discarded if problems are noted. Prepared medias are labeled with lot numbers and expiration and are stored at 4° +/- 2° C.

b) Media Sterilization - Media is sterilized at 121^oC for the minimum time specified. A double walled autoclave maintaining full pressure and temperature in the jacket between loads is used to reduce chances of heat damage. Manufacturer's directions are followed for sterilization of specific medias. Autoclave times other than specified by the manufacturer are in accordance with Standard Methods, 22nd Ed., pg 9-15, Table 9020:IV. Sterilized media is removed from the autoclave as soon as chamber temperature reaches zero. Media is never re-autoclaved. An Autoclave Logbook is kept detailing specifics of each batch, including actual sterilization time and total autoclave time. Effectiveness of sterilization is monitored using indicator tape and an autoclave thermometer. Weekly spore ampule testing for autoclave effectiveness is documented in the Autoclave Logbook. If growth of autoclave spores occurs, then sterilization procedures are deemed inadequate and an authorized service representative is called for

corrective action. As a further check of sterility, media controls are run with each series of test samples and media controls are run for each new lot of media prepared. Results of controls are recorded on raw data worksheets accompanying test samples. If growth occurs on media controls, the source of contamination is investigated, preparation of the media and analytical procedures are reviewed and new samples are requested.

c) Media Use - Melted agars are tempered in a waterbath until use but are not held longer than 3 hours. A control bottle of media containing a thermometer is exposed to the same heating and cooling conditions as the media to be used in pouring plates. Sterile fermentation tubes are carefully handled and examined before use to ensure that gas bubbles are absent. Fermentation tubes stored refrigerated are incubated overnight and checked for the presence of gas prior to use. Tubes showing gas are rejected and a new lot of fermentation media is prepared.

d) Media Storage - Sterile media is prepared in amounts that will be used within holding time limits. If fermentation tube media is refrigerated, the tubes are incubated overnight before use and checked for false positives. Media that can be stored for more than one week is prepared in tightly capped screw top tubes or bottles. Pre-poured media plates are sealed in plastic bags, inverted and refrigerated to retain moisture. Holding time for pre-poured media plates is two weeks, except for NA-mug which can be held for one week. Media containing dyes are protected from light and discarded if color changes are observed. Holding times for prepared media follow those specified in Standard Methods, 22nd Ed., pg 9-16, Table 9020:V.

e) Media Quality Control - Media preparation records are maintained in a bound logbook with the name and date of preparer, name and lot number of the medium, amount weighed, volume and solvent used, pH measurements, balance used and any other adjustments. Sterility and positive and negative control culture checks are included for each lot of media used in sample analysis. Deviations from expected results are investigated by reviewing preparation and analytical procedure and documenting corrective action.

4.3 Dilution Water

Stock phosphate buffer solutions are stored refrigerated in tightly capped 100 mL screw top bottles for up to 6 months. Working phosphate buffer solutions are prepared from the stock, in reagent grade water, and are sterilized by autoclaving 30 minutes. Each batch of dilution/rinse water is checked for sterility by adding 50 mL of sterile, double-strength non-selective broth, which is then incubated at 35 +/- 0.5ºC and checked for turbidity at 24 and 48 hours and the

results recorded. Dilution water is stored at room temperature in screw cap bottles for up to 6 months. Any bottles observed to have turbidity are discarded. Dilution water controls are checked with each use and the lot is discarded if any growth is observed. Dilution water is prepared in accordance with laboratory SOP # 4.13.

Section 5 - Sampling Procedures

5.1 Sampling Containers

Samples are collected in clean, distilled water rinsed, sterilized bottles of borosilicate glass or non-toxic polypropylene plastic, with screw cap closures. Sodium thiosulfate (0.1 N) is added, prior to sterilization, to containers intended for the collection of water samples containing residual chlorine or other halogen requiring neutralization. Sample containers are sterilized by autoclaving for 30 minutes. The sterility of sample containers is verified, per each batch, by adding 25 mL sterile, non-selective nutritive broth to a container, rotating the media within the container and incubating for 24 and 48 hours. Results are recorded at each interval and maintained in the QA Logbook. If growth is observed, the sterilization process is reviewed, with corrective action documented in the QA Logbook.

5.2 Sample Collection

Samples are collected leaving ample air space in the bottle (2.5 cm) to facilitate mixing by shaking prior to examination. Sample ports are flushed or disinfected in order to collect samples representative of the water being tested. Sample bottles are kept closed until ready to be filled. Contamination of the inner surface of the cap or neck of the bottle is avoided. Sample containers are filled and immediately capped. The volume of samples collected is sufficient to carry out all tests required. Sample containers are labeled with an identifying number or location, time and date of collection. Other pertinent sampling information is included on a field data collection form. In collecting samples from recreational areas, samples are taken at a uniform depth of approximately 1 m. Waders and shoulder length disposable gloves are available for sampling in contaminated areas. When sampling from other non-potable sources (rivers, streams, lakes or ponds) samples are taken by holding the bottle near its base in the hand and plunging it, neck downward, below the surface. The bottle is turned until the neck points slightly upward and directed towards the current, or pushing bottle forward in a direction away from the hand. A chain of custody form must accompany all samples including those shipped by mail or courier. Upon receipt of samples in the laboratory, the chain of custody is completed and signed. Samples are then logged in with a unique identification number, which follows the samples throughout

the testing and reporting procedures. For this purpose, a Sample Receipt & Identification Logbook is maintained in the laboratory. The sample ID numbering system uses the month and year of sample receipt with the chronological number of samples received for the month attached.

5.3 Sample Preservation and Storage

Microbiological examination of water samples is initiated as soon as possible after collection. An iced cooler and a temperature control blank is used for transportation to the laboratory if analysis cannot be initiated within one hour. Holding times for specific water types are as follows:

a) Drinking water for compliance purposes - Samples are iced in a cooler during transit to the laboratory and refrigerated upon receipt. Samples are analyzed on the day of receipt. Maximum holding time will not exceed 6 hours from the time of collection to the time of analysis for coliform bacteria.

b) Non-potable water for compliance purposes - Source water, stream pollution, recreational water and wastewater samples are kept iced in a cooler during a maximum transport time of 6 hours. Upon arrival at the laboratory, a temperature control blank is checked for adherence to the storage temperature requirement of 1 – 4ºC. Samples are refrigerated upon receipt at the laboratory and processed within 2 hours.

Samples which fail to meet the preservation and storage requirements are rejected. The sampling entity is notified and new samples are requested.

Section 6 - Laboratory QA/QC Testing

6.1 Microbiological Quality Control Procedures

a) **Plate Count Comparison** - Counts on one or more positive samples is conducted monthly and compared with those of other analysts testing the same samples. Replicate counts for the same analyst should agree within 5% and those between analysts should agree within 10%. Statistical calculation of data precision is performed in accordance with Standard Methods Section 9020B.13b. If replicate counts do not fall within prescribed limits, colony counting equipment is inspected, the plate counting procedure is reviewed and corrective action is documented.

..

b) **Control Cultures/Positive and Negative Controls** - Each new lot of medium is checked by testing with known positive and negative control cultures for the organism(s) under test. Specific control cultures are referenced in Section 3.4 of this manual. If a new lot of medium does not perform appropriately, control cultures are replaced with fresh cultures and the preparation of the media employed is reviewed. Any corrective action taken is documented in the Control Culture Logbook.

c) **Duplicate Analysis** - Duplicate analyses are performed on 10% of samples and on at least one sample per test run - defined as an uninterrupted series of analyses. Calculation of data precision is as described in part e) below. If duplicates do not fall within data precision criteria, the analytical procedure is reviewed and corrective action is documented in the Precision and Accuracy Logbook.

d) **Sterility Checks** - For each series of analyses, the sterility of media, pipets, rinse water and glassware, as appropriate, is checked. If any contamination is indicated, the cause is determined and the analytical data is rejected.

e) **Precision of Quantitative Microbial Methods** - Duplicate analyses is performed on 15 positive samples undergoing quantitative microbial analyses by membrane filtration procedures. The logarithm of each duplicate result is calculated and used to determine the range (R) for each pair of transformed duplicates. The mean range of the transformed duplicates is used in the equation 3.27 (R) to establish precision criterion. If the defined precision criterion is exceeded then analytical results are discarded and the problem is identified and resolved prior to making further analyses. Precision criterion is updated for each new set of 15 duplicate results.

f) **Method Verification** - For membrane filtration procedures with new lots of commercial media, positive samples may be verified by picking at least 10 representative colonies for transfer to selective mediums. Results are scored and counts are adjusted based on percentage of verification.

g) **Laboratory Precision and Accuracy for Physical/Chemical Parameters** - The laboratory establishes acceptance limits for precision and accuracy and maintains quality control charts for the various physical/chemical parameters commonly analyzed in conjunction with microbial analyses (ie; pH, salinity, turbidity, chlorine, etc.) to assess the validity of the analytical process. For precision, duplicate analyses are employed as described in section c) above, for calculation of the closeness of agreement between repeated measurements. Standard deviations are applied to mean percent deviation in establishing upper and lower control limits for acceptable precision criterion. Laboratory

standards are utilized to establish accuracy criterion, as the closeness of a measurement to the true value, in the assessment of possible systematic error. Standard deviations are applied to mean percent recoveries in defining upper and lower control limits. Measurements are acceptably accurate when both systematic and random errors are low. QC results outside the acceptability limits warrant investigative and corrective action prior to further analyses. Equipment and the analytical procedures used are examined and corrective actions are documented in the laboratory Precision and Accuracy Logbook.

h) **Annual Proficiency Tests** - The laboratory participates in annual Proficiency Tests with a DEP approved Proficiency Test Provider, to demonstrate microbiological proficiency in the analysis of total coliform and fecal coliform in potable and non-potable water samples and enterococcus in ambient water samples. The laboratory reports the specific analytical method and media used for each PT round to the Provider, and reports results of each sample analysis as coliform present or absent, and if coliform present, either present or absent for fecal coliform. The acceptability of results is determined by the PT Provider. Results must be sent directly from the PT Provider to the DEP Lab Certification Office (LCO). Acceptable performance for total and fecal coliform is defined as the correct analysis of at least 90% of the samples in each testing round with no false negatives. If a PT round is determined by the PT Provider to be not acceptable, the laboratory, within 30 days of receiving notification of the failure, will determine the cause of error by checking media preparation, reviewing the analytical procedures used and checking for possible contamination. Upon taking corrective action, the DEP LCO will be notified in writing describing the corrective actions taken. In this instance, the laboratory will participate in a new PT round and report results to the Provider and the DEP LCO. The follow up PT round is completed within the same calendar year, from 30-90 days after the initial failed PT notice.

Section 7 - Standard Operating Procedures

7.1

Written Standard Operating Procedures (SOPs) are maintained in a Laboratory SOP Manual, and are provided to each analyst. For analysts in training or for routine reviews an SOP “read and understood” signing sheet is included in the SOP Manual. The SOPs describe in a detailed step-wise fashion all laboratory operations involving instrumentation, reagents, analytical methods, QA/QC, calculations and reporting requirements to assure uniform operations between analysts and analyses. The SOPs specific for recreational water testing are:

SOP # 4.42 - Monitoring New Bedford Bathing Beaches: Collection, Analysis, Quality Assurance, Regulations and Reporting.
SOP # 4.48 - Bacteriological Examination of Environmental and Recreational Waters for 24 Hour Enterococci Using EPA Method 1600 and the Membrane Filtration Technique.
SOP # 4.50 - Data Reduction, Handling and Reporting Specific To Contractual Compliance With The MDPH Beach Program Quality Assurance Project Plan.

The current complete list of Laboratory SOPs are as follows:

1.1 How to Write an SOP
1.2 Data Recording, Handling and Storage
1.3 Ensuring Compliance with GLPs
1.4 Sample Intake Procedures for Well Waters
3.15 Orion Star A211 Series pH Meter
3.16 Sartorius Cubis Model MSE1202S-100-DO Toploader Balance
3.17 SOP for Incubators
3.18 SOP for Waterbaths
3.21 SOP for Thermometers Used in Testing
3.31 Barnstead Mega-Pure Water Still and Barnstead Nanopure II D3077 Series
3.32 Hach DR 2010 Spectrophotometer Test for Free and Total Chlorine
3.34 Use, Calibration and Maintenance of the YSI Model 57 Dissolved Oxygen Meter
3.39 Calibration of Eppendorf Pipettors
3.45 Use of the LaMotte 2020 Turbidometer
3.48 Use of the YSI Model 30 Handheld Salinity, Conductivity & Temperature Meter
4.9 Bacteriological Examination of Shellfish Samples
4.10 Bacteriological Examination of Seawater by the Medium A-1 Method for Fecal Coliform
4.11 Washing Glassware Used in Shellfish and Waters Testing
4.12 SOP for Heterotrophic Plate Counts
4.13 Preparation of Phosphate Buffered Dilution Water (PBW) and Phosphate Buffered Saline (PBS)
4.26 Bacteriological Examination of Potable Water for Members of the Total Coliform Group on mEndo Agar LES, SM 9222B,Using the Membrane Filtration Technique and Rapid Confirmation of E.coli using SM 9222G on NA-Mug medium.
4.27 Bacteriological Examination of Environmental Waters for Members of the Fecal Coliform Group Using the Membrane Filtration Technique
4.28 Bacteriological Examination of Environmental, Recreational, Drinking and Ground Waters for Confirmatory Testing of Total and Fecal Coliforms for E. coli

4.30 Bacteriological Examination of Seawater and Pollution Sources for Fecal Coliforms and E. coli Utilizing the mTec Membrane Filtration Method
4.41 LYFO-DISK Microorganisms for Bacteriological Control Cultures
4.42 Monitoring New Bedford Bathing Beaches, Collection, Analysis, Quality Assurance, Regulations and Reporting
4.43 Bacteriological Examination of Environmental Waters for the 48 hour Fecal Streptococci/Enterococci Using the Membrane Filtration Technique
4.44 Bacteriological Examination of Seawater Holding Tanks by the Double Strength Media MPN Total Coliform Test
4.48 Bacteriological Examination of Environmental, Wastewater and Recreational Waters for 24 hour Enterococci Using EPA Method 1600 and the Membrane Filtration Technique
4.49 Total Suspended Solids EPA Method 160.2
4.50 Data Reduction, Handling and Reporting Specific to Contractual Compliance with the MDPH Beach program Quality Assurance Project Plan

Section 8 - Documentation and Recordkeeping

8.1

The documentation and recordkeeping system provides needed information on sample collection and preservation, analytical methods, raw data, calculations through reported results, and a record of persons responsible for sampling and analyses. All records are kept in bound notebooks, with entries in ink, and corrections made by drawing a single line through any change with the correction entered next to it. Laboratory raw data worksheets are bound with copies of computer generated reports. Reports generated from laboratory raw data are checked for accuracy and are signed and dated. All bound laboratory notebooks are archived by fiscal year and maintained on laboratory premises for a period of ten years. Electronic databases are kept secure in a locked office.

Section 9 – Reporting

9.1 - Representation

When reporting results for an analyte/method in which the laboratory does not hold MassDEP

certification a statement is included on the report which expressly states such analysis was not conducted in accordance with DEP certification standards but rather with pertinent laboratory Standard Operating Procedures.

9.2 – Notifications

Commercial and private clients- Any data indicating an exceedance of a regulatory limit is reported as soon as possible, not to exceed 24 hours after completion of sample analysis. The laboratory will document the date, time and manner of notification on the sample analysis data form.
Municipal drinking water systems- Client town and MADEP are notified by phone and email immediately.

Section 10 – Laboratory Ethics Training

10.1

On an annual basis all personnel will participate in a laboratory ethics training program. Training includes ensuring data integrity, recognition and prevention of improper laboratory practices, promotion of objectivity in the generation and reporting of analytical data and procedures for confidential reporting of data integrity concerns to the laboratory supervisor/director. The Laboratory Ethics Training Program Manual is maintained in the location of the Laboratory Quality Assurance Manual, Lab Safety Manual, and Lab SOP Manual. The ethics training manual includes a signature/date sheet as well as pertinent training materials used.

QUALITY ASSURANCE PROGRAM PLAN

ENVIROTECH LABORATORIES, INC.

8 Jan Sebastian Dr unit 12

SANDWICH, MA 02563

REVISION DATE:
09/28/2017

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SECTION IV -- GENERAL SAFETY

1. Fire Prevention
2. Instructions In Case of Fire
3. General Safety
4. Fire Extinguisher Maintenance

SECTION V—ANALYTICAL METHODS

1. Analyte
2. Method
3. Method Type
4. MDL

SECTION VI—SAMPLING AND HANDLING REQUIREMENTS

1. Determination
2. Container type
3. Minimum sample size
4. Preservation required
5. Maximum storage time
6. Sampling Instructions

Table of Attachments

1. Water Analysis Requisition
2. Chain of Custody
3. Analytical Methods
4. Summary of Special Sampling or Handling Requirements

SECTION I - QUALITY ASSURANCE

1. QUALITY ASSURANCE POLICY

The principal role of Envirotech Laboratories, Inc. is to produce valid data for setting standards determining compliance for drinking water and for monitoring wastewater and groundwater applications.
Envirotech Laboratories is committed to carry out its testing and calibration activity in order to meet the requirements of the Internal Standard Organization (ISO) as referenced in ISO/IEC 17025:2005 and to satisfy the needs of the customers and the regulatory authorities, (Massachusetts Department of Environmental Protection and the U.S. Environmental Protection Agency).

Envirotech Laboratories, Inc. follows a strict Quality Assurance Plan to include the following categories:

A. Sampling and preservation
B. Laboratory analysis
C. Quality control

a. Determining precision and accuracy
b. Evaluating daily performance
c. Evaluating laboratory review
d. Overseeing specific assignments and documentation.
e. Reporting

D. Internal audits to verify that its operations comply with the requirements of its management system, including the testing and calibration activities.

2. SCOPE

This Quality Assurance Manual presents an overview of Envirotech's QA Program. The QA Program is designed to control and monitor the quality of, data generated in the laboratory by:

* Providing information which documents the overall qualifications of the laboratory to perform analyses. This includes a description of the workplace, a summary of the credentials of the technical personnel and a list of all the equipment used for analysis.

* Establishing procedures which measure the laboratory's performance on a daily basis. These include the QA/QC analyses performed. To monitor data quality, preventive maintenance is performed on various analytical instruments. The procedures used monitor equipment such as refrigerators, balances, pH meters and ovens.

* Documenting the quality of items which may in the end affect the quality of the analytical

results.

* Describe the procedures involving data review and internal laboratory auditing.

* To review the Quality Assurance Plan and Standard Operation Procedures on a yearly basis.

* To be enrolled in certified Proficiency test Program and successfully perform unknown analytes.

ORGANIZATION, PERSONNEL, AND RESPONSIBILITIES

The attached organizational chart describes the entire structure of Envirotech Laboratories. All employees play a vital role in assuring the quality of their work and the work generated by the laboratory as a whole. Administering an effective QA program in a full range environmental laboratory demands the commitment and attention of both staff and management. The QA/QC effort of day to day operations is directed by the QA/QC Manager reporting to the Laboratory Director and General Manager. These three make any decisions involving the direction the QA Program may take and any major issues that might require operational modifications. They are ultimately responsible for the development and implementation of the QA Program described in this manual. All personnel shall be free from undo internal and external commercial, financial, or influence that may adversely affect the quality of their work. All personnel shall avoid involvement in any activity that diminishes the confidence in the competence, impartiality judgment or operational integrity. All personnel shall sign a confidentiality agreement.

Responsibilities

GENERAL MANAGER: Controls the daily operations of the corporation and is primarily responsible for client relations, financial management, and personnel issues. The primary QA/QC functions involve balancing business needs and demands with the need to provide the highest quality data based on recommendations from the QA/QC Manager. Specific QA/QC duties

include:

- Give final approval to all QA Program modifications based on recommendations from the QA/QC Manager.
- Supervise and oversee sample collection and sample control personnel to insure that all QA Program guidelines and regulatory agency requirements are followed.
- Determine client specific QA/QC requirements through communication and related regulatory agencies.
- To commit to comply with International Standard and to continually improve the effectiveness of the management system.
- Maintains records of experience, skills, educational and professional qualifications of technical and non technical personnel.

LABORATORY DIRECTOR: Responsible for day-to-day technical operations of the laboratory. The Laboratory Director must integrate the QA/QC requirements and the procedures that are part of the QA Program into the schedule with client work. Specific QA/QC duties include:

- Convey the importance of the QA Program to all technical employees and verify that all employees are aware of and follow the QA Program.
- Ensure that any specific requests from the QA/QC Manager are answered in a timely manner.
- Coordinate personnel to insure that all sample analyses are performed within established holding times.
- Review the final results generated in the laboratory prior to providing results to clients. The Laboratory Director or specific appointee must sign all final results.
- Provide proper training and documentation of employee training to insure that analysts only perform analyses for which they are qualified.
- Schedule and ensure the performance of Minimum Detection Limit Studies (MDLs).
- Require that all personnel concerned with testing and calibration activities within the laboratory familiarize themselves with the quality documentation and to implement the policies and procedures in their work to comply with international Standard.
- Ensure that its personnel are aware of the relevance and importance of their activities and how they contribute to the achievement of the objectives of the management system.
- To schedule periodically and perform internal audits of the laboratory’s activities to verify that the operations continue to comply with the requirements of the management

system and the International Standard. The audit shall address all elements of the management system, including the testing and calibration activities.

- Schedules yearly review of laboratory management system and testing and calibration activities to ensure the continuing suitability and effectiveness and to introduce necessary changes of improvements.

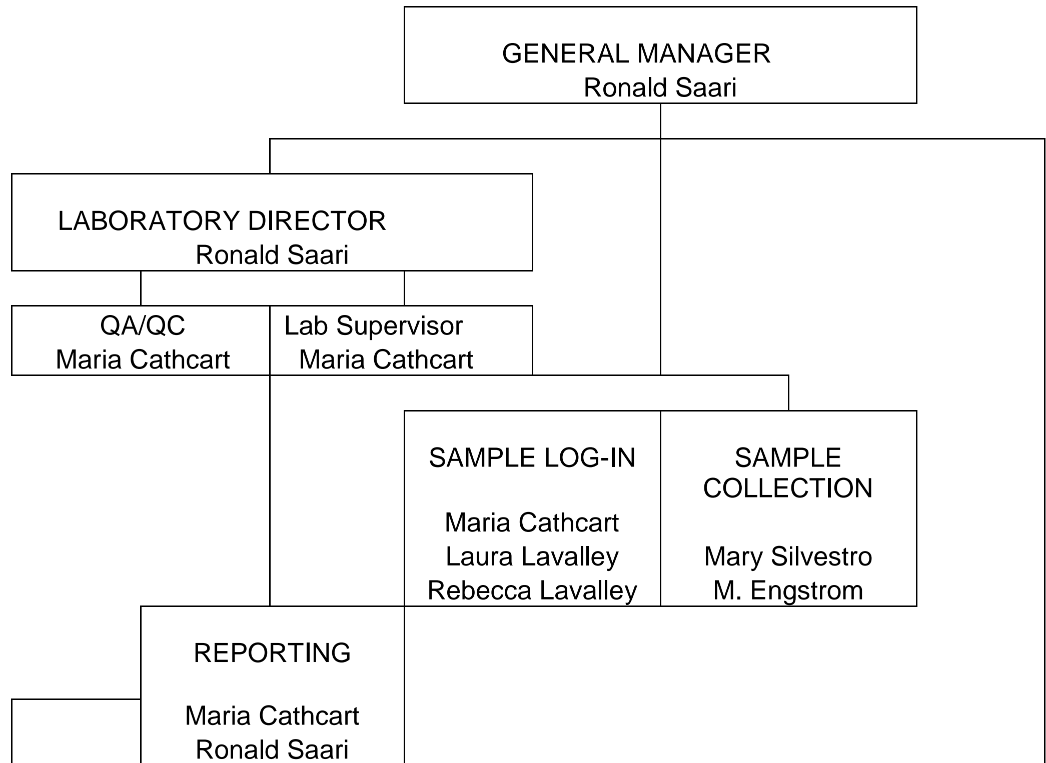
QUALITY ASSURANCE/QUALITY CONTROL MANAGER: Responsible for coordinating and overseeing all QA/QC operations to ensure fulfillment of the QA Program. The QA/QC Manager has the final authority to accept or reject any analysis result. The primary responsibilities are:

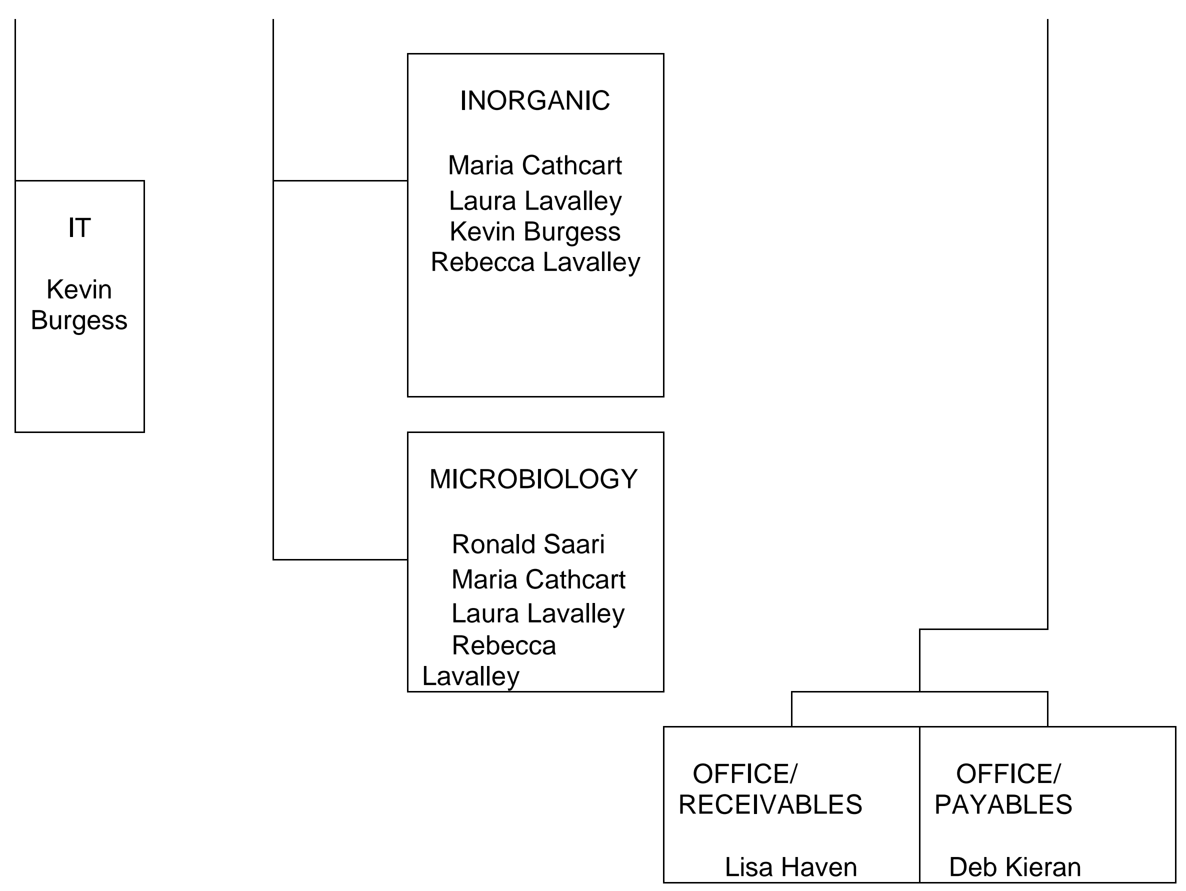
- Monitor the QA program to ensure compliance with the QA/QC objectives.
- Develop and implement new QA/QC procedures within the laboratory to improve Data quality.
- Prescribe and monitor corrective actions throughout the laboratory.
- Coordinate the writing and updating of SOPs and any QA/QC documentation.
- Schedule and conduct internal audits of laboratory operations.
- Report the results of internal audits complete with suggested corrective actions to the Laboratory Director.
- Coordinate the distribution of all Performance Evaluation (PE) samples. Coordinate the review and evaluation of all PE results prior to reporting to ensure timeliness o fall analyses.
- Review method data for any method being developed, work with the Laboratory Director to set reasonable QA/QC goals for the method, and determine final acceptability of the method.

LABORATORY STAFF: The Laboratory staff encompasses all Envirotech employees including sampling, sample control, reporting, financial, and office personnel as well as analysts

and technician. Employees at all levels and in all departments have responsibilities in the QA Program.

- Maintain a working knowledge of the QA Program.
- Ensure that all work performed is in compliance with the QA Program.
- Perform work according to the SOPs. Analytical method modifications must be reported to the QA/QC Manager prior to utilizing the modified procedures.
- Ensure that all documentation related to their work is complete and accurate.
- Provide immediate notification of any quality problems or the potential existence of quality problems.





MANAGEMENT SYSTEM

Envirotech Laboratories maintains a management system that documents its policies, systems, programs, procedures and instructions to assure the quality of the tests and/or calibration results.

The laboratory's management is committed to good professional practice and to the quality of its testing and calibration in servicing its clients and customers.

It is management's responsibility to ensure that the laboratory produces valid data for setting standards in determining compliance to drinking water and wastewater applications.

The management requires that all personnel concerned with testing and calibration activities familiarize themselves with the quality documentation and to implement the policies and procedures in their work. A yearly review (internal audit) is performed.

The management is committed to comply with the International Standard to continually improve the effectiveness of the management system.

To perform a management review at least on a yearly basis

MANAGEMENT REVIEW

A management review is performed yearly to insure continuing suitability and effectiveness of the laboratory’s management system, and testing and calibration activities, and to introduce necessary changes or improvements. The overall objectives shall be established, and shall be reviewed.
The objectives are:

The suitability of policies and procedures;

Reports from managerial and supervisory personnel;

The outcome of recent internal audits;

Corrective and preventive actions;

Assessments by external bodies;

The results of interlaboratory comparisons or proficiency tests;

Changes in the volume and type of the work;

Customer feedback;

Complaints;

Recommendations for improvement;

Quality control activities, resources, and staff training;

CONFIDENTIALITY AGREEMENT

SUMMARY

This policy covers the protection of privileged and confidential information contained in records by Envirotech Laboratories, Inc, Sandwich, MA.

POLICY

It is the policy of Envirotech Laboratories, Inc. to protect the privileged and confidential nature of laboratory information and records. Toward the end, employees may not alter, remove, destroy, view, access, photocopy, discuss or disclose confidential laboratory records without proper authorization. A violation of this policy will result in immediate discipline.

PRACTICE:

1. **Unless specifically designed otherwise by the custodian of records, all laboratory records shall be considered confidential.**

2. **No confidential laboratory information shall be disclosed to or discussed with persons inside or outside Envirotech Laboratories except in the following situations:**

   - **Where required in the regular course of business.**
   - **Where proper authorization has been obtained.**
   - **Where compelled by law, such as by subpoena, court order or search warrant.**

3. **All lab employees are required to sign a confidentiality agreement acknowledging their understanding of this policy requirement as described in the confidentiality agreement.**

CONFIDENTIALITY AGREEMENT

Envirotech laboratory, Inc. acknowledges a legal and ethical responsibility to protect the privacy of laboratory information. It requires that all employees, contractors and consultants of Envirotech Labs with access to personally identifying information on data and any other records refrain from discussing information or providing information or providing copies of reports, regardless of how or where acquired, to family members, friends, professional colleagues, other employees, other clients or any other person unless such person has been authorized to have access to that information.

Employees, contractors and consultants are prohibited from the unauthorized use or disclosure of laboratory data and any other type of information that is privileged, legal confidential information; or any information received from third parties on confidential

conditions; and or any other data or information the use or disclosure of which might reasonably be construed to be contrary to the interests of Envirotech Laboratories.

I understand that as an employee, contractor or consultant of Envirotech Labs, I have a personal, professional, ethical and legal obligation to uphold these principles of confidentiality, privacy and information security, and will abide by the following standards:

1. **I will obey all Envirotech Lab's accreditation standards pertaining to confidentiality and privacy requirements;**

2. **I acknowledge that my work may include access to and the use of information of a proprietary and confidential**

nature. I shall not seek access to, review, discuss, copy, disclose or use such information, directly or indirectly in any way, during or after my term of employment or association with Envirotech Labs, except as authorized or required in the performance of my duties during the regular course of the laboratory's business;

3. **As a person who has a legitimate need for and use of such information, I shall take whatever actions are necessary to protect said information from accidental or unauthorized review, copying, modification, destruction, distribution or disclosure, and I will immediately inform the Laboratory Director if I become aware that such actions are planned or have taken place;**

4. **I shall not retrieve, review, discuss, copy or use laboratory information not related to my work, or otherwise said information to unauthorized persons who do not have a legitimate need to know such information, including other employees, without the written consent of his/her legal representative, except where required or permissible under the law. I will not reveal in oral, written or electronic form, test results or other confidential information to any unauthorized individual;**

I understand that any violation of this confidentiality agreement during the course of my employment or work with Envirotech Labs will be considered a breach of my obligations and may result in disciplinary action, up to and including termination of employment.

I acknowledge that I have read and understand the above statement.

______________________ ______________________ ______________________

Printed Name Signature Date

______________________ ______________________ ______________________

Witness Name Signature Date

Envirotech Laboratories, Inc.
8 Jan Sebastian Dr unit 12
Sandwich, MA 02563

COMPLAINT FORM

CLIENT NAME_____________________________
DATE____________
COMPLAINT TYPE_____________________________

COMPLAINT:

INVESTIGATION:

CORRECTIVE ACTION:

ROOT CAUSE:

REVIEW AND COMMENT:

Signed_______________________

COMPLAINTS

All complaints received by the laboratory from clients or customers shall be reported onto a complaint form which includes the name, date, the particular complaint, the investigation and the corrective action taken.
The completed form is submitted to the Lab Director or General Manager for review and comment.
After review the complaint form is signed and any pertinent documentation is attached and filed.

Envirotech Laboratories, Inc.
8 Jan Sebastian Dr unit 12
Sandwich, MA 02563

DATA INTEGRITY AND TECHNICAL ETHICS

Each employee of Envirotech Laboratories will read, understand, and sign the form below.

Yearly, each employee will take part in the power point presentation in groups.

It is essential that each employee understands the company's commitment to data integrity and the ethical and quality standards required to work in this industry.

Each employee must adhere to the ethical code of conduct adopted by company.

Why unethical behavior occurs:

- Real or perceived pressure
- Lack of understanding of the impact of unethical behavior
- Lack of knowledge or confidence in appropriate ways to solve problems
- Ineffective oversight by management
- Focus on production over quality
- Unrealistic expectations
- Lack of fortitude or personnel standards

What are improper/fraudulent laboratory practices (what should not be done in laboratory?)

- Fabricate information
- Misrepresent QC results
- Manipulate tuning data
- Improperly alter operating conditions
- Use improper calibrations or verifications
- Perform improper manual peak integrations
- Alter samples prior to analysis
- Perform unwarranted software manipulations
- Delete non-compliant data
- "Time travel"
- Conceal a known problem
- Inappropriately delete data points
- Analyze proficiency tests differently than routine samples

How to prevent improper/fraudulent laboratory practices

- Document
- If you think it is wrong: check it out
- What does the SOP say?
- What does method say?
- Talk with your manager or supervisor
- Be conservative, if you think you messed up, just start over.
- Follow the SOP as written

Corrective Action, client notification, disciplinary action

- Corrective action required for investigation findings
- Process changes to prevent reoccurrence
- Client notification for negatively impacted data. Data recall may be necessary
- Disciplinary action consistent with severity
  - No action
  - Retraining
  - Warning
  - Dismissal

Final Comment

- It is OK to make a mistake. They do not lead to fraud unless you try to hide them.
- Short term gains are not worth long consequences
- Traceability is the operative word. Everything you do must be able to be traced back from beginning to end and can be recreated.
- Report inappropriate behavior, unethical or illegal practices to management
- Take pride in what you do

Envirotech Laboratories, Inc.
8 Jan Sebastian Dr unit 12
Sandwich, MA 02563

DATA INTEGRITY TRAINING ACKNOWLEDGEMENT
& ETHICAL CONDUCT AGREEMENT

I understand the high ethical standards required of me with regard to the duties I perform and the data I report in connection with my employment at Envirotech Laboratories.

I have received formal instruction on the code of ethics that has been adapted by Envirotech laboratories and have been informed of the specific procedures:

A data integrity investigation is conducted when data issues are identified that may negatively impact data integrity.

Routine data integrity monitoring is conducted on sample data which may include an evaluation of the data I produce.

I am aware that data fraud is punishable crime that may include fines and /or imprisonment upon conviction.

I shall not intentionally modify data values unless the modification can be technically justified through a measurable analytical process.

I shall not intentionally report dates and times of data analysis that are not the true and actual times the data analysis was conducted.

I shall not condone any accidental or intentional reporting of inauthentic data by other employees and immediately report it's occurrence to my superiors.

I shall immediately report and accidental reporting of inauthentic data by myself to my superiors.

I acknowledge that I have read and understand the above statement.

_____________________ _______________________
____

Printed Name Signature
Date

________________________ __________________________
____

Witness Name Signature
Date

SECTION II - SAMPLE HANDLING PROCEDURES

1. SAMPLING AND PRESERVATION

Samples are brought to the laboratory directly by clients (well drillers, clients, etc.). The appropriate analysis request form (see attachment) should be completed. Information should include the name of the client, the date of sample collection, the date the sample is received at the lab, the time the sample was taken, the name of the sampler, the sample I .D. number, the number of copies required, client phone number, and the analyses required properly checked off. The information received is recorded into the master logbook in the following manner:

DATE RECEIVED/STAFF INITIALS/LAB I.D. # /CLIENT/CLIENT ID #/SOURCE,LOCATION/ COLLECTION DATE/ PRESERVATIVE /SAMPLER/ ANALYSIS REQUESTED/COMMENT

Samples are inspected and verified against the chain of custody or the client requisition for the sample date, the preservation and the holding times for the analyses requested. If any discrepancies are noted, clients are immediately contacted to resolve the discrepancy.

2. CHAIN OF CUSTODY

If a chain of custody form (see attachment) is required, the information will include the sample I.D. number, the sample description, the date and time of the sample collection, the location of the sample, the sample collector. The chain will also have the proper signatures all custodial persons and times of sample exchange written in the proper section.

3. PRESERVATION OF SAMPLES

Recommended preservation of samples at the laboratory is carried out in accordance with the guidelines established by the Department of Environmental Protection (see attachment) and stated in the Standard Methods, 19th Edition. If preservation is required and a preservative is not added to the container first, then the laboratory will add the appropriate preserve to the sample at the time of delivery to the laboratory. See the list of sampling and handling requirements for the parameters being tested (see attachment).

4. STORAGE OF SAMPLES

Samples received at the laboratory will be processed immediately by the technical personnel. If any delay is anticipated, the sample will be refrigerated in a refrigerator designated for samples. Samples that have a chain of custody and need to be secured are stored in a refrigerator, which is secure at all times.

5. SAMPLE HOLDING TIMES

Envirotech Laboratories initiates preparation and/or analysis of samples received at the laboratory immediately or within 12 hours after receipt of the samples to insure proper analysis within the specific holding time. If a sample is delivered to the lab after the holding time has expired; the laboratory will contact the client to determine whether to proceed with the analysis. If the analysis is performed after the holding time has expired, a statement regarding the integrity of the analytical results will be included on the final report.

6. SAMPLE REJECTION

The Laboratory will reject samples received at laboratory for the following reasons:
a. Improperly labeled or unlabeled sample containers. b. insufficient sample volume. c. improper preservation. d. improper holding time. e. improper storage.
In case of sample rejection, the client is notified immediately and the reason for rejection is documented in the comment section of computer sample login. The rejection is also recorded in the Correction Action log book.

8. ANALYSIS LOG-IN

Samples received at laboratory are entered into computer. The program assigns the lab ID # to the sample. Also recorded are the date and time received, the lab personnel receiving the sample, the date and time sample taken, the sampler, client and the requested analysis. The technician labels sample containers with proper lab ID# and requested analysis.

9. PREPARATION OF REPORTS

The laboratory technical staff, from the raw data, enters the results of analysis, date analysis performed, and initial of technician performing analysis into the proper location for that sample ID and client after insuring that the quality control parameters have been met. The laboratory director or supervisor reviews all reports for accuracy. He/she then checks that the information is correct and checks values and comments on the report. The final step is signing the report. The original copy of the report is sent to the client. A copy of the results is attached to the lab requisition or chain of custody and then filed away by month in alphabetical order.

Clients are notified within 24 hrs of any sample that exceeds a DEP or EPA maximum contaminant level (MCL), maximum residual disinfectant level or reportable concentration.

Reports of analyses submitted to the DEP must contain the laboratory's Massachusetts Certification I.D. for analyses performed by Envirotech Laboratories, Inc., the analytical methods utilized to detect and quantify analytes, the name of the sampler, and the date of analysis. The analytical reports specify the Maximum Contaminant Level (MCL) or recommended limits of each analyte on report. If the analyte(s) is/are subcontracted to an outside laboratory for analysis, all the QC/QA sections are recorded on the report including the sampler, sample dates, sampling times, methodologies.

Results of analysis of reagent blanks, fortified blanks, duplicates, recovery data, and lab fortified sample matrices will be reported when requested by the DEP. The format of data submitted to the DEP will include the sample date(s), the sampling time(s), the methodologies used in the analysis and the name of the sampler. Data submitted to the DEP Water Supply division will be reported on forms supplied by the DEP.

Most methods for analytes performed by Envirotech lab or by subtracted laboratories are certified by the Massachusetts Department of Environmental Protection. If a client requests an analyte that the laboratory is not certified, the laboratory will inform client, analyze sample, and indicate on report that the laboratory is not certified for that particular analyte.

Analytical data of samples for regulatory compliance is reported in timely manner to ensure the client's reporting requirements. Analytical data of samples subcontracted to Envirotech by another laboratory is released in a timeline that is suitable for laboratory.

Analytical records are maintained for a minimum of 10 years

10. Shipping SAMPLES

Sample analyses that are subcontracted out to the other labs are shipped out by Federal Express, UPS and the U.S. Mail. A chain of custody, which documents the date of the sample, the sample I.D. number accompanies these samples, the analysis requested and any special reporting required by regulatory agencies. Ie. Please call lab ASAP with any MCL exceedences. A copy of the chain of custody is kept on file. Samples sent out are recorded in the SEND OUT BOOK kept at the front of the lab. The information recorded is the date sent, client name, sample I.D. number, and the laboratory contracted to do the analysis. When the sample results is received, the date of receipt is recorded in the SEND OUT BOOK.

11. SAMPLE RETENTION AND DISPOSAL

The laboratory retains all the samples for 2 weeks after the report has been generated. Envirotech Laboratories will agree to hold samples beyond this period at the client's request. Standard Operating Procedures based on the regulations contained in 310 CMR 30 for small quantity generators.

SECTION III- GENERAL LABORATORY PROECEDURE

The following section describes the *QAIQC* portion of the QA program that affects the daily general operations of the laboratory. This includes method references, operating equipment, preventive maintenance schedules, glassware cleaning, standards and reagent preparation and QC sample analysis.

1. REFERENCES

1. "Standard Methods for the Examination of Water and Wastewater", 21st edition, 2005."
2. "HACH Water Analysis Handbook", 1987.
3. "Determination of Metals and Trace Metals in Water and Wastes by Inductively Coupled plasma-Atomic Emission Spectrometry." Method 200.7 revision 4.4 EMMC Version.
4. "Methods for the determination of Inorganic Substances in Environmental Samples", EPA/600/R/100 Aug 1993.
5. US EPA 821-R-98-002 Feb 1999, "Method 1664A, HEM; Oil & Grease.
6. Internal Standard, General requirements for the competence of testing and calibration laboratories ISO/IEC 17025:2005E.

For the analytical methods see the Standard Operational Procedures.

STANDARD OPERATING PROCEDURES

Parameter **Method** **reference**

BOD SM 5210 "Std Methods for the Examination of Water and Wastewater",21st edition, 2005
Chlorine free SM 4500-CL-G "Std Methods for the Examination of Water and Wastewater",21st edition, 2005
COD Hach 8000 "HACH Water Analysis Handbook", 1987
IC anions USEPA 300.0 "Methods for determination of Inorganic substances", EPA/600/R/100 August 1987
Nitrate
Nitrite
Chloride
Sulfate
Fluoride
ICP metals USEPA 200.7 "Determination of Metals in Water and Wastes by ICP method 200.7 revision EMMC Version
Mantech
pH SM 4500 H-B Std Methods for the Examination of Water and Wastewater",21st edition, 2005
Alkalinity SM 2330 B Std Methods for the Examination of Water and Wastewater",21st edition, 2005
Sp. Conductance USEPA 120.0 EPA 60014-79-020 "Methods of Chem Analysis of Water Wastes"
Turbidity SM 2130 B Std Methods for the Examination of Water and Wastewater",21st edition, 2005
Ammonia-N SM 4500 C Std Methods for the Examination of Water and Wastewater",21st edition, 2005
Oil & Grease USEPA 1664A US EPA -821-R-98-005 Feb 1999 using Horizon 3000 XL Extracter
Total Ortho Phos SM 4500 P-B-E Std Methods for the Examination of Water and Wastewater",21st edition, 2005
TDS SM 2540 C Std Methods for the Examination of Water and Wastewater",21st edition, 2005
TKN SM 4500 C Std Methods for the Examination of Water and Wastewater",21st edition, 2005
TSS SM 2540 D Std Methods for the Examination of Water and Wastewater",21st edition, 2005
TS SM 2540 B Std Methods for the Examination of Water and Wastewater",21st edition, 2005

Standard Operating Procedures for Microbiology

Parameter	Method	reference
Total Coliform	SM 9222B	Std Methods for the Examination of Water and Wastewater", 21st edition, 2005
	SM 9222G	Std Methods for the Examination of Water and Wastewater", 21st edition, 2005
Fecal Coliform	SM 9222D	Std Methods for the Examination of Water and Wastewater", 21st edition, 2005
Fecal Coliform/EC	SM 9221E	Std Methods for the Examination of Water and Wastewater", 21st edition, 2005

Enterococcus	USEPA 1600EPA 821-R-06-001, 2006	
E coli	USEPA 1603EPA 821-R-06-011,2006	
Presence/Absence	SM 9223	Std Methods for the Examination of Water and Wastewater", 21st edition, 2005
Heterotrophic Plate Count (HPC)	SM 9215B	Std Methods for the Examination of Water and Wastewater", 21st edition, 2005

ANALYTICAL METHODS (Revised October 2016)

Analyte	Method	Method Type	MDL	MCL/or limit	Holding times
Recommended Microbiology					
Recommended/Reg					
Enterococcus	1600	Membrane filtration	0/100 ml	<1	6 hr
Colisure	9223B	Presence/Absence	N	A	30 hr
Heterotrophic Plate Count	9215 B	Pour Plate Method	1/ml	500	6 hr
Total Coliform	9222 B	Membrane filtration	0/100 ml	0	30 hr
E coli drinking	9223G	Fluorescence (Filtration)	0/100ml	0	30 hr
Fecal Coliform	9222 D	Membrane filtration	0/100 ml	<1	6 hr
Total Coliform MPN	9221 B	Multiple fermentation	2/100 ml		30 hr
Fecal Coliform MPN	9221 E	Multiple fermentation	2/100 ml		6 hr
E coli salt water	1603	Membrane filtration	0/100 ml		6 hr
Pseudomonas	9213 E	Membrane filtration	0/100 ml		30 hr
E coli ambient	1603	Membrane Filtration	0/100 ml		6 hr

Acidity	2310 B	Titration	1.0 mg/L		24 hr/14 d
Alkalinity	2320 B	Titration	2.5 mg/L		24 hr/ 14 d
BOD-5 day	5210 B	DO meter	2.0 mg/L		6 hr/48 hr
Spec. Conductance	120.1	Wheatstone Bridge	4.0 uhoms/cm		28 d
COD	Hach Meth 8000	Closed Reflux (Col)	5.0 mg/L		7 d/ 28 d
Color	2120 B	Visual comparison	1.0 APC	5.0	48 hr
Chloride	300.0	Ion Chromatography	3.0 mg/L	250	28 d
Chlorine	4500-Cl G	DPD Colormetric	0.03 mg/L		1 hr
Free CO2	4500-CO2 B	Calculation	NA		1 hr
Dissolved Oxygen	4000-O C	DO meter	1.0 mg/L		1 hr
Fluoride	300.0	Ion Chromatography	0.1 mg/L	4.0	28 d
MBAs	5540 C	Methylene Blue	0.01 mg/L		48 hr/NS
Metals	**200.7**	**ICP**	**see attached**		**6 months**
Hardness	3120B	Calculation	1.0 mg/L		
Calcium	200.7	ICP	0.1 mg/L		
Copper	200.7	ICP	0.003 mg/L	1.3	
Iron	200.7	ICP	0.01 mg/L	0.3	
Lead	200.7	ICP	0.006 mg/L	0.015	

ANALYTICAL METHODS (Revised October 2016)

Analyte	Method	Method Type	MDL	MCL	Holding times
Metals	200.7	ICP	see attached		6months
Magnesium	200.7	ICP	0.1 mg/L		
Manganese	200.7	ICP	0.005 mg/L	0.05	
Potassium	200.7	ICP	0.1 mg/L	20.0	
Sodium	200.7	ICP	1.0 mg/L	20.0	
Zinc	200.7	ICP	0.004 mg/L	5.0	
Aluminum	200.7	ICP	0.010 mg/L	0.05-2.0	

Analyte	Method	Method Type	MDL	MCL	Holding times
Antimony	200.7	ICP	0.010 mg/L	0.006	
Arsenic	200.7	ICP	0.010 mg/L	0.010	
Barium	200.7	ICP	0.002 mg/L	2.0	
Beryllium	200.7	ICP	0.002 mg/L	0.004	
Boron	200.7	ICP	0.010 mg/l		
Cadmium	200.7	ICP	0.002 mg/L	0.005	
Chromium	200.7	ICP	0.002 mg/L	0.1	
Cobalt	200.7	ICP	0.005 mg/L		
Molybdenum	200.7	ICP	0.005 mg/L		
Nickel	200.7	ICP	0.005 mg/L	0.1	
Selenium	200.7	ICP	0.010 mg/L	0.05	
Silver	200.7	ICP	0.002 mg/L	0.10	
Strontium	200.7	ICP	0.010 mg/L		
Thallium	200.7	ICP	0.006 mg/L	0.002	
Titanium	200.7	ICP	0.030 mg/L		
Vanadium	200.7	ICP	0.005 mg/L		
Mercury/Subcontracted	245.1	Cold Vapor AA	0.0005 mg/L	0.002	

Nitrogen

Analyte	Method	Method Type	MDL	MCL	Holding times
Ammonia-N	4500 NH3 C	Titrimetric	0.5 mg/L		7 d/ 28 d
Nitrate-N	300.0	Ion chromatography	0.01 mg/L	10.0	48 hr/ 48 h
Nitrite-N	300.0	Ion Chromatography	0.006 mg/L	1.0	48 hr/48 h
TKN (Organic N)	4500 NH3 C	Titrimetric	0.6 mg/L		7 d/28 d
Total Nitrogen	4500-Norg-B,C	calculation	0.6 mg/L		7 d/28 d
Oil & Grease	1664 A	Gravimetric	1.0 mg/L		28 d/28 d
Odor	2150 B	Threshold test	none		6 h
Total Phos (P)	4500-P	Amino Acid	0.005 mg/L		28 d/28 d
Ortho Phos (P)	4500-P	Amino Acid	0.005 mg/L		48 h/ 48 h

Analyte	Method	Method Type	MDL	MCL	Holding times
pH	4500-H-B	Meter	NA		2 hr
Salinity	2520 C	Density	0		6 mo

Solids

Analyte	Method	Method Type	MDL	MCL	Holding times
Suspended Solids	2540 D	103-105 ° C	1.5 mg/L		7 d
Total Solids	2540 B	103-105 ° C	6.0 mg/L		7 d
Dissolved Solids	2540 C	180 ° C	6.0 mg/L		7 d
Volatile Solids	2540 E	500 ° C			

			1.5 mg/L		7 d
Settleable solids	2540 F	Imhoff Cone	0.1 mg/L		7 d
Solids-solid or semisolids	2540 G	103-105	1.5 mg/L		7 d
Sulfide	4500-S 2- D	Methylene Blue	0.2 mg/L		7 d
Sulfate	300.0	Ion Chromatography	3.0 mg/L	250	28 d/28 d
Turbidity	2130 B	Nephlometric	0.05 NTU		24 hr/48 hr
Tannins	5550 B	Colorimetric	0.1 mg/L		7 d
Hydrogen Peroxide	NA	Titration	0.4 mg/L		7 d

NS=not stated in cited reference

2. QUALITY CONTROL SAMPLES

Method Blank:

An aliquot of analyte-free water prepared and analyzed with the analytical batch. The method blank is prepared through the whole procedure as a sample. Analysis of the method blank allows the analyst to identify and quantify any background

contamination levels prior to analysis of samples.

Laboratory Control Spike (LCS):

Also known as a fortified blank, the LCS is an aliquot of reagent water spiked with a known amount of target analytes. The LCS spiking standard should be independent from the standards used to calibrate the instruments. The LCS, like the method blank, is subjected to the entire preparation procedure. The LCS recovery indicates how closely an independent standard can be recovered from preparation relative to the calibration curve where matrix effects should be minimal.

Matrix Spike (MS):

An aliquot of a sample matrix spiked with a known amount of target analytes. The MS spiking solution should be independent of the standards utilized for calibration. The MS is subjected to the entire preparation procedure. The MS recoveries allow the opportunity to determine matrix effects when analyzed compared to a LCS.

Duplicate:

Also known as a laboratory Duplicate, this sample helps measure the precision of analyses. Some analyses, such as organic, prepare matrix spike duplicates instead of MS and duplicates. The main information gained from a duplicate is the % difference value. The duplicate can offer little to no information if any positive results are determined in the sample.

Matrix Spike Duplicate (MSD):

The MSD sample helps measure the precision of analyses is the sample matrix in addition to providing information concerning the recovery. The main precision information gained from an MSD is the % difference value. The MSD can offer little to no information about precision if no positive results is determined in the sample. However, the MSD will include % recovery information, which will assist in determining accuracy of the analysis.

Initial calibration (IC):

A series of standards analyzed to determine response over a range of concentrations, linear range, and reporting limits. In certain cases, where allowable in the referenced method, Envirotech may utilize higher order calibration curve which will more closely approximate response than a linear curve.

Interference Check: (used with ICP metal analysis):

A known series of analytes at high concentrations are analyzed to verify inter element and background corrections are within limits.

DATA ACCEPTANCE CRITERIA

Quality Control Charts are constructed for each analyte for following: Calibration check standard, Duplicate samples, Fortified and Spiked samples, and End Check samples.

Charts are constructed with Upper Control limit (CL), Lower Control limit (CL), Upper Warning limit (WL), and Lower Warning limit (WL). The laboratory uses ±2s and ±3s

limits for WL and CL respectively (s=Standard Deviation).

Corrective Actions based on:

Control Limit: If one measurement exceeds a Control Limit (CL), repeat analysis immediately. If the repeat is within the CL continue analysis; if it exceeds the CL, analysis is discontinued and corrective action taken.

Warning Limit: If 2 out of 3 successive points exceed a Warning Limit (WL) reanalyze. If the next point is less than the WL continue analysis: if the next point exceeds the WL, analysis is discontinued and corrective action taken.

Standard Deviation (s): If 4 of 5 successive points exceeds 1s or is in decreasing or increasing order, another sample is analyzed. If the next point is less than 1s or changes order, analysis is continued; otherwise analysis is discontinued and corrective action taken.

Integration: When integrating chromatography peaks, either automatically or manually, the analyst ensures that the integrations are performed in a correct consistent manner for standards and samples, including quality control samples. Documentation of manual integrations includes the following:

1. The original chromatogram and the manual integrated chromatogram must be documented.
2. The analyst's initials, date of manual integration, and reason(s) for the manual integration are documented.

CORRECTIVE ACTION

1. Res standardize.
2. Standards are checked for degradation.
3. The instrument is checked for hardware and, or software problems.
4. Technical support for instrument is consulted.
5. Any corrective action is recorded with date, problem, and action taken.

3.0 STANDARDS & REAGENTS

A critical element in the generation of quality data is the purity of reagents and the accuracy and traceability of standards and their preparation. All reagents or stock standards are purchased as high quality commercial products from respected sources. Preparation of standards and reagents are done with analytical grade or high stock materials.

Preparation of stock and standards are documented in Lab prep book. Log includes date, tech, volumes and final concentrations achieved for individual standards.

Of all the reagents and chemicals utilized in the laboratory, the deionized water is, by far, the most critical since it is used for everything from method blanks to the final glassware rinse. All reagent water is prepared by passing it through particulate, activated carbon, cation removal, dissolved oxygen removal, membrane and anionic exchange filters. The units are leased from U.S. Filter Corp. and the cartridges are replaced when Spec Conductance is > 2.0 uhmos/cm. Reagent water is checked daily for pH and Specific Conductance. Reagent water is also checked for all analyses in the preparation of method blanks.

Verification for new stock and working standard concentrations are checked against an independent standard. Stocks and working standards are regularly checked for a sign of deterioration, discoloration, precipitate formation or changes in concentration. All containers are labeled as to the compound, concentration, solvent, expiration date, prepares initials, and preparation date.

4. LABORATORY GLASSWARE

All glassware and sample containers are cleaned using a dilute detergent (Fisher FL-70) solution, followed by 3 successive rinses with hot tap water, followed by 3 successive rinses of deionized water. For sample bottles, residual acid or alkali is checked by the addition of one Bromothymol blue tablet (HACH) to l00 ml of deionized water in a newly washed bottle. Observation of light to dark green is recommended. If a green color is not obtained, re rinse glassware and retest.

For glassware for Total Phosphorous analysis, add approximately 50m1 of 0.1N HCL to 250m1 flask and place it on a hotplate at a low temperature setting. After the acid is warmed, remove and pour acid from flasks into 50m1 glass cylinders. Empty the contents of the cylinders and rinse both flasks and cylinders with deionized water. Alternatively acid washed containers are purchased.

Glassware used for trace metal analysis is rinsed with 1:1 Nitric Acid water and rinsed three times with deionized water.

5. INSTRUMENT CALIBRATION PROCEDURES

Metals - The ICP is calibrated prior to any analyses being performed, using established criteria from appropriate methods. Calibration is verified using an interelement check standard at the beginning, mid run, and end of the analytical run.

Wet Chemistry- Calibration and standardization procedures vary depending on the analytical method required for the analysis. In general, the principles of calibration apply universally. Each system is calibrated prior to analyses being conducted. Calibration consists of defining the linear range by use of a series of standards, establishing detection limits and identifying potential interferences. If the ongoing calibration check does not meet established criteria, the system is recalibrated and all samples that

were analyzed since the last acceptable calibration check standard is reanalyzed. See the SOP for particular standards and check standards used for each analysis performed.

6. EQUIPMENT & INSTRUMENT UP-KEEP, MAINTENANCE, REPAIR

Balances - The analytical balance is checked prior to the first use each day. Class S weights are utilized for this check for l00mg, 200mg, 1,000mg, and 5,000mg. Top loader balances are checked monthly with class S weights 10 mg, 20 mg, 50 mg, 100 mg, 150 + 0.100 mg, and 200 mg. Balances are serviced by a qualified service representative (Alert Scientific) and checked against an independent set of NBS weights annually. The accuracy of the reference weights are certified yearly by Alert Scientific.

pH Meters - The pH meters are calibrated daily according to EPA Method 4500-H+B, using analytical grade buffer solution of pH values 4, 7, and 10. The slope is calibrated and must be 98C to 102C. All pH probes are cleaned regularly.

Thermometer - All thermometers used to monitor the temperatures of ovens, refrigerators, incubators, pH meters, and conductivity meters are checked annually against an NBS thermometer in the expected area of temperature measured. A correction factor is applied to the readings and determined at that time.
The accuracy of the certified thermometers is certified yearly by Alert Scientific.

Refrigerators - Are monitored twice daily to ensure that a temperature of 0-4.0°C± 2°C is maintained. If the temperature exceeds the acceptable range, the temperature is rechecked at another time later in the day. If the temperature continues to exceed the limit, corrective action must be performed and all the samples must be removed from the refrigerator. Corrective action may include an adjustment of temperature with continued monitoring on contact of a qualified refrigerator repair professional.

Incubators - Incubators for microbiological analysis are maintained at 35C $\pm$ 0.5, 44.5C±0.2, 41.5±0.5 respectively and are checked twice daily. Corrective action is equivalent to those imposed for refrigerators.

Ovens - The drying oven is monitored with respect to the required temperature for the particular analysis that is performed, (i.e. TDS 180°C $\pm$ 2.0, TSS 104°C $\pm$ 2). The oven temperature is monitored before use and at the end of the required drying time to ensure the proper temperature.

Many of the analytical instruments are on regularly scheduled preventative maintenance programs in addition to careful monitoring of their daily performance. The procedures are either regularly scheduled or are performed based on the instrument failing specific criteria set by the laboratory and documented in the SOP.

ICP - Checks are made daily on the Argon gas supply and the humidifier. Also, pump and hose and sample feed hose are checked for any damage. A 3% Nitric Acid solution is pulled through

the tubing after each run to prevent deposit accumulation. Monthly, the torch is examined for dirt and damage. A spare torch is stored which can be installed if the torch must be cleaned or replaced. The air filters are checked and replaced every two months. The nebulizer and nebulizer chamber are checked weekly for deposits and blockages. The chamber is cleaned with mild detergent once a month. The quality of the spray is also checked monthly. The fiber optics is checked weekly for dirt and damage.

Spectrometers- Wavelength checked annually by Alert Scientific. Linearity checked annually at wavelength 410 and 630.

7. *QAIQC* OVERSIGHT OF LABORATORY PROCESS

Data Reduction and Review - All analytical data generated within the laboratory is checked for accuracy and completeness. The processing of data consists of data generation, data reduction, and four levels of review.

1. Level One Reviews

The analyst who generates the analytical data has the prime responsibility for the correctness and completeness of the data. Each analyst is required to review their own work, based on the following guidelines:

* Sample receipt information.
* Reported dates are correct.
* Sample analyses are within holding time requirements.
* Preparation information is correct.
* All calculations are correctly performed.
* The proper SOP or method has been followed.
* Reagent blanks show no contamination.
* QC samples are within established limits.
* Surrogate recoveries (if applicable) are within QC limits.
* Documentation is complete.

2. Level Two Reviews

A senior level analyst who did not perform the work performs this level of review. The purpose is to insure the technical integrity of the data. The review is conducted according to set guidelines to ensure that:

* Calibration, calculation and results have been properly performed, documented and are quantitatively and qualitatively correct.

* QC samples are within set limits.

* Sample information to include date of receipt, preparation, and analysis are consistent.

* Documentation is complete.

The level two review includes a recalculation of a minimum of 10% of the final results. If any discrepancies are found, the analysis must be reviewed prior to returning the data to the analyst for correction. Following any corrections, the reviewer should confirm that the corrections have been adequately addressed. The data can be entered into the final report, after the level two review is satisfactorily completed.

3. Level Three Review

Following the entering of the data into the final report forms, independent reporting personnel review the data for transcription errors. The reporting personnel entering the data into the final report is primarily responsible for releasing a correct final copy for review. The primary function of the review is for transcription errors. This is then submitted for level 4 review.

4. Level Four Review

Prior to reporting the results to the client, the final results undergo a level four review by the laboratory director. The data is reviewed to insure that the data meets the overall objectives of the client and that the data is consistent with the overall project. If any discrepancies are suspected, the raw data is checked by the director or retested. While each step of the review process involves evaluation of data quality based on the results of the QC data, professional judgement, technical knowledge, and experience of the Director. The level four review is not intended to be a full technical review.

8. STANDARD OPERATING PROCEDURES

Details of analytical and QC protocols are contained in the SOP. SOP preparation and updating are coordinated through the laboratory director. The laboratory manager is responsible for determining which SOP's should be written, based on knowledge of the regulations and an understanding of the laboratory processes. The lab manager is responsible for providing a description of the various portions of the SOP and must be available to assist the technical staff with the SOP. SOP's for performance of an analytical method should include:

* Purpose
* Scope
* Target analytes
* Detection limits
* Applicable matrices
* Linear range
* Analytical time requirements
* Summary of methods
* Common interference’s

* Safety issues
* Sample collection, preservation, container and holding time
* Apparatus
* Reagents and Standards
* Detailed procedure
* Calibration
* Sample analysis

* QC requirement
* Acceptable criteria
* Calculation
* Reporting limits
* Significant figures of results
* References

SECTION IV - GENERAL SAFETY

1. FIRE PREVENTION

FIRE PREVENTION **IS VERY IMPORTANT.** TO ACCOMPLISH **THIS,** EVERYONE MUST BE A “SAFETY BUG" AND LOOK FOR OR SEE THAT THE FOLLOWING RULES ARE FOLLOWED:

1. TRASH SHOULD NEVER BE ALLOWED TO ACCUMULATE.
2. AIR VENTS SHOULD NOT BE BLOCKED.
3. NO SMOKING EXCEPT IN AUTHORIZED AREAS.
4. THE UTMOST CARE SHOULD BE TAKEN IN THE DISPOSAL OF SMOKING MATERIALS.
5. DEFECTIVE WIRING AND EQUIPMENT SHOULD BE REPORTED TO THE SUPERVISOR FOR REPAIR.
6. DO NOT USE EXTENSION CORDS.
7. FLAMMABLE LIQUIDS OF QUANTITIES GREATER THAN 500m1 SHOULD BE STORED IN SAFETY CONTAINERS.
8. EXIT DOORS SHOULD NOT BE BLOCKED.
9. HALLWAYS LEADING TO EXITS MUST BE KEPT CLEAR OF CLUTTER.
10. DO NOT DISPOSE OF CHEMICALS WITHOUT CHECKING THE RECOMMENDED PROCEDURE.
11. A FIRE EXTINGUISHER IS LOCATED IN THE LAB.

2. INSTRUCTIONS IN CASE OF FIRE

IN CASE OF FIRE IN THE LABORATORY AREA IT IS IMPORTANT TO OBTAIN

AS MUCH HELP AS POSSIBLE QUICKLY:

A. THE PERSON DISCOVERING THE FIRE WILL NOTIFY OTHERS IN THE AREA.

B. INFORM THE CLERICAL STAFF IMMEDIATELY FOR NOTIFICATION OF THE FIRE DEPARTMENT IF NECESSARY.

C. CLOSE ALL DOORS AND WINDOWS IN THE AREA OF THE FIRE.

D. CHECK THE INTENSITY OF THE FIRE AND THE PROXIMITY OF FLAMMABLES AND EXPLOSIVES BEFORE PROCEEDING TO EITHER EXTINGUISH THE FIRE OR TO NOTIFY THE FIRE DEPARTMENT.

E. FIGHT FIRE WITH ALL AVAILABLE MEANS. EXTINGUISH OR CONTAIN IT UNTIL THE ARRIVAL OF THE FIRE DEPARTMENT (UNLESS IT IS NECESSARY TO EVACUATE THE AREA).

3. GENERAL SAFETY

GENERAL SAFETY WHEN HANDLING CHEMICALS:

*** REGARD ALL CHEMICALS AS POTENTIALLY DANGEROUS.**
*** DO NOT SMELL OR TASTE CHEMICALS.**
*** AVOID TOUCHING A CHEMICAL WITH BARE HANDS.**
*** DO NOT POUR HOT SOLUTIONS INTO NON-PYREX REAGENT BOTTLES.**
*** USE FUNNELS OR SYPHONS WHEN TRANSFERRING LIQUIDS.**
*** NEVER POUR WATER INTO STRONG ACID - ALWAYS ACID INTO WATER.**
*** WHEN WEARING CONTACT LENSES USE PROTECTIVE GOGGLES OR A FACE SHIELD.**
*** WASH YOUR HANDS AFTER HANDLING CHEMICALS.**
*** STORE CONCENTRATED ACIDS, BASES, OR FLAMMABLE SOLVENTS ON SHELVES BELOW EYE LEVEL.**

A. LABORATORY EQUIPMENT:

a. Chemical spill kits should be in storage and work areas. Size of kits should be according to quantities of acids, alkalies, or solvents being used.

b. Eye Washes: Remove contact lenses if applicable. Flush eyes immediately and thoroughly (15 min.) to prevent sight impairment. Splashes to the face are rinsed off easily with a four-head eyewash.

c. Fire Extinguishers.

1. Dry chemical types are effective against most fires, but particularly those involving flammable liquids and metals and electrical fires.

2. Extinguishers are checked yearly and recharged by the Ralph Perry Co. Maintenance is recorded on page 4.

d. Laboratory fumes hoods to vent operations with hazardous materials. Hood performance is checked yearly by qualified company.

e. Safety containers to minimize consequences of an accident to prevent spread of harmful materials. Use safety containers to transport chemicals, especially concentrated acids and alkalies.

f. Storage of Chemicals: Acids, bases, and solvents are stored in separately marked cabinets.

B. LABORATORY HAZARDS

a. Safety Plan should be developed. This is to provide all personnel working with or near hazardous material with information about them. Include the Material Safety Data Sheets (MSDS's) in a safety plan and describe routine emergency procedures; require that all personnel read and sign that they have read and understood the document.

b. Spills:

1. To avoid splattering, slowly add acids and bases to water. If contact with skin is made, thoroughly flush the contaminated area with water. Seek medical attention if irritation persists. If eye contact is made, flush both eyes for 15 minutes and seek medical. Consult the MSDS for questions concerning chemical information.

c. Waste Containers:

1. Acids and bases are stored in separate 5-gallon plastic containers.

2. Solvents are stored in a 3.5-liter glass bottle.

NOTE: The Beacon company is contracted to pick-up and dispose of waste containers.

C. PERSONAL PROTECTIVE EQUIPMENT:

a. Clothing: Personal clothing creates a barrier between the individual and the hazard. Employees are required to change from street to laboratory clothing when

entering the work area.

b. Gloves frequently are important. Consult the material safety data sheet for a solvent or reagent to determine the e type of glove to be used. Rubber gloves may be used when handling hazardous liquids. Insulated gloves are essential for handling hot objects and extremely cold ones.

c. All laboratory personnel require safety glasses. They protect from splashes, powders, and flying objects.

FIRE EXTINGUISHER MAINTENANCE

ALL FIRE EXINGUISHERS ARE CHECKED ANNUALLY BY THE FOLLOWING COMPANY:

RALPH J. PERRY, INC.
88 FALMOUTH ROAD
HYANNIS, MA 02601

DATE LAST CHECKED	MAINTENANCE PERFORMED

4. LAB SAFETY TRAINING PROGRAM

The laboratory maintains a safety program for the protection of the laboratory personnel from physical, chemical, and biological hazards.

The program includes training of laboratory personnel in the use of fire extinguishers, safety equipment, and protective equipment and clothing.

Training will occur yearly.

All personnel will sign sheet indicating full understanding of training sessions.

The undersigned have attended a safety session and understand the contents of the safety program and manual held on date__________ .

The subjects:

SAMPLING INSTRUCTIONS

Samples should be cooled immediately, refrigerated, and shipped at 4 C.

Typically for most analytes fill bottle or container approximately 1” from top of container except for VOCs. VOCs should be collected in special vials, leaving no headspace or air bubbles. Be Careful when filling VOC containers with acid that it does not splash onto the sampler.

FOR COLIFORM SAMPLING

Faucets used for sampling should not be mixing type faucets, have no purification devices, hose attachments or strainers (Remove from faucet before proceeding).

Do not touch the inside of the bottle, container or the faucet.

A 10% solution of bleach sprayed inside the faucet is a good practice to follow.
Remove any aerators from the faucet before sampling. Aerators can harbor high amounts of bacteria.

Be sure to run faucet 5-15 minutes to clear pipes and to rinse the bleach away. If water at site has not been used in sometime, such as an unoccupied house, than flush no less than 15 minutes.

GOOD RULE OF THUMB FOR SAMPLING FOR ANY TYPE OF ANALYSIS IS NOT TO PLACE ANY COVER DOWN ON THE GROUND OR ONTO COUNTERS IF POSSIBLE.

Use a sterile container. (Thiosulfate preservative if

chlorinated water).

Hold cap in your free hand. Ensure that there is no contact with inside of cap.

Fill container to 1 in from top or the 100ml fill line.

FOR METAL AND INORGANIC ANALYSIS SAMPLING:

As for Coliform sampling run faucet for 5-15 minutes.

Fill proper container (usually plastic) leaving approximately 1-inch air space.

CONTAINERS

Small sterile plastic container is used for coliform (Bacteriological analysis).

Larger plastic containers are used for chemical analysis.

Ensure proper preservative in container. Consult list.

Sample Instructions for VOC Analysis

Each of following steps is important to insure validity of the VOC results

1. Sample from cold water tap only. Let water run for at least 5 minutes if the house is currently occupied and at least 15 minutes if house is vacant. While water is running, vial labels and collection forms can be filled out.

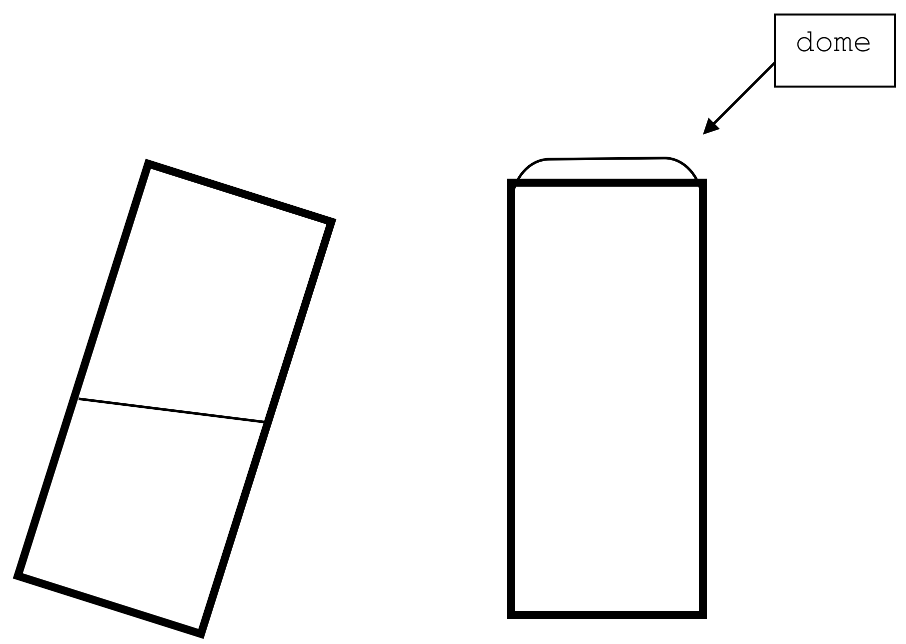
2. Once plumbing has been purged, slow the flow of water to obtain as low a steady stream from the faucet as possible.

3. Place the 40 ml VOC vial (CAUTION: contains hydrochloric acid) at a slight angle under the stream of water so that the water is hitting the inside wall of the vial. As the vial fills, straighten it to a vertical position until a dome of water is observed in the mouth of the vial. See below.

4. Gently remove the vial from the stream of water and cap the vial. There will be a small amount of water that overflows the vial as the cap is screwed on. Tighten cap, invert the vial and check for any air space or air bubbles that may appear. If air bubbles are observed, remove cap, and top off the vial under the gently flowing stream. DONOT EMPTY VIAL (Emptying vial will cause loss of acid preservative). Check for air again.

5. After satisfactory completing sampling, turn off water and refrigerate the samples as soon as possible. The sample should remain refrigerated until transported to laboratory.

Low
water



SAMPLING TECHNIQUES

In the Standard Methods for the Examination of Water and Wastewater it is stated that generally before filling the sample container, rinse it two or three times with the water being collected unless the bottle contains a preservative or dechlorinating agent. You don't want to rinse out any preservatives that are in container.

Many labs have sample containers that are pre-cleaned according to US EPA standards (the are usually purchased)
These types of pre cleaned containers should not be pre rinsed with sample prior to sample collection. This can cause higher results.

Samples should be cooled immediately, stored refrigerated, and shipped at 4 C.

Typically for most analytes Fill bottle or container up to approximately 1" from top of container except for VOCs which is collected with no headspace or air bubbles.

Be Careful when filling containers with acid that it does not

splash onto the
sampler.

FOR COLIFORM SAMPLING

Obviously do not touch the inside of the bottle or container or the inside of the faucet.

A 10% solution of bleach sprayed inside the faucet is a good practice to follow.

Remove any aerators from the faucet before sampling. Aerators can harbor high amounts of bacteria.

Be sure to run faucet at least 5-15 minutes to clear pipes and also to rinse the bleach away.

5 minutes is usually adequate. If water at a site has not been in use for some time, such as an unoccupied house- 15 minutes would be a better flush.

DONOT place the cover down on counter or ground. Good way to contaminate sample.

GOOD RULE OF THUMB FOR SAMPLING FOR ANY TYPE OF ANALYSIS IS NOT TO PLACE ANY COVER DOWN ON THE GROUND OR ONTO COUNTERS IF POSSIBLE.

FOR VOC: See instruction Page 34

The Reason for no air space or bubbles is that Volatile organics dissolved in the water tend to volatilize readily and will fill any air bubble available in the vial.

This will result in a loss or reduction of any volatiles in the sample during lab procedures.

LEAD AND COPPER SAMPLING.

From the sampling site a first draw tap sample is taken

A first draw sample means that

The water must stand motionless in the plumbing system for at least 6 hrs.

The site should be cold water sampled at the kitchen tap or bathroom sink.

If a non-residential building is used, take the sample from a site where the water is typically drawn for consumption, such as a bubbler.

The Sample is collected in a 1-liter plastic container with Nitric Acid as a preservative
To avoid potential problems of residents handling nitric acid,(Some systems allow residents to draw sample) Acidification may be done up to14 days after collection of sample.
Inform the lab that the sample for Lead and Copper has not been acidified.
The Laboratory will acidify the sample at that time.
Lab must let the acidified sample stand for a least 28 hrs before analysis.

The Quality Assurance Plan for Envirotech Laboratories, Inc. has been reviewed

and approved 9/28/17.

Electronically signed
____
Ronald J. Saari
Laboratory Director

Electronically signed
____
Maria Cathcart
QA/QC Manager

Cecilia Manual

Start up:

1. Cecilia refers to the Seal Analytical BOD autosampler.
2. Turn Cecilia on (there is a silver button in the front of the instrument).
3. Turn on the YSI DO probe
4. Slightly turn on the cold faucet. You don't have to fully turn the faucet on but the valve needs to be open.
5. Open "BOD Mini lab" application.
6. Sign into the program
   i. User name: Cecilia
   ii. Password: Seal123
7. Initialize the system
   Tools => Initialize System
8. Calibrate the DO probe

Start a run on Cecilia:

1. Go to the start tab and click "new table by template"
2. Select the BOD template and press open
3. The file's name will automatically generate as the date of the run. You should leave it as this
4. Start loading the samples into the program.
5. Type the sample name in the identification column (you should have a line for each dilution)
   NOTE: to add lines or move down the list pressing enter will not work. The only way to add or advance up and down the column is to use the arrow keys
6. Fill out the volume column for the samples
7. Fill Out if it's a duplicate. If it is a duplicate type true in the appropriate cell.
8. If the sample is a CBOD type true in the appropriate cell
9. Type the seed volume in for all samples (Seed volume for samples is always 4)
10. Flush the dilution liquid through the system (this fish is not automatically times so you have to flush air through it then flush the dilution liquid through it)
11. Flush the additional liquids through the system (this step is timed. You will not need to shut off the additional liquid flush.)
12. Select the "Measure Start DO" in the table tab
13. Right click the individual trays in the measure panel and select " load BOD" tray and select the tray you would like to load
14. Place the BOD bottles in the exact order that is displayed on the screen. Filled with the appropriate sample volume
15. After all trays in the run is loaded or all the trays that can be loaded at that time are loaded select start main run
16. The arm will go through the run and beep loudly after the run is complete.
17. At this point remove the trays from the from Cecilia and load more samples if needed and repeat steps 13-16 as many times as needed till the run is finished
18. Place the dilution liquid tube and the additional liquid tube into DI wash bottles and flush the pumps. Follow steps 10/11 but with DI
19. Close the control panel and exit the application
20. Turn Cecilia off and the DO probe make sure to say "good night Cecilia"

Measure End DO on an Existing Run

1. Turn on the instrument and Follow the Start-Up and Calibration instructions
2. Go to the start tab and select the "Open Active" icon
3. Select the run that you wish to read the end results on and the file name should be the date that the initial DO measurements were made.
4. Open the file. The table should appear on the screen with data in the start DO, Name CBOD, Dup and Seed column.
5. Select measure end DO under the table tab.
6. The measure panel should pop up at the bottom of the screen.
7. Load the BOD trays into the instrument and program.
8. Press Start run
9. At the end of the run close the measure panel
10. Press Calculate under the table tab and print out the final results with all results.

REVISION DATE EFFECTIVE: October 7, 2020

APPROVALS: Leslie Aubut 10/7/2020
Authorized By Date

Standard Operating Procedure

for

Bacteriological Examination of Environmental Waters for Members of the Thermotolerant Fecal Coliform Group Using the Membrane Filtration Technique

OBJECTIVE: This SOP describes the procedures used to examine environmental and waste waters for members of the thermotolerant fecal coliform bacteria group by membrane filtration using mFC agar medium and an incubation temperature of 44.5ºC, according to Standard Methods For The Examination of Water and Wastewater, Section 9222D.

SAMPLE HOLDING TIME AND TEMPERATURE: Samples are held below 10ºC during a maximum transport time of 6 hours. Upon receipt of samples, chain of custody and logging-in at the laboratory, samples are refrigerated and processed within 2 hours.

MEDIA PREPARATION:
Prepare commercial, dehydrated mFC agar with 1% rosolic acid (in 0.2 N NaOH) solution addition according to manufacturer's instructions. Rosolic acid solution is freshly prepared for each new lot of prepared plates. Do not sterilize by autoclaving. Record preparation details and assigned lot number in the laboratory Media and Reagent Preparation Logbook. The final pH should be 7.4 +/- 0.2. Pipet 5 mL of media into each sterile plastic, 47 x 9 mm Petri plate with tight fitting lid. Prepared mFC agar is stored in the refrigerator for up to two weeks, inverted, in sealed containers.
Test each commercially prepared medium lot against a previously acceptable lot for performance using a 24 hour culture of *E. coli* which is diluted to 10^{-6} in sterile phosphate

buffered dilution water, then filtering 1.0 mL and 0.1 mL aliquots to give 20-60 colonies per filter. If using wastewater effluent as the diluent, the effluent is collected in a bottle which has been pretreated with 0.1N sodium thiosulfate (0.5 mL/1000 mL effluent to be collected) prior to autoclaving the bottle. With each new commercial dehydrated lot of medium, verify at least 10 colonies. Test each batch of laboratory-prepared mFC agar medium for performance with positive (*E. coli*) and negative (*Enterobacter aerogenes*) culture controls.

PROCEDURE: Select sample volume to give preferably 20-60 colonies on the membrane filter surface. Multiple dilutions may be necessary depending on the sample source. Use Table 9222:IV in Standard Methods as a guide. Filter sample through a 0.45um, gridded, sterile membrane. For sample dilutions use sterile, phosphate-buffered dilution water. If less than 20 mL of sample is filtered, add buffered dilution water to the funnel in order to aid in colony dispersal. Rinse the sides of the filtration apparatus between successive filtrations with the dilution water. The filtration apparatus is changed between each series of samples or at a minimum of every 30 minutes. Transfer filter to the mFC agar Petri dish, avoiding air bubbles beneath the membrane.
The prepared plates must be submerged in the waterbath within 30 minutes of sample filtration. Prepared plates are sealed with laboratory parafilm, placed inverted inside a glass beaker of appropriate size and placed in a waterbath for incubation at 44.5 +/- 0.2 ºC for a 24 +/- 2 hour period. Plates are weighted down to keep them submerged below the water surface. Following incubation, count all various shades of blue colonies as fecal coliform. Do not count non-fecal coliform colonies which are grey to cream colored. Count colonies using a 10X magnification dissecting microscope and calculate fecal coliform density based on dilution factors applied. Verify positives monthly by picking at least 10 blue colonies from one positive sample for inoculation to LTB and EC broths and adjust counts based on percent verification. To determine false negatives, pick representative atypical colonies of different morphological types for verification.

REPORTING: If a dilution factor was applied, multiply the actual number of colonies counted per plate by the reciprocal of the dilution, and report as CFU/100 mL of sample.

QUALITY CONTROL

Positive and Negative Controls: For each new lot of mFC agar used, samples are analyzed concurrently with known positive and negative pure cultures. The positive control culture used is *E. coli* and the negative control culture is *Enterobacter aerogenes*. The pure cultures are purchased commercially and maintained in-house with records kept in the laboratory Control Cultures Logbook.

Sterility Checks: Check sterility of media, filters and rinse water at the start and at the end of each series of samples. Use sterile reagent water as the sample to check equipment sterility as applicable (filters, flasks, pipets, etc.). Record results of sterility checks on the laboratory raw data sheets.

Duplicate Analyses: Perform duplicate analyses on 10% of samples. Record results in the laboratory Precision Logbook. Calculate precision of duplicate analyses for the quantitative test method according to Standard Methods for the Examination of Water and Wastewater, 22nd Ed., Section 9020B, pg 9-18.

REFERENCES: Standard Methods for the Examination of Water and Wastewater, Section 9222D, 22nd Edition 2012.

Bacteriology

QUALITY ASSURANCE

&

STANDARD OPERATING PROCEDURE

ENVIROTECH LABORATORIES, INC.

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The Quality Assurance Plan and Standard Operating Procedure for Microbiology has been reviewed and approved on 01/06/2023

Electronically signed
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REVISION DATE: 03/15/21

Microbiology

Laboratory Equipment and Supplies

PH Meter

Accuracy and scale graduations are within 0.01.

PH buffer aliquot used only once.

Electrodes are maintained according to manufacturer's recommendations.

Standardize pH meter each use period with pH 7.0 and pH 4.0 standard buffers.
Check with 10.0-pH buffer. PH meter calculates slope.

Balance (top ***loader***) Sartorius Universal

Balance detects 100 mg at a 200-gram load.

Calibrate balance monthly using Class S or S-1 reference weights (10, 20, 50, 100, 200g weights)

Service contract with Alert Scientific. Maintenance conducted annually.
Maintenance records are on file.

Balance (Analytical) Sartorius CPA324S

Balance automatically calibrates each time power is turned on.

Balance is checked each day of use with 100mg, 200mg, 1,000mg, and 2,000mg S class weights.

Service contract through Alert Scientific. Maintenance is conducted annually.

Temperature Monitoring Device

Glass /Mercury thermometers graduated in 0.1°C increments are used for incubator units.
Mercury column in a glass thermometer is not separated.

Glass/Mercury thermometers for refrigerators are graduated in 0.5C increments.

The calibration of thermometers is checked annually, at the temperature used, against thermometers checked against a NIST thermometer (Alert Scientific).

The difference between the lab thermometer and NIST reference thermometer is recorded and lab thermometer is tagged indicating difference and correction.

Incubator Units

One incubator unit has an internal temperature monitoring device and maintains a temperature of 35º ± 0.5 ºC.

One incubator unit has an internal temperature monitoring device and maintains a temperature of 41º ± 0.5 ºC.

One incubator is maintained at 32 º ±0.5 ºC.

One water bath unit with internal temperature monitoring device that maintains the temperature at 44.5 º ± 0.2 °C. Water bath equipped with a gabled cover.

Temperature is recorded for days in use twice daily, separated by at least four hours. The times and Technician's initials are recorded.

Standard Weights

Standard weights are traceable to NIST and recalibrated yearly by a qualified service (Alert Scientific).

Autoclave

Autoclave has a temperature gauge with a sensor on the exhaust, a pressure gauge, and operational safety valve. Autoclave maintains a sterilization temperature during the sterilizing cycle and completes an entire cycle within 45 minutes when 12-15 minute sterilization is used. Autoclave depressurizes slowly to ensure media does not boil over and bubbles do not form in inverted tubes.
Temperature is set at 121 °C.

A sterilization record for each item sterilized is kept. Record indicates item sterilized, date, start up time, time and temperature reached, length of time at temperature, total time in autoclave, sterility check (using Magna Amp containing B. Stearothermophilus), and maximum registry thermometer temperature.

Colony Counter

Colony counters with dark field are used to count heterotrophic plate count colonies. (Fischer & Quebec).

Conductivity Meter

Bench top meter (Mantec Model 4310) is readable to 0.2 umhos/cm.

Meter is checked daily with a 3 levels of KCI solution (see method 120.1 or 2150 B).

Refrigerator

Refrigerators are maintained at a temperature of 4°C ± 2 °C with the thermometer bulb immersed in water.

Temperatures are recorded for days in use twice per day for both upper and lower shelves.

Inoculating Equipment

Sterile 6" wood applicator sticks are purchased from Puritan Co.

Membrane Filtration Equipment

MF units are made of autoclavable plastic. Units are not scratched, corroded and do not leak.

Filtration units are autoclaved each day before use.

Filtration units are sterilized between batches using a germicidal lamps (peak sensitivity approximately 254nm).

Samples analyzed for E. coli, fecal coliform, total coliform, and enterococcus on NSF International samples: Use only Filtration units that are sterilized by autoclave.

Microscope (Stearomaster II) with 10X and 15X magnification device with a fluorescent light source (Baush & Lomb) is used to count sheen type colonies.

Membrane filters are approved by manufacturer for total coliform analysis. See specification sheets in Appendix.

Lot number and date received for membrane filters are recorded.

For each filtration unit the 100 ml graduation mark is checked with 100 ml of water for graduated cylinder and adjusted for accuracy. With use of indelible mark 100 ml volume is etched onto cylinder.

Culture Dishes

Pre sterilized culture dishes are purchased from LeiScitech and are maintained in sealed plastic containers.

Opened packs are resealed between major use periods.

Pipets

Pre sterilized pipettes are purchased from Weber Scientific.

Pipettes have legible markings.

Opened packs are resealed between use periods.

Culture Tubes, Container, and Closures

Tubes and containers are made of borosilicate glass.

Culture tubes and containers are of significant size to contain medium plus sample without being more than three-quarters full.

Tube and container closures are plastic screw caps with non-toxic, autoclavable liners.

Sample Containers

Sample containers consist of wide mouth presterilizied, non-leaking plastic purchased from Greenwood Products. Capacities of both are at least 120 ml (4 oz.).

Sample containers for chlorinated samples are purchased which contain Sodium Thiosulfate tablet.

A sample container is selected at random from each lot of purchased containers and sterility is confirmed by adding approximately 50 ml of sterile Tryptic soy broth. The bottle is capped and rotated so that the TSB broth comes in contact with all surfaces. The bottle is incubated at 35ºC ± 0.5 for twenty-four and forty eight hrs and is checked for growth. Results are recorded. Time in and time out of incubator is recorded.

Ultraviolet lamp

Lamp is contained in box to protect technician from UV rays.

Unit is disconnected monthly and cleaned by wiping with a soft cloth moistened with ethanol.

UV lamp output is checked monthly using an ultraviotet intensity meter (J-225 Blak-Ray).

nm reading of 220-290 nm is acceptable. If reading on meter is below 7.0 on the lamp is replaced.

Stearomaster II microscope(Fisher Scientific)

Scope is checked annually by Alert Scientific.

Scope is maintained on a monthly basis: cleaning ocular, lamps and microscope.
A record of maintenance is recorded.

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General Laboratory Practices

Analytical reports, logs , charts and records

All copies created are stored for 10 years
Reports are filed by year and client.
Logs, charts, and records are filed by year.

Proficiency tests

Proficiency tests are performed yearly.
Results are forwarded to Massachusetts Department of Environmental Protection.
For unsatisfactory results:

Check for transcription error.
Check for misinterpretation of reactions.
Check quality control for all media used.

Order new Proficiency test immediately.

Sterilization Procedures

Autoclave: Tuttnauer 3870

Times for autoclaving materials at 121 degrees Celsius are listed below:

Carbohydrate containing media	12-15 min.
Membrane filter assemblies	15 min.
Sample collection bottles	15 min.
Individual glassware	15 min.
Dilution water blank	30 min.
Rinse water	30 min.

Autoclaved media is removed immediately after completion of sterilization cycle.

Membrane filter equipment is autoclaved at the beginning of each filtration series. Ultra violet light (germicidal lamp, 2537 angstroms) is used alternatively to sanitize equipment after supplies have been pre sterilized by autoclave.

The operation of the autoclave is checked each time of use with the maximum registry thermometer (121ºC).

The operation of the autoclave is checked weekly to verify sterility by use Magna Amp Biological Indicator (by Cross Tech SA1-50-05).

The ampule contains the bacteria Geobacillus stearothermophilis within a growth medium containing Bromocresol Purple as the pH indicator. The acid production associated with growth of the organism causes a color change from purple to yellow.

One ampule is placed in autoclave during normal sterilization procedures. After sterilization cycle is completed it is placed in a dry bath incubator set at 56º C (range 55°-60ºC for 48 hrs. Also a positive control (Ampule not placed in autoclave) is placed in 55ºC incubator.

Ampoules are examined at 24 hrs and 48 hrs.

Positive control should turn yellow.

Results are recorded including date and time ampule placed in dry bath and time ampule read.

A failed sterilization cycle is indicated by turbidity or change to yellow in color. A test ampule that retains its purple color indicates and adequate sterilization cycle.

Reagent Water

Deionizing unit is leased from Siemens Corporation and is used to prepare water.

Quality of reagent water is tested to assure it meets the following:

Parameter	**Limits**	**Frequency**
Residual free chlorine	< 0.03	Monthly

Specific conductance	< 1 umhos / cm	Daily
Pb, Cd, Cr, Cu, Ni, Zn	< 0.05 mg/L (collectively, 0.1)	Annually
Heterotrophic plate count	< 500 / ml	Monthly
Biosuitability	0.8 – 3.0 ratio of growth rate	Each lot

If any of the above parameters exceed the limits listed Siemen Corporation is notified and service is performed immediately on the deionizing units.

Dilution / Rinse Water

Stock buffer solution using reagent water according to standard methods 21st Edition, 2005. They are stored in 100ml volume bottles

$MgCl2$	(80 g/L)	sterilize
K $H2PO4$	34.0 g/500	sterilize – Adjust pH 7.2 with 1N NaOH

Each bottle is labeled which includes date prepared, pH, and analysts initials.
The buffers are stored at 1-5ºC.
Discard if buffer exhibits any turbidity.

Preparation of dilution / Rinse Water

Add 5 ml MgCl, 1.25 ml KH2P 04 in 1000 ml Reagent Water. Sterilize in autoclave.

Each bottle is checked for sterility by adding 50ml of water to 50 mL of a double strength Tryptose Soy Broth and incubated at 35º C for 24 and 48 hrs and checked for growth. The time of incubation and results are recorded.

Glassware Washing

All glassware and filtration units are washed in hot soapy (Liquinox detergent) water, rinsed 3 times with hot water, proceeded by 3 rinses in deionized water. Glassware is checked after each batch of glassware that is washed for residual acid or alkali with Bromothymol blue (0.04%). A Hach Bromothymol powder (cat#20672-99) pillow is added to 100 deionized water in a cleaned bottle. Color should be light green to dark green. If not rinse glassware again and repeat test.

Glassware washed in automatic dishwater using Miele Neodisher UW Detergent is checked after each batch using the same method as above.

Inhibitory Residue Test is performed by the Manufacturer on each lot to ensure that glassware is free of toxic residue. A copy of the Inhibitory Residue Test is on file.

Analytical Media

Dehydrated media is purchased commercially. It is stored in a cool, dry location. Any media showing a caked or discolored characteristic is discarded.

Containers of dehydrated media are dated upon receipt and also when initially opened. Dehydrated media is stored in a desiccator and is discarded 12 months after opening container. Any dehydrated media is discarded that has passed the manufacturer's expiration date.

For media prepared in laboratory:

- The date of preparation, pH verification (slope & control of pH media included) ,manufacturers lot and expiration date, and Technician's initials are recorded.
- Each batch of medium and broth media prepared and pre-prepared is checked with a positive and negative culture.

Total Coliforms

M-endo LES Positive= E coli negative= Ps. aeruginosa

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Fecal Coliforms & E coli Positive= E.coli negative= E. aerogenes

MFc

EC Medium

Modified m-tec

NA MUG

Pseudomonas Agar (MPAC) Positive= Ps. aeruginosa negative= E. aerogenes

MEI mod agar Positive=enterococcus negative=E coli

Brilliant Green Positive=E aerogenes negative= Ps aeruginosa

Layryl Tryptose Broth Positive=E aerogenes negative= Ps aeruginosa

A portion of the stock positive and negative culture to be used to check the performance of the media is diluted in phosphate buffer (may need at least 2 dilutions). A portion of the dilution is filtered onto a membrane filter and placed on the media to be checked, incubated and observed for the correct characteristic.

If the incorrect characteristic is observed, discard plates.

- Plates prepared in the lab are dated and stored in sealed plastic containers and stored in refrigerator at 4 º C± 2 º for no longer than 2 weeks.

- Broth prepared in lab is dated and stored in tightly screw-cap tubes and stored at 4º± 2 C. for no longer than 3 months.

- Agar prepared in the lab is dated and stored in large bottles with tight fitting caps and stored at 4ºC ±2 for no longer than 3 months.

Membrane Filter (MF) Media for total coliform

mEndo LES agar is used. The ethanol used in rehydration procedure is not denatured (Grain alcohol is purchased from Pharmco). Media is prepared in a sterile flask that is heated with frequent agitation. Medium is not boiled. The final pH is 7.2 +/- .2.

Poured MF agar plates are refrigerated and held no more than two weeks. Any plates showing growth or surface sheen is discarded immediately.

Confirmation media for total coliform

Media is sterilized for 15 minutes at 121ºC.

Lauryl Tryptose broth (LTB) is used for the presumptive test and brilliant green lactose bile
(BGLB) broth for the confirmed test. A small inverted tube (6x50mm) is added for gas formation detection.
For the presumptive test media concentration:

Use double strength concentration when using 10ml of sample. This will ensure a final single strength volume. The final pH: LTB is 6.8 +/- 0.2, BGLB broth is 7.2 +/- 0.2.

Following sterilization, tubes are inverted and checked to insure that 6x50mm tubes are covered by media and are free of bubbles.

Media is stored in the dark at 4ºC± 2 in screw cap tubes for no longer than 3 months. Discard media if evaporation exceeds 10% of original volume.

MEI (modified) medium for enterococcus (Method 1600)

Add 72g of MEI agar (difco 214885) to 1 liter of deionized water in a flask and heat to boiling until media dissolves. Autoclave for 15 min. at 121º C and cool in a 44-46-ºC water bath.

After sterilization: mix 0.24 g naladixic acid in 5 ml deionized water, add a few drops of 0.1 N NaOH to dissolve: add to the mEI medium. Then add 0.02 g triphenyl tetrazolium chloride separately to the mEI medium and mix. Final pH 7.1 ± 0.2.

Pour the mEI agar into 50 mm petri dishes to a 4-5 mm depth (approx 5 ml). Final pH should be 7.1 ±0.2. Store refrigerated. Hold plates no more than 2 weeks.

Modified M TEC Agar for E coli (method 1603)

Add 45.6 g. of modified m tec agar (BD 214884) powder to 1 liter in a flask, and heat to boiling until ingredients dissolve. Autoclave at 121ºC for 15 min. and cool in a 50ºC water bath.
Pour the medium into each 9X50 mm culture dish to a 4-5 mm depth (approx 4-6 ml) and allow to solidify. Final pH should be 7.3± 0.2. Store in refrigerator.

Nutrient Agar with MUG NA-MUG (Method 9222G)

Add 23.1 g. of media (Purchased BD 0097498) to 1000 ml of deionized water. Heat medium to boiling with agitation to completely dissolve. Sterilize by autoclaving at 121°C for 15 min. Final pH should be 6.8 ± 0.2 @ 25°C.
Approximately 20 ml is poured into sterile tubes and medium is stored in refrigerator. When needed tube containing agar is melted and 4 ml is dispensed into 50X9 petri dishes. Media tubes are stored for not more than 3 weeks.

E C Medium for Fecal Coliform

Medium is autoclaved for 15 minutes at 121ºC.

Following sterilization, tubes are examines to ensure that the inverted vials are covered by the medium and are free of air bubbles.

EC medium is maintained in tightly closed screw-cap tubes at 4ºC± 2 for no longer than 3 months. Refrigerated medium is incubated overnight at 35ºC before use. Final pH: 6.9 +/- 0.2.

Heterotrophic Plate Count (HPC) Media – Standard Methods Agar

Medium is autoclaved at 121ºC for 15 minutes. Final pH: 7.0 +/- 0.2.

Refrigerated medium is melted and tempered at 44º - 46ºC before pouring. Melted Agar is held no longer than 3 hours. Agar is not melted more than once. Refrigerated media is stored for not more than two weeks.
Agar is checked for sterility and result recorded.

M – FC Agar for Fecal Coliform Membrane Filter

M – FC Broth with Agar is sterilized by bringing it to a boiling point. Don’t over boil or autoclave

Final pH: 7.4 +/- 0.2.

Medium is refrigerated and discarded after two weeks. Medium in plates is discarded if any growth is observed.

Analytical Methodology

General

Sterility check is performed for each filtration series. Result is recorded in workbook.
Samples are analyzed within 30 hr holding time.
Samples from source water (e.g. fecal coliform, HPC, enterococcus and E coli are analyzed within 8 hrs holding time.

Method Precision (Range of logs) for fecal coliform, HPC, Enterococcus, and E coli

1. 10 samples are analyzed in duplicate
2. Calculate the logarithm of each result. If a result is zero add 1 to value before calculating.
3. Calculate the range of logs (R) for each pair of duplicates and the mean range R and determine the precision criterion.
4. Thereafter, analyze 10 % of routine samples in duplicate and calculate the range of log values.
5. If fewer than 10 samples analyzed per week, perform a duplicate on at least one sample each week.
6. If the range of log for the duplicates if greater than the Precision Criterion calculated in step 4 then the method or technique is unacceptable.
7. Discard all analytical results since last precision check. Identify and resolve analytical problem.

MF Procedure 9222 B (for enumeration of total coliforms in source water)

See paragraphs for procedure in drinking water samples.

Use appropriate sample dilutions, which yield 20-80 coliform colonies per membrane.

Initial counts are adjusted up on verification data.

MF procedure 9222D (for enumeration of fecal colonies in source water)

M-FC agar is used to isolate fecal coliform.

Appropriate sample volumes that will yield 20-60 fecal coliforms per membrane are used.

Incubate plates in water bath at 44.5ºC ± .2 for 24 ± 2 hrs.

Count typical blue colonies as fecal.

Confirm by inoculating colony into EC medium (with inverted tube).

Pour Plate Method (for enumerating heterotrophic bacteria) 9215B

The Holding time for HPC analysis is 8 hours for source water (maximum transit time is 6 hours and maximum processing time is 2 hours). If analysis cannot begin within 8 hrs, maintain sample below 4°C but do not freeze. Maximum elapsed time between collection and analysis must not exceed 24 hours.

For most potable water samples, countable plates are obtained by plating 1.0ml and 0.1ml volume of the undiluted sample. Duplicates of each dilution are plated.

Sample is mixed 25 times prior to analysis.

Sample is aseptically pipeted into the bottom of a 100mm x 15mm petri dish. Twelve to fifteen ml of tempered Standard plate agar is added to each petri dish.
The sample and melted tempered agar are mixed carefully to avoid spillage. After agar has solidified on a level surface they are inverted and incubated at 35ºC ± 0.5C for 48 ± 3 hrs. Time in and out of incubator is recorded.

Four plates are used for sterility check with each batch.
Sterility check is performed on each series of samples tested.
Plates-Agar is poured into plate without sample
Dilution water- approximately 1 ml is poured into dish and agar poured in.
Pipet- 1 ml of sterile dilution water is inoculated onto plate and agar poured in.
Air- at start of procedure pour one plate and leave cover off for 15 min.
Agar- if all plates show colonies- agar contaminated and batch discarded.

No more than 20 minutes will elapse between pipetting of sample and pouring of the media.

Plates are stacked in incubator and spaced to allow proper circulation with no more than six plates to a stack.

Colonies on duplicate plates are counted manually using a Quebec colony counter. The readings of each plate are recorded, and the results are reported as the average of the two plates. Plates having 25 to 250 colonies are used in determining plate count except plates

inoculated with 1.0 ml of undiluted sample. Counts less than 25 for such plates are acceptable. **Round off counts to two significant numbers**.
If < 25 colonies report # ESPC
If >250 colonis report # ESPC
If <10 colonies/sq Count 12 sq. take average X 60 X dilution. Report #ESPC.
If 10-100 colonies. Count 4 sq. Take average X 60 X dilution. Report # ESPC.
If > 100 colonies/sq. Report > 6,000 ESPC
example: .1 dil >6,000 1.0 dil > 6,000. Report >6,000 X 10= >60,000 ESPC

Spreaders- When spreaders must be counted, count each of the following types as one: a chain of colonies, a spreader that develops as a film of growth between the agar and bottom of petri dish, and a colony that forms at the edge of the petri dish. If a spreader covers half the plate, count colonies underneath spreader as best as possible. If plates have excessive spreader growth, report as spreaders.

Precision- 10 % of samples are checked for precision by calculating the range of logs. The range should not be greater than the precision criterion determined by laboratory.

SM 9222B and 9222G ***<u>TOTAL COLIFORM MEMBRANE FILTER PROCEDURE (rapid Confirmation of E coli)</u>***

- Using flat sterile forceps, place sterile membrane filter (0.45um) over porous plate GRID SIDE UP. Sterilize forceps with ethyl alcohol and flame between each sample or dilution.

2. Place matched funnel over receptacle and lock in place.

3 Shake sample 25 times up and down in a circular motion.

4. Pass l00 ml of sample through filter under partial pressure. (For samples other than drinking water, less water may be filtered using phosphate buffer to dilute).

5. Rinse inside of funnel with 3 successive 20 ml portions of dilution water.

6. Run control blanks on funnels before and at end of each run. Record time in and out of plates in incubator. Use sterile buffered rinse water. If either shows growth, invalidate all results between blanks.

7. Remove filter and place on an m Endo agar plate, GRID SIDE UP.

8. If more than one dilution of a sample is used rapid decontamination of equipment between each filtration series is accomplished using a U.V. sterilizing lamp.
For NSF samples: Use filtration units that are sterilized by autoclave.
Record volume of sample used, time placed in incubator, and end time plate removed from incubator.

9. Incubate for 22-24 hrs. at 35C $\pm$ 0.5. Plates may be read at 18 hrs of incubation. If no colonies present, plates are re incubated and read after 22-24 hrs for typical and atypical coliforms.

10. COUNTING:
Typical coliform colony on an m Endo has a pink to dark red color with a metallic sheen.
Use Stereo microscope at 10 magnification to enhance accuracy.

11. CALCULATION OF COLIFORM DENSITY
Compute using filters with 20-80 colonies and not more than 200 colonies per membrane.

$$\text{Total coliform/l00ml} = \frac{\text{coliform colonies counted} \times 100}{\text{(vol) ml filtered}}$$

With water of good quality (drinking water) the occurrence of coliform should be minimal, therefore count all coliform colonies and use above formula.

12. RAPID CONFIRMATION OF E COLI

After 18 hrs aseptically transfer the membrane filter containing presumptive coliform colonies on m-endo to a plate containing NA-MUG medium.

Mark each presumptive coliform positive colony (Typical and atypical, sheen and non-sheen) with permanent marker on the lid of the plate. Mark the lid and the
base of plate with a line so that the plate and lid can be realigned with the base. Marking the typical and non-typical colonies is critical as there are other bacteria (pseudomonas) that fluoresce on NA- MUG.

Incubate NA-MUG plate at 35° C for 4 hrs. Record the time in and time out plates in incubator.

Examine plate for fluorescence using ultraviolet lamp (366-nm) with a 6-watt bulb in a darkened area. Any blue fluorescence observed on the outer edge of a colony or from the back side of the plate indicates the presence of E coli.

If E coli colonies are confirmed, no further verification is required—the sample is total coliform and E. coli positive

13. COLIFORM VERIFICATION if colonies are non fluorescent after 4 hr incubation

Swab entire plate with sterile cotton swab and transfer to lauryl tryptose broth (LTB) and brilliant green broth (BGLBB). Incubate for at least 48hrs. Gas formation in both confirmation tubes indicates coliform type bacteria. Record the time in and out of tubes at 24 and 48 hrs.

If Lauryl tryptose broth is positive and Brillant Green broth is negative, take a loopful of growth from LTB and inoculate new BGLBB tube and incubate 48 hrs and observe for gas formation. Invalidate sample exhibiting heavy growth but no gas production in BGLBB and, or LTB broth. Request resample.

14. *****Samples resulting in confluent or TNTC (Too Numerous to Count) growth are recorded as "confluent growth "or "TNTC "and an additional sample is requested from same sampling site. To confirm a streak from bacteria growth is inoculated to a Brilliant Green broth tube and a Lauryl Sulfate broth tube and checked for growth and gas formation. If positive result, sample is reported as TNTC-P. If negative result, sample is reported as TNTC-I. Confluent growth is defined as a continuous bacterial growth, without evidence of sheen colonies, covering the entire membrane filter. TNTC is defined as **greater than 200 colonies** on the membrane filter in the absence of detectable coliforms. Samples with at least one coliform are not invalidated.

*****All sheen colonies (up to at least 10 colonies) are verified using single strength LTB and single strength BGLB broth.

*****Total coliform colonies are tested for fecal or E-Coli by transferring the colonies to NA-MUG medium respectively. Colonies are transferred directly from m Endo plate or LTB positive media.

*If single swab method used for confirmation TRANSFER IN ORDER: NA MUG, LTB, and BGLBB media.

SM 9222D ***<u>FECAL COLIFORM MEMBRANE FILTER PROCEDURE</u>***

1. Using sterile forceps, place sterile membrane filter (0.45um) over porous plate GRID SIDE UP. Sterilize forceps with ethyl alcohol and flame between each sample or dilution.

• Place matched funnel over receptacle and lock in place.

3. Shake sample 25 times up and down in a circular motion.

• Pass l00ml of sample through filter under partial pressure. (For samples other than drinking water, less water may be filtered using phosphate buffer to dilute).Run filtration blanks at the beginning and the end of each filtration series.

5. Rinse filtration unit with 3 successive 20 ml portions of dilution water.

6. Remove filter and place on an m M-FC agar plate, GRID SIDE UP.

• If more than one dilution of a sample is used rapid decontamination of equipment between each filtration series is accomplished using a U.V. sterilizing lamp.
• For NSF samples: Use filtration units that are sterilized by autoclave.

Record volume of sample used, time placed in water bath, and end time plate removed from water bath.

8. Incubate for 22-24 hrs. at 44.5C $\pm$ 0.2.

9. COUNTING:
Typical fecal coliform colony on an M-FC agar has blue color.
Use Stereomicroscope at 10 magnification to enhance accuracy.

10. CALCULATION OF COLIFORM DENSITY
Compute using filters with 20-80 colonies and not more than 200 colonies per membrane.

$$\text{Fecal coliform/l00ml} = \frac{\text{fecal coliform colonies counted} \times 100}{\text{(Vol) mL filtered}}$$

With water of good quality (drinking water) the occurrence of coliform should be minimal, therefore count all coliform colonies and use above formula.

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• CONFIRMATION (P. 17)

  • 9221E using EC medium for fecal coliform
  • 9221F using EC+MUG for E coli (Optional)

• Range of logs performed on 10 % of routine samples to ensure precision of methodology. Corrective action is taken if the analyses do not meet the acceptable criteria. At least one sample is run in duplicate per week if less than 10 samples are run.

SM 9221E Fecal Coliform using EC Medium

- Suspected colonies from m-Endo or mFC agar plates transfer with a sterile loop or wooden stick to EC broth tube (with inverted tube).
- Incubate EC broth tube in water bath at 44.5ºC ± 0.2 for 24 ± 2 hrs.
- EC broth is placed in water bath within 30 minutes of inoculation.
- The water level in water bath must be at least to the top of media.
- Any gas production with growth is considered a positive fecal coliform.
- Failure to produce a gas constitutes a negative reaction indicating coliform is from a source other than the intestinal tract of warm blooded animals.

EPA method 1600 **_ENTEROCOCCUS MEMBRANE FILTER PROCEDURE_**

1. Using flat sterile forceps, place sterile membrane filter (0.45um) over porous plate GRID SIDE UP. Sterilize forceps with ethyl alcohol and flame between each sample or dilution.

2. Place matched funnel over receptacle and lock in place.

3. Shake sample 25 times up and down in a circular motion.

4. Pass l00 ml of sample through filter under partial pressure. (For samples other than drinking water, less water may be filtered using phosphate buffer to dilute). Run filtration blanks at the beginning and the end of the filtration series.

5. Rinse inside of funnel with 3 successive 20 ml portions of dilution water.

6. Remove filter and place on a mEI agar plate, GRID SIDE UP.

- If more than one dilution of a sample is used rapid decontamination of equipment between each filtration series is accomplished using a U.V. sterilizing lamp.
- .For NSF samples: Use filtration units that are sterilized by autoclave

Record volume of sample used, time placed in incubator, and end time plate removed from incubator.

8. Incubate for 24 hrs. at 41ºC $\pm$ 0.5.

9. COUNTING:
   A typical enterococcus colony on mEI agar has a blue halo.
   Use Stereo microscope at 10 magnification to enhance accuracy.

10. CALCULATION OF ENTEROCOCCUS DENSITY
    Compute using filters with 20-80 colonies and not more than 200 colonies per membrane.

$$\text{Enterococcus/l00ml} = \frac{\text{enterococcus colonies counted} \times 100}{\text{(Vol) ml filtered}}$$

11. Range of logs is performed on 10 % of the routine samples to ensure precision of methodology. At least on sample per week is run in duplicate if less than 10 samples are analyzed. Corrective action is taken if analyses do not meet the acceptable criteria.

EPA Method1603 ***E COLI MEMBRANE FILTER PROCEDURE(Modified)***

1. Using sterile forceps, place sterile membrane filter (0.45um) over porous plate GRID SIDE UP. Sterilize forceps with ethyl alcohol and flame between each sample or dilution.

2. Place matched funnel over receptacle and lock in place.

3. Shake sample 25 times up and down in a circular motion.

- Pass l00ml of sample through filter under partial pressure. (For samples other than drinking water, less water may be filtered using phosphate buffer to dilute). Filtration blanks are run at the beginning and the end of each filtration series.

- Rinse filtration unit with 3 successive 20 ml portions of dilution water.

6. Remove filter and place on a modified m-TEC agar plate, GRID SIDE UP.

   If more than one dilution of a sample is used rapid decontamination of equipment between each filtration series is accomplished using a U.V. sterilizing lamp.
   For NSF samples: Use filtration units that are sterilized by autoclave
   Record volume of sample used, time placed in water bath, and end time plate removed from incubator.

7. Place plates in 35ºC ±0.5 incubator for 2 hrs. Time in and out of incubator is recorded.

8. After 2 hrs place in vacuum bag, seal and place in water bath for 22-24 hrs. at 44.2C ± 0.2. Record the time in and out that plates are placed in incubator.

9. COUNTING:
   Typical E coli colony on modified m-TEC agar has red or magenta color.
   Use Stereomicroscope at 10 magnification to enhance accuracy.

10. CALCULATION OF E COLI DENSITY
    Compute using filters with 20-80 colonies and not more than 200 colonies per membrane.

$$\text{E coli/l00ml} = \frac{\text{E coli colonies counted} \times 100}{\text{ml filtered}}$$

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11. CONFIRMATION if required
    a 9221F using EC+MUG for E coli.
    b. 9221E using EC Medium for fecal coliform.

Range of logs performed on 10 % of routine samples to ensure precision of methodology. Corrective action is taken if the analyses do no meet the acceptable criteria. Perform at least on duplicate per week if less than 10 samples are run.

SM 9223B **<u>PRESENCE/ABSENCE</u>**- P/A substrate purchased from IDEXX colisure (cat# WCL200).

Colisure media is used for the simultaneous detection and confirmation of total coliforms and E. coli in water. When total coliforms metabolize Colisure's nutrient-indicator CPRG, the sample turns yellow to re/magenta. When E coli metabolizes Colisure nutrient indicator MUG, the sample fluoresces. Colisure can simultaneously detect these bacteria within 48 hrs.

- Allow sample to equilibrate to room temperature.*
- Shake sample 25 times in circular motion. Containers have 100 ml line.
- If sample is over 100 ml line carefully with a sterile pipet remove excess volume until 100 ml is left. Add contents of one pack to a 100 ml sample into the sterile, transparent, non-fluorescing container. Cap vessel and shake thoroughly.
- Incubate at 35ºC ± 0.5ºC for 24 hrs. Record the time in and out containers placed in incubator.
- Read Results:

Appearance	Result
Yellow/gold	Negative for total coliforms and E coli
Red or magenta	positive of total coliforms
Red/magenta and fluorescence	positive for E coli

- To look for fluorescence a long wave, 366 nm UV light, Model UVL-21 from Blak-Ray is held within 5 inches of sample. Read in a dark room. Wear UVEX safety goggles and face light away from eyes.
- Sample with substrate can be incubated an additional 24 hrs to report valid negative results. Therefore, total incubation time of 48 hrs is valid. Any red or magenta color after 48 hrs could be from heterotrophs and is not a valid positive.
- Report as Presence or Absence.
- Quality Control: each new lot received.

  Inoculate 3 sterile vessels with 100 ml sterile water with the following:
  A. E coli result: red/magenta, fluorescent
  B. E. cloacae result: red/magenta, no fluorescence
  C. Pseudomonas aeruginosa result: yellow, no fluorescence

  Add to sterile vessel containing sterile water. Incubate 24 hr. at 35ºC±0.5 and observe for autofluorescence.

*Laboratory has determined that the time for several cold 100 ml samples to reach 35ºC to be about 2 hrs. therefore add an extra 2 hrs to incubation if samples are placed in incubator from cooler.

UV LIGHT VALIDATION

Prepare a cell suspension of Bacillus subtilis containing approximately 1000 cells/ml
Prepare Brain Heart Infusion(BHI) agar plates
Using a sterile inoculating loop, spread 0.25 ml of suspension on the surface of BHI agar plates. keep one un-inoculated plat as a negative control and one plate, which is inoculated but not,esposed to UV light, as a positive control.
Expose the plates under UV lamps for a defined time intervals. 5 min., 10 min., and 15 min.
After exposure, cover plates and incubate at 35 degrees C for 3 days.
From positive control, count the number of colony forming units as "initial count".
Count number of colony forming units for each exposed plate.
Calculate reduction in count after exposure.
Acceptable Criteria: 99% reduction after UV exposure of 15 Minutes.

SAMPLING, PRESERVATION AND HANDLING

Sample Collection:

1. Sample Collector:

Collector is trained in sampling procedures and, if required, approved by the appropriate regulatory authority or its designated representative.

2. Sampling Icing:

Sample collectors who deliver samples directly to the laboratory should ice samples immediately after sample collection.

3. Sampling Procedures

Keep sample container closed until ready to sample. Do not contaminate inner surface of cap and neck if bottle used to sample. Replace cap immediately after container is full. Do not rinse.

POTABLE WATER:

Remove aerators or screens and let water run for at least 5 min. to clear service line. Apply using a swab containing a solution of sodium hypochlorite (10%) to inside the faucet before sampling. Let water run for an additional 2-3 minutes after treatment.

Take sample from COLD WATER.

Do not collect samples from leaking taps that allow water to flow over outside of tap.

Sample Rejection

The laboratory will reject samples received at laboratory for the following reasons:

a. improperly labeled or unlabeled sample containers.

b. insufficient sample volume

c. Improper holding time

d. improper storage (temperature)

In case of sample rejection, the client is notified immediately and the reason for rejection is documented in comment section of computer log in. The rejection is also recorded in the Corrective Action log book. If holding time exceeds 30 hrs for drinking water samples or exceeds 6 hrs for non potable water the sample is rejected. If client desires result, laboratory will perform examination, but indicate holding time exceeded on the report.

SURFACE WATER:

Select locations to include a baseline location upstream from the study, industrial and

municipal waste outfalls, tributaries except those with a flow less than 10% of the main stream, intake points for municipal or industrial water facilities, downstream samples and downstream recreational areas.

Samples may be collected from a boat or from a bridge near critical study points.

To monitor stream and Lake Water quality, establish sampling at critical sites.

BATHING BEACHES:

Collect samples in the swimming area from a uniform depth of approximately 3 ft. Collect sample 12 inches below water surface. Consider sediment sampling of the water-beach (soil) interface because of exposure of young children at the water's edge.

To obtain baseline date on marine and estuarine bathing water quality include sampling at low, high, and ebb tides.

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Sampling should be done in afternoon (peak hours) and on peak use days.

Time elapsed between collection time and examination should not exceed 6 hrs.

COLLECTION:

Hold bottle near its base and plunge it neck downward below the surface.

Turn bottle until neck points slightly upward and mouth is directed toward the current. If there is no current, create a current artificially by pushing the bottle forward horizontally in a direction away from the hand.

When sampling from a boat, obtain sample from upstream side of boat.

Take care to avoid contacts with bank or stream bed; otherwise, water fouling may occur.

SWIMMING POOL WATER AND HOT TUB WATER

Use containers with sodium thiosulfate preservative.

Collect samples during periods of maximum bather load.

Collect samples by carefully removing cap of sterilized container and holding bottle near base at a 45º angle. Fill in one slow sweep down through water, with the mouth of the container always ahead of the hand. Replace cap. Do not rinse bottle.

Preservation and Handling

Hold temperature of all stream pollution, drinking, and wastewater samples below 10C during a maximum transport time of 6 hrs. If sample cannot be transported to lab in 6 hours, keep refrigerated a 4±2 C.

Time elapsed between collection and examination should not exceed 30 hrs for drinking water samples. For source waters 6-hour holding time is required. For source water Heterotrophic Plate Counts 8 hour holding time recommended.

Date and time of collection are recorded on the chain of custody.

Date and time of examination is recorded by laboratory on chain of custody and sample log sheets.

NEW BEDFORD HEALTH DEPARTMENT LABORATORY

SOP # 4.27 (2020)

REVISION DATE EFFECTIVE: October 7, 2020

APPROVALS: Leslie Aubut 10/7/2020

Authorized By Date

Standard Operating Procedure

for

Bacteriological Examination of Environmental Waters for Members of the Thermotolerant Fecal Coliform Group Using the Membrane Filtration Technique

OBJECTIVE: This SOP describes the procedures used to examine environmental and waste waters for members of the thermotolerant fecal coliform bacteria group by membrane filtration using mFC agar medium and an incubation temperature of 44.5°C, according to Standard Methods For The Examination of Water and Wastewater, Section 9222D.

SAMPLE HOLDING TIME AND TEMPERATURE: Samples are held below 10°C during a maximum transport time of 6 hours. Upon receipt of samples, chain of custody and logging-in at the laboratory, samples are refrigerated and processed within 2 hours.

MEDIA PREPARATION:

Prepare commercial, dehydrated mFC agar with 1% rosolic acid (in 0.2 N NaOH) solution addition according to manufacturer's instructions. Rosolic acid solution is freshly prepared for each new lot of prepared plates. Do not sterilize by autoclaving. Record preparation details and assigned lot number in the laboratory Media and Reagent Preparation Logbook. The final pH should be 7.4 +/- 0.2. Pipet 5 mL of media into each sterile plastic, 47 x 9 mm Petri plate with tight fitting lid. Prepared mFC agar is stored in the refrigerator for up to two weeks, inverted, in sealed containers.

Test each commercially prepared medium lot against a previously acceptable lot for performance using a 24 hour culture of *E. coli* which is diluted to 10^{-6} in sterile phosphate

buffered dilution water, then filtering 1.0 mL and 0.1 mL aliquots to give 20-60 colonies per filter. If using wastewater effluent as the diluent, the effluent is collected in a bottle which has been pretreated with 0.1N sodium thiosulfate (0.5 mL/1000 mL effluent to be collected) prior to autoclaving the bottle. With each new commercial dehydrated lot of medium, verify at least 10 colonies. Test each batch of laboratory-prepared mFC agar medium for performance with positive (*E. coli*) and negative (*Enterobacter aerogenes*) culture controls.

PROCEDURE: Select sample volume to give preferably 20-60 colonies on the membrane filter surface. Multiple dilutions may be necessary depending on the sample source. Use Table 9222:IV in Standard Methods as a guide. Filter sample through a 0.45um, gridded, sterile membrane. For sample dilutions use sterile, phosphate-buffered dilution water. If less than 20 mL of sample is filtered, add buffered dilution water to the funnel in order to aid in colony dispersal. Rinse the sides of the filtration apparatus between successive filtrations with the dilution water. The filtration apparatus is changed between each series of samples or at a minimum of every 30 minutes. Transfer filter to the mFC agar Petri dish, avoiding air bubbles beneath the membrane.

The prepared plates must be submerged in the waterbath within 30 minutes of sample filtration. Prepared plates are sealed with laboratory parafilm, placed inverted inside a glass beaker of appropriate size and placed in a waterbath for incubation at 44.5 +/- 0.2 ^{o}C for a 24 +/- 2 hour period. Plates are weighted down to keep them submerged below the water surface. Following incubation, count all various shades of blue colonies as fecal coliform. Do not count non-fecal coliform colonies which are grey to cream colored. Count colonies using a 10X magnification dissecting microscope and calculate fecal coliform density based on dilution factors applied. Verify positives monthly by

picking at least 10 blue colonies from one positive sample for inoculation to LTB and EC broths and adjust counts based on percent verification. To determine false negatives, pick representative atypical colonies of different morphological types for verification.

REPORTING: If a dilution factor was applied, multiply the actual number of colonies counted per plate by the reciprocal of the dilution, and report as CFU/100 mL of sample.

QUALITY CONTROL

Positive and Negative Controls: For each new lot of mFC agar used, samples are analyzed concurrently with known positive and negative pure cultures. The positive control culture used is *E. coli* and the negative control culture is *Enterobacter aerogenes*. The pure cultures are purchased commercially and maintained in-house with records kept in the laboratory Control Cultures Logbook.

Sterility Checks: Check sterility of media, filters and rinse water at the start and at the end of each series of samples. Use sterile reagent water as the sample to check equipment sterility as applicable (filters, flasks, pipets, etc.). Record results of sterility checks on the laboratory raw data sheets.

Duplicate Analyses: Perform duplicate analyses on 10% of samples. Record results in the laboratory Precision Logbook. Calculate precision of duplicate analyses for the quantitative test method according to Standard Methods for the Examination of Water and Wastewater, 22nd Ed., Section 9020B, pg 9-18.

REFERENCES: Standard Methods for the Examination of Water and Wastewater,

Section 9222D, 22nd Edition 2012.

REVISION DATE EFFECTIVE: ___October 2, 2020

APPROVAL: _Leslie Aubut 10/2/2020

Lab Director Date

Standard Operating Procedure

for

Bacteriological Examination of Potable Water for Members of the Total Coliform Group Using the Membrane Filtration Technique on mEndo Agar LES Medium, According to Standard Methods 9222B and Rapid Confirmation of *E. coli* Using NA-mug Medium, According to Standard Methods 9222G.

Objective: This SOP describes the procedures used to examine and enumerate potable water samples for members of the total coliform bacteria group by membrane filtration in 22 to 24 hours, and rapid confirmation of *E. coli* within 4 hours, in accordance with Standard Methods for the Examination of Water and Wastewater, Sections 9222B and 9222G, respectively, 22nd Edition, 2012.

MATERIALS:

Sample Bottles - sterilized glass or plastic bottles.

Dilution Bottles -sterile borosilicate glass marked with graduations.

Buffered Dilution Water - Refer to SOP # 4.13.

Pipets and Graduated Cylinders - sterile, of appropriate size.

Containers for Culture Medium - sterile flasks or bottles.

Culture Dishes - sterile glass or plastic, 47 x 9 mm, tight fitting to avoid moisture loss during incubation.

Filtration Units - sterile glass or plastic units, marked with volume graduations, autoclaved before each use.

Electric Vacuum Pump - attached to a sidearm catch flask.

Membrane filters - sterile, grid marked, with a pore size capable of retaining coliform bacteria (0.45 um).

Forceps - smooth tipped, flat and without corrugations on the inner side, sterilized before each use by flaming.

Incubator - at 35 +/- 0.5°C, capable of maintaining high humidity.

Magnification Lens and Light Source - 10X-15X magnification and cool light source (Spencer Cycloptic Series 56 Dissection Microscope).

M Endo Agar LES - Difco dehydrated media or equivalent. Prepare according to manufacturer's instructions. Check final pH and dispense in 4 mL amounts to 47 mm Petri dishes. May be stored refrigerated, in sealed containers, for up to two weeks. Dehydrated media stored for 1 year after opening, in a dessicator.

NA-mug medium - Difco dehydrated media or equivalent. Prepared according to manufacturer's instructions. Check final pH and dispense in 5 mL amounts to tightly closed screw cap tubes. The sterile tubed media is stored at 4°C for up to 3 months prior to pouring plates.

E. coli and Enterobacter - Positive control cultures to be used with each new lot of media opened and each new lot of media prepared.

Staphylococcus - Negative control culture to be used for each new lot of media opened and each new lot of media prepared.

Ultraviolet lamp – 366-nm with a 6 watt bulb.

Laurel Tryptose Broth (LTB) and Brilliant Green Bile Broth (BGBB 2%) – Prepared according to manufacturer instructions, in tightly closed screw cap tubes containing an inverted Durham tube. Following sterilization, tubes are stored refrigerated for up to 3 months.

SAMPLE HOLDING TIME AND TEMPERATURE: Preferably samples are held below 10°C (50°F) during a maximum transport time of 6 hours. A temperature control is recorded. Upon receipt of samples, chain of custody and logging-in at the laboratory, samples are refrigerated and processed within 2 hours.

PROCEDURE:

The standard volume to be filtered is 100 mL. Samples are shaken prior to analysis and filtered promptly. The sample is poured to the 100 mL volume mark on the filtration cup. Smaller volumes, or dilutions, may be necessary if water is suspected to contain a high level of total coliforms. Use sterile, flat forceps to place a sterile membrane filter (grid side up) over the porous plate of the receptacle. Lock in place and filter under vacuum. Rinse the used filter with a flow of sterile buffered dilution water from a squeeze bottle. Remove the membrane filter with sterile forceps and place it on the mEndo medium in the dish (avoid air entrapment). If a series of samples is run prepare sterile rinse water control samples (20-30 mL) before and after the series of test samples. Invert the prepared plates and incubate for 22 - 24 hours at 35 +/- 0.5^{o}C. Record the test results of dilution water, media and positive and negative controls on the raw data form.

CALCULATION OF COLIFORM DENSITY:

All bacteria producing a red colony with a metallic sheen within 22- 24 hours incubation at 35° are considered members of the coliform group. Count sheen colonies using the dissection microscope with 10X-15X magnification. Compute the count, using membrane filters with 20 to 80 colonies, and not more than 200 colonies, as per 100 mL of sample, as described in Standard Methods Section 9222 B.6. If confluent or nondistinct growth occurs in exceedence of 200 colonies per membrane, report as invalid and request a new sample as described in SM 9222 B.6.a. Report confluent growth or TNTC with at least one detectable coliform colony (which is verified) as a total coliform positive sample. In the event of a positive coliform sample, within 24 hours of obtaining valid data, the lab shall notify its client (Acushnet Water Department) of all samples which exceed the established maximum contaminant level, ie; zero total coliforms in potable water. The date, time, and manner of notification must be documented on the data sheet and kept on file.

RAPID CONFIRMATION OF *E. COLI*:

1. If coliforms are observed, aseptically transfer a membrane filter containing typical and/or atypical presumptive coliform colonies on the m-Endo medium to a plate containing NA-MUG medium.
2. Mark each sheen colony and each atypical colony with a permanent marker on the lid of the plate. Also mark the lid and base of the plate with a line to enable realignment if the lid is removed. Marking the sheen and atypical colonies is critical since other bacteria (e.g., Pseudomonas spp.) can fluoresce on NA-MUG medium.
3. Inoculated NA-MUG plates are incubated at 35 +/- 0.5°C for four hours.
4. Examine the plate for florescence using a 366-nm ultraviolet lamp with a 6 watt bulb in a darkened area. Any blue florescence observed on the outer edge of a colony or from the back side of the plate indicates the presence of *E. coli*.
5. If *E. coli* colonies are confirmed, no further verification is required – the sample is total coliform and *E. coli* positive.
6. If *E. coli* colonies are not detected following the four hour incubation, the membrane surface is aseptically streaked and transferred with a sterile loop (flamed before each transfer) and used to inoculate tubes of LTB, and BGBB 2% medium for total coliform verification. The broth tubes contain Durham tubes for the detection of gas production. Inoculated LTB and BGBB tubes are incubated at 35°C for an additional

24 hours. The presence (positive) or absence (negative) of gas formation is recorded.

QUALITY CONTROL:

Duplicate Analyses: Perform duplicate analyses on 10% of samples and on at least one sample per test run. Record results in the laboratory Precision and Accuracy Logbook. Calculate precision of duplicate analyses for the quantitative test method according to Standard Methods for the Examination of Water and Wastewater, 21st Ed., Section 9020B, pg 9-10.

Positive and Negative Controls: For each new lot of mEndo and/or NA-MUG medium prepared, samples are analyzed concurrently with known positive and negative pure cultures. The pure cultures are purchased commercially and maintained in-house with records kept in the laboratory Control Cultures Logbook.

Sterility Checks: Check sterility of media, filters and rinse water at the start and at the end of each series of samples. Use sterile reagent water as the sample to check equipment sterility as applicable (filters, flasks, pipets, etc.). Record results of sterility checks on the raw data.

REFERENCES: Standard Methods for the Examination of Water and Wastewater,

Section 9222B and 9222G, 22nd Edition, 2012.

Envirotech Laboratories, Inc.

USEPA 300.0

STANDARD OPERATING PROCEDURE

For

Determination of Anions (Nitrate, Nitrite, Sulfate, Chloride, Fluoride) in Aqueous Samples Using ion Chromatography

01/18/23

		Signature	Date
Analyst:	Kassie Finlayson	electronically signed	01/18/23
Laboratory Director:	Ronald Saari	electronically signed	01/18/23
QA/QC Manager:	Kevin Burgess	electronically signed	01/18/23

EPA METHOD 300.0

DETERMINATION OF INORGANIC ANIONS BY ION CHROMATOGRAPHY
Ref: "Methods for the Determination of Inorganic Substances in Environmental Samples", EPA/600/R/100, Aug 1993.

1.0 SCOPE AND APPLICATION

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

PART A.	
Bromide	Nitrite
Chloride	
Fluoride	Sulfate
Nitrate	

1.2 The matrices applicable to each method are shown below:

1.2.1. Drinking water, surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, solids (after extraction 11.7), leachates (when no acetic acid is used).

1.2.2. Drinking water and reagent waters

1.3 The single laboratory Method Detection Limit (MDL defined in Sect. 3.2) for the analytes listed in Tables IA. The MDL for a specific matrix may differ from those listed, depending upon the nature of the sample.
The MDL is performed on each analyte every 6 months.

1.4 Method A is recommended for drinking and wastewaters.

1.5 This method is recommended for use only by or under the supervision of analysts experienced in the use of ion chromatography and in the interpretation of the resulting ion chromatograms.

1.6 When this method is used to analyze unfamiliar samples for any of the above anions, anion identification should be supported by the use of a fortified sample matrix covering the anions of interest. The fortification procedure is described in Sect. 11.6.

1.7 Users of the method data should state the data-quality objectives prior to analysis.

Users of the method must demonstrate the ability to generate acceptable results with this method, using the procedures described in Sect. 9.0

2.0 SUMMARY OF METHOD

2.1 A small volume of sample, typically 8 to 10 ml, is introduced into an ion chromatograph. The anions of interest are separated and measured, using a system comprised of a guard column, analytical column, suppressor device, and conductivity detector.

2.2 The main differences between Parts A and B are the separator columns and guard columns. Sections 6.0 and 7.0 will elicit the differences.

2.3 An extraction procedure must be performed to use this method for solids (See 11.7).

2.4 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 9.0, Quality Control.

3.0 DEFINITIONS

3.1 CALIBRATION BLANK (CB) -- A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.

3.2 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 FIELD DUPLICATES (FD) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of field duplicates indicate the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.4 INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test

3.5 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or

other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.6 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.7 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.8 LINEAR CALIBRATION RANGE (LCR) The concentration range over which the instrument response is linear.

3.9 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.10 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.11 PERFORMANCE EVALUATION SAMPLE (PE) -- A solution of method analytes distributed by the Quality Assurance Research Division (QARD), Environmental Monitoring Systems Laboratory (EMSL-Cincinnati), U. S. Environmental Protection Agency, Cincinnati, Ohio, to multiple laboratories for analysis. A volume of the solution is added to a known volume of reagent water and analyzed with procedures used for samples. Results of analyses are used by QARD to determine statistically the accuracy and precision that can be expected when a method is performed by a competent analyst. Analyte true values are unknown to the analyst.

3.12 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS

is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.13 STOCK STANDARD SOLUTION (555) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4.0 INTERFERENCES

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

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

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

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

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

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

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

4.8 Any residual chlorine dioxide present in the sample will result in the formation of additional chlorite prior to analysis. If any concentration of chlorine dioxide is suspected in the sample, purge the sample with an inert gas (argon or nitrogen) for about five minutes or until no chlorine dioxide remains.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material. Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1 Sulfuric acid (7.4)

6.0 Equipment and Supplies

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

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

6.2.1 Laboratory is using a Metrohm model 882 IC with an automatic sampler. System is computer directed using the MagIC net 3.3 software.

6.2.2 Anion separator column: This column produces the separation shown in Figures 1 and 2.

6.2.2.1 Anion analytical column (Method A): The separation shown in Figure 1 was generated using a Dionex AS9-SC column (Thermo Scientific P/N 043185). An optional column may be used if comparable resolution of

peaks is obtained, and the requirements of Sect. 9.2 can be met.
6.2.2.2 Dionex Guard column (Thermo Scientific P/N 043186)

6.2.3 Anion suppressor device: The data presented in this method were generated using a Metrohm suppressor module (MSM) anion micro membrane suppressor.

6.2.4 Detector -- Conductivity cell: approximately 1.25 ul internal volume, (Metrohm P/N 1.850.9010,) capable of providing data as required In Sect. 9.2.

6.2.5 The Metrohm MagIC net 3.3 data Chromatography Software is used to generate all the data.

7.0 Reagents and Standards

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

7.2 Reagent water: Deionized water, free of the anions of interest. Water should contain particles no larger than 0.20 microns.

7.3 Eluent solution (Method A) Purchased from Thermo Scientific (Dionex AS4A eluent concentrate). Final concentration after 100 X Dilution is 1.8mM Sodium Carbonate and 1.7mM Sodium Bicarbonate.

7.4 **Regeneration Solution (micro membrane suppressor**:
7.4.1 Stock solution 10N. Dilute 280 ml concentrated sulfuric acid (H_2S0_4) to 1 L with reagent water.
7.4.2 Stock Oxalic Acid solution: 63g of oxalic Acid in 1 L reagent water.
7.4.3 Dilute 20mL of 10N sulfuric acid and 20mL of oxalic acid Stock solution into 1 L reagent water.

7.5 Stock standard solutions: are purchased and proper dilutions are prepared see below:

Sulfate: 1,000 mg/L Agilent cat# ICC-006.
Nitrate: 1,000 mg/L (225.73 mg/L Nitrate-N) Agilent cat# ICC-004A
Chloride: 1,000 mg/L Agilent cat# ICC-002
Nitrite: 1,000 mg/L (304 mg/L Nitrite-N) Agilent cat# ICC 007

8.0 Sample Collection. Preservation and Storage

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis, if required, and minimize waste disposal.

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

Analyte	Preservation	Holding Time
Bromate	None required	28 days
Bromide	None required	28 days
Chlorate	None required	28 days
Chloride	None required	28 days
Chlorite	Cool to 4^0C	immediately
Fluoride	None required	28 days
Nitrate-N	Cool to 4^0C	48 hours
Combined (NO3/NO2)	conc. H_2S0_4 to a pH < 2	28 days
Nitrite-N	Cool to 4^0C	48 hours
0-Phosphate-P	Cool to 4°C	48 hours
Sulfate	Cool to 4°C	28 days

NOTE: If the determined value for the combined nitrate/nitrite exceeds 0.5mg/L as N, a resample must be analyzed for the individual concentrations of nitrate and nitrite.

8.3 The method of preservation and the holding time for samples analyzed by this method are determined by the anions of interest. In a given sample, the anion that requires the most preservation treatment and the shortest holding time will determine the preservation treatment. It is recommended that all samples be cooled to 4°C and held for no longer than 28 days for Method A and analyzed immediately in Method B.

NOTE: If the sample cannot be analyzed for chlorite within < 10 minutes, the sample may be preserved by adding 1mL of the ethylenediamine (EDA) preservation solution (7.6) to 1L of sample. This will preserve the concentration of the chlorite for up to 14 days. This addition of EDA has no effect on bromate or chlorate, so they can also be determined in a sample preserved with EDA. Residual chlorine dioxide should be removed from the sample (per 4.8) prior to the addition of EDA.

9.0 QUALITY CONTROL

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of LCR and analysis of QCS) and laboratory performance (determination of MDL) prior to performing analyses by this method.

9.2.2 Linear Calibration Range (LCR) -- The LCR is determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity uses a blank and five standards. If any verification data exceeds the initial values by ±10%, linearity must be reestablished. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required meeting data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within + 10% of the stated values performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDL or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDL are established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit (6). To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where:

t = value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].

S = standard deviation of the replicate analyses. MDL should be determined every 6 months, when a new operator begins work or whenever there is a significant change in the background or instrument response.

ANALYT E	PEAK #	* RETENTION TIME (MIN)	Spiking solution Concentration (mg/L)	MDL mg/L
Fluoride	1	1.2	0.10	0.1
Chloride	2	1.7	3.0	3.0
Nitrite-N	3	2.0	0.012	0.006
Nitrate-N	5	3.2	0.025	0.01
Sulfate	7	6.9	3.0	3.0

9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1 Laboratory Reagent Blank (LRB) -- The laboratory analyzes at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory analyzes at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory uses the LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

UPPER CONTROL LIMIT = x + 3S
LOWER CONTROL LIMIT = x - 3S

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data is used to establish an on-going precision statement for the level of

concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC) -- For all determinations the laboratory analyzes the IPC (a midrange check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ±10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift, the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The data is kept on file.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory adds a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot is a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.1.1 If the concentration of fortification is less than 25% of the background concentration of the matrix the matrix recovery should not be calculated.

9.4.2 Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculated using the following equation:

$$R = \frac{C(s) - C}{s} \times 100$$

Where, R = percent recovery.
C(s) = fortified sample concentration.
C = sample background concentration.
s = concentration equivalent of analyte added to sample.

9.4.3 Until sufficient data becomes available (usually a minimum of 20 to 30 analysis), assess laboratory performance against recovery limits for method A of 80 to 120% and 75 to 125% for method B. When sufficient internal performance data becomes available develop control limits from percent mean recovery and the standard deviation of the mean recovery.

9.4.4 If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (Sect. 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

10.0 Calibration and Standardization

10.1 Establish ion chromatographic operating parameters equivalent to those indicated in Tables l A or lb.

10.2 For each analyze of interest, prepare calibration standards at a minimum of three concentration levels and a blank by adding accurately measured volumes of one or more stock standards (7.5) to a volumetric flask and diluting to volume with reagent water. If a sample analyte concentration exceeds the calibration range the sample may be diluted to fall within the range. If this is not possible then three new calibration concentrations must be chosen, two of which must bracket the concentration of the sample analyte of interest. Each attenuation range of the instrument used to analyze a sample must be calibrated individually.

10.3 Using injections of 0.1 to 1.0 ml (determined by injection loop volume) of each calibration standard, tabulate peak height responses against the concentration. The results are used prepare a calibration curve for each analyte. During this procedure, retention times are recorded.

10.4 The calibration curve is verified on each working day, or whenever the anion eluent is changed, and after every 20 samples. If the response or retention time for any analyte varies from the expected values by more than ± 10% the test must be-repeated, using fresh calibration standards. If the results are still more than 10 %, a new calibration curve must be prepared for that analyte.

10.5 Nonlinear response can result when the separator column capacity is exceeded (overloading). The response of the detector to the sample when diluted 1:1, and when not diluted should be compared. If the calculated responses are the same, samples of this total anionic concentration need not be diluted.

10.6 Quality Control

10.6.1 Check calibration curve daily, whenever the eluent is changed and after every 20 samples. Use one of the standards for calibration. Concentration of the standard must be within 10% of the expected values. If standard is not within 10%, repeat using fresh calibration standard. If the results are not within 10 %, a new calibration curve must be prepared for the analyte.

10.6.2 Calibration curve, linear calibration range must be determined every 6 months or whenever a significant change in instrument response is observed or expected. The verification of linearity must use a minimum of a blank and three standards.

10.6.3 Instrument Performance is check

10.6.4 Daily Analytical Sequence is listed as follows: see Appendix 1 standard make-up

Batch 1: IPC-1, IPC-2, LRB, Sample 1-10, IPC-1, IPC-2, Blank, LFB, LR, LFM, Sample 10 - 20, IPC-1, IPC-2, LRB, LR, LFM, CCS-5, CCS-10
Batch 2: repeat batch 1 sequence
End of day: CCS 5, CCS 10, LRB, Anions High, 5.0 NO3, 0.76 NO2, MDL sequence, LRB, LRB, LRB

11.0 Procedure

11.1 Check system calibration daily and, if required, recalibrate as described in Sect. 10.

11.2 Load and inject a fixed amount of well mixed sample. Flush injection loop thoroughly, using each new sample. Use the same size loop for standards and samples Record the resulting peak size in area or peak height units. An automated constant volume injection system may also be used.

11.3 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for each analyte. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.4 If the response for the peak exceeds the working range of the system, dilute the sample with an appropriate amount of reagent water and reanalyze.

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

NOTE: Retention time is inversely proportional to concentration. Nitrate and sulfate exhibit the greatest amount of change, although all anions are affected to some degree. In some cases this peak migration may produce poor resolution or identification.

11.6 The following extraction should be used for solid materials. Add an amount of reagent water equal to ten times the weight of dry solid material taken as a sample. This slurry is mixed for ten minutes using a magnetic stirring device. Filter the resulting slurry before injecting using a 0.45 micrometer membrane type filter. This can be the type that attaches directly to the end of the syringe. Care should be taken to show that good recovery and identification of peaks is obtained with the user's matrix through the use of fortified samples.

11.7 It has been reported that lower detection limits for bromate (7g/L) can be obtained using a borate based eluent 7. The use of this eluent or other eluents that improve method performance may be considered as a minor modification of the method and as such still are acceptable.

11.8 Should more complete resolution be needed between peaks (7.3) sample can be diluted. This will spread out the run but cause the later eluting anions to be retained longer. Lab must determine to what extent the eluent is diluted and should not be considered a deviation from the method.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve for each analyte by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

12.2 Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

12.3 Report results in mg/L.

12.4 Report:

NO2 - as N
NO3 - as N

12.5 When integrating chromatography peaks, either automatically or manually, ensure that integrations are performed in a correct and consistent manner for standards and samples, including quality control samples.

Possible reasons for manually integrating a peak might be an nearby peak interfering with the target analyte, or the software not correctly detecting the target analyte peak.

Documentation of manual integrations include the following:

12.5.1 The original chromatogram and the manual integrated chromatogram must be documented.
12.5.2 The analyst's initials, date of manual integration, and the reason(s) for the manual integration must be documented on chromatograph.

13.0 METHODS PERFORMANCE

13.1 Tables lA and 2A give the single laboratory (EMSL-Cincinnati) MDL for each anion included in the method under the conditions listed.

13.2 Tables 2A and 2B give the single laboratory (EMSL-Cincinnati) standard deviation for each anion included in the method in a variety of waters for the listed conditions.

13.3 Multiple laboratory accuracy and bias data (S0) and estimated on single operator values (St) for reagent, drinking and waste water using method A and are given for each anion in Tables 3 through 9. Data from 19 laboratories were used for this data.

13.4 Some of the bias statements, for example chloride and sulfate, may be misleading due to spiking small increments of the anion into large naturally occurring concentrations of the same anion.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency

recommends recycling as the next best option.

14.2 Quantity of the chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3 For information about pollution prevention that may be applicable to laboratories and research institutions consult "Less is Better: Laboratory Chemical Management for Waste Reduction," available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel," available from the American Chemical Society at the address listed in Sect. 14.3

Date:
2016

Analyte: Fluoride

Sample	Concentration (mg/L)	Date
1	0.098	1/7
2	0.103	1/8
3	0.097	1/11
4	0.097	1/12
5	0.088	1/22
6	0.088	1/23
7	0.118	1/26

Sum	0.689
Average	0.098
Standard Deviation	0.010
MDL	0.032
RL	0.10
Method	300.0

Date: Sept 2016

Analyte: CHLORIDE

Sample	**Concentration (mg/L)**	**Date**	**Concentration (mg/L)**	**Date**
1	6.50	1/8/2016	1.66	9/14/2016
2	6.48	1/11/2016	1.63	9/15/2016
3	6.44	1/12/2016	1.61	9/15/2016
4	6.51	1/15/2016	1.65	9/16/2016
5	6.50	1/19/2016	1.61	9/16/2016
6	6.46	1/21/2016	1.62	9/17/2016
7	6.42	1/23/2016	1.60	9/19/2016

Sum	45.31	11.38
Average	6.473	1.626
Standard deviation	0.034	0.022
MDL	0.107	0.070
RL	3.0	3.0
Method	300.0	300.0

Date: Sept 2016

Analyte: NITRATE

Sample	**Concentration (mg/L)**	**Date**
1	0.022	9/14/2016
2	0.022	9/15/2016

3	0.022	9/15/2016
4	0.023	9/16/2016
5	0.021	9/16/2016
6	0.021	9/17/2016
7	0.021	9/19/2016

Sum	0.152
Average	0.022
Standard Deviation	0.001
MDL	0.002
RL	0.01
Method	300.0

Date: Sept 2016

Analyte: Nitrite

Sample	**Concentration (mg/L)**	**Date**
1	0.014	9/14/2016
2	0.017	9/15/2016
3	0.014	9/15/2016
4	0.018	9/16/2016
5	0.016	9/16/2016
6	0.017	9/17/2016
7	0.017	9/17/2016

Sum	0.113
Average	0.016
Standard Deviation	0.002
MDL	0.005
RL	0.006
Method	300.0

16. Operation and Maintenance

16.1 Preparing Eluents

16.1.1 Degassing Eluents

Dionex strongly recommends degassing all eluents and storing them in reservoirs pressurized with filtered inert gas (see Section 3.1.3). This helps prevent bubbles (resulting from eluent outgassing) from forming in the pump and the detector cell. Degassed eluents and pressurized reservoirs are especially important when combining aqueous and non-aqueous components (e.g., water and methanol).

Several degassing procedures can be used, including vacuum degassing, sparging with helium, or sonication without vacuum. Follow the steps below for vacuum degassing:

16.1.2 Prepare the eluent required for your application. Pour it into a clean vacuum flask and attach the flask to a vacuum pump or water aspirator.

16.1.3 Vacuum degas the eluent for 30 minutes while agitating the solution by shaking or sonication.

Note: If using non-aqueous components, do not degas eluents for longer than 5-minutes; volatile compounds may be lost.

16.1.4 Remove the flask from the vacuum. Do not allow water to flow from the aspirator back into the flask.

16.1.5 Pour the degassed eluent into a pressurizable reservoir. Be careful not to shake the eluent.

16.1.6 Install end-line filters and pressurize the reservoirs (see Sections 3.1.2 and 3.1.3).

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3.1.2 Filtering Eluents

Always filter eluents before operation to remove small particulates that may contaminate the pump check valves and cause erratic flow rates or loss of prime. End-line filters *(PIN* 045987) are supplied in the pressurizable reservoir ship kits for this purpose.

Install an end-line filter on the end of the eluent line inside the reservoir. To prevent air from being drawn through the lines, make sure that the end of the filter reaches the bottom of the eluent reservoir.

3.1.3 Pressurizing Eluent Reservoirs

Pressurize eluent reservoirs with filtered inert gas (preferably helium). If helium is not available, argon or nitrogen can be used. Refer to the *Pressurizable Reservoir Installation Instructions* for details.

1. Verify that the gas supply is connected to the HELIUM INPUT connector on the rear panel and is regulated to between 0.14 and 0.69 MPa (20 and 100 psi).

2. Press Eluent **Pressure** to turn on the gas pressure to the eluent reservoir(s). A regulator inside the DX-120 regulates the pressure to 0.06 to 0.07 MPa (8 to 10 psi).

A. CAUTION
Never pressurize the reservoirs above 0.07 MPa (10 psi).
If using glass reservoirs, inspect them periodically for scratches or cracks.

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3.2 Preparing Samples

3.2.1 Collecting and Storing

Collect samples in high density polyethylene containers that have been thoroughly cleaned with deionized water. Do not clean containers with strong acids or detergents because they leave traces of ions on the container walls; these ions may interfere with analysis.

If samples will not be analyzed the same day they are collected, filter them through clean 0.45 ~m filters immediately after collection, otherwise bacteria in the samples may cause the ionic concentrations of ions to change with time. Refrigerating the samples at 4 ^{0}C will minimize, but not eliminate, bacterial growth.

Analyze samples containing nitrite or sulfite as soon as possible. Nitrite oxidizes to nitrate, and sulfite to sulfate, thus increasing the measured concentrations of these ions in the sample. Most samples that do not contain nitrite or sulfite can be refrigerated for at least one week with no significant changes in anion concentrations.

3.2.2 Pretreating

Analyze rain water, drinking water, and air particulate leach solutions directly with no sample preparation (other than possibly filtering and diluting).

Filter groundwater and wastewater ~amples through 0.45 ~m filters before injection if they were not filtered after collection.

Before injection, pretreat samples that may contain high concentrations of interfering substances by putting them through Dionex OnGuardTM cartridges. Refer to the *installation and Troubleshooting Guide for OnGuard Cartridges* (Document No.032943) for instructions on preparing and using the cartridges.

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3.2.3 Diluting

The concentrations of ionic species in different samples can vary widely from sample to sample, so no single dilution factor can be recommended for all samples of one type. In some cases (for example, many water samples) concentrations are sufficiently low so that dilution is not necessary.

Use deionized water or eluent to dilute the sample. When using carbonate/bicarbonate eluents, diluting with eluent minimizes the effect of the water dip at the beginning of the chromatogram. If you use eluent to dilute the sample, also use eluent to prepare the calibration blank and standards. This is most important for fluoride and chloride, which elute near the water dip. To improve the accuracy of early eluting peak determinations, such as fluoride, at concentrations below 50 ppb, dilute standards in eluent or spike the samples with concentrated eluent to minimize the water dip. For example, spike a 100 mL sample with 1.0 mL of a 100 X eluent concentrate.

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4.1 Initial start-up■■■■■■■■■■■■■■■■■■■■■■■50■■■■■■■■■882 Compact IC plus – Anion

4 Start-up

The chapter Start-up is divided into 2 sections:

Initial start-up The initial start-up is carried out during the initial installation.
Conditioning Conditioning is carried out as a final installation step and each time after the system is started.

4.1 Initial start-up

The initial start-up is carried out during the initial installation. The entire system is rinsed before guard column and separation column are installed.

Caution: The separation column and guard column may not be installed for the initial start-up.
Make sure that the coupling (6.2744.040) is being used instead of the columns.

Perform the following steps during the initial start-up:

1 Preparing the software
■Start the PC program MagIC Net™.
■Open the Equilibration tab in MagIC Net™.
■Select (or create) a suitable method.

2 Preparing the instrument
■Ensure that the eluent aspiration tubing is immersed in the eluentand that there is enough eluent in the eluent bottle.
■Ensure that the aspiration tubings for the auxiliary solutions(regeneration solution and rinsing solution) are immersed into therespective solutions and that the is enough solution in the bottles.
■Switch on the instrument.

3Starting equilibration
■In MagIC Net™, start the equilibration.

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4 Deaerate the high pressure pump
■Deaerate the high pressure pump(s) via the purge valve (seeChapter 3.9.2, page 30).

5 Set the contact pressure of the peristaltic pump

Note: This work step needs to be performed only if a peristaltic pump is being used.

▪If peristaltic pumps are used, set the contact pressure (see "Setflow rate", page 41).
6 Rinsing the instrument without columns
▪Rinse the instrument (without columns) with eluent for 5 minutes.

The instrument is now ready for the installation of the columns (see Chap-ter 3.16, page 46).

4.2 Conditioning

After the installation and after switching on the instrument, the system must be conditioned with eluent until a stable baseline is reached.

Note: After a change of eluent (see Chapter 5.4.2.3, page 56), the condi-tioning time can lengthen considerably.

Conditioning the system

1 Preparing the software

Caution: Ensure that the flow set is not higher than the flow permissible forthe corresponding column (see column leaflet and chip data set).

▪Start the PC program MagIC Net™.
▪Open the Equilibration tab in MagIC Net™.

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▪Select (or create) a suitable method.

2 Preparing the instrument
▪Ensure that the column is correctly mounted according to the flow direction indicated on the label (arrow must point in the direction of flow).
▪Ensure that the eluent aspiration tubing is immersed in the eluent and that there is enough eluent in the eluent bottle.
▪Ensure that the aspiration tubings for the auxiliary solutions(regeneration solution and rinsing solution) are immersed into the respective solutions and that the is enough solution in the bottles.

3 Checking leak-tightness
▪In MagIC Net™, start the equilibration.
▪Check all capillaries and their connections from the high pressure pump to the detector for signs of liquid escaping. If eluent escapes anywhere, tighten the corresponding pressure screw or loosen the connection, check the end of the capillary, shorten it with a capillary cutter if necessary, and restore the con-nection.

4 Conditioning the system
Rinse the system with eluent until the required stability of the base-line is attained (normally 30 minutes).

During this time, step the MSM to the next position every 10minutes.

The instrument is now ready for measuring samples.

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5 Handling and maintenance

5.1 General notes

5.1.1 Care

Warning: The instrument housing must not be opened by untrained personnel. The instrument requires appropriate care. Excess contamination of theinstrument may result in functional disruptions and a reduction in the serv-ice life of the sturdy mechanics and electronics.CautionAlthough this is prevented to a great extent by design measures, themains plug should be unplugged immediately if aggressive media haspenetrated the inside of the instrument, so as to avoid serious damageto the instrument electronics. In such cases, the Metrohm Service mustbe informed.On the rear of the instrument, the drainage tubings must be mounted andthe leak sensor must be plugged in and activated as protection againstescaping liquids.Spillages of chemicals and solvents should be cleaned up immediately. Inparticular, the plug connections on the rear panel of the instrument (espe-cially the mains plug) should be protected from contamination.5.1.2 Maintenance by Metrohm ServiceMaintenance of the instrument is best carried out as part of an annualservice, which is performed by specialist personnel from Metrohm. Ifworking frequently with caustic and corrosive chemicals, a shorter mainte-nance interval is recommended. The Metrohm service department offersevery form of technical advice for maintenance and service of all Metrohminstruments.
5.2 Capillary connections■■■■■■■■■■■■■■■■■■■■■■54■■■■■■■■■882 Compact IC plus – Anion5.1.3 OperationCautionIn order to avoid disturbing temperature influences, the entire systemincluding the eluent bottle must be protected against direct sunlight.5.1.4 Shutting downIf the instrument is not used for a longer period, the whole IC system(except the columns) must be rinsed salt free with methanol/ultrapurewater (1:4), in order to prevent eluent salts from forming crystals whichmay cause subsequent damage.Rinsing salt free the IC systemTo rinse the system, proceed as follows:1Remove the separation column from the eluent path. Connect theconnection capillaries directly with each other using a coupling(6.2744.040).2Rinse the IC system with methanol/ultrapure water (1:4) for 15minutes.Rinse with eluent for at least 15 minutes at starting up again and beforeconnecting the guard column and separation column.

5.2 Capillary connections5.2.1 OperationAll connections between injection valve, separation column and detectormust be as short as possible, have a low dead volume and be completelyleak-tight. The PEEK capillary after the detector must be free of blockages.Only use PEEK capillaries with an internal diameter of 0.25 mm in the highpressure range between the high pressure pump and the detector.
■■■■■■■■■■■■■■■■■■■■■■5 Handling and maintenance882 Compact IC plus – Anion■■■■■■■■■555.3 DoorCautionThe door is made of PMMA (polymethylmetacrylate). It must never becleaned with abrasive media or solvents.CautionNever use the door as a handle.5.4 Eluent5.4.1 ProductionThe chemicals used for the production of eluents should have a degree ofpurity of at least "p.a.". Only ultrapure water (resistance > 18.2 MΩ*cm)may be used for dilution (this generally applies for reagents which areused in ion chromatography).Newly produced eluents should always be microfiltered (filter 0.45 µm).The composition of the eluent has a crucial effect on the chromatographicanalysis:ConcentrationAn increase in the concentration generally leadsto shorter retention times and faster separation,but also to higher

on the subject, which also contain Standard Operat-ing Procedures (SOP) for testing analytical measuring instruments forreproducibility and correctness.MaintenanceElectronic and mechanical functional groups in Metrohm instruments canand should be checked as part of regular maintenance by specialist per-sonnel from Metrohm. Please ask your local Metrohm agent regarding theprecise terms and conditions involved in concluding a correspondingmaintenance agreement.NoteYou can find information on the subjects of quality management, vali-dation and maintenance as well as an overview of the documents cur-rently available at www.metrohm.com/com/ under Support.

AS4O Automated Sampler

3. Inspect each cap for damage (nicks, scratches, etc.). Install the caps in the vials. Again, if doing trace-level analyses, use forceps when handling the caps to prevent contamination and avoid touching any surface that will be wetted by sample.

   An insertion tool (P/N 037987) provided in the Ship Kit helps prevent contamination of the cap socket and ensures that the cap is inserted to the proper depth. One end of the tool inserts the cap to the proper depth for a sample (i.e., the top of the cap is flush with the lip of the vial); the other end inserts the cap to the proper depth for a rinse (i.e., the top of the cap extends one-quarter inch above the lip of the vial).

4. After pushing the cap into the vial, shake off any liquid that has been forced into the cap socket.

NOTE

Do not use laboratory wipes to blot liquid from the cap sockets; wipes leave fibers which can accumulate in the liquid flow path and cause increased backpressure.

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3.2 Load the Sample Cassettes

1. Press the HoldiRun switch to set the **sampler to Hold.**

2. Slide the spring-loaded cassette pusher back and hold it. Place the filled cassettes into the tray, with the **black dots to the right.** The tray holds up to 11 sample cassettes in any combination of the two models (0.5 mL size or *5.0* mL size).

NOTE

Vials move under the sample head from right to left. When cassettes are placed correctly in the input tray, vial number 1 (labeled on the top of the cassette) is closest to the sample head.

3. After all the cassettes are in place, allow the pusher to slide forward into place against the last cassette.

NOTE

If the sampler is not in Hold when you release the pusher, the first sample cassette will automatically feed into the sampling mechanism, positioning the first vial under the sampling head.

3.3 Select the Operating Parameters

When you power-up the A540, the Setup switches on the front panel indicate the default operating parameters (see Section B.3). If necessary, use the **Setup** switches to select new operating parameters.

If you change the operating parameters after pressing **Run, the new** settings will take effect only after sampling from the current vial (using the old settings) is complete. If sampling from the current vial has not yet started, the changes take effect immediately.

The following list summarizes the functions of the **Setup switches.** See Section 2.1.2 for details about each switch.

- **Inj Type (LooplConc):** Determines whether **the** sample will be delivered to a sample loop or to a concentrator column.

AS4O Automated Sampler

- **In] Mode (ProplCnst):** Selects either proportional or constant sample delivery.

- **Bleed (OfflOn):** Determines whether trapped air and excess liquid is sent to waste (On) or pushed through the sample loop (Off).

  InjNial: Selects **1, 2, or 3** injections per vial.

IMPORTANT
When loading a concentrator in constant mode, make sure that Bleed is On. Operating with Bleed Off will result in the volume of the first sample being larger than the volume of subsequent samples from the vial.

If you typically run with operating parameters that are different ~om the default settings, you can change the default parameters with the DEFAULT SET switches on the rear panel. See Appendix C for details.

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3. Operation and Maintenance

3.4 Manual Operation

After selecting the operating parameters, you are ready to begin using the A540 to load samples into your chromatography system for analysis.

NOTE
See Appendix D for information about remote operation of the AS4O using relay control.

IMPORTANT

Envirotech Laboratories, INC.
Anions Method EPA 300.0

Revision 01/18/23

The Standing Operating Procedure for Analytical Chemistry for Envirotech Laboratories, Inc. has been reviewed

And approved 01/18/23

Electronically signed____________________
Ronald J. Saari
Laboratory Director

Electronically signed____________________
Kevin Burgess
QA/QC Manager

ISSUE DATE: 01/18/23

Envirotech Laboratories, INC.
Anions Method EPA 300.0

Revision 01/18/23

Appendix 1

see 7.5 for manufacturers of standards

Standard Calibration

			#17		#6	#5	#4	#3	#2	#1
	ULTRA					250(#6)/500	250(#5)/500	250(#4)/500	100(#3)/500	20(#2)/100
(Stock mg/L)	Scientific	Make-up		Make-up						
Chloride(1000)	ICC-002-5	50/500 mls	100	25/500 mls	50.0	25.0	12.5	6.25	1.25	0.25
Nitrate(1000)	ICC-004A-5	5.0/500 mls	10.0	1/500 mls	2.00	1.00	0.50	0.25	0.050	0.010
Sulfate(1000)	ICC-006-5	50/500 mls	100	25/500 mls	50.0	25.0	12.5	6.25	1.25	0.25
			#12	#11	#10	#9	#8	#7		
Daily(stock)	Make-up		50(stock)100	25(#12)100	50(#11)/100	50(#10)/100	50(#9)/100	50(#8)/100		
Nitrite-N(1000)*	1/200ml=1.52		0.760	0.192	0.096	0.048	0.024	0.012		
ICC-007*										

for fortifying and spiking samples			ion Chromatography Spiking Solution					
	Sol B	Nitrite spike standard Peak Performance P/N 4400-132344						
	Sol A	Chloride/Nitrate/Sulfate spike standard Peak Performance P/N 4400-132344					25 ms spike	25 ml low
			Spike	Final			soln/50 ml	check/50 ml
Solution A	Concentration		Solution A	volume	Spike conc	Acceptable range	low check 1	MDL CHECK
Chloride	500	Combine	Take 1 ml	25 mls	10.0	9.0-11.0	1.0	0.50
Nitrate-N	25	10 mls Sol A	Sol A+ B		0.50	0.45-0.55	0.050	0.025
Sulfate	500	and 10 mls	Mixture.		10.0	9.0-11.0	1.0	0.50
Solution B		Sol B						
Nitrite-N	12.5				0.250	0.225-0.275	0.025	0.012

ion Chromatography check standard(IPC-1)					Calibration verification standard				CCS-5
	Make-up		initial	Acceptable		Make-up	see calibration standard 5		
(Stock mg/L)	mls	Concentrati	verification	range		Concentration		Acceptable range	
Chloride(1000)	10/500	20.0	19.0-21.0	18.0-22.0	Chloride		25.0	22.5-27.5	
Nitrate(1000)	1/500	2.00	1.90-2.10	1.80-2.20	Nitrate		1.00	0.90-1.10	
Sulfate(1000)	10/500	20.0	19.0-21.0	18.0-22.0	Sulfate		25.0	22.5-27.5	
***dilute1mls(1000**)/200=5 mg/L					****1 ml(1000*)/200=1.52 6.5(1.52)/100=0.096				
IPC-2								CCS-10	
Nitrite(5.0)***	5/200	0.125	0.119-0.132	0.112-0.138	Nitrite****	****	0.096	0.086-0.10	
ICC-007A**									

Ion Chromatography Standards

All solutions are prepared using deionized water and appropriate stock solutions to obtain concentrations.

see 7.5 for manufacturers of standards

			Standard Calibration			
			#16	#15	#14	#13
	ULTRA		**50(stock)/100**	**25(#16)/100**	**!5(#15)/10(**	**50(#14)/100**
(Stock mg/L)	**Scientific**	**Make-up**				
Fluoride	**QCI 710**		**1.69**	**0.84**	**0.42**	**0.084**
	3.37 mg/L					

Ion Chromatography Spiking Solution

for fortifying and spiking samples

Fluoride spike standard Peak Performance P/N 4400-010012

		Spike	Final		
Stock	**Concentration**		**volume**	**Spike cor**	**Acceptable range**
Fluoride (100mg/L)	**12.5/100**	**1.0**	**25**	**0.50**	**0.45-0.55**

ion Chromatography check standard

	Make-up		initial	Acceptable
(Stock mg/L)	**mls**	**Concentration**	**verification**	**range**
Fluoride (100 mg/L)	**1mL/100mL**	**1.00**	**0.95-1.05**	**0.95-1.05**

Calibration verification standard

Make-up	see calibration# 14
Concentrati	**Acceptable range**
0.42	**0.40-0.44**

Fluoride Check standard Peak Performance P/N 4400-010012

SM 4500-Norg-B-C

STANDARD OPERATING PROCEDURE

For

Determination of Total Kjeldahl Nitrogen (TKN) in Aqueous Samples Using Titrimetric Method

Standard Methods Revision 2017

Method revision 2011

Signature Date

Analyst: Kevin Burgess Electronically Signed 11/17/20

Laboratory Director: Ron Saari Electronically Signed 11/17/20

QA/QC Manager: Kevin Burgess Electronically Signed 11/17/20

STANDARD OPERATING PROCEDURE

FOR

1. Scope and application

1.1 This method covers the determination of total Kjeldahl nitrogen in drinking, surface and saline water, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids , proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds hydrazones, oximes, semicarbazones and some refractory tertiary amines.

1.2 This method determines organic nitrogen concentrations over a wide range.

2. Definitions

2.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion: described below.

2.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free- ammonia value from the total Kjeldahl nitrogen value. This may be determined directly by removal of ammonia before digestion.

3. Summary of method

3.1 The sample is heated in the presence of conc. sulfuric acid, K_2SO_4 and $HgSO_4$ and evaporated until fumes of SO_3 are obtained and the solution becomes colorless or pale yellow. The residue is cooled, diluted, and is treated and made alkaline with hydroxide-thiosulfate solution. The ammonia is distilled and determined after distillation by Nesslerization, titration or potentiometry.

4. Sample Handling and preservation

4.1 Samples May be preserved by addition of 2 ml of conc. H_2SO_4 per liter and stored at 4 °C. Even when preserved in this manner, conversion of organic nitrogen to ammonia may occur. Preserved samples should be analyzed as soon as possible.

5. Interference

5.1 High nitrate concentrations (10x or more than the TKN level) result in low TKN values. The reaction between nitrate and ammonia can be prevented by the use of an anion exchange resin (chloride form) to remove the nitrate prior to the TKN analysis.

6. Apparatus

6.1 Digestion apparatus: Kjeldahl digestion apparatus with 300 ml flasks and suction takeoff to remove $S0_3$ fumes and water, or Buchi digester model K446 with scrubber.

6.2 Distillation apparatus: The macro Kjeldahl flask is connected to a condenser and an adaptor so that the distillate can be collected, or Buchi Distillation unit model K 350.

6.3 Titration buret 50 ml graduated.
6.4 Autotitrator: Metrohm 888 Titrando.

7. Reagents

7.1 Distilled water should be free of ammonia.
7.2 Copper sulfate tablets (For Buchi digester use Buchi tablet (contains 4.98 g K_2SO_4 and 0.02 g $CuSO_4 \cdot 5H_2O$).
7.3 Concentrated Sulfuric Acid.
7.4 Sodium hydroxide-sodium thiosulfate solution: Dissolve 375 gm NaOH and 25 gm $Na_2S_3O_3 \cdot 5H_2O$ in distilled water and dilute to 1 liter.
7.5 Mixed indicator: 0.5 g Methylene Red (Mallincrodt 6250) & 0.25 g Methylene Blue (fisher #M-291) in 500 ml Ethanol. This solution should be prepared fresh every 30 days

NOTE 2: Specially denatured ethyl alcohol conforming to Formula 3A or 30 of the U.S. Bureau of Internal Revenue may be substituted for 95% ethanol.

7.6 Boric acid solution: dissolve 20 gm boric acid, H_3BO_3, in water and dilute to 1 liter

with distilled water.

7.7 Sulfuric acid, standard solution: (0.01N, 1 ml=0.14mg NH_3-N). Prepare a stock solution of approximately 1.0N acid by diluting 28 ml of conc. H_2SO_4 (sp.gr. 1.84) to 1 liter with $C0_2$-free deionized water. Dilute 10 ml of this solution to 1 liter with $C0_2$-free deionized water.

NOTE 3: An alternate and perhaps preferable method is to standardize the approximately 0.1 N H_2S0_4 solution against a 0.100 N Na_2CO_3 solution. By proper dilution the 0.01N acid can then be prepared.

7.7.1 Standardize the approximately 0.01N acid against 0.02N Na_2CO_3 solution (purchased from lab chem. Inc.LC229901). Use 10 ml of 0.02 N NaCO3 where 1 ml=1.00 CaCO3. Use either TKN or NH3 method under sample use factor 280,000 titrate with 0.01 H2SO4. Verify that 0.01N H2SO4 is accurate

7.8 Initial Calibration Standards:

7.8.1 Level 4 = 29.6 mg/L nitrogen:
Weigh 0.80 g of Ammonia p-Toluenesulfonate (Hach#22779-24) and dissolve and dilute to 2000 mL with reagent water.

7.8.2 Level 3 = 14.8 mg/L nitrogen:
Take 100 mL of Level 4 standard and dilute to 200 ml with reagent water.

7.8.3 Level 2= 0.30 mg/L nitrogen:
Take 2 ml of Level 4 standard and dilute to 200 ml reagent water.

7.8.4 Level 1 = 0.15 mg/L nitrogen:
Take 1 ml of level 4 standard and dilute to 200 ml reagent water.

7.9 Laboratory Fortified Blank (LFB) and Laboratory Fortified Sample Matrix (LFM):

7.9.1 A spiking solution is prepared by weighing 1.76 g of Glycine p-Toluene Sulphonate(Hach 22780-24) in 100 ml of reagent water(1ml = 1 mg of Nitrogen).
To 100 ml sample of Reagent Water and Sample spike add 1 ml of spiking solution.

8. Quality Control

8.1 Initial Demonstration of Performance

8.1.1 Acceptances of the recoveries of Four Levels of Standards (Section 7.8) are listed as follows:

	True Concentration (mg/L)	Acceptance Range (mg/L)
Level 1	0.15	0.13-0.18
Level 2	0.30	0.28-0.42
Level 3	14.8	13.3-16.3
Level 4	29.6	26.6-32.6

8.1.1 Method Detection Limits Study: MDL is established by analyzing a TKN standard of the concentration of 0.15 mg/L (1-5 times dilution of Level 1). To determine MDL values, take seven replicate aliquots of this standard and process through the entire analytical method. Perform the calculations as follows and report the concentration values in mg/L:

MDL = (t) x (S)

Where:

T = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].

S = Standard deviation of the replicate analyses.

MDL study is conducted once every six months, and Table1 lists one set of MDL study results.

REPORTABLE LIMIT= 0.20 mg/L.

8.1.2 Quality Control Sample (QCS):

8.1.3.1 TKN Solution:Weigh 0.208 g of Glycine p-Toluenesulfonate (Vendor/Catalog#) and dissolve and dilute to 100 mL with reagent water. 1.0 ml = 0.118 mg/L.
8.1.3.2 Transfer 1.0 ml of the TKN Solution (8.1.3.1) and dilute to 200 ml with reagent water. 0.59 mg/L

Acceptance range of QCS: 0.42 – 0.70 mg/L.

8.1.3 Ending Quality Control Standard (EQCS): One EQCS is run after each batch of samples. Level 4 standard is used as EQCS.
Acceptance range for EQCS is: 26.3-33.0 mg/L

8.2 Assessing Laboratory Performance: – The following items are included in every analysis batch:

8.2.1 Laboratory Reagent Blank (LRB) – A LRB is prepared and treated exactly as a typical field sample including exposure to all glassware, equipment, solvents, filtration and reagents that are used with field samples. Data produced are used to assess lab performance of a blank sample and evaluate contamination from the laboratory environment. The values that exceed ½ the Method Detection Limit (MDL) indicate a laboratory or reagent contamination is present. The source of the contamination must be determined prior to conducting any sample analysis.

8.2.2 Laboratory Fortified Blank (LFB) –The LFB is spiked at a concentration of 1.0 mg/L. The recovery of the spiked standard must fall in the range of 94%-101% prior to analyzing samples. If the LFB recovery does not meet these recovery criteria, the source of the problem must be identified and resolved before continuing any analyses. Laboratory data

8.2.3 Laboratory Fortified Sample Matrix (LFM) – The laboratory adds a known amount of the standard at the concentration of 1.0 mg/L to a minimum of 5% of the collected field samples or at least one with every analysis batch, whichever is greater.

8.2.3.1 The percent recovery of the spiked standard is calculated as follows:

$$\%REC = \frac{(C_s - C)}{S} \times 100 \qquad (1)$$

where:

%REC = percent recovery;

Cs = measured concentration in the fortified sample;

C = measured native sample concentration;

S = concentration of equivalent of standard added to sample.

8.2.3.2 If the recovery falls the outside of 97%-103%, and the laboratory's performance for all other QC performance criteria is acceptable, the accuracy problem encountered with the fortified sample is judged to be matrix related, not system related.

8.2.4 Duplicate Sample – One every ten samples is analyzed twice to check lab precision of the analysis. The precision of the duplicate samples is calculated as follows:

$$Precision\ (\%) = \frac{C - Cd}{(C + Cd)/2} \times 100$$

Where:

C = the sample result;

Cd = the duplicate sample result.

Acceptable % precision is ±10.0 % .

8.3 Daily Analytical Sequence is listed as follows:

Blank, Level 1 std, Level 2 std, QCS, Level 3 std, Sample 1-10, LFB, Sample Duplicate, LFM,QCS, blank, Sample 11, ... Sample 20, Sample Duplicate, LFM, QCS,blank end of the run EQCS(level 4 std), Record all sample preparation procedures, digestion, distillation date, standard lot # and expiration dates in logbook.

9. Procedure

9.1 The distillation apparatus should be pre-steamed before use by distilling a 1:1 mixture of distilled water and sodium hydroxide-sodium thiosulfate (7.4) until the distillate is ammonia-free. This operation should be repeated each time apparatus is out of service long enough to accumulate ammonia (usually 4 hours or more).

9.2 **Macro Kjeldahl system**

9.2.1 Place a measured sample or the residue from the distillation in the ammonia determination (for Organic Kjeldahl only) into an 800 ml Kjeldahl flask. The sample size can be determined from the following table:

Kjeldahl Nitrogen in sample, mg/L	Sample size ml
0-5	500
5-10	250
10-20	100
20-50	50.0
50-500	25.0

Dilute the sample, if required, to 300 ml with distilled water, and add 6.7 ml concentrated Sulfuric acid, 6.7 g K_2SO_4, and 0.365 g $CuSO_4$) to

distillation flask. Evaporate the mixture in the Kjeldahl apparatus until 503 fumes are given off and the solution turns colorless or pale yellow. Continue heating for 30 additional minutes. Cool the residue and add 300 ml distilled water.

9.2.2 Make the digestate alkaline by careful addition of 50 ml sodium hydroxide-thiosulfate solution (7.4) without mixing.

NOTE 4: Slow addition of the heavy caustic solution down the tilted neck of the digestion flask will cause heavier solution to underlay the aqueous sulfuric acid solution without loss of free-ammonia. Do not mix until the digestion flask has been connected to the distillation apparatus

9.2.3 Connect the Kjeldahl flask to the condenser with the tip of condenser or an extension of the condenser tip below the level of the boric acid solution (7.6) in the receiving flask.

9.2.4 Distill 300 ml at the rate of 6-10 ml/min., into 50 ml of 2% boric acid (7.6) contained in a 500 ml Erlenmeyer flask.

9.2.5 Dilute the distillate to 500 ml in the flask. These flasks should be marked at the 350 and 500 ml volumes. With such marking, it is not necessary to transfer the distillate to volumetric flasks. For concentrations above 1 mg/L, the ammonia can be determined titrimetrically.

9.3 **Procedure for Buchi digester Model K 437.**

9.3.1 Use 200 ml of sample

9.3.2 Add 7.8 ml conc H2SO4 and 1 copper tablet (Missouri 11057982).

9.3.3 Follow procedures in Appendix 1

9.4 **Distillation using Buchi K-350 apparatus**

9.4.1 Turn on cold water. turn on Distiller.

9.4.2 Warm up. Set time to 4.25 minutes.

9.4.3 Add 25 mls of reagent water to digestion tubes.

9.4.4 Add 60 mls of the reagent. NaOH/Thiosulfate mix

9.4.5 Press start. Distill approx. 175 ml to a 250 ml erlenmyer flask containing 50 mls of Boric acid.

9.5 Determination of ammonia in distillate: Determine the ammonia content of the distillate titrimetrically as described below.

9.5.1 Titrimetric determination: Add 3 drops of the mixed indicator (7.5) to the distillate and titrate the ammonia with the 0.01N H_2SO_4 (7.7), matching the end point against a blank containing the same volume of distilled water and H_3BO_3 (7.6) solution, or titrate to pH 5.0 with autotitrator.

9.5.2 It is not imperative that all standards be treated in the same manner as the samples. At least 2 standards (a high and a low) are digested, distilled,

and titrated to insure that the digestion-distillation technique is reliable. If treated standards do not fall within 10 % of value, the operator should find the cause of the apparent error before proceeding.

10. Calculation

10.1 Using the titrimetric procedure , calculate Total Kjeldahl Nitrogen, in mg/l, in the original sample as follows:

$$TKN, mg / L = \frac{(A - B) \times N \times F}{S} \times 1000$$

where:

A =milliliters of standard 0.010N H_2S0_4 solution used in titrating sample.
B =milliliters of standard 0.010N H_2S0_4 solution used in titrating blank.
N = normality of sulfuric acid solution.
F = milliequivalent weight of nitrogen (14 mg).
S = milliliters of sample digested

10.2 If the sulfuric acid is exactly 0.01N, the formula is shortened to:

$$TKN, mg / L = (A - B) \times 140$$

11. Reference

11.1 American Public Health Association, American Water Works Association, and Water Environment Federation, Standard Methods for the Examination of Water and Wastewater, 21st Edition 2005.

12. Appendix 1

TKN PROCEDURE USING BUCHI 437 DIGESTION UNIT (SM 4500org-C)

1. To 200 ml of sample add 7.8 ml concentrated sulfuric acid and Kjeldahl Copper tablet(Buchi cat# 11059782).
2. Turn on heater and preheat to 200°C. Place tubes on heater. Turn on Chiller (WKL-600).
3. When sample begins to boil turn on scrubber. Increase temperature to 400ºC. Periodically check temperature of the digestion unit, temperature should read 400+/-25 ºC.
4. Continue heating to fumes. End tubes will fume first. Wait for all tubes to reach fumes and turn back heat to level 8.

5. Digest for 30 min.

6. Turn off heat and wait 15-20 min.

7. Raise tubes to cooling position.

8. Wait 15 min or until no more fumes seen in tube.

9. Turn off scrubber.

10. Leave cooling water on for distiller.

11. Disconnect suction tube and tap tube.

12. Add 25 ml of reagent water water to each tube.

13. Swirl each tube and place tube rack back into the digester to keep warm.

14. Distill sample and perform ammonia analysis using Buchi K 350 distiller apparatus to obtain TKN value.

13. Appendix 2

Tiamor 2.3 Auto titrator

1. Click file open –click Envirotech
2. click workplace
3. Calibrate pH probe: Pick method –pH Calibration Envirotech. Follow promps for pH 4, 7, 10 calibrations. If slope is within limits 95-100%, program will read "regular with remarks".
4. Place an empty beaker on the stirrer and place H_2SO_4 burret tip in it, click on to manual then dosing device, then prepare. This will dispense 20 mLs of H_2SO_4 to prepare for sample titration and eliminate any air bubbles in the lines.

5. Pick method- TKN sample or Ammonia sample
6. log in Lab ID#, Sample ID, sample size in mls and sample unit mg/L.
7. Place empty beaker on the stirring plate and place pH probe and 0.01 N H_2SO_4 burret tip in beaker.
8. Start
9. After sample titration is complete, program will read regular with remarks.
10. Click on Database to view results.
11. After all samples have been titrated, press File, print determination overview, landscape and click OK. Daily run will print.

SM 4500-P-B,E

STANDARD OPERATING PROCEDURE

For

Determination of Total Phosphorus, and Ortho-Phosphate in Aqueous Samples

Using

Ascorbic Method

Ref: Standard Methods 22nd Edition, 2012
Method revision 2011

		Signature	Date
Analyst:	Iris Pickard	Electronically signed	2/1/23
QA/QC Manager:	Kevin Burgess	Electronically signed	2/1/23
Laboratory Director:	Ronald Saari	Electronically signed	2/1/23

STANDARD OPERATING PROCEDURE

For

Determination of Total Phosphorus, and Ortho-Phosphate in Aqueous Samples Using Ascorbic Method

1. Scope and application

1.1 Methods for the determination of specified forms of phosphorus in drinking, surface and saline waters, domestic and industrial wastes.

1.2 Methods are based on reactions that are specific for the ortho phosphate ion. Thus, depending on the prescribed pre-treatment of the sample, the various forms of phosphorus in may be determined. These forms are defined in Section 4.

1.2.1 Except for in-depth and detailed studies, the most commonly measured forms are phosphorus and dissolved phosphorus, and orthophosphate and dissolved orthophosphate. Hydrolyzable phosphorus is normally found only in sewage-type samples and insoluble forms of phosphorus are determined by calculation.

1.3 The methods are usable in the 0.003 to 1.33 mg/L P range.

2. Summary of method

2.1 Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

2.2 Only orthophosphate forms a blue color in this test. Polyphosphates (and some organic phosphorus compounds) may be converted to the orthophosphate form by sulfuric acid hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by persulfate digestion (2)

3. Sample handling and preservation

3.1 If benthic deposits are present in the area being sampled, great care should be taken not to include these deposits.

3.2 Sample containers may be of plastic material such as cubitainers, or of Pyrex glass.

3.3 For Total Phosphorous analysis, if the analysis cannot be performed the day of collection, the sample should be preserved by the addition of 2 ml conc. H_2SO_4 per liter to pH <2 and refrigeration at 4 °C . For Ortho-phosphorous, if the sample cannot be performed on the day of collection, preserve by cooling to < 6ºC and analyzed within 48 hours.

- **Definitions**

  - TOTAL Phosphorus (P) - all of the phosphorus present in the sample, regardless of form, as measured by the persulfate digestion procedure.

4.1.1 Total Orthophosphate (P, ortho) - inorganic phosphorus$[(PO_4)^{-3}]$ in the sample as measured by the direct colorimetric analysis procedure.

4.1.2 Total Hydrolyzable Phosphorus (P, hydro) -phosphorus in the sample as measured by the sulfuric acid hydrolysis procedure, and minus pre-determined orthophosphates. This hydrolyzable phosphorus includes polyphosphorus, [(P2O7)4 $(P_3 0_{10})^{5.}$ etc.] plus some organic phosphorus.

4.1.3 Total Organic Phosphorus (P, org) - phosphorus (inorganic plus oxidizable organic) in the sample measured by the persulfate digestion procedure and minus hydrolyzable phosphorus and orthophosphate.

4.2 Dissolved Phosphorus (P-D) - all of the phosphorus present in the filtrate of a sample filtered through a phosphorus-free filter of 0.45 micron pore size and measured by the persulfate digestion procedure.

4.2.1 Dissolved Orthophosphate (P-D, ortho) - as measured by the direct colorimetric analysis procedure.

4.2.2 Dissolved Hydrolyzable Phosphorus (P-D, hydro) -as measured by the sulfuric acid hydrolysis procedure and minus pre-determined dissolved orthophosphates.

4.2.3 Dissolved Organic Phosphorus (P-D, org) - as measured by the persulfate digestion procedure and minus dissolved hydrolyzable phosphorus and ortho-phosphate.

4.3 The following forms, when sufficient amounts of phosphorus are present in the sample to warrant such consideration may be calculated.

4.3.1 Insoluble orthophosphate (P-I,ortho)=(P,ortho)-(P-D,ortho).

4.3.2 Insoluble Hydrolyzable Phosphorus (P-I,hydro)= (P,hydro)-(PD,hydro).

4.3.3 Insoluble Organic Phosphorus (P-I,org)=(P-D,org).

4.4 All phosphorus forms shall be reported as (P) mg/l, to the third place.

5. Interferences

5.1 No interference is caused by copper, iron, or silicate at concentrations many times greater than their reported concentration in sea water. However, high iron concentrations can cause precipitation of and subsequent loss of phosphorus.

5.2 The salt error for samples ranging from 5 to 20% salt content was found to be less than 1%.

5.3 Arsenate is determined similarly to phosphorus and should be considered when present in concentrations higher than phosphorus. However, at concentrations found at sea water, it does not interfere.

6. Apparatus

6.1 Spectrophotometer for measurement at 880 NM with light path of 5 cm (UNICO 2150)

6.2 Acid-washed glassware: All glassware used should be washed with hot 0.1 HCl and rinsed with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove the last traces of phosphorus that might be adsorbed on the glassware. This glassware should be used only for the determination of phosphorus and after use it should be rinsed with acidified distilled water and kept covered until needed again. If this is done, the treatment with 0.1 N HCl and reagents is only required occasionally. Commercial detergents should never be used.

7.0 Reagents

7.1 Dilute Sulfuric acid solution: Dilute 30 ml of conc. H_2SO_4 with distilled water to 100 ml.

7.2 5N Sulfuric Acid, Dilute 70 ml conc H_2SO_4 to 500 ml Deionized water.

7.3 Potassium Antimony Tartrate solution: Purchased from GFS cat #2032 ,store in glass amber bottle.

7.4 Ammonium Molybdate solution: Purchased from GFS cat #2024. Store in glass bottle.

7.5 Ascorbic acid: 0.01 M: Dissolve 8.8 g ascorbic acid in 500 ml Deionized water. Solution is stable for 1 week at 4°C.

7.6 Combined reagent: Mix the above reagents in the following proportions for 100 ml of the combined reagent: 50 ml 5N H_2SO_4, 5 ml Potassium Antimony Tartrate solution, 15 ml Ammonium Molybdate solution, and 30 ml Ascorbic Acid solution. Mix after addition of each reagent.

- 1 N Sodium Hydroxide: Dissolved 40 gm NaOH in 600 ml deionized water. Cool and dilute to 1 liter. If turbidity forms in combined reagent let stand for a few minutes until turbidity disappears before proceeding. Reagent is stable for 4 hr.

7.8 Stock Standard phosphorus solution: 5.00 mg/L (P) standard purchased from HACH cat# A6083.

7.9 Dilute 10 ml of 5.00 mg/L in 150 mL final concentration is 0.33mg/L P.

7.10A Initial Calibration Standards: Total Phosphate Expressed as (P).

7.10.1 Level 1 = 0.003 mg/L: Dilute 1 mL of 0.333 mg/L (P) standard to 100 mL reagent water.
7.10.2 Level 2 = 0.013 mg/L: Dilute 4.0 mL of 0.333 mg/L (P) standard to 100 mL reagent water.
7.10.3 Level 3 = 0.033 mg/L: Dilute 10.0 mL of 0.333mg/L (P) standard to 100 ml reagent water.
7.10.4 Level 4 = 0.165 mg/L: Dilute 50.0 ml of 0.333 mg/L (P) standard to 100 ml reagent water.
7.10.5 Level 5 = 0.333 mg/L: Dilute 100 ml of 0.333 mg/L (P) standard to 100 ml reagent water.

- P Standard: 1000 mg/L (P) (3000 mg/L (PO4) Ultra Scientific, Cat# ICC-005A).
- Quality Control Standards (Second Source):

7.12.1 100 mg/L (P) Standard: Dilute 10 mL of 1000 mg/L (P) standard (Ultra Scientific, Cat# ICC-005A) to 100 mL with reagent water.
7.12.2 0.40 mg/L (P) Check Standard: Dilute 0.20 mL of 100 mg/L (P) Standard (7.12.1) to 100 mL with reagent water.

7.13 Spiking solution standard for LFB and LFM: 1000 mg/L (PO4) GFS 758

7.13.1 100 mg/L (PO4) Standard: Dilute 10 mL of 1000 mg/L (GFS 758) in 100 mL reagent water.
7.13.2 Spike Standard 0.200 mg/L (PO4): Add 0.2 mL of 100 mg/L (PO4) standard to 100 ml reagent water (LFB) and to 100 mL Matrix spike sample (LFM).

7.10B Initial Calibration Standards: Ortho Phosphate Expressed as (P).

7.10.1 Level 1 = 0.003 mg/L: Dilute 0.5 mL of 0.333 mg/L (P) standard to 50 mL reagent water.
7.10.2 Level 2 = 0.013 mg/L: Dilute 2.0 mL of 0.333 mg/L (P) standard to 50 mL reagent water.
7.10.3 Level 3 = 0.033 mg/L: Dilute 5.0 mL of 0.333mg/L (P) standard to 50 ml reagent water.
7.10.4 Level 4 = 0.165 mg/L: Dilute 25.0 ml of 0.333 mg/L (P) standard to 50 ml reagent water.
7.10.5 Level 5 = 0.333 mg/L: Dilute 50 ml of 0.333 mg/L (P) standard to 50 ml reagent water.

- P Standard: 1000 mg/L (P) (3000 mg/L (PO4) Ultra Scientific, Cat# ICC-005A).
- Quality Control Standards (Second Source):

7.12.1 100 mg/L (P) Standard: Dilute 10 mL of 1000 mg/L (P) standard (Ultra Scientific, Cat# ICC-005A) to 100 mL with reagent water.

7.12.2 0.40 mg/L (P) Check Standard: Dilute 0.20 mL of 100 mg/L (P) Standard (7.12.1) to 50 mL with reagent water.

7.13 Spiking solution standard for LFB and LFM: 1000 mg/L (PO4) GFS 758

7.13.1 100 mg/L (PO4) Standard: Dilute 10 mL of 1000 mg/L (GFS 758) in 100 mL reagent water.

7.13.2 Spike Standard 0.40 mg/L (PO4): Add 0.2 mL of 100 mg/L (PO4) standard to 50 ml reagent water (LFB) and to 50 mL Matrix spike sample (LFM).

8. Quality Control

8.1 Initial Demonstration of Performance

8.1.1 There are six levels of initial calibration standards (Section 7.10). Absorbance of each standard at 880 nm wavelength is measured and recorded. Linear regression between Absorbance and concentration is used for initial calibration curve. The equation of the linear regression is expressed as follows: Concentration of each standard will be back calculated and be within +/10%.

$$y = ax + b \qquad (1)$$

where:

x: Absorbance of each standard

y: Concentration of each standard.

a: Coefficient.

b: Coefficient.

8.1.1.1 Calculation of Correlation Coefficient (r):

$$r = \frac{\sum_i \{(x_i - \bar{x})(y_i - \bar{y})\}}{\left\{\left[\sum_i (x_i - \bar{x})^2\right]\left[\sum_i (y_i - \bar{y})^2\right]\right\}^{1/2}} \qquad (2)$$

where:

$\bar{x}$: Average of x_i.

$\bar{y}$: Average of y_i.

x_i : Measured value of x.

y_i : Measured value of y.

8.1.1.2 The correlation coefficient (r) must be equal to or greater than 0.995.

8.1.2 Method Detection Limits Study: MDL is established by analyzing phosphorus standard of the concentration of 0.0033 mg/L (P). To determine MDL values, take seven replicate aliquots of this standard and process through the entire analytical method. Perform the calculations as follows and report the concentration values in mg/L: MDL sample should be analyzed as a control sample not a calibration standard.

$$\text{MDL} = (t) \times (S) \qquad (3)$$

Where:

T = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].

S = Standard deviation of the replicate analyses.

MDL study is conducted once every six months, and Table1 lists one set of MDL study results.

8.1.3 Quality Control Sample (QCS): 0.4 mg/L (P) standard (Ultra Scientific) is run right after the initial calibration curve. Acceptance limit must be within ± 10%.

8.1.4 Ending Quality Control Standard (EQCS):
Level 5 standard = 0.333 mg/L (P) is used as theEQCS. Acceptance limit must be within ± 10%.

8.2 Assessing Laboratory Performance: – The following items are included in every analysis batch:

- Laboratory Reagent Blank (LRB) – A LRB is prepared and treated exactly as a typical field sample including exposure to all glassware, equipment, solvents, filtration and reagents that are used with field samples. Data produced are used to assess lab performance of a blank sample and evaluate contamination from the laboratory environment. The values that exceed ½ the Method Detection Limit (MDL) indicate a laboratory or reagent contamination is present. The source of the contamination must be determined prior to conducting any sample analysis. Method blank will be performed for every analytical batch.

- Laboratory Fortified Blank (LFB) –The LFB is spiked at a concentration of 0.20 mg/L (PO4). The recovery of the spiked standard must fall in the range of between 95%- 104% prior to analyzing samples. If the LFB recovery does not meet these recovery criteria, the source of the problem must be identified and resolved before continuing any analyses.

- Laboratory Fortified Sample Matrix (LFM) – The laboratory adds a known amount of the standard at the concentration of 0.20 mg/L (PO4) to a minimum of 5% of the collected field samples or at least one with every analysis batch, whichever is greater.

    - The percent recovery of the spiked standard is calculated as follows:

$$\%REC = \frac{(C_s - C)}{S} \times 100 \qquad (2)$$

where:

%REC = percent recovery;

Cs = measured concentration in the fortified sample;

C = measured native sample concentration;

S = concentration of equivalent of standard added to sample.

    - If the recovery falls outside of 95-104%, and the laboratory's performance for all other QC performance criteria is acceptable,

the accuracy problem encountered with the fortified sample is judged to be matrix related, not system related.

- Duplicate Sample – One every ten samples is analyzed twice to check lab precision of the analysis. The precision of the duplicate samples is calculated as follows:

$$Precision\ (\%) = \frac{C - Cd}{(C + Cd)/2} \times 100 \qquad (3)$$

Where:

C = the sample result;

Cd = the duplicate sample result.

Laboratory Precision (%) = 4.1%

8.3 Daily Analytical Sequence is listed as follows:

Blank, Level 1,Level 2,Level 3,Level 4,Level 5,QCS, Sample 1-10,Blank, LFB, Sample 1 Duplicate, Sample 1 LFM, QCS,Sample 1, ... Sample 20, EQCS,

9. **Procedure**

9.1 Total Phosphorus

9.1.1 Add 1 ml of H_2SO_4 solution (7.1) to a 100 ml sample in a 125 ml Erlenmeyer flask.
9.1.2 Add 0.5 gm of Potassium Persulfate.
9.1.3 Boil gently on hot plate. This will make sample read 90ºC. Boil 30-40 minutes or until a final volume of about 25 ml is reached. Do not allow sample to go to dryness. Alternately, heat for 30 minutes in an autoclave @ 121 °C (15-20 psi).
9.1.4 Cool and dilute the sample to about 30 ml and adjust the pH of the sample to 7.0 ± 0.2 with 1N Na OH (7.10) using phenolphthalein as an indicator. If sample is not clear at this point; add 2-3 drops of acid (7.6) And filter. Dilute to 50 ml.
9.1.5 Determine phosphorus as outlined in 9.3.2 ortho-phosphate.

9.2 Hydrolyzable Phosphorus

9.2.1 Add 1 ml of H_2SO_4 solution (7.1) to a 100 ml sample in a 125 ml Erlenmeyer flask.
9.2.2 Boil gently on a pre-heated hot plate for 30-40 minutes or until a final volume of about 10 ml

is reached. Do not allow sample to go to dryness.

9.2.3 Cool and dilute the sample to about 30 ml and Adjust the pH of the sample to 7.0+0.2 with 1N NaOH (7.10) using phenolphthalein as an indicator. If sample is not clear at this point, add 2-3 drops of acid (7.6) and filter. Dilute to 50 ml.

9.2.4 The sample is now ready for the determination of phosphorus as outlined in 9.3.2.

9.3 Orthophosphate

9.3.1 To 50 ml of sample add I drop of phenolphthalein indicator. If red color develops add 5N H_2SO_4 drop wise to discharge color.

9.3.2 Add 8.0 ml of combine reagent and mix thoroughly. After at least 10 min but no greater than 30 min. measure absorbance of each blank, standard and sample at 880 nm. Use disposable cuvettes. Prepare standard curve.

- Equation (1) is used to calculate the concentration

- **Reference**

10.1 American Public Health Association, American Water Works Association, and Water Environment Federation, Standard Methods for the Examination of Water and Wastewater, 22nd Edition 2017.

The Standing Operating Procedure for Analytical Chemistry for Envirotech Laboratories, Inc.
has been reviewed

And approved 02/02/23

Electronically signed______________________
Ronald J. Saari
Laboratory Director

Electronically signed_______________________
Kevin Burgess
QA/QC Manager

ISSUE DATE: 2/1/23

STANDARD OPERATING PROCEDURE

For

Determination of (Alkalinity, Conductance, pH and Turbidity) in Aqueous Samples Using Mantec PC-Titrate System

Alkalinity SM 2330B, Spec. Conductance USEPA 120.1
pH SM 4500-H-B, Turbidity SM 2130B

(Method Revision 2011)

Standard Methods Revision 2017

Signature Date

Analyst: Kassie Finlayson electronically signed 02/01/23

Laboratory Director: Ronald Saari electronically signed 02/01/23

QA/QC Manager: Kevin Burgess electronically signed 02/01/23

STANDARD OPERATING PROCEDURE
FOR
ALKALINITY

METHOD 2320B (Titrimetric,)
Ref: Standard Methods, 22th edition, 2017
Method revision 2011

1. **Scope and application**

   1.1 This method is applicable to drinking, surface and saline waters, domestic and industrial wastes.

   1.2 The method is suitable for all concentrations of ranges of alkalinity; however, appropriate aliquots should be used to avoid a titration volume greater than 50 ml.

2. **Summary of method**

   2.1 An unaltered sample is titrated to and indicator 4.5 and 8.3. The sample must be not filtered, diluted, concentrated, or altered in any way.

3. **Comments**

   3.1 The sample should refrigerate at 4^{0}C and analyze as soon as practical. Do not open sample bottle before analysis.

   3.2 Substances, such as salts of weak organic and inorganic acids present in large amounts, may cause interference.

4. **Apparatus**

   4.1 Mantec PC-Titrate System.

CONDUCTANCE

EPA METHOD 120.1 (Specific Conductance, umhos/cm @ 25 °C.)

Ref: EPA 600/4-79-020 “Methods for Chemical Analysis of Water and Wastes”.

1.0 Scope and Application
1.1 This method is applicable to drinking, surface, and saline water, domestic and industrial wastes and acid rain (atmospheric deposition).
2.0 Summary of Method
2.1 The specific conductance of a sample is measured by use of a self-contained conductivity meter, Wheatstone bridge-type, or equivalent. 2.2 Samples are preferable analyzed at 25°C. If not, temperature corrections are made and results reported at 25°C.
3.0 Comments
3.1 Instrument must be standardized with KCl solution before daily use.
3.2 Conductivity cell must be kept clean.
3.3 Field measurements with comparable instruments are reliable.
3.4 Temperature variations and corrections represent the largest source of potential error.
4.0 Sample Handling and Preservation
4.1 Analyses can be performed either in the field or laboratory.
4.2 If analysis is not completed within 24 hours of sample collection, sample should be filtered through a 0.45 micron filter and stored at 4°C. Filter and apparatus must be washed with high quality distilled water and pre-rinsed with sample before use.

Apparatus

3.1 Mantec PC-Titrant System
3.2 Jenway 4510 Conductivity meter

STANDARD OPERATING PROCEDURE FOR pH

METHOD SM 4500-H-B (Electrometric)
Ref: Standard Methods, 22th edition, 2017
Method revision 2011

1. **Scope and application**
   1.1 This method is applicable to drinking, surface and saline waters, domestic and industrial wastes and acid rain (atmospheric deposition)

2. **Summary of method**
   2.1 The pH of a sample is determined electrometrically using a pH meter and a combination electrode with auto temp probe.

3. **Sample Handling and Preservation**

3.1 Samples should be analyzed as soon as possible preferably in the field at the time of sampling.

3.2 High -purity waters and waters not at equilibrium with the atmosphere are subject to changes when exposed to the atmosphere; therefore, the sample containers should be filled completely and kept sealed prior to analysis.

4. Interferences

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

4.2 Temperature effects on the electrometric measurement of pH arise from two sources. The first is caused by the change in electrode output at various temperatures. This interference is controlled with the temperature compensation probe. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled. It should, therefore, be noted by reporting both the pH and temperature at the time of analysis.

5. Apparatus

5.1 pH meter : Mantec PC tirant system
5.2 Automatic temperature compensation probe
5.3 Combination electrode
5.4 Magnetic stirrer and Teflon-coated stirring bar

6. Reagents

6.1 Certified pH buffer standard solutions are purchased from Weber Scientific and Laboratory Sales.

7. Quality Control

7.1 Each sample run pH meter is calibrated with 3 buffers (4,7,10), and a pH 7 buffer is analyzed after initial calibration.. Lot number and expiration date of buffer solutions are recorded in calibration output. Slope of the pH meter must be within the range of 95-105%. If the calibration falls outside of the limits recalibrate the pH meter. If pH meter is unable to be calibrated, check probe and probe filling solution, if necessary replace probe. pH 7.0 must be within the range of 6.90-7.10, if the buffer falls outside the range, recalibrate. Buffer solution should be reported to the nearest 0.01 pH units. Every 10 samples a duplicate sample and a pH 7.0 buffer will be analyzed. At the end of the daily run reanalyze pH 7.0 buffer.

STANDARD OPERATING PROCEDURE
FOR
TURBIDITY

METHOD SM 2130 B (Nephelometric)
Ref: Standard Methods, 22th edition, 2017

1. **Scope and application**

1.1 This method is applicable to drinking, surface and saline waters in the range of turbidity from 0 to 180 nephelometric turbidity units (NTU). Higher values may be obtained with dilution of the sample.

2. **Summary of method**

2.1 The method is based upon a comparison of the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, the higher the turbidity. Readings,

in NTU's, are made in a nephelometer designed according to specifications outlined in Apparatus. A standard suspension of Formazin, prepared under closely defined conditions, is used to calibrate the instrument.

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

3. **Sample Handling and Preservation**

3.1 preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to **4**° C., to minimize microbiological decomposition of solids, is recommended.

4. **Interferences**

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

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

5. **Apparatus**

5.1 Mantec PC-titrant system.

Standard Operating Procedure for PC-Titrate System

Alkalinity SM 2330B, Spec. Conductance USEPA 120.1
pH SM 4500-H-B, Turbidity SM 2130B

START UP:

1. Start computer
2. Press PC titrate V3 ICON
3. Press Purge 5 ml buret Icon from Main Menu and system preparation.
4. Wait for Established Communication

5. Press start button on timetable.
6. After purge is complete press OK button.
7. At main menu press Daily Run Icon.

DAILY RUN:

1. Go to Daily Run Template and follow Daily Run Prompts
2. PH Cal. PH 4.00/7.00/10
3. WAIT FOR PRINT OUT FOR CALIBRATION VALIDITY. IF INVALID RECALIBRATE.
4. PH QC 7.00- check cal
5. Alkalinity QC 2.5 and 50.0 mg/L
6. Turbidity QC 1.0 NTU
7. Turbidity QC 5.0 NTU
8. Turbidity QC 10.0 NTU
9. Conductivity QC 50 umhos/cm
10. Conductivity QC 100 umhos/cm
11. Conductivity QC 500 umhos/cm
12. Conductivity, pH, Alkalinity on DI water
13. Conductivity QC 10 umhos/cm
14. Log in Samples with Lab ID #s and choose appropriate analytical profile.
15. Add samples to proper labeled plastic beakers. USE MINIMUM OF 60 ml of SAMPLE FOR ANALYSIS.
16. **After every 10th samples run:**
17. PH 7.00
18. Conductivity 141 umhos/cm
19. Turbidity 10 NTU
20. Alkalinity 50 mg/L, 250 mg/L if necessary.
21. Sample Duplicate
    - Turbidity Meter is calibrated twice (2) a year unless standards are not within acceptable range. See attached calibration procedure.

DAILY MAINTENANCE

1. Add deionized water to the rinse water supply
2. Empty waste container
3. Fill pH electrode with proper filling solution.

END OF DAY

1. Add Deionized water to lower rinse station for Conductivity Probe storage
2. Add 4 ml pH 4.00 buffer for pH probe storage.

GENERAL MAINTENANCE

1. Clean rinse station beaker and titrator once a month or as needed.
2. Each year replace all tubing

STANDARDS (Calibration and Check)

TURBIDITY

Check Standards:

1. 100 NTU: Stock (Purchased from Aurical).
2. 10 NTU: take 100 ml of 100 NTU STD in 1000 mls DI water.
3. 1.0 NTU: take 100 ml of 10 NTU STD in 1000 mls DI water.

Calibration Standards:

1. Levels of 0.02, 10.0, and 100.0 NTU standards purchased from Mantec.
2. 10 NTU: Take 10 ml of stock (100 NTU) in 1000 mls.
3. 0.02 NTU: take 2 ml of 10 NTU std in 1000 ml.

SPECIFIC CONDUCTANCE

Check Standards:

1. 500 umhos/cm: take 50 mls of a ~10,000 umhos/cm traceable stock standard (Purchased from Lab Chem LC18777.2) in 1,000 ml of DI water
2. 100 umhos/cm: take 10 mls of the 10,000 umhos/cm std in 1,000 mls of DI water.
3. 50 umhos/cm: take 50 mls of the 10,000 umhos/cm std in 1,000 ml of DI water.
4. 10 umhos/cm: take 100 mls of the 100 umhos/cm STD in 1,000 ml of DI water.
5. 140 umhos/cm check std for every 10 samples: take 14 ml of a 10,000umhos/cm traceable stock standard (Purchased from Laboratory Sales LS-1170007) in 1000 ml reagent water.

Calibration Standard:

1. The Conductivity probe purchased from Mantec has an internal standard set at the factory. It is in the range of k=0.99-1.07.

PH

1. PH buffers 4.00, 7.00, 10.00 purchased from Lab Chem Inc..

ALKALINITY

1. 250 mg/L as $CaCO_3$ check standard: take 10 ml of a 25,000-mg/L stock standard (purchased from HACH 0.5N $CaCO_3$) in 1,000 ml volumetric flask.
   50 mg/L standard: take 200 mL of 250 mg/L standard solution into 1 L.
   2.5 mg/L standard: take 50 mL of 50 mg/L standard into 1 L.
2. Titrant check: Each new preparation of 0.02N Sulfuric acid titrant is standardized by titration of 10 ml of $NaCO_3$ (0.045 N purchased from HACH cat# 83049) where 1 ml=1.00 $CaCO_3$. This is recorded in TKN analysis workbook.
3. Mantec software will use low level alkalinity calculation for alkalinity values below 20 mg/L. Instrument will use pH 4.2 and 4.5 to calculate alkalinity value.
4. A second source check standard: take 50 mL of Agilent QCI-710 into 100 mL.

October 2016 **MANTEC-PC TITRATE**

All solutions are prepared using deionized water and appropriate stock solutions to obtain concentrations.

QUALITY CONTROL LIMITS

Alkalinity	**Stock Hach Alkalinity Std Sol Ampule 25,000 mg/L CaCO3**				**Recovery**	
	Initial Check	**As needed**	**Low check**		**Duplicate**	**End Check**
standard	50.0 mg/L	250 mg/L	2.5 mg/L			50.0 mg/L
Range	45.0-55.0 mg/L		2.25-2.75			45.0-55.0 mg/L
Make-UP	200 mL(250 mg/L) /1 L	10 ml Ampule/L	50 ml(50mg/L)/L			
Percent recovery	90-110%	90-110%	90-110%		80-120%	90-110%

Conductivity	****Stock Lab Chem Cond Std 10,000 uhmos/cm**				**Recovery**	
	Initial Check(umhos/cm)			**Low check**	**Duplicate**	**End Check***
standard	50	100	500	10		140 uhoms/cm
Range	45-55	90-110	450-550	9-11		126-155
Make-UP**	5 ml/L	10mL/L	50 mL/L	100 mL(100 mg/L)/1L		14 mL/L*
Percent recovery	90-110%	90-110%	90-110%		80-120%	90-110%

***Laboratory Sales LS-1170007 Traceable**

Turbidity	**Aurical Turbity Std 100 NTU**				**Recovery**	Mantec Turb Std
	Initial Check(NTU)			**Low Check**	**Duplicate**	**End Check**
standard	1.0	5.0	10	1.0		10 NTU
Range	0.90-1.10	4.5-5.5	9.0-11.0	0.90-1.1		9.0-11
Make-UP	10 mL/L	50 mL/L	100 mL/L			10 mL/L
Percent recovery	90-110%	90-110%	90-110%		80-120%	90-110%

pH	**Lab Chem Inc**				**Recovery**	
	Initial Check(pH units)				**Duplicate**	**End Check**
standard	7.00	4.00	10.00			7.00 pH units
Range	6.80-7.20	3.80-4.20	9.80-10.20			6.80-7.20
Percent recovery					98-101%	

The Standing Operating Procedure for Analytical Chemistry for Envirotech Laboratories, Inc. has been reviewed

And approved 02/01/23

Electronically signed______________________

Ronald J. Saari

Laboratory Director

Electronically signed_______________________

Kevin Burgess

QA/QC Manager

ISSUE DATE: 02/01/23

Method 1664 A
Oil and Grease Using
Horizon 3000XL Extractor Systems

METHOD 1664A, HEM; OIL AND GREASE

Ref: US EPA-821-R-98-005, Feb 1999

Approved By: electronically signed ______________________________ Date: 01/18/23
Ronald Saari, Laboratory Director

Approved By: electronically signed ______________________________ Date: 01/18/23
Kevin Burgess, QA Manager

Oil and Grease Using a Horizon 3000XL Extractor Systems

1 SCOPE AND APPLICABILITY

1.1. This method is for determining mg/L oil and grease in aqueous samples using the Horizon 3000XL Extractor System. This method is prepared in conjunction with guidelines from EPA Method 1664, Revision A.

1.2. The laboratory method detection limit (MDL) for this procedure is 1.0 mg/L for HEM (n-hexane extractable material), based upon an internal study. The lower reportable detection limit for this method application is 1.0 mg/L, which is validated using a laboratory control sample.

2 SUMMARY OF METHOD

2.1. A sample of approximately 1000mL is acidified to a pH of less than (<) 2 and serially extracted with n-hexane by the Horizon 3000XL extractor, which results in two layers. The bottom layer is aqueous material, and the top layer is n-hexane extractable. The n-hexane extract is separated from the water layer and transferred into an aluminum pan, where the n-hexane is evaporated. The remaining material is then weighed Gravimetrically, and the resulting weight is used to calculate “oil and grease.”

2.2. This procedure is used in place of the traditional manual extraction method for oil and grease using a reparatory funnel.

2.3. Quality is assured in the method by the analysis of laboratory control samples, sample spikes, method blank(s), and a quality control sample. These results are recorded and statistically examined to assure method accuracy and precision.

3 DEFINITIONS

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

3.1. Analyte = the component of interest, in this case, “oil and grease.”

3.2. n-Hexane Extractable Material (HEM) = material that can be extracted from a sample using n-hexane and measured gravimetrically to give “oil and grease.”

3.3. Laboratory Control Sample (LCS) = a sample containing a known concentration of

analyte that is used to assess the performance of the total analytical system, including all preparation and analysis steps. 2 mg/L (reporting limit validation) at the beginning every Oil and Grease run.

3.4. Laboratory Pure Water = reagent water meeting purity characteristics of ASTM Type II laboratory distilled water (daily conductivity <1.0µmhos/cm).

3.5. Matrix Spike = a portion of an aqueous sample that has a known amount of the analyte of interest added to it. The matrix spike can also be referred to as a lab-fortified matrix(LFM). A 4 mg/l concentration is used for every 10 Oil and Grease batch analysis.

3.6. Method Blank = a sample of laboratory pure water containing no target analyte that is taken through the entire sampling and analytical procedure. The analysis of a method blank helps identify any contamination introduced in the analysis process.

3.7. Method Detection Limit (MDL) = the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

3.8. Oil and Grease = materials that may be determined by extracting with n-hexane, evaporating, and performing a gravimetric measurement. These materials include nonvolatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases, and related materials. Oil and grease is not an absolute quantity of a specific substance; rather, it represents groups of substances with similar physical characteristics that can be determined quantitatively on the basis of their common solubility in an organic extracting solvent.

3.9. Quality Control Sample (QCS)= standard from a secondary source, a concentration of 80 mg/L is used at the end of every Oil and Grease run. The QCS is used to meet the ongoing precision and recovery (OPR) requirement of EPA Method 1664A.

4 HEALTH AND SAFETY WARNINGS

4.1. Lab Safety – Due to various hazards in the laboratory, safety glasses must be worn at all times. Always use a safety carrier to transport bottles of hexanes, methanol, and acetone to and from the chemical closet. Use latex gloves to prevent skin contact with solvents.
Please refer to the MSDS file for any other information about personal protective equipment and other safety considerations.

4.2. Chemical Hygiene – n-Hexane has been shown to have increased neurotoxic effects over other hexanes and some other solvents. Inhalation of n-hexane can be minimized by venting the extractors into a hood. Please refer to the MSDS file for any further questions concerning a chemical's toxicity and the necessary safety precautions.

4.3. Waste Disposal – Spent n-hexane, acetone, and methanol are disposed of in labeled containers under the hood in the oil and grease area.

4.4. Pollution Prevention – As long as the solvents used are disposed of and handled properly, the materials used in this method pose little direct threat to the environment.

5 INTERFERENCES

5.1. Oil from fingers or other sources can affect results in this analysis. Be careful to avoid contamination when handling samples, filters, extraction vials, and evaporating pans.

5.2. Interferences extracted from samples will vary considerably from source to source; direct inquiries to the provider of the test samples if there are any questions concerning their content. A high concentration of particulates in a sample will interfere with extraction using this method, possibly rendering the sample useless. Samples may have to be split several times to yield a reportable result. The addition of a Fast-Flow™ pre-filter to the extraction filter can also help with difficult samples.

5.3. Samples preserved with sulfuric acid (H_2SO_4) can be extracted in these units, but the number of samples must be limited, especially using the 3000XL extractors.

6 PERSONNEL QUALIFICATIONS AND RESPONSIBILITY

6.1. General Responsibilities – This method is restricted to use by or under the supervision of analysts experienced with the method. Each analyst must be trained and able to read and understand the SOP.

6.2. Laboratory Analysts and Technicians – It is the responsibility of analyst/technicians to:

6.2.1. Read and understand this SOP and follow it as written.
6.2.2. Produce quality data that meets all laboratory and customer requirements.
6.2.3. Complete the required demonstration of proficiency before performing this procedure without supervision.
6.2.4. Enter laboratory sample and QC results into the LIMS data system for laboratory supervisor review.
6.2.5. Repeat the required initial demonstration of laboratory capability each time a modification is made to the method.

6.3. Lab Supervisor – It is the responsibility of the Lab Supervisor to:

6.3.1. Ensure that all analysts/technicians have the technical ability and have received adequate training required to perform this procedure.
6.3.2. Ensure analysts/technicians have completed the required demonstration of proficiency before performing this procedure without supervision.
6.3.3. Produce quality data that meets all laboratory and customer requirements

7 APPARATUS AND MATERIALS

7.1. Horizon SPE-DEX™ 3000XL series extractors with bottle adaptors and extraction accessories(28mm cap adaptor, 45 and 90 mm disk holders)
7.2. Horizon Speed-Vap™ III
7.3. Vacuum pump
7.4. Analytical balance, capable of weighing to four decimal places
7.5. Bottles for spent solvent
7.6. 500-1L wide and narrow mouth glass sample bottles, .bottles should be lot-certified by date washed and analyzed as a method blank and recorded, or purchased pre cleaned, certified by the manufacturer, to be free of oil and grease.
7.7. Forceps
7.8. 0.5mL, 1.0 mL and 5 mL glass volumetric pipettes, class A
7.9. 1000mL graduated cylinder, class A
7.10. 125mL separatory funnels– for extract containment
7.11. Purge accessories, including o-rings, purge adaptors, vials, bottles
7.12. Valve check tools, for clearing check valves
7.13. 47mm fiber SPE disks, purchased
7.14. 90mm fiber SPE disks, purchased
7.15. Horizon Fast-Flow™ pre-filters
7.16. Aluminum weighing dishes, 70mm
7.17. Plastic disposable transfer pipettes, various sizes
7.18. Plastic squeeze bottle, for dispensing n-hexane
7.19. Top loader Balance.

8 REAGENTS, GASES, AND STANDARDS

8.1. Laboratory Pure Water

8.2. n-Hexane, 95% High Purity grade, Pharmo Catalog # 35995HPLC

8.3. Methanol, ACS reagent grade – Filter pre wet only. Diverted to waste.

8.4. 1 + 1 Hydrochloric Acid (HCl): Prepare this reagent in a 1L volumetric flask. Measure 400mL of laboratory pure water into a graduated cylinder and transfer into the volumetric flask. Then, measure 500 mL of concentrated HCl into a graduated cylinder and slowly and carefully transfer it to the volumetric flask. Add a stir bar to the flask and stir on a stir plate to mix. Bring to volume with lab pure water. CAUTION: This process will generate some heat. Use until expiration date.

8.5. Food Coloring
8.6. Nitrogen Gas Source: for extractor operation
8.7. Compressed Air Source: for Speed-Vap™ operation

8.8. Ultra Method 1664 Precision, Accuracy and Recovery Standard Cat # RGO-102X: 2 mg/mL stearic acid and 2 mg/mL n-hexadecane in acetone. Use until expiration date. Used for 1 mg/L, 2 mg/L standard, and 4 mg/L fortified matrix sample.

8.9. NSI solutions Method 1664 Oil and Grease PAR surrogate 0.4% n-Hexadecane and 0.4% Stearic acid standard cat # QC-003SNIP used for 80 mg/L QCS standard.

8.10. Oil & Grease Low standard , 1 mg/L: Prepare this sample in an empty, clean 1L wide-mouth sample bottle. Pipette 0.25 mL of Oil & Grease standard from 8.8 into 900 mL of lab pure water (measured with a graduated cylinder) that has been acidified with 1+1 HCl. This sample is prepared and analyzed with every batch, and used to validate the laboratory reporting limit.

8.11. Oil & Grease Laboratory Control Sample (LCS), 2 mg/L: Prepare this sample in an empty, clean 1L wide-mouth sample bottle. Pipette 0.5mL of Oil & Grease standard from 8.8 into 900 mL of lab pure water (measured with a graduated cylinder) that has been acidified with 1+1 HCl. This sample is prepared and analyzed with every batch, and used to validate the laboratory reporting limit

8.12. Oil & Grease Quality Control Sample (QCS), 80 mg/L: Prepare this sample in an empty, clean 1L wide-mouth sample bottle. Add 10 ml tube of standard from 890 into 900 mL of lab pure water (measured with a graduated cylinder) that has been acidified with 1+1 HCl. This sample is prepared and analyzed with every batch.

8.13. Oil & Grease Matrix Spike Sample, 4 mg/L spike: Pipet 1 mL of standard from 8.9 Grease standard into the chosen spike sample. This sample is analyzed along with an identical, un spiked sample. Prepare one spike sample for every 10 samples in an analytical batch, with at least 1 spike sample per batch.

9 SAMPLE COLLECTION, HANDLING, AND PRESERVATION

9.1. Aqueous samples are collected in 500-1L narrow and wide-mouth glass bottles, acidified to a pH of less than (<) 2 with HCl, and stored at <4ºC.

9.2. The holding time for oil and grease is 28 days following sample collection.

10 METHOD CALIBRATION

10.1. Standard Preparations: See the REAGENTS, GASES, AND STANDARDS section for the preparation of standards and matrix spike samples.

10.2. Calibration Procedure:

10.2.1. A balance calibration/check is necessary before and after each analysis batch. 1 mg, 2 mg, 1 g and 5 g weights are used to perform the balance check for oil and grease analysis.

11 SAMPLE PREPARATION AND BATCH ANALYSIS

11.1. Aqueous Sample Preparation:

11.1.1. Prepare laboratory control samples and matrix spikes as defined in the

REAGENTS, GASES, AND STANDARDS section of this procedure. All samples Should be acidified (can be checked with pH paper). (NOTE: pH is checked by transferring a small amount of sample using a glass rod to a portion of pH paper. The glass rod is then rinsed with hexane back into the sample container. The use of a glass rod prevents loss of sample.)

11.1.2. A method blank is prepared by adding 900 mL of lab pure water to a 1 L wide mouth glass bottle. Add approximately 2mL of 1+1 HCl to acidify the sample to a pH of <2. A method blank is analyzed with every run.

11.1.3. A 1 mg/L, 2 mg/L and 80 mg/L laboratory control samples are analyzed with every run.

11.1.4. A matrix spike 4 mg/L and duplicate sample is performed after every 10 samples or for every batch size.

11.1.5. All samples and QA to be analyzed are fitted with a bottle adaptor placed under the screw-top lid. Secure the adaptor and its lid by screwing them on hand-tight.

11.1.6. The sample and container to be analyzed is weighed and recorded. Depending of sample matrix, 250-1000 ml glass sample wide mouth bottles are used for collection of samples. If the sample is anticipated to have a high concentration of oil and grease, a smaller sample volume is collected. After analysis, the sample bottles are weighed. The volume is a calculation of the difference in weight of sample and container minus the weight of the empty container.

A typical analytical batch is as follows:

- Method Blank beginning of run
- 1mg/L low standard beginning of run
- 2 mg/L LCS beginning of run
- Sample #1-#10
- Sample duplicate for every sample batch of 10
- Matrix Spike (4 mg/L) for every sample batch of 10
- Method blank fore every batch.
- 80 mg/L QCS or end of run standard

11.1.7. Record all sample batch information in the oil and grease logbook. If samples were submitted in duplicate, record which sample, A or B, will be extracted in the logbook. Extractor information is also recorded.

11.2. Preparation and Purging:

11.2.1. First, check all solvent levels (methanol, and n-hexane) and fill if necessary. Check solvent and water waste recovery bottles, and empty if necessary. Make sure all necessary method blanks and QA has been prepared (see REAGENTS, GASES, AND STANDARDS section for information). NOTE: Spikes should be prepared only after the initial sample extraction is complete in the event that there is an extraction problem.

11.2.2. Turn on the corresponding hood, nitrogen source, vacuum pump, and power up the 3000XL controller. Make sure that the bottle-top on the water waste bottle has a good seal. Check all system pressures. The pressure gauge on the waste solvent recovery bottle should be between 10-15 psi. The solvent bottle pressure (gauge near the solvent bottles) should be about 8-10 psi. The SPE extractor pressure should be approximately 35 psi.

11.2.3. Check the valves on all 3000XL extractors using the valve check tool to be sure that all valves are open and functioning properly.

11.2.4. It is necessary to purge/clean each system before use to remove debris and contamination. Screw a water sample inlet valve assembly on top of the purge bottle/adaptor assembly. Make sure that a disk holder is in place on the extractor, and then lift the sensor arm and place in the disk holder cup. Pull the water sample holder arm down into place. Orient the aluminum shaft of the water sample inlet valve so that it points toward the system. Invert the bottle and load the water sample inlet valve assembly and bottle onto the sample holder arm. Guide the water sample inlet valve shaft onto the actuator key (groove) on the extractor.

11.2.5. Connect a purge separatory flask adaptor assembly on to the bottom of the valve body. Turn the adaptor a quarter of a turn to make sure that the flask fits tightly (any air in the system will interfere with extraction/purging). The collection flask holder will keep the flask in place. From the Horizon screen of the 3000XL controller, hit “Status.” Click on the appropriate station button. Load Method 30 (purge). Press “Start”, then press “Start” again.

11.2.6. Repeat steps 11.2.4-11.2.5 for each extractor until they have all been purged. Each extractor will revert to STANDBY when the purge method is complete. If the purge method is not successful (solvent is not completely extracted), try clearing the check valves again and repeating the purge method. If there is still a problem, refer to the extractor manuals for maintenance/cleaning steps, or call Horizon technical support.

3000XL model Extraction:

11.2.7. Remove the purge flask/adaptor assembly from the appropriate extractor. Unscrew the purge bottle/adaptor assembly from the water sample inlet valve. Store these items.

11.2.8. Remove the disk cup holder from the extractor. With the metal SPE disk support screen into the center of the disk holder, place a 47mm (or 90mm) fiber SPE disk on the support screen using forceps. Place the disk collar with the groove side facing up into the disk holder cup. Firmly screw the aluminum locking ring onto the disk holder cup. (NOTE: Horizon Fast-Flow™ pre-filters can be used for "dirty" samples. This is not necessary for standards, blanks, and samples that are relatively free of solids.) Load the disk holder assembly onto the disk holder platform and press down firmly to ensure a tight seal.

11.2.9. Fit the sample/standard to extract with a bottle adaptor extraction flask. Screw the flask adaptor on the top. Connect the flask to the extractor in the same way that the purge flask/adaptor assembly was connected in step 11.2.4.

11.2.10. Screw a water sample inlet valve assembly on top of the sample bottle/adaptor assembly. Lift the sensor arm and place in the disk holder cup. Pull the water sample holder arm down into place. With the aluminum shaft on the valve assembly facing the analyst and a finger over the hole on the right of the valve assembly, invert the sample bottle. Orient the aluminum shaft of the water sample inlet valve so that it points toward the system. Load the water sample inlet valve assembly and bottle onto the sample holder arm. Guide the water sample inlet valve shaft onto the actuator key (groove) on the extractor.

11.2.11. From the Horizon screen of the 3000XL controller, hit "Status." Click onto the appropriate station button. Load one of the following methods:
Method 26 - ("Clean" samples – 47mm Pacific disks)
Method 27 - ("Dirty samples – 47mm Pacific disks)
Method 28 - ("Clean" samples – 90mm Pacific disks)
Method 29 - ("Dirty samples – 90mm Pacific disks)

11.2.12. Press "Start", and then press "Start" again. When the extraction is complete, the extractor will switch to the STANDBY mode. (NOTE: Methanol solvent is used for prewet only. This solvent is diverted to waste prior to extraction).

11.2.13. When extraction is complete, remove the reparatory funnel/adaptor from the extractor and unscrew the adaptor, transfer pipette. NOTE: Add a drop of food coloring to better define the hexane and aqueous layers. GO TO 11.3.6

11.2.14. Lift the liquid sensor arm out of the disk holder and remove the holder Remove the used filter from the holder. Remove the sample bottle/adaptor from the top of the extractor. Unscrew the valve assembly and adaptor from the sample bottle. Fill the sample bottle with water up to the volume mark on the bottle. Pour this water into a 1000mL graduated cylinder, and record the volume in the oil & grease log book.

11.2.15. Repeat steps 11.2.8-11.2.14 for each blank, QA, and sample extraction. When all extractions are complete, purge each system as described in steps 11.2.4 – 11.2.5. Turn off the controller, vacuum source, and nitrogen source. Empty the solvent recovery bottle into the spent hexane container stored under the oil and grease hood. Empty the contents of the water waste recovery bottle into the sink.

11.3. Gravimetric Determination:

11.3.1. Calibrate an analytical balance to four decimal places prior to use by following the instructions in the "General Instructions for Balance Calibration" laboratory SOP. Weights of 1 g and 2 mg must be used to check the four-place calibration for oil and grease analysis.

11.3.2. Number an aluminum pan to correspond with each sample/standard number in the logbook NOTE: Be careful not to get fingerprints on the pans. Always handle aluminum pans with laboratory forceps or Kim wipes. Place the pans in a tray and let them sit in a desiccator for approximately 20 minutes to remove moisture.

11.3.3. Weigh each pan to four decimal places and record the initial weight in the oil and grease logbook. Put the pans back in the desiccator for 10 minutes, and reweigh the pans. Record the second weight in the oil and grease logbook. The two weights should be within 0.0005g of each other; if not, place the pans back in the desiccator for another 10 minutes and weigh them a third time.

11.3.4. Power on the Speed-Vap™ III and wait for the temperature to adjust to 39ºC. Record this temperature in the oil and grease logbook. Turn on the air source.

11.3.5. Place nine of the weighed aluminum pans inside the Speed-Vap III™.

11.3.6. Remove the top n-hexane solvent layer from the sample extract collection separatory funnel,. Drain the aqueous layer from the separatory funnel, and place the contents in the corresponding aluminum dish. Be careful not to remove any of the bottom aqueous layer; this will adversely affect analysis results. After most of the n-hexane layer has been removed, use a squeeze bottle of n-hexane

to wash the inside wall of the reparatory flask several times. Continue to remove the rest of the n-hexane layer, and place the contents in the corresponding aluminum dish. If a sample was split for extraction, the contents of the first flask should be added to the aluminum dish and evaporated before the contents of the second flask are added.

11.3.7. Repeat step 11.3.5 until all nine dishes are filled with the corresponding sample/standard n-hexane layer. When this is complete, turn on the compressed air by turning the valve on the lab countertop. Close the cover of the Speed-Vap™, turn on the vacuum switch, and adjust the air pressure dial on the Speed-Vap™ to approximately 1 psi. The n-hexane will evaporate from each of the dishes.

11.3.8. Once the solvent has completely evaporated from all nine dishes, remove them from the Speed-Vap™ with forceps and place them back in a tray. Allow them to sit in a desiccator for 20-30 minutes and repeat step 11.3.3 to get two acceptable final weights for each pan. Record all weights in the oil and grease logbook.

11.3.9. Repeat steps 11.3.5-11.3.8 for every sample/standard in the analysis batch. Perform a balance check using 1 g and 2 mg weights once all pan weights are recorded. If the calibration is not verified after measurement of the entire batch, recalibrate the balance and reweigh the batch.

11.3.10. Turn off the evaporator and air source. Dispose of any excess aqueous waste from the vials into the spent hexane container stored under the hood. Turn off the hood.

12. DATA ACQUISITION, CALCULATIONS, & DATA REDUCTION

12.1. Data from the oil and grease analysis is currently entered manually into the laboratory reporting system. QC analysis is entered into a quality control EXCEL spreadsheet to determine analytical batch is under control. All results are reported to 2 significant digits.

12.2. Oil and Grease Calculation:

Calculate "oil and grease" for blanks, samples, and standards using the following equation:

$$\text{Oil and Grease (mg/L)} = \frac{(WI - WF) \times 1{,}000{,}000}{VS}$$

Where:
WI = initial weight of pan (second value)
WF = final weight of pan and extract (second value)
VS = volume of original sample/standard

12.3. QC Calculations:

Calculate % recovery (%R) for LCS and QCS using the following equation:

%R = Test Result X 100
True Result
Where:
Test Result = the value obtained from the analysis

True Result = the actual value of LCS/QCS

Calculate % recovery (%R) for matrix spike samples using the following equation:

%R = (SSR – SR) X 100
SA
Where:
SSR = spiked sample result
SR = sample results (unspiked)
SA = spike amount added

13 QUALITY CONTROL, ACCEPTANCE CRITERIA, AND CORRECTIVE ACTION

13.1. Method Blank:

13.1.1. Frequency: A method blank will be analyzed at the beginning and after every analytical batch.

13.1.2. Acceptance Criteria: The method blank result must be below the detection limit of the method. In this case, it is less than (<) 2 ppm.

13.1.3. Corrective Action: If the method blank result does not meet the acceptance criteria, the system has to be evaluated for possible errors. All samples must be reanalyzed, or accepted based on other criteria to be determined by the QA officer. If there is not an extra sample for reanalysis or the holding time has expired, the samples must be reported as "No Result." In all cases, the out-of-control result must be recorded in the logbook in the QA office and the QA officer must be notified.

13.2. Laboratory Control and Quality Control Samples:

13.2.1. Frequency: A 2 ppm (LCS) and 80 ppm (QCS) will be prepared and analyzed with each run 2 ppm at the beginning of the batch and the 80 ppm at the end.

13.2.2. Acceptance Criteria: The established control limits for laboratory control sample %recoveries are 78-114%. All LCS data is recorded and plotted in an oil and grease EXCEL spreadsheet .

13.2.3. LCS% Recovery Failure Corrective Action: If the LCS %recovery does not meet the acceptance criteria, the system has to be evaluated for possible errors. All samples must be reanalyzed, or accepted based on other criteria to be determined by the QA manager. If there is not an extra sample for reanalysis or the holding time has expired, the samples must be reported as "No Result." In all cases, the out-of-control result must be recorded in the logbook in the QA office and the QA manager must be notified.

13.3. Matrix Spike Samples:

13.3.1. Frequency: Matrix spike samples are prepared and analyzed at least once per 10 or less samples in an analytical batch. A minimum of 5 percent of all samples from a given sampling site are used as matrix spike samples (see step 11.1.4).

13.3.2. Acceptance Criteria: The established control limits for matrix spike (LFM) %recoveries are 78-114%. All matrix spike data is recorded and plotted in EXCEL spreadsheet.

13.3.3. Matrix Spike Recovery Failure Corrective Action: If the matrix spike % Recovery does not meet the acceptance criteria, the system has to be evaluated for possible errors. All samples must be reanalyzed, or accepted based on other criteria to be determined by the QA manager. If there is not an extra sample for reanalysis or the holding time has expired, the samples must be reported as "No Result." In all cases, the out-of-control result must be recorded in the logbook in the QA manager and the QA manager must be notified.

13.4 Duplicate samples:

13.4.1. Duplicate Samples are prepared and analyzed at least one per 10 or less samples in an analytical batch.

13.4.2. Acceptance Criteria: The established control limits for duplicate (LR) %recoveries are +/-7 % determined from 2018 precision data
. All duplicates data is recorded and plotted in EXCEL spreadsheet

14 REFERENCE SECTION

14.1. EPA Method 1664-Revision A, "N-Hexane Extractable Material (HEM) and Silica Gel
Treated n-Hexane Extractable Material (SGT-HEM) by Extraction and Gravimetry (Oil and Grease and Total Petroleum Hydrocarbons)

14.2. SPE-DEX 1000/3000XL Extractors User's Manual

14.3. EPA Federal Code of Regulations 40

oil & Grease (mg/L)	Precision/Bias for a single concentration			
		2018		
		Actual		Squared
Date	High std	High std	Difference	Difference
6-Jan	36.8	40	3.2	10.24
27-Jan	35.2	40	4.8	23.04
3-Feb	38.4	40	1.6	2.56
16-Feb	38.7	40	1.3	1.69
3-Mar	34.8	40	5.2	27.04
17-Mar	36.8	40	3.2	10.24
21-Mar	35.3	40	4.7	22.09
19-Apr	37.9	40	2.1	4.41
1-May	35.4	40	4.6	21.16
12-May	34.1	40	5.9	34.81
22-May	35.8	40	4.2	17.64
31-May	37.7	40	2.3	5.29
6-Jun	32.5	40	7.5	56.25
18-Jun	35.7	40	4.3	18.49
30-Jun	35.3	40	4.7	22.09
6-Jul	37.6	40	2.4	5.76
20-Jul	31.8	40	8.2	67.24
Sum			70.2	350.04
Bias			4.1294	
Range			4.1294	20.5906
Standard Deviation			3.6608	18.2541
Precision				4.68
Std range			51.368773	

Bias=sum of the differences / # measurements
Precision= Square root of the(squared differences / #of measurement-1)
Std Range=Standard Deviation(Precision)X 3

Precision/Bias for a single concentration				
O&G (mg/L)		**2018**		
		Actual		**Squared**
Date	**Low std**	**Low std**	**Difference**	**Difference**
6-Jan	1.8	2	0.2	0.04
27-Jan	1.6	2	0.4	0.16
3-Feb	1.5	2	0.5	0.25
16-Feb	1.9	2	0.1	0.01
3-Mar	1.7	2	0.3	0.09
17-Mar	1.6	2	0.4	0.16
21-Mar	1.7	2	0.3	0.09
19-Apr	1.8	2	0.2	0.04
1-May	1.9	2	0.1	0.01
12-May	2.1	2	0.1	0.01
22-May	2.1	2	0.1	0.01
31-May	2.4	2	0.4	0.16
6-Jun	1.7	2	0.3	0.09
18-Jun	1.6	2	0.4	0.16
30-Jun	1.8	2	0.2	0.04
6-Jul	1.8	2	0.2	0.04
20-Jul	1.9	2	0.1	0.01
Sum	30.9		4.3	1.37
Bias			0.2529	
Precision				0.2926
Std Dev.			0.1328422	
Range			0.1166159	**1.8-2.1**

Bias=sum of the differences / # measurements

Precision= Square root of the(squared differences / #of measurement-1)

O&G Date	Precision Calculation using Duplicates First Result	2018 Second Result	Difference	% Precision
6-Jan	37.9	34.8	3.1	8.528
27-Jan	80.9	81.7	0.8	0.984
27-Jan	20.5	18.1	2.4	12.435
27-Jan	0.1	0.1	0	0
3-Feb	6.5	6.8	0.3	4.511
16-Feb	10.5	9.8	0.7	6.897
3-Mar	18.2	20.3	2.1	10.909
3-Mar	0.1	0.1	0	0
3-Mar	0.1	0.1	0	0
17-Mar	20.1	17.5	2.6	13.83
31-Mar	23.7	21	2.7	12.081
31-Mar	0.1	0.1	0	0
19-Apr	26.3	25.9	0.4	1.533
19-Apr	66	56.6	9.4	15.334
19-Apr	5.8	5.4	0.4	7.143
1-May	0.1	0.1	0	0
12-May	11.7	10	1.7	15.668
22-May	18.1	15.4	2.7	16.119
31-May	15.3	13.1	2.2	15.493
31-May	13	12.6	0.4	3.125
6-Jun	4.2	3.9	0.3	7.407
6-Jun	27	21	6	25
18-Jun	18.2	17.4	0.8	4.494
30-Jun	0.1	0.1	0	0
6-Jul	25.9	22.7	3.2	13.169
6-Jul	36.5	31.1	5.4	15.976
6-Jul	24.7	21.7	3	12.931
6-Jul	0.1	0.1	0	0
20-Jul	12.4	10.6	1.8	15.652
20-Jul	26.2	22.1	4.1	16.977
Sum			56.5	256.2
Range			1.45	6.57
Standard Deviation			1.28	5.82
Precision			1.45	6.57

Range=sum of the differences / #of measurements
Standard deviation=Range/1.128
Precision=standard deviation

O&G	Precision Calculation from Known Additions	2018			Table 5
Date	Sample Spike	Sample	Calculated Recovery	Concentration Know Addition	Deviation from Expected
6-Jan	3.5	0	3.5	4	0.5
27-Jan	3.8	0.1	3.7	4	0.3
27-Jan	4	0.2	3.8	4	0.2
27-Jan	3.9	0	3.9	4	0.1
3-Feb	4.1	0.6	3.5	4	0.5
16-Feb	3.8	0.2	3.6	4	0.4
3-Mar	3.4	0.1	3.3	4	0.7
3-Mar	3.7	0.1	3.6	4	0.4
3-Mar	3.4	0.1	3.3	4	0.7
17-Mar	3.7	0.1	3.6	4	0.4
31-Mar	3.5	0	3.5	4	0.5
31-Mar	3.7	0	3.7	4	0.3
19-Apr	3.6	0.7	2.9	4	1.1
19-Apr	3.5	0	3.5	4	0.5
19-Apr	3.7	0.1	3.6	4	0.4
1-May	3.4	0	3.4	4	0.6
12-May	4	0.3	3.7	4	0.3
22-May	3.9	0.6	3.3	4	0.7
31-May	4.6	1.3	3.3	4	0.7
31-May	3.8	0.4	3.4	4	0.6
6-Jun	3.9	0.5	3.4	4	0.6
6-Jun	3.4	0.2	3.2	4	0.8
18-Jun	3.8	0	3.8	4	0.2
30-Jun	3.4	0.2	3.2	4	0.8
6-Jul	3.7	0	3.7	4	0.3
6-Jul	5.2	2.1	3.1	4	0.9
6-Jul	3.3	0.2	3.1	4	0.9
6-Jul	3.7	0.2	3.5	4	0.5
20-Jul	13.2	10	3.2	4	0.8
20-Jul	3.6	0.2	3.4	4	0.6
Sum					16.3
standard deviation(Precision)					0.237
Range					**3.8-4.2**

15 METHOD PERFORMANCE

15.1. See the oil and grease QC EXCEL spreadsheets for recent data on method performance.

The following personnel have read, understood, and will follow this Standard Operating Procedure:

Signature Date

The Standing Operating Procedure for Analytical Chemistry for Envirotech Laboratories, Inc. has been reviewed

And approved 11/13/20

Electronically signed______________________

Ronald J. Saari

Laboratory Director

Electronically signed_______________________

Kevin Burgess

QA/QC Manager

ISSUE DATE: 01/18/23

STANDARD OPERATING PROCEDURE

For Method SM 5210 B

Determination of Biological Oxygen Demand (BOD, BOD5,c-BOD) in Aqueous Samples Using Dissolved Oxygen Meter (Method SM 4500-O-G)

Reference Standard Methods 23rd Edition online

Method revision 2016

		Signature	Date
Analyst:	Iris Pickard	electronically signed	02/01/23
Laboratory Director:	Ron Saari	electronically signed	02/01/23

EPA METHOD 200.7

DETERMINATION OF METALS AND TRACE METALS BY INDUCTIVELY COUPLED PLASMA-ATONIC EMISSION SPNCTROMETRY

Ref:"Determination of Metals and Trace Metals in Water and Wastes by Inductively Coupled plasma-Atomic Emission Spectrometry", Method 200.7 revision 4.4 EMMC Version.

STANDARD OPERATING PROCEDURE

02/01/2023

		Signature	Date
Analyst:	Kevin Burgess	electronically signed	02/01/23
Laboratory Director:	Ronald Saari	electronically signed	02/01/23
QA/QC Manager:	Kevin Burgess	electronically signed	02/01/23

STANDARD OPERATING PROCEDURE EPA METHOD 200.7

APPLICATION:

1. This method is applicable to the following analytes:

Analyte

Aluminum	Magnesium
Antimony	Mercury
Arsenic	Molybdenum
Barium	Nickel
Beryllium	Phosphorus
Boron	Potassium
Cadmium	Selenium
Calcium	Silica
Chromium	Silver
Cobalt	Sodium
Copper	Strontium

Iron	Thallium
Lead	Tin
Lithium	Vanadium
Magnesium	Zinc

Table 1 lists are the recommended wavelengths for these analytes including adjacent locations for background correction. Also listed are typical instrument detection limits (IDL's).

2. Instrumental operating conditions are given in *Table 4.*

3. When using this method for determination of boron and silica in aqueous samples, only plastic, Teflon or quartz labware should be used from time of sample collection to completion of analysis. For accurate determinations of boron in solid sample extracts at concentrations below 100 mg/kg, only quartz beakers should be used in the digestion with immediate transfer of an extract aliquot to a plastic centrifuge tube following dilution of the digestate to volume. For these determinations, borosilicate glass must not be used in order to avoid sample contamination of these analytes from the glass.

4. This method is applicable to analysis of drinking water for the determination of primary and secondary contaminant metals. However, it can only be used for compliance monitoring of a drinking water contaminant when listed in the Federal Register as an approved method and laboratory performance data meet the required method detection limit (MDL) or practical quantification limit (PQL). All drinking water samples must be pretreated with acid prior to analysis. Certain analytes require 4X preconcentration prior to analysis instead of the 2X preconcentration procedure at the time the method is promulgated.

SUMMARY OF METHOD

The basis of the method is the measurement of atomic emission by an optical spectrometric technique. Samples are nebulized and the aerosol that is produced is transported to the plasma torch where desolvation and excitation occur. Characteristic atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma (ICP). The spectra are dispersed by a grating spectrometer, and line intensities are monitored by a photosensitive device (e.g., photomultiplier tube).

DEFINITIONS:

A. *DISSOLVED* - The concentration of analyte that will pass through a 0.45-um membrane

filter assembly, prior to sample acidification.

B. *TOTAL RECOVERABLE* - The concentration of an analyte determined in an unfiltered sample following treatment by refluxing with hot, dilute mineral acid.

C. *INSTRUMENTAL DETECTION LIMIT(IDL)*- The concentration equivalent to the analyte signal which is equal to three times the standard deviation of a series of ten replicate measurements of a reagent blank signal at the same wavelength.

D. *METHOD DETECTION LIMIT(MDL)* - The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero (Sect. 10.2.2).

<u>INTERFERENCES</u>:

Several types of interference effects outlined below may contribute to inaccuracies in the determination of an analyte by ICP.

A. *Spectral interferences* - Can be categorized as (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) background contribution from stray light from the line emission of high concentration elements

Given in Table 3 is a listing of the interelement spectral interferences that can occur between method analytes when using the recommended wavelengths and locations for background corrections listed in Table 1.

The correction factors given in Table 3 were determined by analyzing single element solutions of each interfering element.

B. *Physical interferences* - Are generally considered to be effects associated with the sample nebulization and transport processes. Such properties as change in viscosity and surface tension can cause significant inaccuracies especially in samples which may contain high dissolved solids and/or high acid concentrations. The use of a peristaltic pump may lessen these interferences. If these types of interferences are operative, they must be reduced by sample dilution and/or utilization of standard addition techniques.

C. *Chemical interferences* - Can be minimized by careful selection of operating conditions (i.e., incident power, observation position, etc.), by buffering the sample, matrix matching, or standard addition procedures.

D. *Memory interferences* - Result when analytes in a previous sample contribute to the signals

in a current sample.

The occurrence of interferences described in A, B, and C above are primarily attributed to the sample matrix. The following tests can be used to ensure the analyst that neither positive nor negative interference effects are operative on any of the analyte elements thereby distorting the accuracy of the reported values.

a. Serial dilution
b. Analyte addition
c. Comparison with alternative method of analysis
d. Wavelength scanning of analyte line region

REAGENTS AND STOCK SOLUTIONS:

1. All chemicals and acids purchased is reagent ACS or trace metal grade.

2. Water - For all sample preparation and dilutions, deionized distilled ASTM type 1 water is used.

3. Standard Stock Solutions - Are purchased from FISHER SCIENTIFIC Co.

4. Refer to EPA 200.7 for specific preparation of standard solutions.

SAMPLE COLLECTION, PRESERVATION AND STORAGE:

A. For determination of dissolved elements; the sample must be filtered through a 0.45-um membrane filter. Use a portion of the filtered sample to rinse the filter flask, discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) nitric acid immediately following filtration to a pH <2.

B. For the determination of total recoverable elements in aqueous samples, acidify with (1+1) nitric acid at the time of collection to a pH (2 (normally, 3 ml of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples). The sample should not be filtered prior to analysis. Use trace metal grade Nitric and Hydrochloric Acid.

C. Samples are held at least 24 hrs after addition of acid preservation before analysis or digestion of sample.

D. Solid samples require no preservation prior to analysis other than storage at 4º C.

CALIBRATION AND STANDARDIZATION:

1. Recommended wavelengths and background correction locations are listed in Table 1. In Table 4 specific instrument operating conditions are recommended.

2. Allow the instrument to become thermally stable before beginning. This usually requires at least 30 mm. of 9peration prior to plasma optimization, plasma tuning and/or calibration.

3. Plasma optimization- Prior to the use of this method optimizes the plasma operating conditions using ICAL solution produced be Inorganic Ventures Co. The purpose of plasma optimization is to provide a maximum signal to background ratio for the least sensitive element in the analytical array.

The instrument operating condition selected, as being optimum, should provide the lowest reliable IDLs and MDLs.

4. **Calibration** - Calibrate according to Spectro Genesis ICP outline using the prepared calibration blank and CAL solutions. Run Calibration blank immediately after calibration standards. The following operational steps should be used for both CAL solutions and samples. See Calibration Standard and QC Solution sheet.

   a. Using a peristaltic pump introduces the standard or sample to nebulizer at a uniform rate (e.g., 1.2 ml/min).
   b. To allow equilibrium to be reached in the plasma, aspirate the standard or sample solution for 60 sec. after reaching the plasma before beginning integration of the background corrected signal.

   c. Use the average value of four 5-sec. background corrected integration periods as the atomic emission signal to be correlated to analyte concentration.

   d. Between each standard and sample, flush the nebulizer and solution uptake system with the rinse blank acid solution.
   e.

QUALITY CONTROL

1. LABORATORY REAGENT BLANK (LRB): Run at least one blank with each 20 or fewer samples of the same matrix. When the LRB value is 2.2 times the analyte's MDL, fresh aliquots of samples are prepared and reanalyzed.

2. LABORATORY FORTIFIED BLANK (LFB): At least one LFB is analyzed per batch of samples. Recovery of any analyze must be within 85-115% recovery.

3. INSTRUMENT PERFORMANCE CHECK (ICP check): Check is run after daily calibration. Level should be within 5%. After every 10th sample check should be within 10%. End ICP Check Levels should be within 10%. If values fall outside the control limits, the instrument is recalibrated and samples following last acceptable ICP check solution are reanalyzed.

4. LABORATORY FORTIFIED MATRIX (LFM): SPIKED SAMPLE: Spiked sample is run after every 10 samples. Recovery should be 85%-115%. Use a duplicate of the aliquot used for replicate sample. Use the same concentration used for the laboratory fortified blank.

5. REPLICATE SAMPLE: A replicate of a sample used in routine analysis is performed after every 10 samples. Recovery should be 80%-120%.

6. INTERFERENCE CHECK: Check is run at beginning, in the middle, and at end of run. If correction factor is within 10% of the mean for 5 consecutive days, then the interference check is run weekly to check corrective factor interferences.

7.

TABLE 1. RECOMMENDED WAVELENGTHS WITH LOCATIONS FOR BACKGROUND CORRECTION AND
ESTIMATED INSTRUMENT DETECTION LIMITS (IDL)

Analyte	Wavelength, nm[1]		Location for BKG. Correction	Estimated LDLs mg/L [2]
Ag	328.068		+0.070 nm	0.005
Al	308.215		+0.070 nm	0.05
As	193.696		+0.070 nm	0.03
B	249.678x2	249.773	+0.035 nm	0.006
Ba	493.409		-0.064 nm	0.001
Be	313.042		-0.064 nm	0.0007
Ca	315.887		+0.070 nm	0.02
Cd	226.502		+0.070 nm	0.002
Co	228.616		-0.064 nm	0.007
Cr	205.552x2		-0.032 nm	0.007
Cu	324.754		-0.064 nm	0.003
Fe	259.940		+0.070 nm	0.007
Hg	194.227x2		-0.032 nm	0.02
K	766.491		-0.064 nm	0.7
Li	670.784		+0.070 nm	0.005
Mg	279.079		-0.064 nm	0.03
Mn	257.610		+0.070 nm	0.0008
Mo	203.844		-0.064 nm	0.02
Na	588.995		+0.070 nm	0.03
Ni	231.604x2		+0.035 nm	0.009
P	214.914x2		+0.035 nm	0.09
Pb	220.353		-0.064 nm	0.03
Sb	206.833		+0.070 nm	0.03

Se	196.090		+0.070 nm	0.08
SiO_2	251.611		-0.064 nm	0.02
Sn	189.980x2		-0.032 nm	0.02
Sr	421.552		+0.070 nm	0.0006
Tl	190.864		-0.064 nm	0.03
V	292.402	311.071	+0.070 nm	0.009
Zn	213.856x2		+0.035 nm	0.002

(1) Wavelength x 2 indicates wavelength is read in second order.

(2) The IDLs were estimated from three times the standard deviation of 10 replicate measurements of the calibration blank. The calculated IDL was rounded upward and reported to a single digit.

TABLE 3. LISTING OF POTENTIAL INTERELEMENT SPECTRAL INTERFERENCE

		Interfering	Correction	
	Interfering	Correction		
Anal vte	Wavelength, nm	Element	Factor	Analyte
	Wavelength, nm	Element	Factor	
Ag	328.068	Fe	-0.0002	Co
228.616	Ba	0.0009		
		Mn	0.0001	
	Cr	0.0002		
		V	-0.0001	
	Mo	0.0001		
	Ni	0.0003		
Al	308.215	Co	-0.0020	
				Cr
205. 552x2	Be	0.0014		
		Mo	0.0107	
	Cu	-0.0004		
		V	0.0082	
	Fe	-0.0009		
	Mo	0.0009		
As	193.696	Al	0.0067	
	Ni	0.0006		
		Be	-0.0007	
		Co	0.0004	Cu
		324.754	Mo	0.0005

Fe 0.0003
Mo -0.0012
Ni 0.0001 Fe
259.940 None
V 0.0120
B 249. 678x2 None
Hg
194.227x2 Mo 0.0004

V 0.0030
Ba 493.409 None
K
766.491 None
Be 313.042 V 0.0041
Li
670.784 None
Ca 315.887 Co 0.0016
Cr -0.0002 Mg
279.079 Mn -0.0030
Mo 0.0033

Mo -0.0029
Cd 226.502 Co -0.0012 Mn
257.610 Fe -0.0004
Fe -0.00004
Ni 0.0004 Mo
203.844 Al -0.0002
Sn -0.0003
9.0
Fe -0.0001

Mn -0.0041

TABLE 3. (Continued)

INTERFERING

CORRECTION

Analyte	Wavelength; nm	Interfering Element	Correction Factor	Analyte	
	Wavelength ,nm	Element	FACTOR		
Na	588.995	None		Si	
251.611	None				
Ni	231.604x2	Co	0.0011	Sn	189.980x2
Fe		0.0004			

Mo -0.0016
Mn 0.0004

Ti 0.0005

Mo

-0.0114

Sb -0.0009

Si

0.0002
P 214.914x2 Al -0.0019
Ca -0.0014
Cu 0.0121
Mo 0.0060 Sr
421.552 None -------

Pb 220.353 Al 0.0013 Ti
190.864 Co 0.0054
Co -0.0332
Fe 0.0008
Cr -0.0021
Mn 0.0021
Cu 0.0005
Mo 0.0057
Fe -0.0002
P 0.00008
Ni -0.0012
V 0.0038
V -0.0016

Sb 206.833 Co -0.0030 V
292.402 Cr 0.0006
Cr 0.0114
Fe 0.0005
Fe 0.00008
Mo 0.0026
Mo 0.0082
Ni -0.0092 Zn
213 .856x2 Cu 0.0011
Sn 0.0024

Fe

.0001

Ni

0.0034

Se	196.090	As	-0.0025
		Co	-0.0047
		Fe	0.0004
		Mo	-0.0152
		V	-0.0022

TABLE 4. INDUCTIVELY COUPLED PLASMA INSTRUMENT OPERATING

Incident rf power	1300 watts
Reflected rf power	< 5 watts
Viewing height above work coil	NA end on view
Injector tube orifice i.d	1 mm
Argon supply	liquid argon
Argon pressure	100 psi min. @ tank
Coolant argon flow rate	12 L/min
Aerosol Carrier argon Flow rate	0.8-1.0 ml/min
Auxiliary (plasma) Argon flow rate	1.0 L/min
Sample uptake rate controlled to	2.0 ml/min

Background Positions - Background Corrections

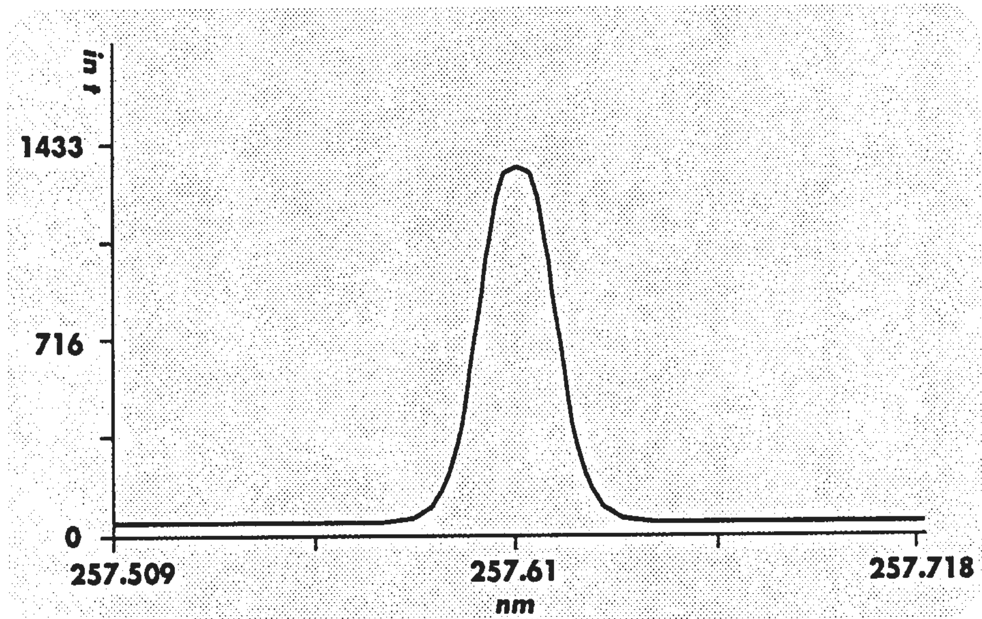
The varying salt contents in samples lead to varying background intensity signals. For this reason a background correction is necessary for almost all matrices (see example 1).

The scans maybe examined to determine whether or not the measurements must be measured with background correction. If the background intensity remains the same for the blank sample, the standards and the unknown samples, it is not necessary to measure with background correction.

If the intensities are not the same, then one or, when necessary, two background positions must be set.

2A. 1 Procedure

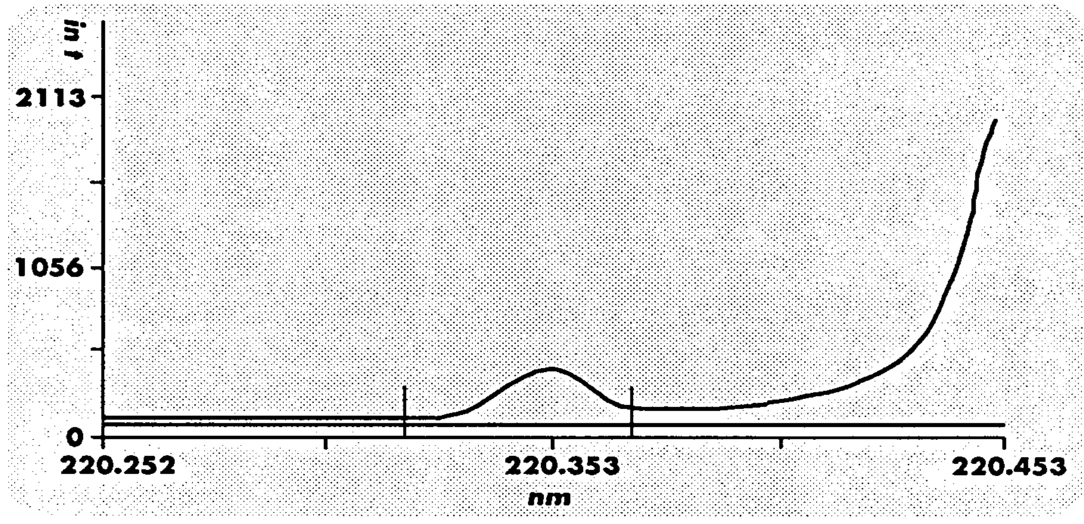
The scan must be examined. It must be determined whether or not the background intensities are approximately the same on both sides of the peak.
The Spectrometer program suggests a position equal to two halfwidth values. In most cases this may be accepted. There are, however, exceptions as shown in the following examples.



The background intensities are about the same on both sides of the peak. In this case only one background position is required.

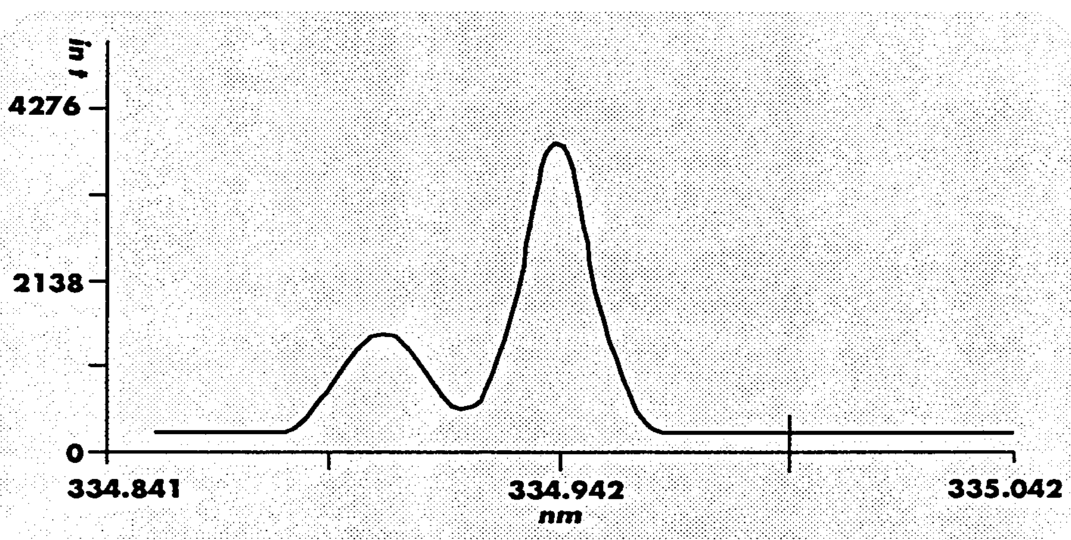
Example 1: Mn 257.61 nm

Example 2: Pb 220.35 nm in a soil/slurry matrix



The background is increased on one side by a remote aluminum peak in this matrix. In this case, background positions must be set on both sides of the peak to eliminate the influence of the aluminum peak. The right background position must be set close to the flank of the peak (see diagram).

Example 3: Ti 334.94



This peak is a doublet and for this reason only one background position is set, to the right of the peak. A background position set between the two peaks leads to errors in the measurement.

7.1.3 Monthly Checks

Check	*Note*	*Done*
• Torch	outer tube and aerosol tube should be examined for dirt and damage; clean or replace	
• Air filter in the generator cube	examine for blockages replace every two months	
• Air filter in instrument	examine for blockages replace every two months	
• Nebulizer chamber	clean, remove grease (use a mild detergent)	
• Nebulizer	clean and check the quality of the spray	

7.1.4 *Semiannual Checks*

Check	*Note*	*Done*
• Air filter under the power unit	examine for blockages replace every two months	

7.1 Service Checklist

7.1.1 Daily Checks

Check	*Note*	*Done*
• Argon supply	should be > 40 bar	
• Argon humidifier	must be filled to the SPECTRO logo with Deionised water	
• Pump hose and sample feed hose	check for any damage	

peristaltic tubing	check for wear. Replace as needed

7.1.2 Weekly Checks

	Check	***Note***	***Done***
•	Fiber optic	check for damages and dirt, clean if necessary	
•	Nebulizer	check for blockages or deposits, clean if necessary	
•	Nebulizer Chamber	check for deposits, clean if necessary	
•	Store analytical data	in folder on raw data sheets	

DIRECT ANALYSIS PROCEDURE (for potable water samples only)

All potable water samples are analyzed for turbidity by the Mantec PC Titrant Analyzer.

Samples with turbidity values of < 1 NTU are acidified to pH < 2 with HNO3 and are aspirated directly into nebulizer and analyzed according to the procedure.

Direct Analysis can only be used on potable water samples.

The pH value is recorded.

AQUEOUS SAMPLE PREPARATION - TOTAL RECOVERABLE ELEMENTS:

Hold acidified (<2) non-potable water samples for a minimum of 24 hrs before digestion procedure.

For determination of total recoverable elements in water or wastewater, other than marine and estuarine water, take a 50 ml (+ 1 ml) aliquot from a well mixed, acid preserved sample and transfer it to a 75-ml Digestion vial. [For drinking water compliance monitoring certain analytes require 4X preconcentration prior to analysis. Add 2m1 of trace metal grade (1+1) nitric acid and 1.0 ml of (1+1) hydrochloric acid. Heat the sample block

digester set at 85ºC. Sample will read 70-80^{0}C. Heat until the volume has been reduced to approximately 20 ml, ensuring that the sample does not boil. (A spare beaker containing 50 ml of water can be used as a gauge.)*

Slight boiling may occur but vigorous boiling should be avoided. Allow to cool and quantitatively transfer to either a 50-ml volumetric or a 50-ml class A stoppered graduated cylinder. Dilute to volume with ASTM type 1 water and mix. Allow to stand overnight to separate insoluble material or filter sample if insoluble material present. The sample is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, samples should be analyzed as soon as possible after preparation.

*50 ml of deionized water in digestion vial is placed at the center of the Block digester. The Bloc digester is adjusted to no higher than 95ºC. This is recorded. Digestion logbook will be maintained including date of digestion, pH measurement, turbidity, lot # of standands and expiration date for samples in that batch for analysis.

SOLID SAMPLE PREPARATION - TOTAL RECOVERABLE ELEMENTS:

For determination of total recoverable elements in solid samples (sludge, soils, and sediments), mix the sample thoroughly to achieve homogenity and weigh accurately a 1.0 + 0.01 g portion of the sample. Transfer to a 250-ml Phillips beaker. Add 4 ml (1+1) nitric acid and 10 ml (1+4) hydrochloric acid. Cover with a watch glass. Heat the sample on a hot plate and gently reflux for 30 mm. Very slight boiling may occur, however, vigorous boiling must be avoided to prevent the loss of the HCl-H_20 azeotrope.

Allow the sample to cool and quantatively transfer to 100-ml volumetric flask. Dilute to volume with ASTM type 1 water and mix. Centrifuge the sample or allow to sit overnight to separate insoluble material. - The sample is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, samples should be analyzed as soon as possible after preparation.

SAMPLE ANALYSIS:

1. Analyze the samples by the procedural routine described in the calibration procedure. Perform the method of standard addition if required. Samples having concentrations higher than the established linear dynamic range (LDR) should be diluted into range and reanalyzed. The sample may first be analyzed for trace analytes providing the elements in high concentration do not cause a severe matrix effect and any interelement spectral interference or shift in background intensity can be properly corrected.

2. For drinking water compliance monitoring, if the concentration of a primary contaminant is determined to be 90% of its MCL. or above and the combined Mg and Ca concentration equals 500 mg/L, the sample should be analyzed by the standard addition technique

CALCULATIONS:

1. Sample data should be reported in units of mg/L for aqueous samples and mg/kg dry weight for solid samples. Do not report element concentrations below the determined MDL.

2. For aqueous samples prepared by total recoverable procedure multiply solution concentrations by the dilution factor 0.5. Round the data to the thousandth place and report the data in mg/L up to three significant figures.

3. For solid samples prepared by total recoverable procedure round the solution concentrations (ug/L in the analysis solution) to the thousandth place and multiply by the dilution factor 100. Report the data to a 0.1 mg/kg up to three significant figures taking into account the percent solids as noted in Sect. 11.3 when the data are reported on a dry weight basis.
4.

ICP Genesis Operation outline

START UP

1. Turn on exhaust switch.
2. Turn on chiller. HT150-1000
3. Turn on gas supply-Argon
4. Go to system
5. Switch plasma on/off
6. After flame is on wait 1 hour.
7. Go to ICALization. Check Genesis ICAL solution
8. Pick a profile needed.
9. Click File
10. Click Open
11. Choose file sequence

TO PERFORM ANALYSIS

1. Standardize instrument. Press start program (green button).
2. Wait for calibration.
3. If cal OK, start testing samples.
4. After every run sequence check spectra of all samples.
5. Click Spectra.
6. Click file.
7. Click Operation
8. Click sample to view
9. Check for split peaks. If peak split dilute accordingly and reanalyze.

SAVE FILE TO

1. Click job parameters
2. Sample sequence preview
3. Copy.
4. Start.
5. Wordpad
6. Paste.
7. Print.
8. File save as.

TURN OFF SYSTEM

1. Switch plasma on/off. Wait till plasma stops.
2 Turn chiller off.
3. Turn vent off.
4. Turn gas off.

CALIBRATION AND QUALITY CONTROL SOLUTIONS

1. Calibration Standards (Series 1)

1.1 Stock standards. ICM 203 and ICM 205 (Ultra Scientific)

1.2 Standard preparation:

Std. 100: To 250 ml of deionized water in a acid washed 500 ml volumetric flask add 4 ml of conc. HNO3. Add 50 ml stock ICM 203 and 50 ml ICM 205. Fill to 500 ml with DI water.

Std. 50: To a 500ml volumetric flask add 100 ml deionized water, 2.5 ml conc. HNO3 and 250 ml of standard 100. Fill to 500 ml with DI water..

Std 25: To a 500ml volumetric flask add 100 ml deionized water, 2.5 ml conc. HNO3 and 250 ml of standard 50. Fill to 500 ml with DI water.

Std 12.5: To a 500ml volumetric flask add 100 ml deionized water, 2.5 ml conc. HNO3 and 250 ml of standard 25. Fill to 500 ml with DI water.

Std 6.25: To a 500ml volumetric flask add 100 ml deionized water, 2.5 ml conc. HNO3 and 250 ml of standard 12.5. Fill to 500 ml with DI water.

Std 3.12: To a 500ml volumetric flask add 100 ml deionized water, 2.5 ml conc. HNO3 and 250 ml of standard 6.25. Fill to 500 ml with DI water.

Std 0.312: To a 500ml volumetric flask add 100 ml deionized water, 2.5 ml conc. HNO3 and 250 ml of standard 3.12. Fill to 500 ml with DI water.

Final Calibration Standard Concentrations – Series 1

	Std 100	Std 50	Std 25	Std 12.5	Std 6.25	Std 3.12	Std 0.312
Ag	50	25	12.5	6.25	3.125	1.56	0.156
Al	100	50	25	12.5	6.25	3.125	0.313
Ba	10	5	2.5	1.25	0.625	0.312	0.0312
Be	10	5	2.5	1.25	0.625	0.312	0.0312
Ca	100	50	25	12.5	6.25	3.125	0.313
Co	20	10	5	2.5	1.25	0.625	0.0625
Cr	50	25	12.5	6.25	3.125	1.56	0.156
Cu	10	5	2.5	1.25	0.625	0.312	0.0312
Fe	100	50	25	12.5	6.25	3.125	0.313
K	100	50	25	12.5	6.25	3.125	0.313

Mg	100	50	25	12.5	6.25	3.125	0.313
Mn	10	5	2.5	1.25	0.625	0.312	0.0312
Na	100	50	25	12.5	6.25	3.125	0.313
Ni	50	25	12.5	6.25	3.125	1.56	0.156
V	10	5	2.5	1.25	0.625	0.312	0.0312
Zn	50	25	12.5	6.25	3.125	1.56	0.156

2. Calibration Standards (Series 2)

2.1 Stock standards.

CPI P/N 4400-ICP-MSCS

2.2 Standards preparation:

Std. 2.00 : stock solution – to a 500 ml acid washed volumetric flask add 100 ml ICP-MSCS P/N 4400 , 4.0 ml HNO3 and add deionized water to 500 ml volumetric.

Std 1.00*:* To a 500 ml volumetric flask add 100 ml of deionized water, 2.5 ml conc.HNO3, and 250 ml of Std 2.00. Fill to 500 ml with DI water.

Std 0.50*:* To a 500 ml volumetric flask add 100 ml of deionized water, 2.5 ml conc.HNO3, and 250 ml of Std 1.00. Fill to 500 ml with DI water.

Std 0.10*:* To a 500 ml volumetric flask add 100 ml of deionized water, 4.0 ml conc.HNO3, and 100 ml of Std 0.50. Fill to 500 ml with DI water.

Std 0.01*:* To a 500 ml volumetric flask add 100 ml of deionized water, 4.5 ml conc.HNO3, and 50 ml of Std 0.10. Fill to 500 ml with DI water.

Std 0.005*:* To a 500 ml volumetric flask add 100 ml of deionized water, 2.5 ml conc.HNO3, and 250 ml of Std 0.01. Fill to 500 ml with DI water.

	Std 2.0	Std 1.0	Std 0.50	Std 0.10	Std 0.01	Std 0.005
Ag	2.0	1.0	0.50	0.10	0.01	0.005
Al	2.0	1.0	0.50	0.10	0.01	0.005
As	2.0	1.0	0.50	0.10	0.01	0.005
B	2.0	1.0	0.50	0.10	0.01	0.005
Ba	2.0	1.0	0.50	0.10	0.01	0.005
Be	2.0	1.0	0.50	0.10	0.01	0.005
Cd	2.0	1.0	0.50	0.10	0.01	0.005
Cr	2.0	1.0	0.50	0.10	0.01	0.005
Cu	2.0	1.0	0.50	0.10	0.01	0.005
Mn	2.0	1.0	0.50	0.10	0.01	0.005
Mo	2.0	1.0	0.50	0.10	0.01	0.005
Ni	2.0	1.0	0.50	0.10	0.01	0.005
Pb	2.0	1.0	0.50	0.10	0.01	0.005
Sb	2.0	1.0	0.50	0.10	0.01	0.005
Se	2.0	1.0	0.50	0.10	0.01	0.005
Sr	2.0	1.0	0.50	0.10	0.01	0.005
V	2.0	1.0	0.50	0.10	0.01	0.005

3.0 Quality Control (ICP-1 check sample)

3.1 Stock solutions

Inorganic Ventures QCP-QCS-1

Inorganic Ventures CGK10

Inorganic Ventures CGMG10

3.2. Standard Preparation:

3.2.1. To a 1000 mL volumetric flask, add approximately 300 mL DI water.

3.2.2. Add 10 mL HNO3

3.2.3. Add 2.0 mL of QCP-QCS-1

3.2.4. Add 4.0 mL of CGK10

3.2.5. Add 1.0 mL of CGMG10

3.2.6. Fill to volume with DI

4.0 Quality Control (ICP-2 check sample)

4.1 Stock solutions

CPI Prod# 4400-010118-A

Hach Fe Std Cat# 14049

Ultra QCI-710

Ultra QCI-720

4.2. Standard Preparation:

4.2.1. To a 1000 mL volumetric flask, add approximately 300 mL DI water.

4.2.2. Add 10 mL HNO3

4**.2.3.** Add 25 mL of CPI 4400-010118-A

4**.2.4.** Add 50 mL Hach #14049

4**.2.5.** Add 100 mL Ultra QCI-710

4**.2.6.** Add 100 mL Ultra QCI-720

4**.2.7.** Fill to volume with DI

5.0. Spiking standards

5.1. Stock solutions.

Agilent ICM-212

Agilent ICM-213

Agilent ICM-223

5.2. Standard preparation

5.2.1. Spike #1 – no preparation required

5.2.2. Spike #2

5.2.2.1. To a 100 mL volumetric flask, add approximately 50 mL DI water

5.2.2.2. Add 9 mL HNO3

5.2.2.3. Add 10 mL Agilent ICM-213

5.2.2.4. Fill to volume with DI water

5.2.3. Spike #3

5.2.3.1. To a 100 mL volumetric flask, add approximately 50 mL DI water

5.2.3.2. Add 9 mL HNO3

5.2.3.3. Add 10 mL Agilent ICM-223

5.2.3.4. Fill to volume with DI water

5.3. Laboratory Fortified Blank (LFB) preparation

5.3.1. LFB1

5.3.1.1. To 50 mL of acidified DI, add 0.20 mL of Spike #1

5.3.2. LFB2

5.3.2.1. To 50 mL of acidified DI, add 0.10 mL of Spike #2

5.3.3. LFB3

5.3.3.1. To 50 mL of acidified DI, add 0.20 mL of Spike #3

6.0. Internal Standard

6.1. Stock standard

Inorganic Ventures Yttrium Product# CGY1

6.1.1. Standard preparation

6.1.1.1. To a 1 liter volumetric flask, add approximately 500 mL of DI water.

6.1.1.2. Add 5 mL of nitric acid

6.1.1.3. Add 0.5 mL of Yttrium stock standard

6.1.1.4. Fill to 1000 mL with DI water

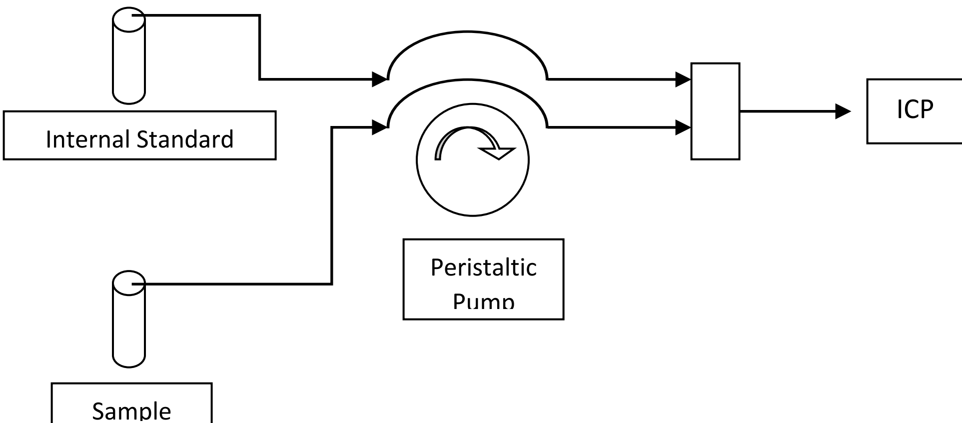
6.1.2. Internal standard implementation setup

6.1.2.1. Place orange-orange peristaltic tubing on the pump

6.1.2.2. Insert lengths of capillary tubing into both sides of the peristaltic tubing

6.1.2.3. Place the sample uptake side in the internal standard

6.1.2.3. Insert the capillary tubing carrying the internal standard into the 3-way T fitting as depicted below.



7.0 Interference Check solution: ICM 223 and ICM 224

Fe	**110 mg/L**
Al	**300 mg/L**
Be	**1.00 mg/L**
Ag	**60.0 mg/L**
Cr	**60.0 mg/L**
Cu	**60.0 mg/L**
Ni	**60.0 mg/L**
V	**60.0 mg/L**
Zn	**60.0 mg/L**
Co	**30.0 mg/L**
Ba	**3.00 mg/L**
Mn	**10.0 mg/L**
Tl	**50.0 mg/L**
Cd	**15.0 mg/L**

The Standard Operating Procedure for Analytical Chemistry for Envirotech Laboratories, Inc. has been reviewed and approved 02/01/23.

Electronically signed____________________

Ronald J. Saari

Laboratory Director

Electronically signed_____________________

Kevin Burgess

QA/QC Manager

ISSUE DATE: 02/01/23

STANDARD OPERATING PROCEDURE

For Method SM 5210 B

Determination of Biological Oxygen Demand (BOD, BOD5,c-BOD) in Aqueous Samples Using Dissolved Oxygen Meter (Method SM 4500-O-G)

Reference Standard Methods 23rd Edition online

Method revision 2016

		Signature	Date
Analyst:	Iris Pickard	electronically signed	02/01/23
Laboratory Director:	Ron Saari	electronically signed	02/01/23

STANDARD OPERATING PROCEDURE

FOR

BIOCHEMICAL OXYGEN DEMAND

METHOD SM 5210 B (5-DAY)

1.0 SCOPE AND APPLICATION:

1.1 The biological oxygen demand (BOD) test is used to determine the oxygen requirement of municipal and industrial wastewaters.

1.2 Application of the test to organic waste discharges allows calculation of the effect of the discharges on the oxygen supply of the receiving waters. Data from BOD tests are used in engineering calculations in designing wastewater treatment plants.

2.0 SUMMARY OF METHOD:

2.1 The method consists of filling a 300 ml BOD bottle to almost overflowing, with the appropriate dilution of the sample and dilution water. The sample is incubated in the dark for 5 days at 20 ° C. The reduction in dissolved oxygen concentration during the incubation period from the dilution water yields a measure of the biological oxygen demand.

3.0 APPARATUS AND REAGENTS:

3.1 Incubator. Thermostatically controlled at 20 °C±1, closed to exclude all light.

3.2 3000 ml container for dilution water preparation.

3.3 300 ml BOD bottles.

3.4 plastic sealing caps

3.5 graduated pipettes

3.6 graduated cylinders

3.7 vacuum bubbler

3.8 HACH BOD buffer pillows cat no. 14861-98 and cat no. 1416066

3.9 Polyseed (InterLAb) BOD Seed Inoculum cat no. P-110

3.10 HACH BOD standard solution ampule 300 mg/L glucose cat. No. 14865-10

3.11 Iodate-Iodide Standard Solution 0.00125N cat no. 401-49

3.12 Hach HQ 40d DO meter and probe

3.13 Distilled water

3.14 Sodium thiosulfate 0.0125 N for dechlorination

3.15 Dissolved Oxygen Meter HACH HQ 40d

3.16 Sodium Sulfite, Cobalt chloride for Zero Std.

3.17 Hach Nitrification Inhibitor Formula 2533 Cat 253334

3.18

4.0 Washing

4.1 The BOD test is a semi-sterile procedure.

4.2 All glassware used for test are washed thoroughly with biodegradable detergent, and rinsed three time with distilled water.

1. BOD bottles are sterilized in autoclave.

5.0 SAMPLE SIZE

A. Practically all wastewater plants operate within a predictable BOD range for various sampling points based on previous in-house and State analyses of record. These must be consulted prior to using this method.

B. Select earlier HIGHEST and LOWEST recorded BOD values for types of samples desired, e.g., Raw Influent, Primary and Final Effluents.

C. Determine a reasonable day-to-day BOD value from the above and consult Table 1 - A Guide to Sample Size Selection.

D. For example, a plant has had a high Raw BOD value of 350 and a low of 110. The usual value has been about 180. If desired, proceed to Step H.

E. Inspection of the Table shows that for a BOD range of 60-420 a sample size of five (5) ml is recommended. This is a 1:60 dilution.

F. Influent (Raw) BOD dilution for this plant prepared with a 5 ml sample should show a 5-day D.O. depletion of about 2-6 mg/l corresponding to a BOD of 120-360.

G. The D.W., if prepared and stored as described below, (Section 6), will have a D.O. well in excess of 8 mg/L so that the above depletions can be easily measured.

H. A simple formula for determining BOD sample sizes may be used if we take the value from Step D and also assume that the ideal 5-day D.O. depletion for any BOD sample is about 4 mg/L or approximately *50%* of the available D.O.

I.

BOD = Depletion X Factor

$$\text{BOD} = \frac{4 \times 300}{\text{Sample Size}}$$

$$\text{Thus, Sample Size} = \frac{1200}{\text{BOD}}$$

In the example of Step D,

$$\text{Sample Size} = \frac{1200}{180} = 6.7 \text{ ml}$$

2b Using Table 1, the nearest practical volume would be 6 or 7.5 ml.

I. Sample sizes may be varied periodically as required by plant operating conditions and as judged by Suspended Solids and Turbidity values.

J. Table 2 gives additional general information on BOD's and sample sizes for various water types.

6. Dilution Water (D.W.)

A. Allow about seven gallons of dilution water per BOD run.

B. Add 1 HACH BOD buffer pillows cat no. 14861-98 to each of three gallons of distilled water. Measure DO if below 7.5 aerate.

C. Aerate the D.W. vigorously for five (5) minutes (or more) by drawing clean air through or by shaking and place in the incubator at 20+/1 C.

D. If the dilution water is shaken to maximize the D.O. it should be allowed to sit for a minimum of 15 minutes to remove pin-point air bubbles.

Preferably, one to 24 hours will reduce the chance for oxygen super-saturation, high D.O. values initially and otherwise unexplainable D.O. losses after incubation.

E. The aeration step should be repeated before each new BOD run.

F. The D.W. should be kept in the incubator, particularly during the summer months. Exposure to bright light must be avoided to forestall algae growth.

G. D.W. should not be used for the test if the D.O. is not at least 7.5 mg/L or temp not 20°±3 C.

7. Procedure

A. Dechlorination of Effluent

1. Test the effluent sample for residual chlorine using test strip (Micro check HF Scientific). Record results on BOD worksheet.
2. If the test shows that no chlorine, proceed directly to Part B.

In this case, seeding (Section 8) is not required because it may be assumed that sufficient wastewater organisms remain to exert the full BOD.

If chlorine is present, add one ml of 0.025N Sodium Thiosulfate to one liter of effluent, stir well and allow to stand for a short time until a second residual chlorine test is negative. It may then be used for the BOD test. This step will dechlorinate up to about 7 mg/l total residual chlorine.

Alternatively proceed as follows:

a) Measure out 100 ml of the effluent sample, add 0.5 g potassium iodide and one ml. of conc. sulfuric acid. Swirl until dissolved.
b) Add 5-10 drops of starch indicator solution and titrate dropwise with 0.025N (N/40) sodium thiosulfate to the disappearance of the blue color. Record the volume of "thio" used (per 100 ml effluent).
c) Using a fresh sample of effluent, measured carefully in multiples of 100 ml (depending on sample volume requirements for the BOO test), add from a buret, the calculated amount of "thio". Mix well.

This mixture is used for the BOD determination.

NOTE: PAO (phenyl arsine oxide) MUST NOT BE USED FOR DECHLORINATION OF

BOD EFFLUENT SAMPLES SINCE PAO IS A POWERFUL AND PERMANENT BACTERIOSTAT. BOD VALUES OF ZERO WILL RESULT!

B. Check pH of each sample .Record on worksheet. pH should be in range of 6.0-8.0. If not in range, adjust pH with $NaOH/H_2SO_4$ to pH of 7.0-7.2.

C. Dissolved Oxygen meter calibration.

a. Press Calibrate. Place probe in DI water that has been saturated with air.

b. Press Calibrate. Allow to stabilize. Record slope. Save calibration.

c. Measure D.O. of Zero Standard (1 g Na_2SO_3 and few crystals of Co2Cl crystals.

d. Check Standard (0.00125N Iodate-Iodide Standard 10.0 mg/L, purchased from HACH)

e. Record on worksheet

D. <u>Set-Up of Run</u>

1. Fill 2 BOD bottles with D.W. by pouring without bubbling or splashing. Measure D.O. and Record as initial D.O. Stopper all of them and fill the flared bottle collars with distilled water, then attach plastic caps to two of the bottles. These are the D.W. blanks. Place in BOD incubator.

2. Seeding

Quality control samples: fill 10 BOD Bottles up to half way with DW

as in Step 1. a) to four(4) BOD bottles add 15,20,25, and 30 mL respectively with the polyseed solution that was previously prepared. Polyseed solution: 1 capsule of Inter Lab Polyseed (Cat # LS2001047) in 500 mL of DW. Mix for 1 hour and let stand for 15 minutes. To the remaining 5 bottles add 3 mL of Polyseed solution.

3. BOD standard: to the 6 BOD bottles with the 3 mL of Polyseed solution add 4, 4, 4, 3, 2, and 1 mL of the BOD standard (300 mg/L of glucose and glutamic acid ampule purchased from Hach Cat #14865-10.

4. Fill the BOD bottles to volume and measure the Initial DO and record. For each wastewater sample, fill BOD bottles up to half way with DW. as in Step 1,

then add appropriate volume of the sample using a wide-tip pipet or graduated cylinder the same way. Add 3 mL of the Polyseed solution.

For c-BOD samples add 0.16 g or 2 shots of Nitrification Inhibitor to each bottle. Use 2-4 bottles with different volumes. Measure the D.O. of each bottle and record as initial D.O. Fill the bottles with D.W., stopper and fill the bottle collars with distilled water, then attach plastic caps to two of each set. Put these in the BOD incubator (20°C±1).

NOTES: a) For a 200 ml or greater sample the BOD bottles filled with the sample and DW to volume if necessary as in Step 1 and a concentrated buffer pillow is added to the sample (Hach Nutrient Buffer Pillow Catalogue #1416066)

b) BOD runs which use samples of 50 ml or more should be made with effluent which has been freshly aerated in a similar manner to the dilution water. These aerations provide for the maximum oxygen reserve for use during the incubation by the micro-organisms.

c) All samples and the D.W. should be brought to a temperature of 20°C before setting up the BOD run.

d) After 5-day incubation, analyze D.O. in all dilutions of each sample and record value as final D.O.

3. Determine the D.O. of one seeded blank and set aside two (2) BOD seed blank bottles as usual for five (5) days in the incubator.

4. Set-up three (3) BOD bottles containing a sample volume of dechlorinated effluent in addition to one ml of seed. Proceed as in Step 3.

5. After 5-day incubation determine the D.O. of the seed blanks and seeded

effluent samples.

NOTES: a) At least two bottles of each kind are incubated for five days to provide insurance against their loss by careless D.O. measurement or accident. If a BOD sample is lost in Step 3 (initial D.O.) another may be promptly prepared instead.

b) Dilution Water D.O. depletions of over 0.2 mg/l not only invalidate the entire run for reporting purposes but require a careful check of the BOD dilution water or of BOD technique. The most common causes are dirty (bacteria, mold and algae) equipment and reagents used for the test and blowing air into the dilution water from the pressure outlet of a mechanical vacuum pump.

c) B.O.D. bottles which deplete less than 2.0 mg/l D.O. over five days will probably not give reliable B.O.D. results. Larger samples should be used for future runs.

d) All B.O.D bottles having a 2.0 mg/L minimum depletion and at least a 1.0 mg/L residual DO must be used in calculations.. In case of no alternative, it may be reported by taking the initial D.O. value of the sample, multiplying by the dilution factor and reporting the value obtained with a + sign following.

For example: Raw Sample of 5 ml

Dilution Factor = 60

Initial D.O. = 8.2 mg/l

5-day D.O. = 0.6 mg/L (IGNORE)

Reportable BOD = 8.2 x 60 = 492+

e) BOD bottles must have at least 1.0 mg/L D.O. residual to ensure insufficient D.O. does not affect the rate of oxidation of the sample.

8. <u>Quality Control</u>

To check the lab technician's overall BOD methodology, 4 seeding blanks are used and the average is used to calculate a factor for the BOD concentrations. A seeded 300 mg/L BOD standard solution ampule

(Purchased from HACH cat # 1486166) is used with a dilution factor of 75(add 4.0 ml std), 100(add 3.0 ml std). A 300 dilution (add 1.0 ml std) is used for MDL. The BOD value should be between 167.5 and 228.5 mg/L. Dilutions are performed for each run. Sample duplicates are performed every 10 samples. (±30%).

9. Calculations - REPORT ALL VALUES TO NEAREST WHOLE NUMBER.

BOD precision may vary up to ±25%. Changes in D.O. of the D.W. blank quality controls are NOT to be used for correcting any wastewater sample D.O. results. The D.W. blanks serve ONLY as a rough check on D.W. quality.

A. Regular (Unseeded)

BOD (mg/l) (Initial D.O. - 5 day D.O.) x Factor Depletion

$$\text{Factor} = \frac{300}{\text{sample size}}$$

I

B. Seeded: **Calculations done on Excel spreadsheet**

1. Using Polyseed

$$\text{Seed Correction (S.C.)} = \frac{\text{depletion of Influent}}{\text{ml per influent sample used for regular BOD run}}$$

BOD (Seeded) (mg/L) = (Depletion - S.C.) X Factor

2. Using SEED BLANK

Seed Correction (S.C.) per ml = Depletion of Seed Blank

BOD (Seeded) (mg/L) = (Depletion - S.C.) X Factor

C. KHP Quality Control

S.C. is same as in Part B-I above.

BOD of KHP (mg/L) = (Depletion - S.C.) x 60 D.

D. %Removal

Percent Removal of BOD by WPC =

$$\frac{\text{(mg/L Influent BOD-m g/L Effluent BOD) X 100}}{\text{mg/L Influent BOD}}$$

Table 1. Guide to Sample Size Selection for BOD's

The dilution water is assumed to have D.0. in excess of 8 mg/L (store in incubator during warm weather).

BOD bottle volume is taken as 300 ml

$$\text{Factor} = \frac{300}{\text{Sample size}}$$

BOD = (5 day D.O depletion) X Factor

BOD. Range Measurable	Sample Size ml.	Factor	% Dilution
300,000-2,100,000	0.001	300000	0.00033
30,000-210,000	0.01	30000	0.0033
10000-70000	0.03	10000	0.01
3000-18000	0.1	3000	0.033
1000-7000	0.3	1000	0.1
600-4200	0.5	600	0.17
300-2100	1	300	0.33
150-1050	2	150	0.67
100-700	3	100	1
75-525	4	75	1.33
60-420	5	60	1.67
50-300	6	50	2
40-280	7.5	40	2.5
30-210	10	30	3.33
25-175	12	25	4
20-140	15	20	5
15-105	20	15	6.67
12-84	25	12	8.33
10-70	30	10	10
7.5-52.5	40	7.5	13.33
6-42	50	6	16.67
5-30	60	5	20
4-28	75	4	25
3-21	100	3	33.33
2-14	150	2	50
1-7	300	1	100

NOTE: Allowable D.O. depletion (5-day) is 1-7 mg/L range per bottle.

TABLE 2 Biochemical Oxygen Demands of Various Types of Water

Type	BOD	Required Dilution Factor (300 m. bottle)	ml sample to be taken	Measurable BOD Range mg/L
Potable, Surface Waters	1 – 20	1-5	60-300	1- 35
Trickling fi1ter	10- 100)	1- 20	15-300	1-140
OR Activated Sludge Effluents	5- 50)))			
Influent Sewage OR Primary Effluent	50 – 500	20 – 100	3-15	20-700
Digested Sludge Supernatant OR Filtrate	500 - 2500	100 - 600	0.5 – 3	100 -4200
Industrial Wastes	500-5000	300-1000	0.3 – 1	300 - 7K
Process Chemicals	30K – 2M	10K - 300K	0.001 -0.03	10K - 2.1M

K=1000 M=Million

'I The Standing Operating Procedure for Analytical Chemistry for Envirotech Laboratories, Inc. have been reviewed

And approved 02/01/23

Electronically signed____________________

Ronald J. Saari

Laboratory Director

Electronically signed______________________

Kevin Burgess

QA/QC Manager

ISSUE DATE: 2/1/23

Appendix B. Water Quality Grab Sample Collection Standard Operating Procedure

1.0 Scope and Application

This standard operating procedure establishes the protocol to be followed for collecting water quality grab samples for both the wet and dry weather monitoring events. Samples are collected for bacteria (*E. coli*, Enterococci and Fecal Coliform) and possibly other optional parameters (Nitrates as Nitrogen, Orthophosphate, Total Kjeldahl Nitrogen, and Oil and Grease).

2.0 Method Summary

- **Sample Location**

  When feasible, surface water samples are collected directly from the outfall of the discharge pipe into the laboratory analysis bottles. If the discharge pipe is inaccessible or if tidal water is back flowing into the discharge pipe, the Field Teams move to the next available upstream manhole (or catchbasin if no manholes exist in the drainage system). The sample in the manhole is taken at the inlet of the main storm drainpipe. If the sample cannot be taken under free-flowing conditions, the Field Teams take the sample as close to the inlet pipe as possible and make a notation on the comment section of the field data sheet (preferably with a photograph) as to the problems encountered at the site (tidal back flowing, inlet/outlet elevations prevent free-flowing conditions, etc.)

- **Sample Collection**

  To minimize potential sediment contamination, samples to be analyzed for bacteria are collected first using a sterile sampling container. Once the bacteria samples are collected, they are immediately placed in a cooler on blue ice. Separate bottles are then used to collect water samples for all field tests (surfactants, ammonia, nitrates, and total chlorine) and then any other laboratory testing (followed by appropriate storage protocol). If the sample location is not accessible at arm's length, samples are collected using a sampling pole holding either a laboratory analysis bottle or a clean sampling container (rinsed in the field with de-ionized water or purified water from the laboratories). If available, the Field Teams may also use a hand-held vacuum pump to collect samples. Once collected, the container and/or tubing from the vacuum pump are used to distribute the sample directly to the appropriate lab bottles. The collection container and or tubing are decontaminated (rinsing with de-ionized water or purified laboratory water) prior to use at the next grab sample site to prevent cross contamination.

3.0 Safety, Restrictions, and Limitations

When accessing all monitoring sites, Field Teams must be aware of accessibility and safety issues, especially during adverse weather conditions. At outfall pipes, Field Teams should not attempt collecting samples if the gradient is too steep or slippery, water velocity too high, or water depth is over three feet. Field Teams must follow all safety precautions as outlined by the local Department of Public Works when lifting manholes/catchbasins grates or when entering/exiting manholes or catchbasins.

While collecting grab samples, Field Teams should wear clean latex gloves to minimize their contact with contaminated water and to prevent sample contamination. The Field Teams should never touch the inside of any bottle or cap, especially the sterilized containers for the bacteria samples. If the sample is collected using a sampling pole or vacuum pump, the sampling pole bottle or tubing must be decontaminated in the field prior to use.

The laboratories should supply the proper size sample bottles to meet the required volume for testing. If the containers are not adequate, multiple samples are needed to supply the necessary volume. If possible, each laboratory analysis bottle should be filled from a single grab sample. In addition, the QA/QC duplicate samples should be filled from the same grab as the original sample bottles.

4.0 Sample Collection, Handling, and Preservation

Samples to be analyzed for bacteria are collected first using a sterile sampling container. Following the bacteria

collection, a second sample is collected for the analysis of the on-site "In the field" (pH, temperature, conductivity, salinity, chlorine, and ammonia) parameters. Once the "In the field" analysis has been completed, this second bottle is placed on ice and used for the "In house" analysis (surfactants and nitrates). Additional samples are then collected for any remaining laboratory parameters. Once collected, all samples are handled and preserved in accordance with Tables 3 through 7 in the QAPP.

The preferred method of sample collection is to fill the sample containers directly from the discharge under free-flowing conditions. If a free-flowing sample is not feasible, the Field Team collects the sample as close to the discharge as possible. If the discharge is inaccessible, a sampling pole container or vacuum pump are used to collect the sample. Once the sample is retrieved, it is completely mixed and then poured into the analysis containers. If the water quality sample cannot be collected under free-flowing conditions and/or must be collected with a sampling pole, the Field Team documents the testing conditions/methods on the field data sheets and the chain of custody forms so that the laboratory results can be flagged for review.

5.0 Equipment and Materials

coolers with blue ice
pens and permanent markers
field logbooks
data collection sheet
chain of custody forms
sampling pole or hand-held vacuum pump
pre-labeled laboratory bottles
sampling pole with clean collection containers
de-ionized water or laboratory purified water
hip-waders
manhole hook
latex gloves
paper towels
sealable bags
measuring tape
camera
safety glasses

6.0 Procedures

- Analysis laboratory should be contacted prior to sampling date.
- Laboratory analysis bottles and equipment should be organized prior to each grab at the site.
- New, clean, latex gloves should be always worn when handling the sample collection bottles and obtaining samples in the field.
- The caps from the lab analysis bottles should be removed just prior to collecting or receiving a sample. They should be re-capped just after a sample is collected or received. The amount of time an opened bottle is exposed to the environment should be minimized.
- The bacteria samples should be collected first, followed by the field measurements and then any other laboratory samples.
- If the discharge pipe is free flowing, obtain the laboratory sample directly from the pipe while minimizing sediment disturbance. If the discharge pipe is not free flowing, take the sample as close to the pipe as possible. Note on the monitoring data sheet the conditions under which the sample was taken (not free-flowing, tidal backflow, manhole, etc.). There should be a minimum of two people collecting samples at all times, one to take all notes, fill out labels and forms, etc. while the other collects the samples.

Appendix C. Field Water Quality Measurements Standard Operating Procedure

1.0 Scope and Application

This standard operating procedure addresses the procedures to collect field water quality measurements using Hach Pocket Pro, Multi 2 (pH, temperature, conductivity, salinity), Hach test strips (ammonia), Chemetrics K-9400 (surfactants), LaMotte Nitrate-Nitrogen test kit (nitrate) and Hannah Colorimeter (chlorine).

2.0 Equipment Inspection, Maintenance, and Calibration

Prior to each sampling event, all test kits and equipment are inspected to ensure the availability of testing materials (Hach strips, test kits, and Chemetrics) and operability of equipment (Hach meter and Colorimeter). Calibration of the Hach Pocket Pro, Multi 2 meter and Colorimeter are in accordance with manufacturer's instructions. If any parts of the testing equipment need to be repaired or replaced, this are noted in the standard Equipment Inspection, Testing and Maintenance Log Sheet.

Calibration checks on the Hach Pocket Pro, Multi 2 meters and colorimeter are performed by the Field Team prior to each sampling event with the equipment being re-adjusted as needed in accordance with manufacturer's instructions. Calibration checks are recorded on the standard Equipment Calibration Form and in the data logbook.

3.0 Field Measurement Procedure

Field measurements are collected at each sampling location after the filling of analysis bottles. Whenever possible, field measurements samples are taken at the center of the discharge flow, at half of the depth and upstream of the sample collector. For the Hach Pocket Pro, Multi 2 meter, the Field Team is careful not to allow the probe to contact any accumulated sediment.

As with the bacterial samples, if free-flowing conditions do not exist, the samples are taken as close to the discharge as possible or moved upstream at the discretion of the Stormwater Coordinator (or designee). If the discharge pipe is inaccessible, water quality samples are collected using a sampling pole or handheld vacuum pump. The sample collection point, collection conditions, and accessibility are noted on the field data sheet. Data collection in the field is conducted in accordance with manufacturer's instructions.

The Stormwater Coordinator or Field Team Leader are responsible for equipment maintenance and cleaning following each sampling event. Any issues regarding the equipment or other testing materials are reported immediately to the Stormwater Coordinator.

4.0 Quality Assurance

For quality assurance purposes, each Field Team duplicates at least one sample or ten percent of all samples collected (whichever is more) as part of each sampling event. A duplicate is a second reading of the same water sample.

5.0 Field Water Quality Measurements Standard Operating Procedure

Prior to each sample round each of the HachPocket Pro, Multi 2 meters are calibrated by the Field Team Leader in accordance with the manufacturer's instructions. The time and date of the calibration are recorded on the standard Equipment Quality Assurance Form.

6.0 References

HachPocket Pro, Multi 2 https://www.hach.com/pocket-pro-multi-2-tester-for-ph-cond-tds-salinity-with-replaceable-sensor/product-downloads?id=17990686217

LaMotte Nitrate Nitrogen Test Kit (2013) www.lamotte.com/en/industrial/individual-test-kits/3615-01.html

Pocket Colorimeter II User Manual, Edition 1 (2014) www.hach.com/pocket-colorimeter-ii-chlorine-free-and-total/product-downloads?id=7640442953

Chemetrics Instructions www.chemetrics.com/Detergents+(anionic+surfactants,+MBAS)/Visual+Kits/K-9400/R-9400

Hach test strips www.hach.com/teststrips

Appendix D. Wet and Dry Weather Field Data Sampling Sheet

Buzzards Bay Stormwater Collaborative - Water Quality Sampling Sheet

Check if back used for additional information: ( )

Section I

VisitID:		FacilityID:		Location:			
Sample Type:	Wet ( ) Dry ( )	CatchmentID:					
Date:		Weather:		Town:			
Time Arrive:		Time Depart:		Collectors:		Facility Type:	

Section II

Station	**SampleID** (Bottle Label)	Flow Type	Flow Area	Sensory	Turbidity (Y/N)	pH	Temp. (C)	Cond. (specify)	Sal. (ppt)	NH_3 (ppm)	Cl (specify)	NO_3 (ppm)	Surf. (ppm)
A													
B													
C													
D													
E													
SUMP													

Flow Type: **P** = Free Flowing Pipe
F = Surface Flow or Weir
W = Water Body / Wetland / Stream
S = Sump or Submerged Pipe

Flow Area: From Pipe = diameter x depth in pipe (ie. 12"D x 2")
Surface or Weir = width x depth (ie. 20" x 0.25")
Measurements rounded to 1/4 in

Sensory: **C**=Color, **O**=Odor, **W**=Waste Products, **T**=Trash

Units: Conductivity: **u** for µS/cm or **m** for mS/cm
Chlorine: **b** for ppb or **m** for ppm (mg/l)

Section III

Location Sketch (plan view):	Station Sketch:
	Comments:

Lab: Use three dilutions for bacteria

Six metals: Al, As, Cd, Cr, Cu, Pb

Laboratory Work	Fecal	EColi	Entero	TN	TP	TSS	Turbidity	VOCs	DO	BOD	Metals
Check all that Apply:											
Sampling Container:	100ml sterile	100ml sterile	100ml sterile	500ml plastic	250ml plastic	1000ml plastic	125ml plastic	100ml glass w/R	300ml glass	500ml plastic	500ml plastic
Custody 1:				Custody 2:				Custody 3:			

Form Version: 15 February 2023

Reviewed ( ) Entered ( ) Verified ( ) Scanned ( )

Appendix E. Data Collection Equipment and Equipment Calibration

- **Calibration of Pocket Pro™+ Multi 2** (*for Conductivity, Salinity, pH, and Temperature measurements)*

  Calibrate as needed for Conductivity *and* pH.
  Safety precautions: The calibration solution can cause skin and eye irritation and should be well flushed if exposed. Wear gloves and eye protection.
  Disposal: Calibration solution can be disposed into a sink with running water to a sewer system.
  Calibration: Conductivity mode using potassium chloride (KCl) solution 1,413 µS/cm; pH using a pH 4.01 buffer solution.
  Steps for Conductivity:
  1. Set the power to on.
  2. Remove the cap from the sensor.
  3. Push and hold → until Conductivity shows.
  4. Push to go to calibration mode, the auto-recognition standard (KCL-1413) to measure shows on the bottom line. *Note: If "C1" shows on the bottom line, do not continue. Set the meter to auto calibration mode. Refer to Users Guide*
  5. Rinse the sensor and cap with de-ionized water and blot dry.
  6. Pour potassium chloride (KCl solution 1,413 µS/cm) into the cap to the fill line.
  7. Put the sensor fully into the cap.
  8. When the measurement is stable, push to save the calibration and go to continuous measurement mode. The measured value will flash 3 times and then stop.
  9. Push and hold to go to continuous measurement mode. "END" shows on the display.
  10. Once calibrated, rinse the electrode area of the meter and the sample cup three times with de-ionized water, blot dry, Leave meter on.

  Steps for pH

  11. Push and hold → until pH shows.
  12. Push to go to calibration mode, the auto-recognition standard (pH buffer solution - 7.0) to measure shows on the bottom line. *Note: If "C1" shows on the bottom line, do not continue. Set the meter to auto calibration mode. Refer to Users Guide*
  13. Follow steps 5-10 above but substituting the pH buffer solution 7.0 for the KCl
  14. Once all calibrations are completed, replace cap and return to monitoring storage bin.

- **Use of Pocket Pro™+ Multi 2 Use** (*for Conductivity, Salinity, pH, and Temperature measurements)*

This multi-range sensor for measuring Conductivity, Salinity, Total Dissolved Solids (not monitoring) and Temperature. Do not touch the inner surfaces of the conductivity electrodes. Touching the surface of the electrodes may damage and reduce the life of the probe. Store the electrode dry in the storage cap. **Note: this meter always measures total dissolved solids, which is not currently being monitored.**

1) Shake the tester from side to side to remove air bubbles as bubbles under the probe tip when submerged can cause slow stabilization or cause an error in measurement.
2) Set the power to on.
3) Remove the cap from the sensor.
4) If the lock icon shows on the display, push to go to continuous measurement mode.
5) Push and hold → to select the parameter to measure (i.e., Conductivity).
6) Rinse the sensor and cap with deionized water and blot dry.
7) Pour the water sample into the cap to the fill line.
8) Put the sensor fully into the cap. The measured value shows on the top line.
9) To keep the measured value on the display when the sensor is removed from the sample, push .

   ***Note:** The lock icon shows on the display when the measurement is stable.*
10) To measure another sample, do steps 4–9.

11) When completed, rinse the electrode area of the meter and the sample cup three times with de-ionized water and blot dry before next use, put the cap on the meter and turn off.

- **Hach Ammonia and Chlorine Test Strips** *Store container of testing strips in a cool dry place with no exposure to moisture.*
  1) Triple rinse the ammonia vial and cap with sample water. Fill sample vial to top line with water.
  2) Remove a test strip out of the test strip container and replace the cap tightly.
  3) Place test strip in vial and vigorously move up and down for 30 seconds. Make sure all pads are always submerged.
  4) Remove test strip, shake of excessive water.
  5) Hold test strip level, with pad side up, for 30 seconds.
  6) To read result, turn test strip over so both pads are facing away from you.
  7) Compare the color of the small pad to the color chart on bottle. Read result through the clear plastic of the test strip.
  8) Record results on Sampling Sheet
  9) Rinse vial and cap with de-ionized water.
  10) Dispose of Hach strip in trash bag.

Appendix F. Buzzards Bay Impaired Waters Categories 4A and 5

Category 4a waters listed alphabetically by major watershed "TMDL is completed"

Waterbody	AU_ID	Description	Size	Units	Impairment	ATTAINS Action ID
Unnamed Tributary	MA73-31	Headwaters, outlet of Massapoag Lake, Sharon to mouth at inlet of Hammer Shop Pond, Sharon (not depicted on 1987 Mansfield USGS quad).	0.30	Miles	Fecal Coliform	2592
Willet Pond	MA73062	Walpole/Westwood/Norwood (at northern end, includes former 2008 segment: Unnamed Tributary MA73-13).	205.00	Acres	Mercury in Fish Tissue	33880
Boston Harbor: Weymouth & Weir						
Mill River	MA74-04	Headwaters, west of Route 18 and south of Randolph Street, Weymouth to inlet Whitmans Pond, Weymouth (portions culverted underground).	3.40	Miles	(Fish Passage Barrier*)	
					Escherichia Coli (E. Coli)	R1_MA_2019_01
					Fecal Coliform	R1_MA_2019_01
Old Swamp River	MA74-03	Headwaters just west of Pleasant Street and north of Liberty Street, Rockland to inlet Whitmans Pond, Weymouth.	4.60	Miles	(Fish Passage Barrier*)	
					Escherichia Coli (E. Coli)	R1_MA_2019_01
					Fecal Coliform	R1_MA_2019_01
Buzzards Bay						
Back River	MA95-47	Estuarine portion, west of County Road, Bourne to confluence with Phinneys Harbor (excluding Eel Pond), Bourne.	0.09	Square Miles	Fecal Coliform	36172
Bread and Cheese Brook	MA95-58	Headwaters north of Old Bedford Road, Westport to confluence with East Branch Westport River, Westport.	4.90	Miles	Enterococcus	36170
					Fecal Coliform	36170
Broad Marsh River	MA95-49	Headwaters in salt marsh south of Marion Road and Bourne Terrace, Wareham to confluence with the Wareham River, Wareham.	0.17	Square Miles	Fecal Coliform	36172
Buttonwood Brook	MA95-13	Headwaters, Oakdale Street, New Bedford to mouth at Apponagansett Bay, Dartmouth (excluding the approximately 0.2 miles through Buttonwood Park Pond segment MA95020).	3.60	Miles	Enterococcus	36170
					Escherichia Coli (E. Coli)	36170
					Fecal Coliform	36170
Cape Cod Canal	MA95-14	Waterway between Buzzards Bay and Cape Cod Bay, Bourne/Sandwich.	1.17	Square Miles	Fecal Coliform	36171
Cedar Island Creek	MA95-52	Estuarine portion southwest of the intersection of Parker Drive and Camardo Drive, Wareham to the mouth at Marks Cove, Wareham.	0.01	Square Miles	Fecal Coliform	36172
Crooked River	MA95-51	Estuarine portion east of Indian Neck Road, Wareham to the confluence with the Wareham River, Wareham.	0.04	Square Miles	Enterococcus	36172
					Fecal Coliform	36172
East Branch Westport River	MA95-40	Headwaters, outlet Noquochoke Lake, Dartmouth to Old County Road bridge, Westport (mileage includes length of braid).	2.40	Miles	Enterococcus	36170
					Fecal Coliform	36170
Eel Pond	MA95-48	Salt water pond that discharges to the Back River, Bourne.	0.03	Square Miles	Fecal Coliform	36172
Great Sippewisset Creek	MA95-23	From the outlet of Beach Pond in Great Sippewisset Marsh, Falmouth to the mouth at Buzzards Bay, Falmouth (including Quahog Pond and the unnamed tributary from the outlet of Fresh Pond).	0.03	Square Miles	Fecal Coliform	36172
Harbor Head	MA95-46	The semi-enclosed body of water south of the confluence with West Falmouth Harbor, south of Chappaquoit Road, Falmouth.	0.02	Square Miles	Estuarine Bioassessments	34284
					Fecal Coliform	36172

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CN 505.1

*TMDL not required (Non-pollutant)

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Category 4a waters listed alphabetically by major watershed "TMDL is completed"

Waterbody	AU_ID	Description	Size	Units	Impairment	ATTAINS Action ID
Hiller Cove	MA95-10	The water landward of a line drawn between Joes Point, Mattapoisett and the second boat dock northeast of Hiller Cove Lane, Mattapoisett.	0.04	Square Miles	Fecal Coliform	36172
Little Bay	MA95-64	From the confluence with the Nasketucket River, Fairhaven south to the confluence with Nasketucket Bay at a line from the southernmost tip of Mirey Neck, Fairhaven (~latitude 41.625702, ~longitide 70.854045) to a point of land near Shore Drive (~latitude 41.621994, ~longitude 70.855415), Fairhaven.	0.33	Square Miles	Fecal Coliform	36172
Little Sippewissett Marsh	MA95-24	From headwaters north of Sippewissett Road and east of Maker Lane, Falmouth to the mouth at Buzzards Bay southwest of end of Saconesset Road, Falmouth.	0.02	Square Miles	Fecal Coliform	36172
Long Pond	MA95097	Rochester.	32.00	Acres	Mercury in Fish Tissue	33880
Mattapoisett River	MA95-60	From the Mattapoisett River Dam (#MA02447) at Fairhaven Road (Route 6), Mattapoisett to the mouth at Mattapoisett Harbor, Mattapoisett.	0.04	Square Miles	Fecal Coliform	36172
Nasketucket Bay	MA95-65	From the confluence with Little Bay, Fairhaven to Buzzards Bay along Causeway Road, Fairhaven (on the south) and along a line from the southern tip of Brant Island, Mattapoisett to the eastern tip of West Island, Fairhaven.	3.69	Square Miles	Fecal Coliform	36172
Oyster Pond	MA95927	west of Route 28A, Falmouth.	0.01	Square Miles	Dissolved Oxygen	34331
					Estuarine Bioassessments	34331
Phinneys Harbor	MA95-15	From the confluence with the Back River, to the mouth at Buzzards Bay (demarcated by a line from the southeastern point of Mashnee Island to the northwestern point of Tobys Island), Bourne (includes the "north facing embayment of Tobys Island").	0.72	Square Miles	Estuarine Bioassessments	35069
					Fecal Coliform	36172
					Nitrogen, Total	35069
Pocasset River	MA95-16	From the outlet of Mill Pond, Bourne to the mouth at Buzzards Bay, Bourne.	0.05	Square Miles	Fecal Coliform	36172
Sippican River	MA95-07	County Road, Marion/Wareham to confluence with Weweantic River, Marion/Wareham.	0.08	Square Miles	Fecal Coliform	36172
Snell Creek	MA95-44	Headwaters west of Main Street, Westport to Drift Road, Westport.	1.50	Miles	Enterococcus	36170
					Escherichia Coli (E. Coli)	36170
					Fecal Coliform	36170
Snell Creek	MA95-45	Drift Road, Westport to 'Marcus' Bridge', Westport (prior to 2004 this segment included estuarine portion).	0.40	Miles	Enterococcus	36170
					Escherichia Coli (E. Coli)	36170
					Fecal Coliform	36170
Snell Creek	MA95-59	'Marcus' Bridge', Westport to confluence with East Branch Westport River, Westport (formerly part of 2002 segment: Snell Creek MA95-45).	0.01	Square Miles	Fecal Coliform	36172
Snipatuit Pond	MA95137	Rochester.	711.00	Acres	Mercury in Fish Tissue	33880
Turner Pond	MA95151	New Bedford/Dartmouth.	86.00	Acres	Mercury in Fish Tissue	33880

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"TMDL not required (Non-pollutant)

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Category 4a waters listed alphabetically by major watershed "TMDL is completed"

Waterbody	AU_ID	Description	Size	Units	Impairment	ATTAINS Action ID
Wankinco River	MA95-50	From outlet of Parker Mills Pond, south of Elm Street, Wareham to the confluence with the Agawam River (at a line between a point south of Mayflower Ridge Drive and a point north of the railroad tracks near Sandwich Road (forming headwaters of the Wareham River)) just north of Route 6 bridge, Wareham.	0.05	Square Miles	Fecal Coliform	36172
West Falmouth Harbor	MA95-22	From the confluence with Harbor Head at Chappaquoit Road, Falmouth to the mouth at Buzzards Bay at a line connecting the ends of the seawalls from Little Island and Chappaquoit Point, Falmouth (including Inner West Falmouth Harbor, Outer West Falmouth Harbor, Snug Harbor, and Mashapaquit Creek).	0.29	Square Miles	Estuarine Bioassessments	34328
					Estuarine Bioassessments	34332
					Fecal Coliform	36172
					Nitrogen, Total	34328
					Nitrogen, Total	34332
					Nitrogen, Total	34917
					Nitrogen, Total	34918
Westport River	MA95-54	From the confluences of the East Branch Westport River and the West Branch Westport River to Rhode Island Sound (at a line from the southwestern tip of Horseneck Point to the easternmost point near Westport Light), Westport (includes Westport Harbor and Hulda Cove).	0.74	Square Miles	Fecal Coliform	36172
Cape Cod						
Baker Pond	MA96008	Orleans/Brewster.	26.00	Acres	Mercury in Fish Tissue	33880
Barnstable Harbor	MA96-01	From the mouths of Scorton and Spring creeks, Barnstable east to an imaginary line drawn from Beach Point to the western edge of the Mill Creek estuary, Barnstable.	3.20	Square Miles	Fecal Coliform	36771
Bass River	MA96-118	"Grand Cove" portion of Bass River, north of Main Street (Route 28), Yarmouth.	0.12	Square Miles	Nitrogen, Total	68003
					Nutrient/Eutrophication Biological Indicators	68003
Bass River	MA96-12	Headwaters outlet Kelleys Bay, Route 6, Dennis/Yarmouth to mouth at inlet Nantucket Sound, Yarmouth (excluding Grand Cove, Dennis).	0.69	Square Miles	Estuarine Bioassessments	68003
					Fecal Coliform	36771
					Nitrogen, Total	68003
Bearse Pond	MA96012	Barnstable.	64.00	Acres	(Fanwort*)	
					Mercury in Fish Tissue	42393
Bournes Pond	MA96-57	west of Central Avenue, Falmouth outlet to Vineyard Sound, including Israels Cove, Falmouth.	0.24	Square Miles	Estuarine Bioassessments	32535
					Estuarine Bioassessments	32638
					Fecal Coliform	36772
					Nitrogen, Total	32535
					Nitrogen, Total	32638
Bucks Creek	MA96-44	Outlet Harding Beach Pond (locally known as Sulfur Springs), Chatham to mouth at inlet Cockle Cove, Nantucket Sound, Chatham.	0.02	Square Miles	Enterococcus	36772
					Fecal Coliform	36772
					Nitrogen, Total	36230
Bumps River	MA96-02	From pond outlet, Bumps River Road, Barnstable through Scudder Bay to mouth at Main Street/South Main Street bridge (confluence with Centerville River), Barnstable.	0.07	Square Miles	Fecal Coliform	36771

*TMDL not required (Non-pollutant)

Category 5 waters listed alphabetically by major watershed
The 303(d) List – "Waters requiring a TMDL"

Waterbody	AU ID	Description	Size	Units	Impairment	ATTAINS Action ID
Weymouth Back River	MA74-13	From the base of the fish ladder north of Commercial Street, Weymouth to mouth between Lower Neck, Weymouth (to the west) and Wompatuck Road, Hingham (area associated with Weymouth Back River ACEC designated as ORW).	0.85	Square Miles	Cause Unknown [Contaminants in Fish and/or Shellfish]	
					Fecal Coliform	R1_MA_2019_01
					PCBs in Fish Tissue	
Weymouth Fore River	MA74-14	Commercial Street, Braintree to mouth (eastern point at Lower Neck, Weymouth and western point at Wall Street on Houghs Neck, Quincy).	2.29	Square Miles	Cause Unknown [Contaminants in Fish and/or Shellfish]	
					Enterococcus	R1_MA_2019_01
					Fecal Coliform	R1_MA_2019_01
					PCBs in Fish Tissue	
Whitmans Pond	MA74025	Weymouth.	176.00	Acres	(Curly-leaf Pondweed*)	
					(Fanwort*)	
					DDT in Fish Tissue	
Buzzards Bay						
"Inner" Sippican Harbor	MA95-70	The waters landward of a line from Allen Point, Marion around the southeastern tip of Ram Island, then westerly from the southern tip of Ram Island to the point of land south of Nyes Wharf, Marion excluding Hammett Cove (formerly reported as a portion of segment MA95-08).	0.57	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
Acushnet River	MA95-31	Headwaters, outlet New Bedford Reservoir, Acushnet to Hamlin Street culvert, Acushnet.	2.90	Miles	Dissolved Oxygen	
					Enterococcus	36170
					Escherichia Coli (E. Coli)	36170
					Fecal Coliform	36170
					Nutrients	
Acushnet River	MA95-32	Hamlin Street culvert, Acushnet to culvert at Main Street, Acushnet.	1.10	Miles	Benthic Macroinvertebrates	
					Dissolved Oxygen	
					Enterococcus	36170
					Escherichia Coli (E. Coli)	36170
					Fecal Coliform	36170
					Nutrients	
Acushnet River	MA95-33	Outlet Main Street culvert, Acushnet to Coggeshall Street/Howland Road bridge, New Bedford/Fairhaven.	0.31	Square Miles	(Debris*)	
					Color	
					Dissolved Oxygen	
					Enterococcus	36171
					Fecal Coliform	36171
					Metals	
					Nitrogen, Total	

*TMDL not required (Non-pollutant)
[ ] provided as further explanation of ATTAINS impairment code

Category 5 waters listed alphabetically by major watershed
The 303(d) List – "Waters requiring a TMDL"

Waterbody	AU ID	Description	Size	Units	Impairment	ATTAINS Action ID
					Nutrient/Eutrophication Biological Indicators	
					Odor	
					Oil and Grease	
					Polychlorinated Biphenyls (PCBs)	
					Trash	
Agawam River	MA95-29	Wareham WWTP outfall, Wareham to confluence with Wankinco River (forming headwaters of the Wareham River) just north of the Route 6 bridge, Wareham.	0.16	Square Miles	Algae	
					Fecal Coliform	36171
					Nitrogen, Total	
Angeline Brook	MA95-83	Perennial portion south of Charlotte White Road, Westport to mouth at West Branch Westport River (Angeline Cove), Westport.	4.40	Miles	Enterococcus	
Apponagansett Bay	MA95-39	From the mouth of Buttonwood Brook, Dartmouth to a line drawn from Ricketsons Point, Dartmouth to Samoset Street near North Avenue, Dartmouth.	1.06	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
					PCBs in Fish Tissue	
Aucoot Cove	MA95-71	From the confluence with Aucoot Creek, Marion to the boundary of Division of Marine Fisheries designated shellfishing growing area BB31.1, north and southwest from Haskell Island, Marion (formerly part of 2006 segment: Aucoot Cove MA95-09).	0.03	Square Miles	Dissolved Oxygen	
					Fecal Coliform	36172
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
Aucoot Creek	MA95-72	Estuarine portion east of Holly Pond Road, Marion to confluence with Aucoot Cove, Marion (formerly part of 2006 segment: Aucoot Cove MA95-09).	0.02	Square Miles	Dissolved Oxygen	
					Fecal Coliform	36172
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
Beaverdam Creek	MA95-53	Estuarine portion just south of the outlet from cranberry bog southeast of Route 6, Wareham to confluence with Wewantic River, Wareham.	0.04	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nitrogen, Total	
Butler Cove	MA95-77	Just south of Buttermilk Bay, Wareham.	0.05	Square Miles	Estuarine Bioassessments	
Buttermilk Bay	MA95-01	Bourne/Wareham.	0.67	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nutrient/Eutrophication Biological Indicators	

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Waterbody	AU ID	Description	Size	Units	Impairment	ATTAINS Action ID
Buzzards Bay	MA95-62	Open water area encompassed within a line drawn from Wilber Point, Fairhaven to Clarks Point, New Bedford to Ricketson Point, Dartmouth to vacinity of Samoset Street, Dartmouth down to Round Hill Point, Dartmouth and back to Wilber Point, Fairhaven.	8.07	Square Miles	Fecal Coliform	36172
					PCBs in Fish Tissue	
Clarks Cove	MA95-38	The semi-enclosed waterbody landward of a line drawn between Clarks Point, New Bedford and Ricketsons Point, Dartmouth.	1.90	Square Miles	Enterococcus	36172
					Fecal Coliform	36172
					PCBs in Fish Tissue	
Copicut Reservoir	MA95175	Dartmouth/Fall River.	596.00	Acres	Mercury in Fish Tissue	
Copicut River	MA95-43	Headwaters, outlet of Copicut Reservoir, Fall River to mouth at inlet of Cornell Pond, Dartmouth.	1.30	Miles	Mercury in Fish Tissue	
					PCBs in Fish Tissue	
Cornell Pond	MA95031	Dartmouth.	12.00	Acres	Mercury in Fish Tissue	33880
					PCBs in Fish Tissue	
Crane Brook Bog Pond	MA95033	Carver.	37.00	Acres	(Non-Native Aquatic Plants*)	
					Algae	
					Phosphorus, Total	
Dunham Pond	MA95044	Carver.	43.00	Acres	Chlorophyll-a	
					Transparency / Clarity	
East Branch Westport River	MA95-41	Old County Road bridge, Westport to the mouth at Westport Harbor/Westport River, Westport (excluding Horseneck Channel).	2.65	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36171
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
Eel Pond	MA95-61	Coastal pond at the head of Mattapoisett Harbor, Mattapoisett.	0.04	Square Miles	Fecal Coliform	36172
					Nutrient/Eutrophication Biological Indicators	
Fiddlers Cove	MA95-79	cove south off Megansett Harbor, Falmouth.	0.01	Square Miles	Dissolved Oxygen	
					Estuarine Bioassessments	
					Fecal Coliform	
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
Halfway Pond	MA95178	Plymouth (formerly reported as 1996 segment: Halfway Pond MA94057).	215.00	Acres	Harmful Algal Blooms	
Hammett Cove	MA95-56	Borders Sippican Harbor (along a line from the southwestern most point of Little Neck to the end of the seawall on the opposite point), Marion.	0.07	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nitrogen, Total	

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Waterbody	AU ID	Description	Size	Units	Impairment	ATTAINS Action ID
Herring Brook	MA95-21	Estuarine portion northeast of Dale Drive and west of Route 28A, Falmouth to the mouth at Buzzards Bay, Falmouth.	0.01	Square Miles	Chlorophyll-a	
					Fecal Coliform	36172
					Nitrogen, Total	
Kirby Brook	MA95-82	Headwaters just south of Old County Road, Westport to the mouth at East Branch Westport River, Westport.	2.00	Miles	Enterococcus	
Leonards Pond	MA95080	Rochester.	49.00	Acres	(Aquatic Plants (Macrophytes)*)	
					(Non-Native Aquatic Plants*)	
					Chlorophyll-a	
					Transparency / Clarity	
Little Buttermilk Bay	MA95-76	off of Buttermilk Bay, Bourne.	0.16	Square Miles	Estuarine Bioassessments	
					Nutrient/Eutrophication Biological Indicators	
Mattapoisett Harbor	MA95-35	From the mouth of the Mattapoisett River, Mattapoissett to a line drawn from Ned Point to a point of land between Bayview Avenue and Grandview Avenue, Mattapoisett.	1.12	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nutrient/Eutrophication Biological Indicators	
Mattapoisett River	MA95-36	Headwaters, outlet Snipatuit Pond, Rochester to Mattapoisett River Dam (#MA02447) at Fairhaven Road (Route 6), Mattapoisett.	10.40	Miles	Enterococcus	
					Escherichia Coli (E. Coli)	
Megansett Harbor	MA95-19	From the outlet of Squeteague Harbor, Falmouth to Buzzards Bay at a line from the western tip of Scraggy Neck, Bourne south to the tip of Nyes Neck, Falmouth.	1.44	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	
					Nutrient/Eutrophication Biological Indicators	
Nasketucket River	MA95-67	Estuarine portion, from the boundary of the salt water wetland south of Route 6, Fairhaven to the mouth at Little Bay, Fairhaven (includes connector to Little Bay on the east side of the river).	0.03	Square Miles	Nitrogen, Total	
New Bedford Inner Harbor	MA95-42	Coggeshall Street/Howland Road bridge, New Bedford/Fairhaven to hurricane barrier, Fairhaven/New Bedford.	1.25	Square Miles	(Debris*)	
					Dissolved Oxygen	
					Enterococcus	36171
					Fecal Coliform	36171
					Metals	
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
					Odor	
					Oil and Grease	
					PCBs in Fish Tissue	
					Polychlorinated Biphenyls (PCBs)	
					Trash	

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Waterbody	AU ID	Description	Size	Units	Impairment	ATTAINS Action ID
New Bedford Reservoir	MA95110	Acushnet.	210.00	Acres	(Aquatic Plants (Macrophytes)*)	
					(Non-Native Aquatic Plants*)	
					DDT in Fish Tissue	
					Dissolved Oxygen	
					Mercury in Fish Tissue	
					Nutrient/Eutrophication Biological Indicators	
					Phosphorus, Total	
Noquochoke Lake	MA95113	(Main Basin) Dartmouth.	88.00	Acres	(Non-Native Aquatic Plants*)	
					Aquatic Plants (Macrophytes)	
					Enterococcus	
					Mercury in Fish Tissue	33880
					PCBs in Fish Tissue	
					Turbidity	
Noquochoke Lake	MA95170	(South Basin) Dartmouth.	13.00	Acres	(Non-Native Aquatic Plants*)	
					Aquatic Plants (Macrophytes)	
					Mercury in Fish Tissue	33880
					PCBs in Fish Tissue	
					Turbidity	
Noquochoke Lake	MA95171	(North Basin) Dartmouth.	17.00	Acres	(Non-Native Aquatic Plants*)	
					Aquatic Plants (Macrophytes)	
					Mercury in Fish Tissue	33880
					PCBs in Fish Tissue	
					Turbidity	
Onset Bay	MA95-02	Wareham.	0.78	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
Outer New Bedford Harbor	MA95-63	From the hurricane barrier, Fairhaven/New Bedford to a line drawn from Wilbur Point, Fairhaven to Clarks Point, New Bedford (formerly part of 2000 segment: Outer New Bedford Harbor MA95-27).	5.78	Square Miles	Dissolved Oxygen	
					Enterococcus	36172
					Fecal Coliform	36172
					Metals	
					Nitrogen, Total	
					Other Organics	
					PCBs in Fish Tissue	
Parker Mills Pond	MA95115	Wareham.	73.00	Acres	(Non-Native Aquatic Plants*)	
					Phosphorus, Total	
Paskamanset River	MA95-11	Headwaters, outlet Turners Pond, Dartmouth/New Bedford to confluence with Slocums River (Rock O'Dundee Road), Dartmouth.	10.50	Miles	Combined Biota/Habitat Bioassessments	
					Enterococcus	
					Escherichia Coli (E. Coli)	

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Waterbody	AU ID	Description	Size	Units	Impairment	ATTAINS Action ID
Pocasset Harbor	MA95-17	From the confluence with Red Brook Harbor near the northern portion of Bassetts Island and Patuisset, Bourne to the mouth at Buzzards Bay between the western portion of Bassetts Island and Wings Neck, Bourne.	0.33	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
Queen Sewell Pond	MA95180	Bourne (formerly reported as 2000 segment: Queen Sewell Pond MA96253).	18.00	Acres	Harmful Algal Blooms	
Quissett Harbor	MA95-25	The semi-enclosed body of water landward of a line drawn between The Knob and Gansett Point, Falmouth.	0.17	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
Rands Harbor	MA95-78	harbor south off Megansett Harbor, Falmouth.	0.02	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
Red Brook Harbor	MA95-18	From the confluence with Pocasset Harbor between the northern portion of Bassetts Island and Patuisset, Bourne to the mouth at Buzzards Bay between the southern portion of Bassetts Island and Scraggy Neck, Bourne (including Hen Cove).	0.92	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nutrient/Eutrophication Biological Indicators	
Sampson Pond	MA95125	Carver.	295.00	Acres	(Non-Native Aquatic Plants*)	
					(Non-Native Fish/Shellfish/Zooplankton*)	
					DDT in Fish Tissue	
					Mercury in Fish Tissue	
Shingle Island River	MA95-12	Outlet of small unnamed pond northeast of Flag Swamp Road, Dartmouth to mouth at inlet Noquochoke Lake (north basin), Dartmouth.	5.00	Miles	Enterococcus	
Sippican River	MA95-06	Headwaters, outlet Leonards Pond, Rochester to County Road, Marion/Wareham.	3.00	Miles	Chlorophyll-a	
					Dissolved Oxygen	
					Enterococcus	
Slocums River	MA95-34	Rock O'Dundee Road (confluence with Paskamanset River), Dartmouth to mouth at Buzzards Bay, Dartmouth.	0.66	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
Squeteague Harbor	MA95-55	Waters landward of the confluence with Megansett Harbor, Bourne/Falmouth.	0.15	Square Miles	Nutrient/Eutrophication Biological Indicators	
Tihonet Pond	MA95146	Wareham.	87.00	Acres	Dissolved Oxygen	

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Waterbody	AU ID	Description	Size	Units	Impairment	ATTAINS Action ID
Wareham River	MA95-03	From confluence of Wankinko and Agawam Rivers at Route 6 bridge, Wareham to Buzzards Bay (at an imaginary line from Cromeset Point to curved point east/southeast of Long Beach Point), Wareham. Including Marks Cove, Wareham.	1.18	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nitrogen, Total	
West Branch Westport River	MA95-37	West of Quail Trail, Westport to mouth at Westport Harbor/Westport River, Westport.	1.29	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
Weweantic River	MA95-04	Headwaters confluence of Rocky Meadow and South Meadow brooks, Carver to the inlet of Horseshoe Pond, Wareham (through former 2014 segment: Tremont Mill Pond MA95150).	11.50	Miles	(Non-Native Aquatic Plants*)	
					Enterococcus	
Weweantic River	MA95-05	Outlet Horseshoe Pond, Wareham to mouth at Buzzards Bay, Marion/Wareham.	0.62	Square Miles	Enterococcus	36172
					Estuarine Bioassessments	
					Fecal Coliform	36172
					Nitrogen, Total	
Wild Harbor	MA95-20	Waters landward of an imaginary line from Crow Point to Nyes Neck (excluding Wild Harbor River), Falmouth.	0.13	Square Miles	Estuarine Bioassessments	
					Fecal Coliform	36172
					Nitrogen, Total	
					Nutrient/Eutrophication Biological Indicators	
Wild Harbor River	MA95-68	Headwaters, Falmouth to mouth at Wild Harbor, Falmouth.	0.03	Square Miles	Fecal Coliform	36172
					Nutrient/Eutrophication Biological Indicators	
Cape Cod						
Allens Harbor	MA96-95	south of Lower County Road, Harwich to Doanes Creek, Harwich.	0.02	Square Miles	Fecal Coliform	
					Nitrogen, Total	65883
					Nutrient/Eutrophication Biological Indicators	65883
Areys Pond	MA96-70	Orleans.	0.02	Square Miles	Nitrogen, Total	33786
					Nutrient/Eutrophication Biological Indicators	
Ashumet Pond	MA96004	Mashpee/Falmouth.	203.00	Acres	Abnormal Fish Deformities, Erosions, Lesions, Tumors (DELTS)	
					Dissolved Oxygen	
					Mercury in Fish Tissue	33880
					Phosphorus, Total	
Bassing Harbor	MA96-48	Excluding Crows Pond and Ryder Cove, Chatham.	0.13	Square Miles	Estuarine Bioassessments	

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