Massachusetts Alternative Septic System Test Center
Barnstable, Massachusetts

Quality Assurance Project Plan

Title: EPA Science to Achieve Results (STAR) STAR Grant: Human Virus and Viral Surrogates as Measures of Water Reuse Potential from Centralized and Decentralized Wastewater Treatment Grant #84025901

Effective Date: 2021-12-20	**Number:** MASSTC-QAP-002	**Revision:** 000

Authors

(See internal cover page)

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EPA Science to Achieve Results (STAR) STAR Grant: Human Virus and Viral Surrogates as Measures of Water Reuse Potential from Centralized and Decentralized Wastewater Treatment Grant #84025901	**Document ID#:** MASSTC-QAP-002 **Revision#:** 000 **Released Date:** 2021-12-20 **Released By:** Brian Baumgaertel

Revision History

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the MASSTC Director on the MASSTC Sharepoint Site.

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Quality Assurance Project Plan – Revision #2 November 2021

1.1 Title and Approval Page

EPA Science to Achieve Results (STAR) STAR Grant: Human Virus and Viral Surrogates as Measures of Water Reuse Potential from Centralized and Decentralized Wastewater Treatment Grant #84025901

A collaborative project with

Barnstable County Department of Health and Environment
Massachusetts Alternative Septic System Test Center

And

Massachusetts Department of Environmental Protection
William X. Wall Experiment Station

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	US EPA Signatory			

2.3 Project Organization

Project Manager is the responsible official for this project overseeing the overall project and budget, as well as tasking contractors and staff with work required to complete this project.

Principal Investigators are responsible for the technical aspects of their respective tasks. There are two Co-Principal Investigators. George Heufelder is responsible for the proper collection, conveyance, and storage of all biological samples and the collection and proper conveyance to the laboratories of concurrently collected analytes. Dr. Oscar Pancorbo is responsible for the method development for polymerase chain reaction (PCR) tests to be performed at the Division of Environmental Laboratory Sciences, Wall Experiment Station, Massachusetts Department of Environmental Protection.

Quality Assurance Manager (QAM) is responsible for reviewing and approving the Quality Assurance Project Plan (QAPP). The QAM may provide technical input on proposed sampling design, analytical methodologies, and data review. The QAM may assist with coordinating laboratory services. The QAM is also responsible for transferring and reviewing all data prior to its inclusion into the project datasets. This includes the acquisition of laboratory reports, inspection of data derived from laboratory notes, downloading and inspection of data from the field instrumentation and assisting in the correction of conditions that prevent quality data from being attained and used toward project objectives. The QAM is responsible for writing, reviewing, and making recommendations to the Project Manager in relation to changes in Standard Operating Procedures as they apply to the maintenance of quality data.

Facility Operator is responsible for the routine operation and management tasks necessary to ensure the proper function of the critical MASSTC infrastructure. The Operator also monitors and reports on the various treatment technologies being tested and maintains all written records that facilitate backward inspection of data relating to the site operation. These tasks include but are not limited to wastewater supply pumps, conveyance systems, sampling and maintenance equipment.

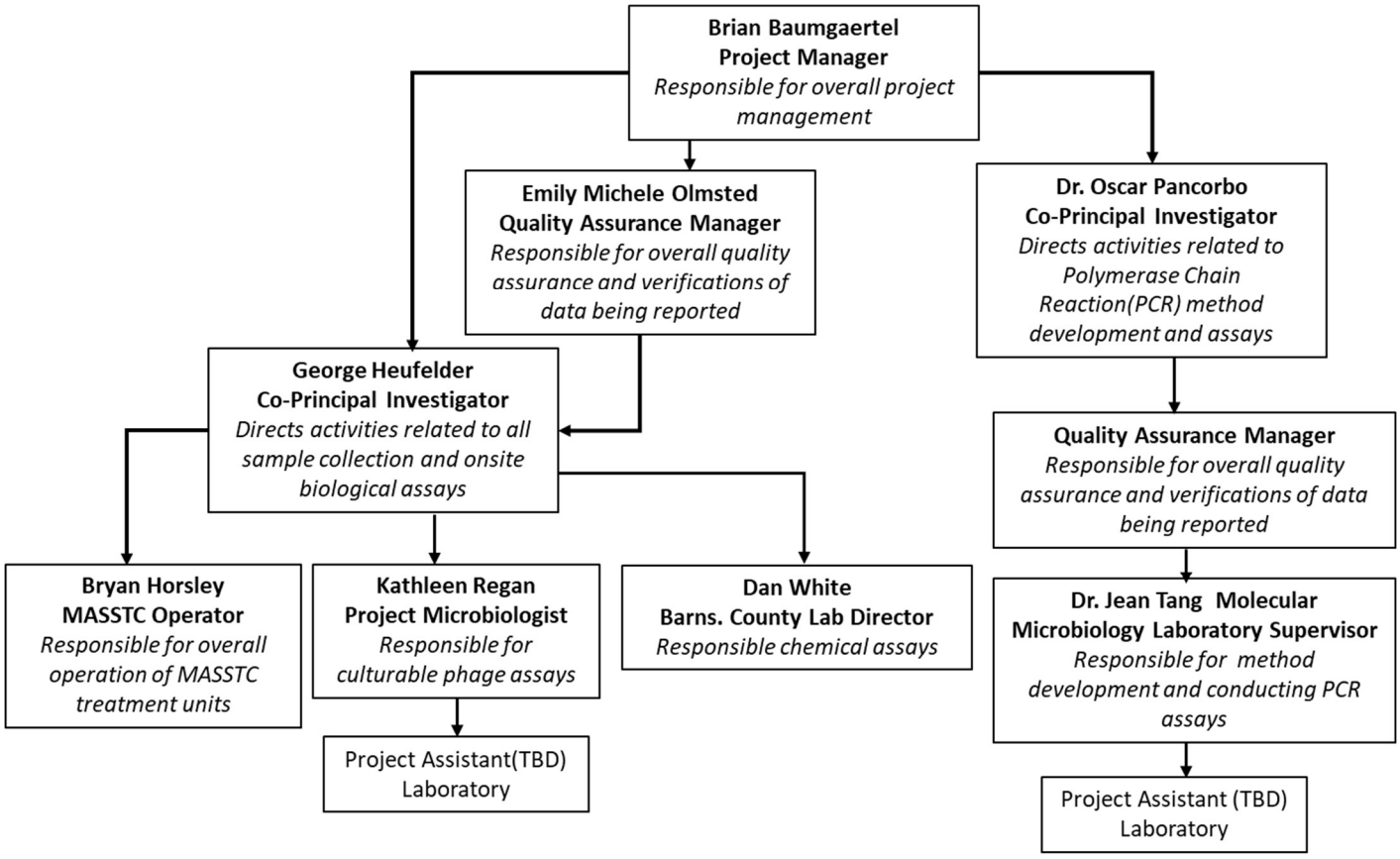
Project Microbiologist is responsible for the analysis of biological parameters that include bacteria and coliphage viruses. The microbiologist is responsible for the maintenance records on all equipment and supplies ancillary to these assays including but not limited to refrigerators, incubators, water baths, temperature control devices, autoclaves and ovens. In addition, this position is responsible for that traceability of all media and critical data relating to instrumentation use.

Barnstable County Laboratory Director is responsible for oversight of all analytical laboratory operations including review and integration of all data originating from the occasional subcontracting of assays through a non-county laboratory.

Project Assistants are responsible for providing support to the assays and ancillary tasks such as media/sample preparations and data compilation in their respective laboratories under the direction of the microbiologist and the molecular biologist respectively.

The organization schema of project participants for Barnstable County Department of Health and Environment (includes MASSTC) and Massachusetts Department of Environmental Protection (MassDEP-WES) is presented in Figures 1 and 2.

Figure 1 Organizational Structure EPA STAR Grant #84025901



A detailed organizational representation for the Division of Environmental Laboratory Sciences, Senator William X. Wall Experiment Station, Massachusetts Department of Environmental Protection (MassDEP-WES) is presented in Figure 2.

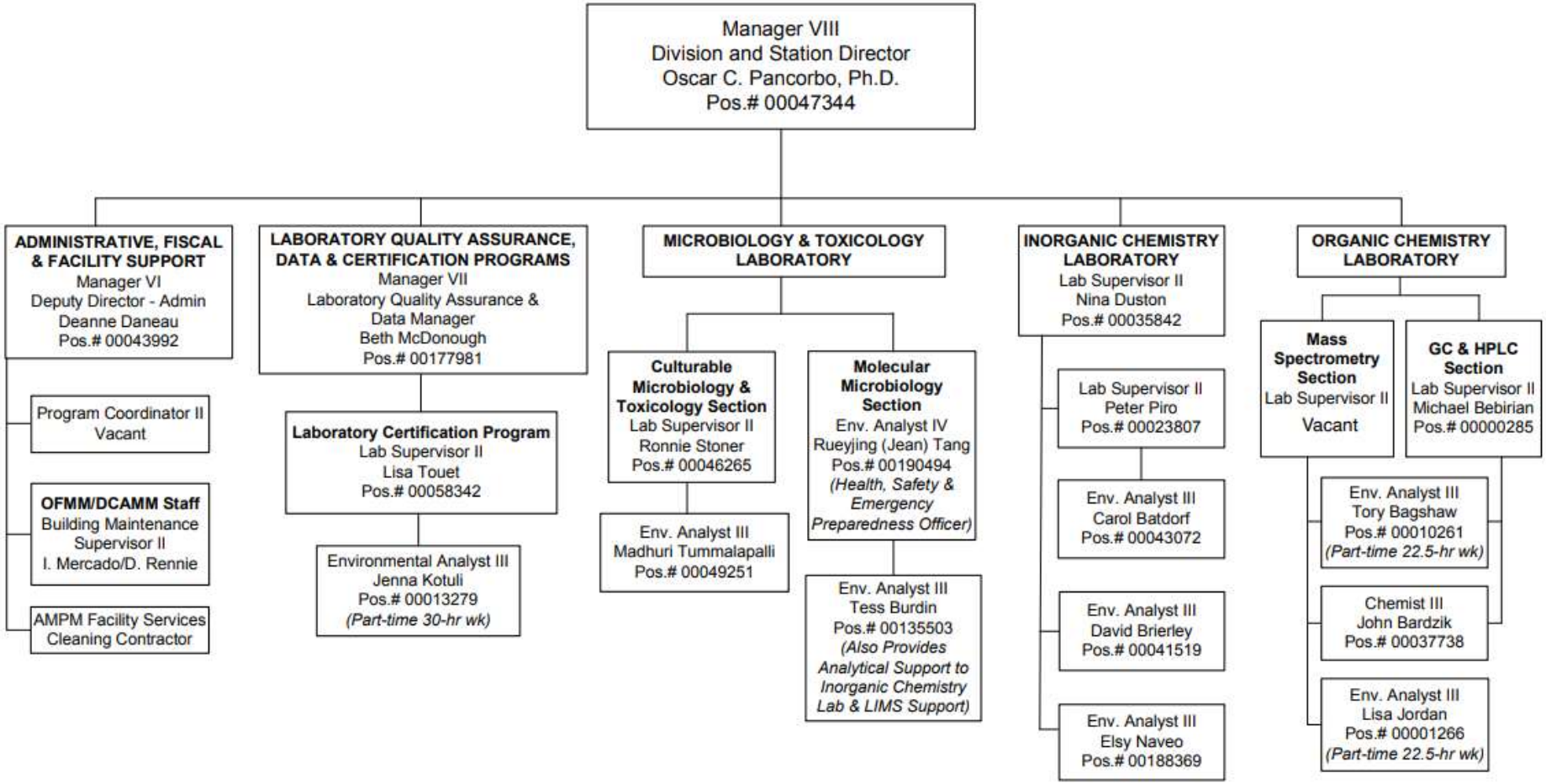


Figure 2 Organizational Structure MassDEP-WES as relating to EPA STAR Grant #84025901

2.4 Problem Definition/Background

Human pathogenic viruses in residential wastewater can pose significant public health risks in both unintended reuse scenarios, where wastewater discharges intercept drinking water sources and recreational and shellfish harvesting areas, as well as intended reuse situations. Problematic is the fact that culture assays for the suite of human enteric viruses have not been fully developed, which hinders both the ability to evaluate the public health risk of incidental exposure as well as the assessments for the proper level of treatment by various wastewater treatment processes. This project endeavors to validate the use of two culturable coliphage viruses (i.e., somatic and male-specific F+) and three other viral surrogates (i.e., pepper mild mottle virus - PMMoV, crAssphage, and Bacteroides HF183 human fecal genetic marker) assayed by droplet digital polymerase chain reaction (ddPCR) methods as possible predictors of human enteric viruses by conducting parallel measures of these five surrogates with the human enteric pathogenic viruses, adenoviruses and noroviruses GI and GII, also monitored by ddPCR. In addition, the project will assess the efficacy of selected decentralized and centralized treatment technologies for the removal of human enteric viruses using the techniques investigated and allow for a determination of the level of fitness for reuse. This information is vital to support efforts that seek to repurpose and reuse wastewater and additionally will serve researchers and regulators engaged in performing quantitative microbial risk assessments (QMRA).

2.5 Project/Task Description and Schedule

Six unit-wastewater treatment processes from decentralized wastewater treatments systems and three unit-processes from municipal wastewater treatment plants will be used to validate the surrogate indicators as predictors of human pathogenic viruses. The ultimate goal is to produce a predictive tool to determine the appropriate level of wastewater reuse based on the virus reductions across various treatment processes.

During the initial six months of the project, necessary staff additions will be recruited and hired and trained to assist in culturable coliphage assays (at MASSTC) and polymerase chain reaction (PCR) assays at MassDEP-WES to ensure completion of the project timelines. PCR method development, validation and standard operating procedures will be completed during this period. During the initial three months of this study, MASSTC will arrange for the collection of samples from decentralized technologies presently in place at MASSTC and the three existing municipal wastewater treatment plants. Selected additional decentralized technologies will be installed at MASSTC and readied for sampling and inclusion in the study during this period. The objective is to obtain data from all participating technologies for twenty months. In addition to human enteric virus and viral surrogate sampling, selected chemical parameters will be measured at all sampling locations coincident with biological analyses. These parameters were chosen for their particular influence on the persistence or attenuation of viruses. Data synthesis and interpretation will be ongoing, but final analyses of data and write-up of results will occur in the final three months of the project.

2.6 Quality Objectives and Criteria for Measurement Data

The general quality objectives of MASSTC for any study include the production of quality data for the assessment of wastewater treatment technologies for the range of contaminants present. This includes the use of approved methods of analysis, the integrity of the chain of custody for all collected samples, a continuous assessment of the quality of the data produced, the maintenance of accurate records, and the continuous vigilance to adherence of standard operating procedures (SOP) and principles of good test center and laboratory practice.

Parameter-specific data quality objectives are provided in Table 1. Should data not meet the required qualities of precision, accuracy, representativeness, completeness, and sensitivity as defined below, the data collected may be subject to qualification or censoring during the post monitoring quality control review.

2.6.1 Accuracy

Accuracy is the value of a measurement in relation to a true or expected value. The accuracy of a measurement is defined in the SOP for each method and is expressed as deviations from known prepared standards, matrix spikes and performance evaluation samples. In the case of chemical parameters, accuracy can be expressed as a percent or value (plus or minus) the concentration. In the case of coliphage measurements the accuracy is expressed as a percentage of recovery from spiked matrix samples or fortified laboratory blanks (Ongoing Precision and Recovery samples). Coliphage assays typically have wide acceptance criteria for recoveries (0 – 100% for male-specific phage and 59 – 406% for somatic phage), compared with the chemical analytes.

2.6.2 Precision

Precision is a measure of the degree of agreement among repeated measurements and is estimated by assays of replicate samples. Procedures for laboratory precision which assay the same sample are described in the respective SOPs, and generally less than 3% of the Relative Standard Deviation (RSD). Coliphage lab assays measuring the same sample vary more widely and typically have 16% – 31% precision as determined by the recoveries in spiked samples or fortified blanks. Overall precision is quantified using duplicate field samples and typically range form 10 – 25% relative percent difference (RPD). For this project, the MASSTC will maintain a continuous records for the computation of the RSD and subsequent evaluation of precision and accuracy. The methods and computations are described in the two methods used (EPA 1643 for unconcentrated raw wastewater samples and EPA 1642 for concentrated treated wastewater samples).

2.6.3 Representativeness

Representativeness is the extent to which the data collected characterize the true range of environmental conditions for which statements of cause and effect will be postulated. In the present study, samples taken across variations in temperature and maturity of wastewater treatment biota are considered essential to properly characterize the overall and expected treatment for viruses. These objectives are accommodated by the 20-month sampling duration. In addition, the range of meteorological conditions should be experienced for those treatment modalities influenced by such

(soils'-based treatment units exposed to the elements). The frequency of sampling is considered adequate to meet the goal of representativeness, however they are adjusted by the costs of sampling as well as the capacity of the laboratories to complete assays within the specified holding times.

2.6.4 Completeness

Completeness refers to the amount of valid data collected that meets the objectives of the study. Although 100% of all collections are anticipated, we set as our objective at least 80 - 100% of collection events realizing that conditions beyond control can occasionally arise and prevent sampling.

A primary objective of this study is to complement existing information regarding the ability of selected and diverse wastewater treatment processes to remove the tested human enteric viruses in both the onsite and municipal wastewater treatment setting. The results will help determine the appropriate level for wastewater reuse from these unit processes. Since it is presently not practical to culture the actual human enteric viruses, we endeavor to use culturable virus surrogates (coliphages) as well as the selected other three markers of human wastewater assayed by ddPCR and relate their occurrence/concentration to the occurrence/concentration of human adenoviruses, and noroviruses GI and GII as indicated by the genomic materials concurrently detected using ddPCR The genomic copies of these human enteric viruses detected by ddPCR are assumed to indicate the possible existence of pathogenic human viruses[1]. In brief, the removal of a culturable viral surrogate (coliphage) and/or the other three anthropogenic genetic viral surrogates (HF183, CrAssphage and PMMoV) through a treatment process, correlated with the removal of genomic material from actual human adenoviruses and noroviruses in that treatment process will serve as an indicator of the overall treatment efficacy and suggest the appropriate mode and level of reuse.

The most common metric used to evaluate treatment levels for viruses is the $\log_{10}$ reduction or LRV ($\log_{10}$ reduction value) where:

LRV = (Log_{10} virus in the wastewater before treatment) – (Log_{10} virus in the wastewater following treatment)

All analyses in this study will report using this metric for both comparability with published studies and the wastewater reuse standards adopted by various organizations (such as the World Health Organization[11]) and various regulatory bodies. Although there are no universally accepted action limits for virus removal, using the near-universal metric of LRV, various regulatory or advisory entities will enable comparison of these study results with respective guidelines and requirements. Expressing the data in LRV will enable comparisons with many of the published studies[2–5] and aid in the use of the data in quantitative microbial risk assessment(QMRA) models[3,6–10] employed by water reuse managers as well as regulators.

An important initial task in this study will be the enteric virus and viral surrogate concentration range-finding effort. Although the common presence of coliphage and HF-183 in wastewater sources for all the decentralized wastewater treatment systems has been confirmed from our previous work, we will

[1] PCR techniques detect genomic material that may or may not have originated from a viable infective virus.

initially need to confirm the presence and densities of crAssphage, PMMoV, and the human enteric viruses. This initial effort will direct further refinement of the effort.

As referenced above, this study involves collection of influent and treated effluent samples from various treatment units. In broad summary, the culturable phage male-specific (F+) coliphages and somatic coliphages will be assayed at the MASSTC laboratory, while concurrently collected and concentrated samples will be assayed for the other three viral surrogates and human adenoviruses and noroviruses GI and GII at the Senator William X. Wall Experiment Station, Massachusetts Department of Environmental Protection using droplet digital PCR. In addition to the value of this study as referenced above, we have chosen a number of chemical parameters to measure in the samples coincident with microbiological assays. These selected parameters of temperature, pH alkalinity, turbidity, nitrate-N, nitrite-N, total Kjeldahl nitrogen (TKN), total organic carbon (TOC), specific conductance, and dissolved oxygen all been shown to possibly impact the survival and entrainment of viruses within the treatment processes. Establishing any significant correlations of these parameters with the treatment process will further our understanding of virus transport in treatment process and may explain differences among the published studies. For a portion of this study, fecal indicator bacteria (FIB), E. coli, enterococci and fecal coliform will also be monitored at selected sites under separate funding. These are measured because they continue to be used un regulatory programs to indicate the status of drinking water sources as well as recreational waters.

Table 1 Parameters to be measured as part of EPA STAR Grant #84025901

Parameter	EPA Method	Standard Method	Units	Preservative	Collection	Holding Time	Laboratory	Precision	Minimum Detection Limit	Minimum Reportable Limit
Nitrate	EPA 300.0		mg/L	Ice	250-ml plastic	48 hours	BCDHE	0.033 %RSD	0.019 mg/L	0.10 mg/L
Nitrite	EPA 300.0		mg/L	Ice	250-ml plastic	48 hours	BCDHE	0.037 %RSD	0.005 mg/L	0.05 mg/L
Ammonia-Nitrogen	EPA 350.1		mg/L	ice (<6 C)H_2SO_4	250-ml plastic	28 days	BCDHE	0.60 %RSD	0.15 mg/L	0.25 mg/L
Total Kjeldahl Nitrogen	EPA 350.2		mg/L	ice (<6 C)H_2SO_4	250-ml plastic	28 days	BCDHE	1.9 %RSD	0.10 mg/L	0.25 mg/L
Alkalinity		2320-B	mg/L	ice (<6 C)	250-ml plastic	14 days	BCDHE	1.8 %RSD	0.95 mg/L	2.0 mg/L
Total Organic Carbon		5310 - B	mg/L	ice (<6 C)H_2SO_4	40 ml amber glass vial	28 days	BCDHE	2.0 %RSD	0.34 mg/L	1.0 mg/L
Dissolved Organic Carbon		5310 - B	mg/L	ice (<6 C)H_2SO_5	40 ml amber glass vial	28 days	BCDHE	2.0 %RSD	0.34 mg/L	1.0 mg/L
MS2-bateriophage	EPA 1643		PFU	ice (<4 C)	100 ml plastic	6 hours	MASSTC	35% RSD	1 PFU/L	1 PFU/L
MS2-bateriophage	EPA 1642		PFU	ice (<4 C)	4 liter plastic	6 hours	MASSTC	20% RSD	1 PFU/2L	1 PFU/2L
Somatic Phages	EPA 1643		PFU	ice (<4 C)	100 ml plastic	6 hours	MASSTC	30% RSD	1 PFU/L	1 PFU/L
Somatic Phages	EPA 1642		PFU		4 liter plastic	6 hours	MASSTC	18%RSD	1 PFU/2L	1 PFU/2L
Escherichia coli		9223B	MPN	ice (<4 C)	101 ml plastic	6 hours	MASSTC	TBD	1/100 ml	1/100 ml
Fecal coliform		9223B	MPN	ice (<4 C)	102 ml plastic	6 hours	MASSTC	TBD	1/100 ml	1/100 ml
Enterococcus		9230D	MPN	ice (<4 C)	103 ml plastic	6 hours	MASSTC	TBD	1/100 ml	1/100 ml
Human viruses, HF183, CrAssphage and/or PMMoV		TBD		-80°C	2 ml cryovials 15 ml conical tubes	28 days	MassDEP-WES			
Ozone		45000-O_3 B.	mg/L	run immediately	grab	N/A	MASSTC			
Dissolved Oxygen		4500-O H	mg/L	none	grab(ProDSS meter)	N/A	MASSTC			
pH		4500-H^+	Std. Units	none	grab(ProDSS meter)	N/A	MASSTC			
Turbidity	EPA 180.1		NTU	ice (<6 C)	250-ml plastic	48 hours	MASSTC			
Temperature		2550	C°	none	grab(ProDSS meter)	N/A	MASSTC			

2.7 Special Training Requirements/Certification

MASSTC maintains a comprehensive record of training for all personnel involved in projects. In general, all staff are trained in sample collection and the use of field instrumentation, and the tracking and recording of all critical operational and monitoring requirements. A verification of all training is maintained using an approved standard form (MASSTC-FRM-011 – Training Log, see Appendix 1) which is updated and changed, when necessary, under a document control system described below. All sampling procedures specific to this project have been added to the updated sampling SOP for MASSTC and are presently part of the Controlled Document System (MASSTC-SOP-001 – Document Control Procedure, see Appendix 2).

Prior to sampling, the responsible staff member reviews SOPs specific to each sampling location as well as sampling from common sites and use of instruments and equipment common to many sampling events (such as autosamplers, field instruments, sample handling). All forms and documentation of training as well as the continuing verification records are maintained under a Document Control system described below. MASSTC has been informally classified as a Grade 4 Treatment Facility under the Massachusetts Department of Environmental Protection, which requires that all individuals maintaining treatment systems like those being used in the present study, be Grade 4 or higher licensed operators. MASSTC maintains, at minimum, two Grade 4 Wastewater Treatment Plant Operators for the operation of the Massachusetts Alternative Septic System Test Center. At least two staff are also Registered Sanitarians or Registered Environmental Health Specialists and are required to maintain continuing education training on a yearly basis. Certified or licensed staff oversee all critical operational features of MASSTC and the sampling under the various protocols.

To enable coliphage assays at the MASSTC, one staff member was sent to the EPA ORD Laboratory in Cincinnati to receive hands-on training and instruction on the EPA Method 1642 and 1643 methodologies from individuals there who were responsible for developing these methods. Using Standard Operating Procedures from EPA ORD Staff and in addition using EPA methodology, staff at MASSTC have gained proficiency on the method as defined by the method's initial precision and recovery (IPR) and continue to verify proficiency through ongoing precision and recovery (OPR) procedures specified in the methods. MASSTC-SOP-039 – Virus Analysis (see Appendix 3) was internally developed by adapting the SOP from the EPA Cincinnati Laboratory and was formalized into the methodology for the MASSTC laboratory setting.

The Division of Environmental Laboratory Sciences (DELS), Senator William X. Wall Experiment Station (MassDEP-WES), Massachusetts Department of Environmental Protection (MassDEP), being the certifying agency for all drinking water and non-potable water laboratories in the Commonwealth, has a comprehensive training program for their analytical staff. This is presented in context with its Quality Assurance Plan, Revision #8, February 2021 (see Appendix 4) and referenced Forms 6 and 7 which are part of the MassDEP-DELS-WES document control system and available upon request.

2.8 Documents and Records

MASSTC employs a document management and control system compliant with ISO.IEC 17025 (2017) to ensure that appropriate staff have access to quality management documents where and when they need it but ensuring that no unauthorized or unrecorded changes are made to the contents of documents under control (See Appendix 2, MASSTC-SOP-001 – Document Control Procedure). Documents under this system include forms and datasheets used to collect critical data and information on testing. A list of documents under the controlled document system (as of November 2021) is provided in Appendix 5. The Director of MASSTC and the Quality Assurance Manager are the only persons allowed to approve and make changes to any document in this control system and all previous versions of any document are maintained indefinitely in the event of a need for future reference.

For procedures relating to the maintenance of records and archives see Appendix 6 – MASSTC-SOP-003 – Data and Records Management. Upon approval, this QAPP will be entered into the document control system as MASSTC-PLN-002 and all project participants will be supplied copies and be required to sign off as having read and be willing to adhere to it.

The MassDEP-DELS-WES document/records management, retention and security procedures are described in Section 18.0 of its Laboratory Quality Assurance Plan, Revision #8, February 2021 (see Appendix 4).

3 Data Generation and Acquisition

3.1 Sampling Design

The sampling design for this project is modulated somewhat by the expense and time-requirements for the assays and the capacity of the laboratories involved to conduct the necessary timely analyses. Fundamentally, the sampling design is purposed to compare influent and effluent levels of viruses and viral surrogates from various centralized and decentralized wastewater treatment systems. In addition to the treatment system proficiency evaluations, the sample design was formulated to validate the use of the various viral surrogates (coliphages and human/sewage markers) as efficient cost-effective ways to predict/evaluate the presence of the range of human viruses for which there are few direct culture techniques. A summary of the sampling numbers and frequencies are in the Table below. For decentralized wastewater technologies, selected chemical assays will be performed concurrent with microbiological analyses. Field parameters of temperature, pH, dissolved oxygen, and turbidity will be measured during every sampling event. What follows are detailed descriptions of sample locations at each of the technologies.

Table 2. Schedule summary for testing of parameter (microbiology and chemical) classes during the performance of EPA STAR Grant #84025901. Field parameters of temperature, pH and dissolved oxygen are taken during all scheduled sampling events.

	Microbiological Testing				Lab Chemistry Testing			
	Sampling Frequency per month	Number of Locations	Number of Months	Total Samples	Sampling Frequency per month	Number of Locations	Number of Months	Total Samples
Location								
Immediate influent levels	7	1	20	140	3.5	1	20	70
Leachfield 2' sand depth gravity dosed	1	3	20	60	0.5	1	20	10
Leachfield 3' sand depth gravity dosed	1	3	20	60	0.5	1	20	10
Leachfield 4' sand depth gravity dosed	1	3	20	60	0.5	1	20	10
Leachfield 5' sand depth gravity dosed	1	3	20	60	0.5	1	20	10
Leachfield 2' sand depth LPD	1	3	20	60	0.5	1	20	10
Leachfield 3' sand depth LPD	1	3	20	60	0.5	1	20	10
Leachfield 4' sand depth LPD	1	3	20	60	0.5	1	20	10
Drip Dispersal	1	3	20	60	0.5	3	20	30
Membrane Bioreactor	1	3	20	60	0.5	3	20	30
Ozone treatment following reactor	1	2	20	40	0.5	2	20	20
Ultraviolet following secondary treatmer	1	3	20	60	0.5	3	20	30
Cellulosic Treatment Systems	1	5	20	100	0.5	5	20	50
Municipal Treatment 1	0.5	2	20	20	0.5	2	20	20
Municipal Treatment 2	0.5	3	20	30	0.5	2	20	20
Municipal Treatment 3	0.5	3	20	30	0.5	2	20	20
Total Samples				960				360

All viral and viral surrogate samples, along with concurrently taken fecal indicator bacteria (FIB) and field and chemical samples are procured as grab samples due to the short holding times required prior to microbiological analyses. Generally, samples taken at decentralized systems located at MASSTC will begin the coliphage assays within one to two hours of collection, with intermediate storage at 4°C or lower temperature prior to assay. Samples taken at each of the three municipal treatment facilities in Massachusetts (Chatham, Barnstable, and Falmouth) will also be taken as grab samples and transported to the laboratory and processed within four to six hours of collection, again being held at 4°C or lower temperature until processed.

Two general matrix types will be assayed for the project targets: influent or raw wastewater and treated effluent. Raw wastewater is assayed for culturable coliphages without concentration using EPA Method 1643[11]. An aliquot of the raw wastewater sample will also be concentrated using centrifugal ultrafiltration using methods described by Qiu et al.[12], with the resulting concentrate immediately stored at -80°C, and later transported to MassDEP-WES on dry ice for genomic analyses. This method of raw wastewater concentration and the associated SOP will be developed during the first month of sampling.

The treated effluent from all systems will be concentrated by ultrafiltration as described in EPA Method 1642[13], and culturable coliphages will be analyzed in accordance with that method and associated SOP (Appendix 3 - MASSTC-SOP-039 – Virus Analysis). An aliquot of this concentrated sample will also be immediately stored at -80°C and later be transported to MassDEP-WES on dry ice for genomic analyses. Note that the ddPCR methods to be used for the quantitation of human adenoviruses, human norovirus

GI and GII, crAssphage, PMMov, and HF183 in all project samples will be developed and validated during the first six months of this project.

Field parameters of dissolved oxygen, pH, specific conductance, turbidity and temperature will be collected using a YSI ProDSS field meter for each sampling event. FIB samples will be collected at selected soils'-based treatment systems under a different funding source. To assess the influence of carbon and nitrogen on viral and viral surrogate attenuation, samples will also be taken on alternate sampling dates for the selected chemical parameters of alkalinity, nitrate-N, nitrite-N, ammonia-N, total Kjeldahl nitrogen (TKN), total organic carbon (TOC), and dissolved organic carbon (DOC). Our decision to add organic carbon as a project analyte arises from the general assumption of its impact on virus attenuation[14], while nitrogen in its various transformations during wastewater treatment can be responsible for shifts in pH, which is a major determinant of virus sorption and concentrations at soil- and air-water interfaces[15].

3.1.1 Common Influent Wastewater (Decentralized Treatment Technologies)

All decentralized wastewater treatment technologies tested in this project are supplied with a common raw wastewater originating from residential housing (over 250 residences), discharge from the Barnstable County Jail, and discharge from various other light commercial and temporary housing use facilities. Wastewater is accurately dispensed at prescribed volumes to each unit process test using programmable logic controllers, timers, and accurately calibrated dosing buckets. Correct operation for accuracy and precision is ensured by electronic and manual verification procedures outlined in the Standard Operating Procedures that are part of the MASSTC Quality Management Plan. The selected physical parameters of temperature, pH, conductivity, turbidity, and dissolved oxygen will be measured concurrent with all viral and viral surrogate assays. Nitrate-N, nitrite-N, ammonia-N, total organic carbon, dissolved organic carbon, and alkalinity will be assayed using Standard Methods in samples taken concurrently with every other viral and viral surrogate sampling event (Standard Methods for the Examination of Water and Wastewater, 23rd. Ed. (https://doi.org/10.2105/ SMWW.2882.003). The daily flow pattern to each system is standardized to mimic typical household use and is similar to that used in the National Sanitation Foundation (NSF) verification tests (Standards 40, 245, 350 and others): 35% of hydraulic loading between 0600 – 0900 h, 25% of hydraulic loading between 1100 – 1400 h, and 40% of hydraulic loading between 1700 – 2000 h.

3.1.2 Process 1. Soil Treatment as Part of an Onsite Septic System

The soil absorption system portion (leachfield) is the most common unit process in the decentralized wastewater setting and is often the source of "unplanned" water reuse as it enters drinking water aquifers and adjacent surface water bodies. In this study we have configured seven different scenarios that will determine the optimum depth of sand and distribution mode for virus removal and hence would have the highest level of treatment for reuse.

Thirty-five small scale leaching trenches were installed at MASSTC atop separately lined containment units, permitting discrete sampling of each of the 35 cells. The test cells have two different modes of wastewater application (i.e., low pressure distribution and gravity), each with three depths of sand media beneath them (i.e., 2, 3, and 4 feet); each treatment has five replicate cells. In addition, the gravity-delivered wastewater serves an additional 5-replicate set of cells with 5-feet of sand beneath the trench. Low pressure distribution and gravity delivery modes were chosen as they are the two most commonly applied in decentralized wastewater settings. Wastewater to be delivered to these cells will

pass through a septic tank and then be dispersed to the cells. The prescribed hydraulic loading rate of 0.74 gal/sq. ft/day will be applied to all 35 cells (approximate hydraulic loading rate used in a number of states). Three cells in each five constructed replicate sets were chosen for the collection of samples using a random number generator.

Each of the seven treatments (three replicates each) will be sampled monthly for 20 months concurrently with the common wastewater influent source. Because the seven permutations examined here will be measured at minimum in triplicate, this technology will generate the highest number of assays and will represent a statistically robust dataset. Data from this technology will complement data from these cells taken since May 2021, thus allowing a more comprehensive assessment of the variability over time.

3.1.3 Process 2. Drip Irrigation of Septic Tank Effluent

The drip irrigation system in this study will allow discrete monthly sampling beneath two separate portions of the drip dispersal layout. One half of the layout will be planted with edible food plants such as lettuce and selected non-food landscape plants, while the other half will be planted with grass, as is typical for the majority of installations allowed. Both will be irrigated with septic tank effluent. These efforts are designed to demonstrate the efficacy of reusing wastewater in this mode for the purpose of producing edible food or landscape vegetation. A similar sized area will be planted and irrigated with potable water at similar rates to compare growth rates. Viral and viral surrogate samples will be assayed monthly for 20 months in conjunction with the pump chamber supplying septic tank effluent (again originating from a common raw wastewater source as all the decentralized units tested) to the drip area.

3.1.4 Process 3. Membrane Bioreactor

We propose to test two different membrane bioreactors (one of which has passed the Standard 350 – Wastewater Reuse Standard) supplied with typical residential household flows. Since this technology has been shown to be efficacious for removal of bacteria and nutrients, it is paramount to demonstrate viral pathogen and surrogate removals as well as to determine its fit-for-use purpose. Monthly samples will be taken at each of these two units concurrently with raw wastewater influent samples. It is interesting to note that the NSF Standard 350 presently does not have a virus monitoring requirement but is presently deliberating on the inclusion of virus monitoring. Data collected herein may inform these deliberations.

3.1.5 Process 4. Ozone Process Following Membrane Treatment

We propose to test the only product presently purporting to integrate ozone production and treatment into a decentralized treatment system. A fortunate timing of events may allow some simultaneous testing during the NSF/ANSI Standard 350 Water Reuse certification with this present effort. Monthly samples will be taken at this unit at the same time as raw wastewater influent samples are collected. In conjunction with the physical and chemical measurements referenced above, we will conduct grab samples for residual ozone. Ozone residual in water will determined by the indigo method [SM 4500-O3 OZONE (RESIDUAL) (2017) - Standard Methods for the Examination of Water and Wastewater].

3.1.6 Process 5. Ultraviolet Light Following Secondary Treatment

Secondary treatment is perhaps the most common type of advanced treatment system used in the onsite setting and hence may offer a large opportunity for wastewater reuse. To determine the fitness

level for reuse, the viral pathogen and surrogate infectious components of the treated wastewater requires proper assessment. Under this workplan we will sample a typical secondary treatment unit at three locations in the treatment train: one raw wastewater influent, one ahead of the ultraviolet light exposure element, and one following the ultraviolet light exposure. Due to the known impact of turbidity on ultraviolet light penetration through liquid, measurements for turbidity will be conducted for correlation purposes using a calibrated field meter.

3.1.7 Process 6. Cellulose-Based Denitrification Systems

Two cellulose-based systems, operating at MASSTC with residential flows between 330 and 450 gallons per day, will be tested under this project. One technology uses a slow rate sand filter in the nitrification sequence. We will sample three locations in this system monthly for 20 months: one sample at the raw wastewater influent, one sample following the sand filter, and one sample at the final discharge following passage through an upflow woodchip bioreactor. The mid-process sample at this technology also provides an opportunity to assess the viral pathogens and surrogate removing capability of a shallow placed leachfield similar to the drip system. The cellulose-based system, commercially available, is a self-contained single unit that follows a septic tank; this will be sampled monthly for 20 months at two locations (raw wastewater influent and discharge location).

3.1.8 Municipal Treatment Technologies

Two municipal treatment technologies will also be evaluated in this project for their removal of viral surrogates and pathogens: Modified Ludzack-Ettinger process (from the Chatham and Barnstable Wastewater Treatment Plants) and Sequencing Batch Reactors (from the Falmouth Wastewater Treatment Plant). These plants ultimately discharge to the groundwater, and samples will be taken at the primary settling tanks or storage areas, and at the discharge locations prior to disinfection, every two months over the 20-month period. In addition to taking samples for viral pathogens and surrogates, selected physical and chemical parameters (temperature, pH, conductivity, turbidity, dissolved oxygen, nitrate-N, nitrite-N, ammonia-N, total organic carbon, total dissolved carbon, and alkalinity) will be grab-sampled and assayed using Standard Methods.

3.2 Sampling Methods

Samples will be collected in accordance with MASSTC-SOP-037 – Sample Collection (Appendix 8).

3.3 Sample Handing and Custody

Sample preparation and transportation for the site and off site is described in MASSTC-SOP-015 – Sample Preparation and Transportation (Appendix 9). All samples collected are initially stored on ice prior to pre-processing and preparation for transport to the primary certified laboratory at Barnstable County Department of Health and Environment (BCDHE). Chain of Custody Procedures and handling of samples at the laboratories are described in Section 9 of the Quality Assurance Plan of the BCDHE laboratory provided (see Appendix 10) and in Section 6 of the Laboratory Quality Assurance Plan for MassDEP-DEL-WES (see Appendix 4).

For the molecular microbiological assays (ddPCR), MASSTC will store concentrated samples at -80^{o}C until transported to MassDEP-WES. MASSTC will transport all samples for MassDEP-WES in insulated

containment with dry ice. Transport time is approximately 2.5 hours between MASSTC and MassDEP-WES. A sample Chain of Custody Form for transport of concentrated samples from MASSTC to MassDEP-WES is presented in Appendix 11.

3.4 Analytical Methods

Analyses for this project will be performed at three different locations. Staff at the MASSTC location (4 Kittridge Rd, Sandwich MA 02536) will be performing the culturable somatic and male-specific (F+) coliphage assays and all field measurements. Chemical analyses will be performed at the Barnstable County Department of Health and Environment Laboratory, located at 3195 Main Street, Barnstable, MA 02630. FIB assays will be performed at MASSTC. Finally, all genomic ddPCR assays will be performed at Massachusetts Department of Environmental Protection, Division of Environmental Laboratory Sciences Senator William X. Wall Experiment Station, 37 Shattuck Street, Lawrence, MA 01843.

3.4.1 Analytical Methods – MASSTC

3.4.1.1 Field Measurements

Field measurements will be taken concurrently with all viral pathogen and viral surrogate samples include pH, dissolved oxygen, temperature, specific conductance and turbidity. Field measurements will be taken using a YSI ProDSS field meter using MASSTC-SOP-16 (Appendix 12) which also describes calibration and acceptance procedures.

3.4.1.2 Culturable Coliphage

Culturable coliphages, male-specific (F+) and somatic, will be assayed using EPA Method 1643 and EPA Method 1642. Following range finding exercises that will determine the dilutions of raw wastewater necessary to obtain countable plaque-forming units culture plates, diluted samples will be assayed using EPA 1643 and in accordance with MASSTC-SOP-039 – Virus Analysis (Appendix 3). Treated wastewater will be assayed using EPA Method 1642. This method specifies the concentration of the sample by ultrafiltration. Data qualification and acceptance measures are specified within the method and basically involve the initial precision and recovery verification (IPR - already satisfactorily completed) and ongoing precision and recovery verification (OPR). OPR exercises are performed each day that samples are run. For each treated wastewater sample, approximately two liters of effluent is concentrated using a hollow-fiber ultrafilter, the filter is then eluted resulting in a final sample volume of approximately 200 mL which is split into two 100 mL aliquots. Approximately 24 mL of concentrate is placed in cryo-vessels and placed in the -80°C freezer for transport to MassDEP-WES laboratory for genomic analyses.

3.4.2 Analytical Methods – Barnstable County Department of Health and Environment Laboratory

All chemical analyses will be performed at the Barnstable County Department of Health and Environment Laboratory using standard or EPA methods referenced in Table 3 below. SOPs for all chemical analytes for this study are presented in Appendix 13. These methods and SOP prescribe all aspects of the quality control including calibrations, QC acceptance criteria, corrective actions, etc.

3.4.3 Analytical Methods – MassDEP-WES

Indigenous human adenoviruses, human noroviruses GI and GII, and the viral surrogates crAssphage, pepper mild mottle virus, and human-associated Bacteroides HF183 target gene sequence will be quantitated at the MassDEP-WES Laboratory in all project concentrated samples using droplet digital polymerase chain reaction (ddPCR). The ddPCR methods for the project targets will be developed and

validated at the MassDEP-WES Laboratory within the first 6 months of the grant and the associated SOPs for these methods will be added to this QAPP and shared with the EPA at that time.

After the raw influent wastewater and treated effluent samples are concentrated by ultrafiltration (UF), the concentrated samples are stored at -80°C for no more than 12 months prior to nucleic acid extraction. After nucleic acid extraction, the resulting extracts are stored at -20°C and are analyzed as soon as possible within a maximum extract holding time of 30 days. A summary of assays to be conducted at MassDEP-WES is presented in Table 4.

Table 3. Summary of chemical and biological assay methods used in the project EPA STAR Grant #84025901.

Parameter	EPA Method	Std Meth	Units	Preservative	Collection	Holding Time	Laboratory
Nitrate	EPA 300.0		mg/L	Ice	250-ml plastic	48 hours	BCDHE
Nitrite	EPA 300.0		mg/L	Ice	250-ml plastic	49 hours	BCDHE
Ammonia-Nitrogen	EPA 350.1		mg/L	ice (<6 C)H_2SO_4	250-ml plastic	28 days	BCDHE
Total Kjeldahl Nitrogen	EPA 350.2		mg/L	ice (<6 C)H_2SO_4	250-ml plastic	28 days	BCDHE
Alkalinity		SM 2320-B	mg/L	ice (<6 C)	250-ml plastic	14 days	BCDHE
Total Organic Carbon		5310 - B	mg/L	ice (<6 C)H_2SO_4	40 ml amber glass vial	28 days	BCDHE
Dissolved Organic Carbon		5310 - B	mg/L	ice (<6 C)H_2SO_5	40 ml amber glass vial	28 days	BCDHE
MS2-bateriophage	EPA 1642-1643		PFU	ice (<4 C)	4 liter plastic	6 hours	MASSTC
Somatic Phages	EPA 1642-1643		PFU	ice (<4 C)	4 liter plastic	6 hours	MASSTC
Human viruses, HF183, CrAssphage and/or PMMoV		TBD		-80°C	2 ml cryovials 15 ml conical tubes	28 days	DEP-WES
Fecal Coliform		SM 9223B 40CFR Part	MPN/100 ml	ice	100 ml sterile plastic	12 hours	MASSTC
E. coli		SM 9223B	MPN/100 ml	ice	100 ml sterile plastic	12 hours	MASSTC
Enterococcus		SM9230	MPN/100 ml	ice	100 ml sterile plastic	12 hours	MASSTC
Ozone		45000-O_3 B.	mg/L	run immediatel	grab	N/A	MASSTC
Dissolved Oxygen		4500-O H	mg/L	none	grab(ProDSS meter)	N/A	Field
pH		4500-H^+	Std. Units	none	grab(ProDSS meter)	N/A	Field
Turbidity		ISO 7027	NTU	none	grab(ProDSS meter)	N/A	Field
Temperature		2550	C°	none	grab(ProDSS meter)	N/A	Field

Table 3. Summary of droplet digital polymerase chain reaction (ddPCR) methods for project targets in UF-concentrated raw influent wastewater and treated effluent samples.

Parameter	Published Reference Method	Preservative	Sample Collection	Holding Time	Laboratory
Human adenoviruses	Droplet digital PCR quantification of norovirus and adenovirus in decentralized wastewater and graywater collections: Implications for onsite reuse[17]	-80°C	2-mL cryovials 15-mL conical tubes	12 months	MassDEP-WES
Human noroviruses GI		-80°C	2-mL cryovials 15-mL conical tubes	12 months	MassDEP-WES
Human noroviruses GII		-80°C	2-mL cryovials 15-mL conical tubes	12 months	MassDEP-WES
HF 183	Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment[17]	-80°C	2-mL cryovials 15-mL conical tubes	12 months	DEP-WES
CrAssphage	Co-Occurrence of crAssphage with Antibiotic Resistance Genes in an ref #Impacted Urban Watershed[18] Comparative fate of CrAssphage with culturable and during activated sludge wastewater treatment molecular fecal pollution[19]	-80°C	2-mL cryovials 15-mL conical tubes	12 months	DEP-WES
PMMoV	Pepper mild mottle virus as an indicator of fecal pollution[20]	-80°C	2-mL cryovials 15-mL conical tubes	12 months	DEP-WES

3.5 Quality Control Requirements

3.5.1 MASSTC – Quality Control Procedures

3.5.1.1 Field Measurements

Continuing Calibration Verification (CCV) is performed every ten samples per Section 10.12 of MASSTC-SOP-016 – YSI ProDSS Field Meter (Appendix 12). This CCV is part of the permanent record of use and is verified daily by the MASSTC Operator upon the meter closeout procedure of instrument data download (Section 11.9 MASSTC-SOP-016 – YSI ProDSS Field Meter). Corrective actions and other aspects of calibration and meter use are contained in the SOP.

3.5.1.2 Coliphage Assays

Somatic and male-specific (F+) coliphage analyses conducted in this project conform to EPA Method 1643 (raw wastewater) and EPA Method 1642 (treated wastewater – not chlorinated). Each of these methods requires an initial precision and recovery (IPR) procedure to demonstrate laboratory capability. This procedure was successfully completed in accordance with Section 8.3 of each of these methods at MASSTC in March 2021.

Continuing assurance of data quality for these methods is demonstrated by analyzing ongoing precision and recovery (OPR) samples, matrix spikes (MS), duplicate samples, and method blanks with field sample batches. The spiking suspension that serves as reference for these quality control (QC) samples is formulated using the Double Agar Layer (DAL) procedure described in Section 13.4 (Flowchart Section 18) in both of these EPA Methods. New spiking suspensions are formulated monthly from stock solutions. Each method notes the difficulties in assessing the "true" concentrations of the prepared spiking suspensions[2]. The acceptable spike recovery percentages are shown in Table 4. The OPR will be assayed once for each sampling run day. As part of the laboratory QA program, results for OPR and IPR samples will be charted, and updated records maintained in order to monitor ongoing method performance. The laboratory will statistically derive analytical accuracy for Method 1643 and 1642 by calculating the average percent recovery (R) and the standard deviation of the percent recovery (sr). The acceptable accuracy is the % recovery interval from R - 2sr to R + 2sr.

[2] Source Methods 1643 and 1642 "Note: Recovery and relative percent difference are based on each laboratory's enumeration of the referee-prepared spiking suspensions using the DAL procedure (Section 13) and enumeration of the recovery by SAL during the multi-laboratory validation study. Using the SAL procedure to enumerate coliphage spiking suspensions may affect recoveries of somatic (phi-X174) and malespecific (MS2) coliphage and is being investigated."

Table 5 Acceptance Criteria for coliphage analyses based on a multi-laboratory validation study performed in the development of EPA Methods 1642 and 1643 to serve as the basis for qualification and ongoing precision and recovery validations.
[a]Initial Precision and Recovery, [b]Ongoing Precision and Recovery.

A

Method	Phage	IPR[a] Mean Recovery (%)	IPR[a] RSD[c] (%)	OPR[b] Recovery (%)
Method 1643	Somatic (phi-X174)	139 - 278	160000%	134 - 283
	Male-specific (MS2)	9 - 100	170000%	9 - 100

B

Method	Phage	IPR[a] Mean Recovery (%)	IPR[a] RSD[c] (%)	OPR[b] Recovery (%)
Method 1642 (2 liters)	Somatic (phi-X174)	68 - 397	280000%	59 - 406
	Male-specific (MS2)	Detect - 100	3100%	Detect - 100

MASSTC staff frequently consults with the EPA Laboratory in Cincinnati, Ohio to get advice on performing these methods and on any problematic issues encountered, particularly in reference to any discrepancies between enumerations of spiking suspensions using DAL and those observed by SAL during the OPR enumeration.

Matrix spikes will be performed in accordance with Section 8.5 of the Methods on 5% of the samples from each matrix sampled. An un-spiked sample taken concurrently from the same location as the spiked sample will determine background densities somatic and male-specific coliphages and will be used for calculating MS recoveries in accordance with Section 13.5 of EPA Methods 1642 and 1643. Acceptance criteria for recoveries will be based on Table 4 Section 8.5.4.

Method blanks are analyzed each day when sampling and analyses are being conducted. Media sterility checks are performed on each batch of media used.

3.5.2 Barnstable County Department of Health and Environment Laboratory – Quality Control Procedures

Quality assurance measures for the Barnstable County Department of Health and Environment Laboratory are presented in Section 5 of the BCDHE Quality Assurance Plan (Appendix 10) and associated SOPs (Appendix 13).

3.5.3 MassDEP-WES – Quality Control Measures

Quality assurance measures for MassDEP-WES are described in Section 10 – Section 13 of the MassDEP-DELS-WES Quality Assurance Plan (Appendix 4).

3.6 Instrument/Equipment Testing, Inspection, and Maintenance

3.6.1 Massachusetts Alternative Septic System Test Center

All field sampling equipment is inspected and cleaned following and before each outing. The field dissolved oxygen/pH/temperature/conductivity meter is checked for calibration at the opening and closing of each day using three pH standards and air saturation for dissolved oxygen. A record is maintained for both the opening and closing of the meter as discussed in the SOP (Appendix 12 – MASSTC-SOP-016 – YSI ProDSS Field Meter, and opening and closing forms, MASSTC-FRM-033 – ProDSS Calibration Checklist, MASSTC-FRM-034 – ProDSS End of Day Checklist) used for verification of meter performance and maintenance), and these records are maintained for five years. As described in MASSTC-SOP-016 – YSI ProDSS Field Meter, field data and all confirming initial calibration verification (ICV) and continuing calibration verification (CCV) are directly uploaded at the end of each use and data are recorded as an Excel™ worksheet which is later correlated with any samples taken and reported through the laboratory information management system (LIMS). Anomalies in operation, when observed are reported to the Quality Assurance Manager and, if necessary, technical support is notified. The instrument is returned for service if problems are not rectified. MASSTC maintains a service contract of the instrument and two identical instruments are rotated through use patterns to facilitate continuation of operations in the event of instrument failure.

All refrigerators, incubators, freezers (-23^{o}C and -80^{o}C), and autoclaves used for microbiological processing or storage are checked daily for compliance with operational fitness. All thermometers and temperature indicators are calibrated annually with an NIST reference thermometer and offsets are printed and dated on a tag near any display platform. Calibrated thermometers are placed in all cooling/freezing units. All micropipettes and balances are calibrated annually.

Maintenance of all infrastructure elements necessary for the completion of this project are maintained in accordance with manufacturer's specifications. Small sampling pumps, composite samplers, and sampling receivers are regularly cleaned and checked for defects before and after each use. Conveyance and internal tubing (inside of peristaltic pumps) is changed regularly when showing any discoloration or indication of biofilm and growth.

All tasks described above are the primary responsibility of the Test Center Operator, however all staff participate in these maintenance tasks as required. Samples of controlled forms used by the Operator (MASSTC-FRM-010 – Weekly Quality Assurance Checklist and MASSTC-FRM-018 – Weekend Quality Assurance Checklist) are provided in Appendix 14. These forms and checklists are updated as needed and as project proceed.

3.6.2 Barnstable County Department of Health and Environment Laboratory

See Section 11 of the Barnstable County Department of Health and Environment Laboratory Quality Assurance Plan (Appendix 10).

3.6.3 MassDEP-WES

Equipment maintenance procedures for the MassDEP-WES Laboratory are summarized in Table 7 of the MassDEP-DELS-WES Laboratory Quality Assurance Plan, Revision # 8.0, February 2021 (Appendix 4). Maintenance procedures for the specific equipment used in the MassDEP-WES Molecular Microbiology Laboratory for the quantitation of project targets by ddPCR are summarized in the Table below.

3.7 Instrument/Equipment Calibration and Frequency (EPA QA/R-5 B7)

3.7.1 Massachusetts Alternative Septic System Test Center

In addition to the single field instrument (maintenance and calibration described Section 2.6), dosing to the systems at MASSTC is completed by first triggering a pump to fill a bucket to a prescribed volume of raw wastewater. The discrete volume is set by the adjustment of an overflow weir which allows overflow to go to void. A delay-relay float system triggers the "spill" of the wastewater volume to the system following the adequate fill of the bucket while at the same time triggering the advancement of an electronic counter. These devices are checked three times daily by reconciling expected spill counts with observed counts. Counts indicating a divergence of more than ten percent of the required flow initiates action to correct the problem. These checks are electronically recorded, and records are maintained indefinitely. Since the overflow weir is set, calibration of volumes is only conducted when the buckets are disturbed for repair. The Facility Operator is responsible for these tasks and is assisted by other staff as needed and available.

For this project, all dosing bucket fill/spills are electronically recorded for possible use in explaining data. These data are checked weekly by the Facility Operator to ensure that all buckets were dosed according to prescribed volumes. Record of these events is stored indefinitely. Composite samplers are checked for function and readiness prior to each use. These checks include internal and external tubing, battery strength, and desiccant and overall soundness.

3.7.2 Barnstable County Department of Health and Environment Laboratory

See Section 10 of the Barnstable County Department of Health and Environment Laboratory Quality Assurance Plan (Appendix 10).

3.7.3 MassDEP-WES

Equipment calibration procedures for the MassDEP-WES Laboratory are summarized in Table 6 of the MassDEP-DELS-WES Laboratory Quality Assurance Plan, Revision # 8.0, February 2021 (Appendix 4). Calibration procedures for the specific equipment used in the MassDEP-WES Molecular Microbiology Laboratory for the quantitation of project targets by ddPCR are summarized in the Table below.

3.8 Inspection/Acceptance Requirements for Supplies and Consumables

3.8.1 Massachusetts Alternative Septic System Test Center

All consumables and supplies used such as calibration standards, media ingredients, premade media, etc.) are logged in (registered) with information regarding receipt date, expiration date, lot number, and identification of the person logging it in. All media and supplies are purchased from recognized biological or scientific supply sources (Fisher Supply, Thermo-Fisher, Hach, Sigma-Aldrich and others). Prior to opening or using these materials, the expiration date is inspected, and the date opened is recorded on the registration sheet. Controlled Document MASSTC-FRM-014 – Chemical Receipt Log (Appendix 15) is used for this purpose and records are maintained indefinitely. Any staff member receiving or opening the material is responsible for the appropriate recording of the required information.

3.8.2 Barnstable County Department of Health and Environment Laboratory

See Section 5.11 of the Barnstable County Department of Health and Environment Laboratory Quality Assurance Plan (Appendix 10).

3.8.3 MassDEP-WES

All consumables and other supplies used by the MassDEP-WES Laboratory such as calibration standards, chemical reagents, media ingredients, pre-made media, molecular microbiology reagents, plates and other labware, pipettes and tips, etc., are recorded at receipt with information regarding receipt date, expiration date, lot number, and identification of the person receiving it. All consumables and supplies are purchased from approved biological or scientific supply sources (e.g., Thermo-Fisher Scientific, VWR Scientific, GSS, Bio-Rad, and others). Prior to opening or using these materials, the expiration date is inspected, and the date opened is recorded. Certificates of Conformance (Quality) and Certificates of Analysis from manufacturers are scanned, and the electronic scanned files are stored indefinitely on the MassDEP-WES shared drive as part of our quality control documents.

3.9 Data Acquisition Requirements (Non-Direct Measurements)

We anticipate no need for the use of external data sources.

3.10 Data Management

Data collected under this project derive from four main sources: indigenous culturable somatic and male-specific (F+) coliphage data from the MASSTC Laboratory for project samples, field data collected simultaneously with field sample collections, water chemistry data for samples collected with concurrent virus/coliphage samples, fecal indicator bacteria data for samples collected with concurrent virus/coliphage samples, and genomic (ddPCR) data for enteric viruses and viral surrogates for concurrent samples assayed at the MassDEP-WES Laboratory.

3.10.1 MASSTC - Culturable coliphage and fecal indicator bacteria (FIB) data

Culturable somatic and male-specific coliphage will be assayed at the MASSTC Laboratory. Although conducted under an SOP and published EPA Methods, we recognize the importance of the preservation and inspection of the laboratory observations and notes. To determine original coliphage, other viral surrogate, and enteric virus densities in samples before concentration, it is extremely important to accurately record and track initial and final concentrated volumes. In addition, any anomalies in the analyses need to be recorded for reference as data are inspected. Accordingly, the following procedure for record preservation and error checking is in place.

- The MASSTC microbiologist maintains a detailed laboratory notebook to record original volume concentrated by ultrafiltration, total concentrate volume, concentrate volume used in the coliphage analyses at MASSTC, and the concentrate volume preserved at -80^{o}C for the ddPCR enteric virus and viral surrogate assays to be conducted at MassDEP-WES.
- The MASSTC microbiologist records coliphage plaque-forming unit (PFU) counts and other relevant data, such as concentration volumes and dilutions, in the laboratory notebook and later enters these data into a Quality Assurance Manager (QAM)-approved Microsoft Excel™ spreadsheet.

- The MASSTC QAM saves copies of the field notebook electronically and examines the entries for any anomalies and illegible entries and confers with MASSTC microbiologist as needed.
- The MASSTC QAM enters the data into a QAM-approved spreadsheet that is identical to that used by the MASSTC microbiologist, looking for any discrepancies with the microbiologist's spreadsheet. When necessary, the QAM confers with the microbiologist to clarify and document any reasons for any discrepancies. This QAM-approved spreadsheet has locked calculation and reference cells that can only be changed by the project QAM.
- Following an inspection and verification of culturable coliphage data and FIB, the project QAM integrates various field collected data, water chemistry, fecal indicator bacteria, and genomic enteric virus and viral surrogate data from MassDEP-WES into the spreadsheet (see Section 2.10.2 below).
- All data from this spreadsheet are made available to Principal Investigators and other relevant staff through the shared network drive.
- Principal Investigators may request changes in this main spreadsheet such as the addition of columns that can accommodate transformed data (log transformations most common).
- Principal Investigators are encouraged to create their own spreadsheets for their regular use and data analyses that have linked and updatable references.
- Because of the complexity of the data spreadsheet, there is frequent and regular communication between the PIs and the QAM. Any additions or modifications to the spreadsheet are discussed at weekly meetings of the project team.

3.10.2 MASSTC - Field parameter data

The one field instrument for this project is the YSI ProDSS field meter with four probes on the sonde (dissolved oxygen by optical measurement, conductivity-temperature, turbidity, and pH). MASSTC has a rigorous daily tracking and accountability regimen that includes daily calibration checks and daily off-loading of data using proprietary manufacturer's software (KorDSS™) data storage and output options (See Section 2.6). Data from this instrument, including initial and continuing calibration verification is downloaded daily to a comma separated file (*.CSV) using an option within the software. This function is performed by the MASSTC Operator, who also integrates these data into the overarching SQL database of MASSTC. An example output from the instrument is presented below. MASSTC maintains three YSI ProDSS instruments for redundancy and rotates their use weekly. Each individual field meter is designated by one of three colors and the KorDSS™ software designates, recognizes and displays this indicator during each data download.

Field data from the SQL database is integrated into the coliphage spreadsheet and other parameter by the QAM. During this process there are programmed checks to verify that station designators match.

EASUREMENT DATA FILE EXPORT

9/7/2021 15:10

TIME	SITE	DATA ID	Baromete	pH	pH (mV)	Cond (µS/	Sp Cond (µ	Sal (psu)	nLFCond (	TDS (mg/L	Temp (°F)	Sigma-T (s	Sigma (s)	ODO (% Sa	ODO (mg/
7:49:18 AM	1Check 7 pH		757.1	6.98	-14.5	5935.6	6308.3	3.45	6344.5	4100	71.4	0.4	0.4	96	8.24
7:58:27 AM	2Check ODO		757.2	7.09	-21	0	0	0	0	0	69.9	-2	-2	99.3	8.84
[redacted]			757.1	5.38	77.5	490.4	524	0.25	527.2	341	71	-2	-2	41.9	3.68
			757.3	5.41	75.8	566.7	604.6	0.29	608.2	393	71.1	-1.9	-1.9	18.1	1.59
			757.3	5.79	53.9	481.6	513	0.25	516	333	71.2	-2	-2	69.9	6.13
			757.4	6.2	30.3	537.3	573	0.28	576.4	372	71.1	-2	-2	82.4	7.23
			757.4	6.28	25.9	465.4	498.1	0.24	501.3	324	70.8	-1.9	-1.9	38.1	3.36
			757.4	7.48	-43	498.7	531.9	0.26	535.1	346	71.1	-2	-2	54.4	4.78
9:16:51 AM	Virus Pump Chambe		757.4	7.03	-17.3	600.6	653.7	0.32	658.8	425	69.3	-1.7	-1.7	-1.2	-0.11
[redacted]	DCWmeter		757.6	7.08	-20	594.7	640.4	0.31	644.8	416	70.3	-1.8	-1.8	-0.9	-0.08
9:21:40 AM	DCW Virus		757.6	7.08	-20	594.7	640.4	0.31	644.8	416	70.3	-1.8	-1.8	-0.9	-0.08
9:30:40 AM	1Check 7 pH		757.5	7.02	-16.7	5824.1	6305.1	3.45	6350.9	4098	69.8	0.6	0.6	85.8	7.49
10:06:06 AM	2Check ODO		757.6	7.12	-22.3	-0.1	-0.1	0	-0.1	0	69.9	-2	-2	99.1	8.83
10:17:13 AM	G3-1		757.7	6.57	8.8	410.6	446.7	0.22	450.1	290	69.4	-1.8	-1.8	93.8	8.39
10:19:14 AM	G3-2		757.7	6.56	9.7	412.6	448.9	0.22	452.4	292	69.4	-1.8	-1.8	94.4	8.44
10:21:38 AM	G3-4		757.7	6.8	-4.3	429.8	469.4	0.23	473.1	305	69.1	-1.7	-1.7	96.4	8.65
10:25:45 AM	P3-2		757.7	6.65	4.7	413.7	447.7	0.22	450.9	291	69.8	-1.8	-1.8	95.2	8.47
10:29:13 AM	P3-4		757.7	5.67	60.8	611.6	660.4	0.32	665	429	70.1	-1.8	-1.8	93.5	8.29
10:31:16 AM	P3-5		757.7	6.35	21.6	630.3	680.5	0.33	685.3	442	70.1	-1.8	-1.8	95.8	8.51
10:37:18 AM	1Check 7 pH		757.7	7.09	-20.5	5839.8	6299.6	3.44	6343.5	4095	70.1	0.6	0.6	82.7	7.2
10:54:13 AM	2Check ODO		757.4	7.05	-18.6	0	0	0	0	0	69	-1.9	-1.9	99.2	8.92

Figure 3 Downloaded data from The YSI ProDSS field instrument provided by YSI KorDSS. (non-virus project identifiers redacted)

3.10.3 Chemical Analyses – Barnstable County Department of Health and Environment Laboratory

Chemical analyses for this project are performed at the Barnstable County Department of Health and Environment (BCDHE) Laboratory which, similar to the Massachusetts Alternative Septic System Test Center (MASSTC), is a division of the Barnstable County Department of Health and Environment. Data from analyses performed at MASSTC are directly accessed weekly from the Laboratory Information Management System (LIMS) of BCDHE Laboratory. Data from BCDHE Laboratory are stored and maintained as described in the QAP for the laboratory (Appendix 10). All data from analyses on this project will be directly accessed from the laboratory information management system (LIMS) by the QAM. These data are then stored in a Structured Query Language (SQL) file where they are manipulated to produce various internally developed outputs. Data are organized in the MASSTC Database by project to assist in graphing and charting and are capable of being exported as files compatible with Microsoft Excel™ and other character delimited files. If, during the access and integration process errors in the data are suspected, the Laboratory Director is contacted and asked to verify the data. Scanned copies of all laboratory reports are maintained in an organized database on the MASSTC server and are accessible by all staff. If upon inspection of the data by any staff working on a particular project there appears to be an error, the Laboratory Director is notified for comment and resolution.

3.10.4 Genomic Assays – MassDEP-WES Laboratory

Data management for MassDEP-DELS-WES Laboratory is summarized in Section 9 of the Laboratory Quality Assurance Plan, Revision # 8.0, February 2021 (Appendix 4). The MassDEP-WES Laboratory uses a cloud-based Laboratory Information Management System (LIMS), called WinLIMS, from Quality Systems International Corporation, Ramsey, NJ, to manage all samples received for analysis from prelogin through results reporting. All analytical work on samples is documented in instrument printouts, bench sheets, and directly captured analytical data which become the documentary linkage between analysis and final data reports. The analyst initials the top of the first page of each document

or report or document/report subsection where applicable. When analyses are completed, any non-instrument reported final analytical data are transferred to the sample bench sheet. Information from the sample bench sheet or from the instrument report are then entered into LIMS for the generation of the final analytical report directly from LIMS. The final sample laboratory reports generated by Mass-WES include laboratory login #, laboratory sample ID, client sample ID, client sample description, date of sample collection, date of sample receipt, date of sample analysis, name of sample collector, analytical test(s)/method(s) performed, sample analyte concentration results, and supporting quality control data. After the analytical results have undergone 1st and 2nd level verification and have been reported, the laboratory report becomes part of the MassDEP-WES records system and is stored indefinitely in electronic format and in paper copy retained for a minimum of 10 years.

4 Assessment and Oversight

4.1 Assessments/Oversight and Response Actions

The Project Manager (Brian Baumgaertel) holds weekly meeting specifically on this project. Attending is the Principal Investigator (George Heufelder), the Microbiologist (Kathy Regan), the Quality Assurance Manager (Emily Michele Olmsted) and occasionally MASSTC Operator (Bryan Horsley). Regular discussions range from laboratory scheduling of samples, data reduction activities and quality assurance of data, chemical analyses update, sample collection scheduling, review of initial data. Any corrective actions or adjustments are discussed at these meetings and assignments are given to appropriate staff. These meetings are essentially audits of the project progress. Notes are taken and posted on the shared digital drive and preserved for future reference.

Performance and Systems Audits, and Corrective Action Procedures for the MassDEP-WES Laboratory are summarized in Sections 11 and 14, respectively, of the MassDEP-WES Laboratory Quality Assurance Plan, Revision # 8.0, February 2021 (Appendix 4).

4.2 Reports to Management

This project involves the coordination of efforts of two government agencies. We have chosen the management structure that places a Project Manager at MASSTC who will guide and coordinate efforts at MASSTC, the Barnstable County Department of Health and Environment Laboratory, and MassDEP-WES. To limit the interruptions at MassDEP-WES, which has responsibilities state-wide and is involved in many such collaborative efforts, the Principal Investigator at MassDEP-WES (Dr. Oscar Pancorbo) will be requested to participate in update meetings with the other Principal Investigator (George Heufelder) and appropriate staff only as needed. Since MASSTC is supporting this grant effort with a laboratory staff person who will work as MassDEP-WES, the Project Manager will receive more frequent updates on the progress toward all molecular microbiology assays performed as part of this project.

This project constitutes a major part of the MASSTC mission objectives and hence will be subject of weekly updates by all staff involved in collection of samples, conducting coliphage assays, general support of treatment operations, quality assurance and data management and updates on data acquisition and reporting.

5 Data Review and Usability

5.1 Data Review, Verification, and Validation Requirements

5.1.1.1 Chemistry Data - Barnstable County Department of Health and Environment Laboratory

Chemical data review in projects listed herein begins in the BCDHE Laboratory. The data review, verification and validation requirements are presented in Section 13 of the QAP (Appendix 10).

Once approved at the analytical laboratory level, MASSTC Quality Assurance Manager (QAM) has access to download these data into the data management system at MASSTC. The QAM checks at this point relate to ensuring proper connection with the appropriate project as well as timeliness of the received data. The laboratory is notified if the expected date of receipt of any data has exceeded five business days. Once these data are properly merged into the MASSTC Structured Query Language (SQL) setting, graphing and data analyses can be performed by any investigators that are required to report and interpret the data. The graphing routines use allow the visual representation of trends, correlations and outliers. Any suspect data at this point results in an inquiry back to the Laboratory Director who can, verify all operational and analytical correctness down to an appropriate level. The QAM may ask for detailed description of the extent of the review.

5.1.1.2 Coliphage Data – MASSTC

Review, verification and validation of coliphage data is accomplished as data passes through the data management process described above in Section 2.10.1.

5.1.1.3 Molecular Microbiology Data – MassDEP-WES

Data review and verification procedures for MassDEP-WES Laboratory are found in Section 9 of the MassDEP-WES Laboratory Quality Assurance Plan, Revision #8.0, February 2021 (Appendix 4).

5.2 Verification and Validation Methods

5.2.1.1 MASSTC, Barnstable County Department of Health and Environment Laboratory, and MassDEP-WES Laboratory

A number of Standard Operating Procedures are in place at MASSTC that aid in the verification and validation process. From the inspection and verification of calibration standards, checklists to ensure their proper use and calibration of instruments, to daily and weekly checklists for routine maintenance and upkeep of the facility operational features that support the test center venue, all details can be easily accessed by investigators to retrace the conditions under which the data reported by the laboratory are collected. The SOPs include Daily and Weekly Quality Assurance Checklists (also weekend checklists) for the Facility Operator, calibration and close-out procedures that are electronically verified and retrievable, daily electronically retrievable checks on each of the test cells, electronically retrievable weather and site condition data, and a complete historic record of field observations.

If laboratory data appear to be suspect, verification and validation of all associated data will proceed expeditiously. All the above conditions can be reviewed for proper procedural elements. Were all correct sampling techniques used? Do the field notes reveal any anomalies? Were there any operational anomalies on the day of sampling? Were the systems being tested receiving the correct doses? Were there any anomalous weather conditions? Were all field measuring instruments checked for operation?

These and others can be verified either by inspection of hand-written records or the electronic retrieval of recorded operational elements.

In parallel, an inquiry is made to the Laboratory Director (BCDHE or MassDEP-WES Laboratory) who can, in the instance of a suspect datapoint, inspect analysts' bench notes, calibration records, and instrument records. In some instances where samples are still within holding time, the Director can have the suspect sample analyzed again.

5.3 Reconciliation with User Requirements

The study objectives require that data produced enable the comparison of treatment methods relative to removal of viruses using widely accepted metrics. Further we endeavor to add significantly to the existing knowledge base such that performance data from this study can be used both to compare against any existing standards as well as enable the data to be used in conducting quantitative microbial risk assessments (QMRA). QMRA is a mathematical modelling that relies in part on pathogen reduction across various treatment trains to indicate a suitable level of reuse for wastewater following treatment.

We have chosen perhaps the most commonly used metric, the log reduction value (LRV), to express our results for both an evaluation of the technologies being tested as well as to allow for the wider use of the data by other researchers and QMRA modelers.

Log reduction values (LRVs) are simply the $\log_{10}$ transformation of the percent reduction of virus densities in wastewater from the raw wastewater value to a defined point in the treatment process (usually the effluent). The use of LRVs can allow for an easier comparison of systems using standard statistical tests, such as analysis of variances (ANOVA) coupled with post-hoc tests to compare performance of a group of treatment systems. In the case of this study where many variables will be controlled (all systems have the same influent, are taken at the same temperatures and in the same time period), we can compare groups of treatment modalities by comparing their mean LRVs or a transformation of the data that will fit the assumptions of the particular statistical tool chosen.

In addition to the determination of treatment efficacies, another objective in this study is to evaluate the use of the culturable coliphages and genomic copies of other viral surrogates (i.e. pepper mild mottle virus and crAssphage) and Bacteroides HF183 human fecal genetic marker as indicators of human enteric viruses in water reuse applications. Along with all the chemical parameters and fecal indicator bacteria measured, we will perform appropriate regression analyses to determine relationships between the coliphages and viral surrogates and the human enteric viruses assayed by ddPCR.

Human enteric viruses, by their nature, defy a simple means of detection, prediction and assessment of risks. This project endeavors to compare the performance of various treatment means for enteric virus removal/attenuation so that appropriate reuse of wastewater, shown to be a known conveyor of human disease, can be determined. Each study conducted has limitations defined chiefly by the conditions where they were conducted. We will convey to data users and decision makers the limitations of our data by:

- clearly defining the conditions (physical and chemical) under which our samples were collected.
- defining and expressing mathematically the variability of the data.
- defining clearly the limitations of the assay technologies that we used and the time periods in which we used them.

There is perhaps no other time in history that the technologies for enteric virus detection have seen more advancement. The Principal Investigators realize that, although the techniques used in this study are present state-of-the-art, they remain limited in their ability to adequately equip risk modelers with definitive tools for prediction of risk. We only endeavor by this project to add appreciably to the literature of other studies that will incrementally improve the models and allow for the reuse of a precious resource in a sustainable and safe manner.

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Appendix 1

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Massachusetts Alternative Septic System Test Center

Barnstable, Massachusetts

Form

Title: Training Log

Effective Date: 2020-02-11	**Number:** MASSTC-FRM-011	**Revision:** 002

Authors

Name: Brian Baumgaertel
Title: MASSTC Director

Signature: DocuSigned by: Brian Baumgaertel A809A6344B57407... Date: 8/21/2020

Approvals

Name: Brian Baumgaertel
Title: MASSTC Director

Signature: DocuSigned by: Brian Baumgaertel A809A6344B57407 Date: 8/21/2020

Training Log

Document ID#: MASSTC-FORM-011
Revision#: 001
Released: 2020-02-11
Released By: Brian Baumgaertel

Revision History

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the MASSTC Director on the MASSTC Sharepoint Site

History	Effective Date
Revision #002: Added new items and removed outdated items. Edits by BB	2020-02-11
Revision #001: Reformatted. Edits by BB.	2018-08-04
Revision #000: Original Issue	2018-02-12

Training Log

Document ID#: MASSTC-FRM-011
Revision#: 001
Released: 2020-02-11
Released By: Brian Baumgaertel

Trainee Name: _____ Service Start Date: _____ Staff Category: (P)ermanent / (T)emporar

Task	SOP Reference	Req'd For	Initial Training Date	Trainee Initials	Trainer Initials	Verification Date	Trainer Initials
Documentation and Data							
MASSTC Quality Manual - where to find, how to read	MASSTC-PLN-001	P,T					
Daily log books	MASSTC-SOP-003	P,T					
Electronic journal	MASSTC-SOP-003	P,T					
Manual entry of sample data	MASSTC-SOP-003	P					
Importing of sample data	MASSTC-SOP-003	P					
Site security (Locking Gate, etc)	MASSTC-SOP-020	P,T					
Weekday quality assurance checklist	MASSTC-SOP-021	P					
Friday quality assurance checklist	MASSTC-SOP-021	P					
Weekend quality assurance checklist	MASSTC-SOP-021	P					
System Dosing							
Morning shed checks (sign-off sheet, dosing QA sheet)	MASSTC-SOP-018	P,T					
Afternoon shed checks (sign-off sheet)	MASSTC-SOP-018	P,T					
Daily shed checks (cleaning, etc)	MASSTC-SOP-018	P,T					
Dosing pump calibration	MASSTC-SOP-018	P					
Dosing pump scheduling	MASSTC-SOP-018	P					
Sampling and Samplers							
Sample pouring technique	MASSTC-SOP-008	P,T					
Bacterial grab samples	MASSTC-SOP-007	P,T					
Programming auto samplers for uniform time	MASSTC-SOP-017	P					
Programming auto samplers for non-uniform time	MASSTC-SOP-017	P					
Programming auto samplers for flow proportion	MASSTC-SOP-017	P					
Auto sampler calibration	MASSTC-SOP-017	P					
Auto sampler maintenance	MASSTC-SOP-017	P,T					
Creating Chains of Custody	MASSTC-SOP-015	P					
Printing, applying bottle labels	MASSTC-SOP-015	P,T					

Training Log

Transportation of samples to lab	MASSTC-SOP-015	P					
Cleaning auto sampler jugs	MASSTC-SOP-017	P,T					
Field Meters and Electronic Recording Devices							
Calibration of ProDSS	MASSTC-SOP-016	P,T					
Use of ProDSS	MASSTC-SOP-016	P,T					
Closeout of ProDSS	MASSTC-SOP-016	P,T					
Ozone meter calibration	MASSTC-SOP-014	P					
Ozone meter use	MASSTC-SOP-014	P					
Power meter use	MASSTC-SOP-011	P					
Sound meter use	MASSTC-SOP-012	P					
Moisture sensor use	MASSTC-SOP-024	P					
D-Box tipper trays	MASSTC-SOP-023	P					
Facility and Equipment Maintenance							
Lift station maintenance	MASSTC-SOP-019	P					
Influent channel maintenance	MASSTC-SOP-019	P					
Effluent sumps maintenance	MASSTC-SOP-019	P					
Effluent discharge network maintenance	MASSTC-SOP-019	P					

Training Log

Document ID#: MASSTC-FRM-011
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Released: 2020-02-11
Released By: Brian Baumgaertel

Appendix 2

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Massachusetts Alternative Septic System Test Center
Barnstable, Massachusetts

Standard Operating Procedure

Title: Document Control SOP

Effective Date: 2020-02-06	**Number:** MASSTC-SOP-001	**Revision:** 001

Authors

Name: Brian Baumgaertel
Title: MASSTC Director
Signature: DocuSigned by: Brian Baumgaertel A809A6344B57407... Date: 3/8/2021

Approvals

Name: Brian Baumgaertel
Title: MASSTC Director
Signature: DocuSigned by: Brian Baumgaertel A809A6344B57407... Date: 3/8/2021

Revision History

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the MASSTC Director on the MASSTC Sharepoint Site.

History	*Effective Date*
002 – Added reference to MASSTC-FRM-007 for tracking deployment of new/revised controlled documents. Removed reference to watermarking archived documents.	2021-03-08
001 – Removed section 4 – Document Revision History, added new page with revision history for consistency with other Controlled Documents. Added "OSH" document type. Revised revision method. Revisions by Brian Baumgaertel.	2020-02-06
000 - Initial Release	2019-02-11

Contents

1 General Information

1.1 Purpose

The documents that form the MASSTC Quality Management System (either internally generated or from external sources) are managed as controlled documents and information as defined in ISO/IEC 17025-2017. This procedure describes the process for development, review, authorization, control, and distribution of controlled documents.

1.2 Scope/Application

MASSTC's field quality management documents include internally generated documents which provide information regarding how to conduct business and documents which provide a format for recording information.

Quality management documents also include those of external origin used in the implementation of the quality system such as standards, regulations, equipment software, and manufacturers' manuals.

1.3 Documentation/Verification

The official copy of this procedure resides on the MASSTC SharePoint Site. The Document Control Coordinator is responsible for ensuring the most recent version of the procedure is placed on the MASSTC SharePoint Site and for maintaining records of review conducted prior to its issuance.

1.4 Definitions

1.4.1 Document Control Coordinator

The Document Control Coordinator (DCC) is a quality management position appointed by management to maintain documents that form the MASSTC Quality Management System.

1.4.2 Subject Matter Expert

For the purposes of this procedure, subject matter experts (SMEs) are persons deemed competent, experienced, and knowledgeable by MASSTC management in the topic of the procedure, standard, guidance, or other subject matter for which the document is intended.

2 Internal Documents

2.1 Document Development

Internally issued MASSTC Quality Management System documents include standards, management plans, policies, manuals, operating procedures, test methods and guidance. Once the need for a new document has been identified, management assigns an author. A subject matter expert is usually assigned as either the author or a reviewer.

The following steps are used to develop, review, authorize, control, and distribute internal documents related to the MASSTC Quality Management System:

1. Author will prepare the first draft and submit it to the reviewers

2. Reviewers will provide comments to author.
3. Author will address comments and submit second draft to reviewers.
4. Reviewers will provide comments to author.
5. Steps 2-3 are repeated until all comments are addressed.
6. Once all comments have been addressed, the document will be submitted to the DCC for a format check and effective date assignment.
7. The document will then be submitted to the Director for final review and approval.

2.2 Document Format

Flexible formatting is permitted for plans, manuals and methods, depending on the applicable program. Standard Operating Procedures should follow a defined template. All internally issued documents, regardless of format, must contain the following elements:

1. Document number
2. Release Date
3. Released By Name
4. Page numbering indicating total number of pages

2.3 Document Approval

All documents generated by MASSTC, which form part of the Quality Management System, will be reviewed, and approved for use by the Director.

2.4 Review

Document review is the process through which persons with subject matter knowledge contribute to the development of internal documents. Documents are periodically reviewed (see Section 2.8) and, where necessary, revised to ensure continuing suitability and compliance with applicable requirements. Document review includes grammatical, editorial and technical assessment.

2.5 Control

The official copy of all MASSTC Quality Management System documentation resides on the MASSTC SharePoint Site. All other electronic or printed copies are unofficial.

A master list identifying the current revision status of Quality Management System documents is available on the MASSTC SharePoint Site. The list is maintained by the DCC.

When internal documents have completed final review, they are forwarded to the DCC for a final format check, authorization and distribution, and placement in electronic form (read only) on the MASSTC SharePoint Site. The DCC will ensure that the all procedures on the MASSTC SharePoint Site are updated.

Document control numbers are assigned to MASSTC quality system documents using the following alpha-numeric scheme: MASSTC-Document type-sequential#

Example: MASSTC-SOP-001

Revisions are tracked with a 3-digit revision number, starting from “000”.

External Documents are assigned using the following alphanumeric scheme: MASSTC-EXT-Document Type-sequential#

Example: MASSTC-EXT-MAN-001

2.5.1 Document Types:

- FRM – Forms and Checklists
- INS – Insurance Form
- MAN - Manual
- MTH – Method
- OSH – Occupational Health and Safety
- PCY – Policy
- PLN - Plan
- SDS – Safety Data Sheet
- SOP – Standard Operating Procedure
- STD - Standard

2.6 Authorization

MASSTC Quality Management System documents are subject to approval by the MASSTC Director.

2.7 Distribution

The Document Control Coordinator is responsible for ensuring that all internally issued documents that form the MASSTC Field Quality System are readily available. The official copy of all Quality Management System documents resides on the MASSTC SharePoint Site. The DCC will notify all personnel within the via email of document updates and will maintain a copy of the notification. Tracking of distribution notifications will be recoded on the Controlled Document Deployment Tracking Form (MASSTC-FRM-007).

It is the responsibility of the individual to ensure that all hard and/or electronic copies of documents in their possession are the most recent version.

When documents are revised or retired, the DCC will move obsolete copies to the “Archived” folder of the MASSTC SharePoint Site.

2.8 Periodic Review and Revision

Internal documents are subject to periodic review, and where necessary, revised to ensure continuing suitability and conformance with applicable requirements. Internal documents will be reviewed at least once every four years. This schedule will apply to MASSTC internal documents issued after the effective date of this operating procedure.

In January of each year, the DCC will develop and maintain a document review schedule for the upcoming review period. The schedule will include the effective date of the most recent version of the document and the review date. The DCC will update the review schedule as additional reviews are conducted or new documents are developed. The following procedure will be followed:

1. The DCC will notify the author of the need for the review.
2. The author will review the procedure to determine if updates are needed. If so, the author will notify the DCC and proceed to step 3 below. If no updates are needed, the author will notify the DCC.
3. The DCC will consult with the Director to assign reviewers

4. The author will update the procedure and provide the first draft to the reviewers.
5. The reviewers will provide comments to the author.
6. Steps 4-5 are repeated until all comments are addressed.
7. Once all comments have been addressed, the document will be submitted to the DCC for a format check and effective date assignment.
8. The document will then be submitted to the Director for final review and approval.

Changes Quality Management System documents will be clearly indicated in the revision history of the document, except for forms which will be indicated in a separate record. MASSTC's document control system does not allow for the temporary amendment of Quality Management System documents by hand

3 External Documents

Documents of external origin referenced in the implementation of the MASSTC Quality Management System may include national and international standards, EPA manuals and directives, manufacturer's manuals, equipment software, and other associated types of information. External documents will be reviewed for context to determine their applicability in the MASSTC Quality Management System

When applicable, national and international standards, and EPA manuals and directives will be controlled by documenting, as appropriate, the title, document number (if any), and date of publication. Documents will be assigned document control numbers as described in Section 2.5 of this procedure. The DCC will maintain a list of all controlled external documents. The DCC will review the list each January and consult with personnel to determine if updates are available. If they are available, the obsolete copy will be removed from service and the updated version will be labeled with the next revision of the document control number. On a case by case basis, the DCC in consultation with affected personnel will determine if it is necessary to maintain a copy of the previous version.

Appendix 3

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Massachusetts Alternative Septic System Test Center
Barnstable, Massachusetts

Standard Operating Procedure

Title: Virus Analysis SOP

Effective Date: 2021-11-18	**Number:** MASSTC-SOP-039	**Revision:** 000

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This SOP includes adaptations from U.S. Environmental Protection Agency Office of Research and Development, National Exposure Research Laboratory's
D-EMMD-MEB-009-SOP-01 and D-EMMD-MEB-011-SOP-01
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Revision History

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the MASSTC Director on the MASSTC Sharepoint Site.

History	*Effective Date*
Revision 000 - Initial Release	2021-11-18

CONTENTS

1 PURPOSE

1.1 The purpose of the procedure is to concentrate water samples (tap, environmental, wastewater, etc.) ≥ 1L in volume for subsequent detection and enumeration of microorganisms via culture-based or molecular methods.

1.2 The purpose of the procedure is to enumerate culturable somatic and F+ coliphage from 100 mL sample volumes (tap water, environmental water, wastewater, elution solution, etc).

2 SCOPE AND APPLICATION:

2.1 This protocol describes the standard operating procedure (SOP) used for virus analysis via EPA Method 1602, EPA Method 1642, and EPA Method 1643.

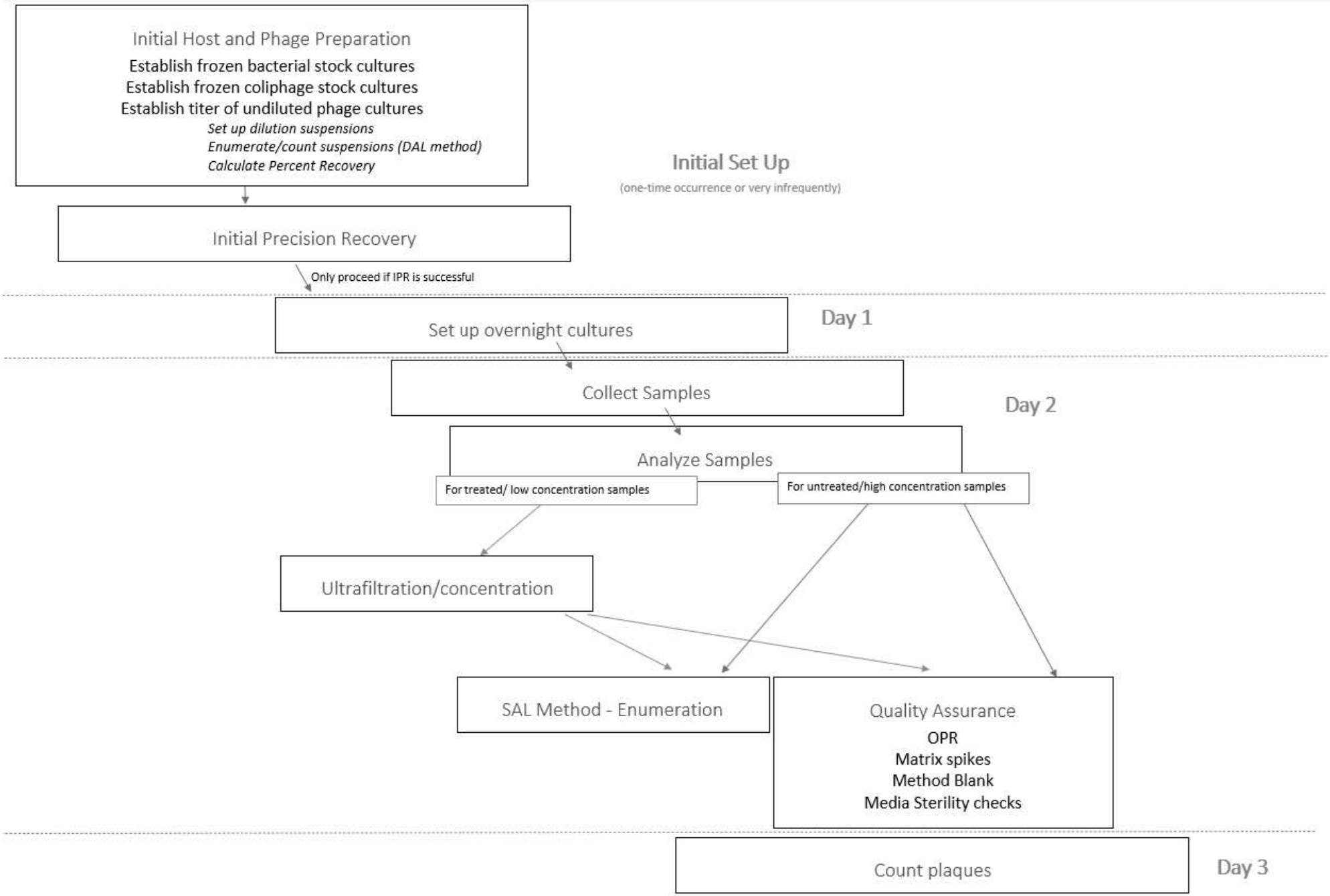
2.2 This SOP applies to all Somatic and Male Specific coliphage analyses conducted at MASSTC and is to be adhered to by all MASSTC staff and all others utilizing this method.

3 SUMMARY

3.1 Single agar layer (SAL) procedure entails mixing 100 mL of sample (or sample concentrate) with an equal volume of molten media, followed by the addition of $MgCl_2$, , appropriate antibiotic, and bacterial host. The mixture is poured over five large petri dishes, incubated at 37°C overnight, then the resulting plaque forming units (PFU) are counted.

3.2 Dead end hollow fiber ultrafiltration employs the principle of size exclusion to trap particles and microorganisms larger than the pore size of the filter fibers as a water sample is passed through the filter using a peristaltic pump. Elution of the filter washes off trapped particles and microorganisms and concentrates them in a smaller volume.

3.3 See the following flowchart for summary:



4 DEFINITIONS AND ABBREVIATIONS:

4.1 **Antibiotic**: an antibacterial substance that is used to prevent microbial growth (and, in this case, allows only one particular species of *E. coli* to grow)

4.2 **Coliphages**: a group of viruses (bacteriophages) that infect *E. coli* and are indicators of fecal contamination.

4.2.1 This method is capable of detecting two types of coliphages: male-specific (F^+) and somatic.

4.3 **D-HFUF**: dead end hollow fiber ultrafiltration

4.4 **F-factor**: the fertility factor in certain strains of *E. coli*. It is a plasmid that, when present, codes for the formation of a pilus termed the F-pilus. This F-pilus allows for transfer of nucleic acid from one bacterium to another.

4.5 **Filtered tap water:** water taken from the sink but filtered through a 1 micron filter

4.6 **Male-specific coliphages (F^+):** ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) viruses that infect via the F-pilus of male strains of *E. coli*.

4.7 **MS2:** a strain of F^+ RNA (group I) coliphage.

4.8 **PBS:** phosphate buffered saline

4.9 **PFU**: plaque forming unit

4.10 **SAL**: single agar layer

4.11 **Somatic coliphages:** DNA viruses that infect host cells via the outer cell membrane.

4.12 **Sample concentrates**: water samples concentrated using dead-end hollow fiber ultrafiltration (See Section 10).

4.13 **Strep/amp:** streptomycin/ampicillin antibiotic combination

4.14 **TSA**: tryptic soy agar

4.15 **TSB**: tryptic soy broth

4.16 **1X TSB**: single-strength tryptic soy broth

4.17 **2X TSA**: double-strength tryptic soy agar

4.18 **Temperature flask**: a 500 ml Erlenmeyer flask filled with 200 ml of filtered tap water

4.19 **Vortex**: to move a mass of fluid in a whirling or circular motion, in this case, through use of special equipment

4.20 **Ultrapure water (ASTM Type I Reagent Grade Water):** water that has been purified to strict chemical and biological specifications, containing, by definition, only H_20, and H+ and OH- ions in equilibrium. Conductivity for ultrapure water is about 0.055 µS/cm at 25°C, also expressed as resistivity of 18.2 MΩ.

5 HEALTH AND SAFETY WARNINGS:

5.1 **Physical Hazards** – The laboratory has multiple hazards, including glassware and chemicals, that have the potential to cause injury. **PPE Required: Closed-toe shoes/boots, lab coat, eye protection, gloves.**

5.2 **Infectious Materials** – Even the cleanest wastewater can contain pathogens, toxins, or toxicants. Proper precautions should be taken to protect oneself. **PPE Required: gloves, goggles.**

5.3 **Fire Hazard** – Procedure involves lit propane torch. **Long hair is required to be tied back, and any loose items must be removed.**

5.4 **Skin Corrosion/Serious Eye Damage** - Some of the chemicals required for these solutions could be hazardous under some conditions; therefore, the standards should only be prepared by qualified

chemists in laboratories where proper safety precautions are possible. **PPE Required: goggles, gloves, lab coat, pants.**

5.5 **Autoclave Hazards** – There is the potential for workers to be burned or cut while handling or sorting hot, sterilized items or sharp instruments. Use care when removing items from autoclave; use oven mitts when handling hot items. Ensure the autoclave door is closed and locked before beginning the cycle. Do not remove items from the autoclave until they are cool. Avoid handling the sharp ends of instruments; use tools or forceps if necessary.

5.6 Mouth-pipetting is prohibited.

5.7 The user should obtain and read the Safety Data Sheet (SDS) for each chemical and should follow the required instructions regarding handling and disposal of these chemicals. SDSs can be found electronically in Sharepoint's MASSTC – Safety Data Sheets, and in print in the colored binder in the autoclave room.

6 CAUTIONS:

6.1 Turn off propane when not in use.

6.2 Close fridge and incubators promptly to prevent overworking the equipment.

6.3 Visually inspect filtration tubing for any tears.

6.4 Stop filtration when the first large air bubble reaches the pump.

6.5 Always stay with and pay close attention to agar while heating to ensure no boil-over.

7 INTERFERENCES:

7.1 Always clean lab surfaces with 70-80% ethanol before using to minimize contamination.

7.2 Do not dip the inoculation loop into the bacterial cultures more than once without re-sterilizing.

7.3 When using vortex, the cyclone should reach the bottom of the test tube.

7.4 Close reagents and return to fridge in a timely manner.

7.5 Close up bacterial colonies on plates as soon as possible to minimize contamination from the air.

7.6 Plates should be stored inverted.

7.7 Do not hold sample in the water bath for an extended time (longer than a few minutes) as this may inactivate the phage.

7.8 Water samples with high turbidity or algae may clog the filter, preventing filtration. High background levels of microorganisms may prevent the host bacteria from producing a confluent lawn of growth.

7.9 The highly variable levels of coliphage (male-specific and somatic) in advanced treatment wastewater effluents and recreational waters should be taken into consideration when determining the volume of sample that may be needed for analyses. Range-finding analyses should be conducted to determine appropriate sample volumes. If smaller sample volumes (e.g., 100 mL) are more appropriate due to high background levels of coliphage, refer to the procedures in EPA Method 1643.

8 PERSONNEL QUALIFICATIONS

8.1 **Each laboratory and analyst using this method must first demonstrate the ability to generate acceptable results using the procedures in Section 21 prior to analyzing field samples.**

8.2 Personnel are required to understand the procedures in this SOP.

8.3 Personnel should be trained in the proper use of Personal Protective Equipment (PPE).

8.4 Personnel performing this analysis are required to review relevant Safety Data Sheets.

8.5 At least one laboratory analyst using this method must first demonstrate the ability to generate acceptable results using the procedures in Section 21 – Initial Precision Recovery (IPR) prior to analyzing field samples.

8.6 All personnel must complete initial and annual laboratory safety training refreshers. All personnel will be trained by a qualified staff member until proficient with the procedure.

8.7 Personnel are required to be knowledgeable of the procedures in this SOP and all referenced SOPs.

8.8 Personnel are required to perform Quality Control procedure specified in Section 21.

9 Reagent and Solution Preparation

9.1 Chemicals :

9.1.1 Phosphate buffered saline (PBS)

a. Dissolve 0.58 g NaH_2PO_4, 2.5 g Na_2HPO_4 and 8.5 g NaCl in 1 L ultrapure water.

b. pH should be 7.4±0.2 SU.

c. Autoclave at 121°C and 15 PSI for 15 minutes. *Some autoclaves have only automatic liquid sterilization settings. In these cases, the automatic liquid setting is used.

d. Label with chemical name (not chemical abbreviation or formula), initials, and date of preparation.

9.1.2 Glycerol

a. Autoclave glycerol (Sigma #G6279 or equivalent) at 121°C and 15 psi for 15 minutes, store at room temperature. *Some autoclaves have only automatic liquid sterilization settings. In these cases, the automatic liquid setting is used.

b. Label with chemical name (not chemical abbreviation or formula), initials, and date of preparation.

9.1.3 Streptomycin/ampicillin (strep/amp) stock solution

a. Dissolve 0.15 g ampicillin sodium salt (SIGMA A9518 or equivalent) and 0.15 g streptomycin sulfate (SIGMA S6501 or equivalent) in 100 ml ultrapure water.

b. Filter-sterilize through 0.22 µm filter.

c. Label with chemical name (not chemical abbreviation or formula), initials, and date of preparation.

d. Store at 4°C for up to 6 months or freeze at -20°C in smaller volume aliquots. Use sterile 15 ml falcon tubes to freeze aliquots.

e. Mix well prior to use.

9.1.4 Nalidixic Acid stock solution

a. Dissolve 1 g nalidixic acid sodium salt (Sigma-Aldrich N4382 or equivalent) in 100 ml ultrapure water.

b. Filter-sterilize through 0.22 µm filter.

c. Label with chemical identification, initials, and date of preparation.

d. Store at 4°C for up to 6 months or freeze at -20°C in smaller volume aliquots. Use sterile 15 ml falcon tubes to freeze aliquots.

e. Mix well prior to use.

9.1.5 Four-Molar Magnesium Chloride (4M $MgCl_2$)

a. Dissolve 203.5 g $MgCl_2$ * $6H_2O$ in no more than 100 ml ultrapure water, stir to dissolve, then bring to 250 ml total final volume. (**CAUTION!** Volume of solution increases when $MgCl_2$ is added.)
b. Autoclave 15 min at 121°C and 15 psi or on autoclave preset liquid setting.
c. Label with chemical identification, initials, and date of preparation.
d. Store at 4°C for up to 6 months.

9.1.6 Elution Solution

a. Fill 2 L beaker with 1 L ultrapure water and begin mixing on stir plate.
b. Add 0.1 g sodium hexametaphosphate.
c. Add 0.1 ml Tween 80 (extremely viscous; clear pipet tip as well as possible).
d. Add 0.01 ml Antifoam Y-30.
e. After mixing, pour solution into 22 µm rapid-flow filtration unit.
f. Filter through 22 µm filtration unit using vacuum pump.
g. Label filtrate "Elution Solution" and include the date and initials.
h. Store at room temperature for up to 1 month.
i. 200 ml elution solution is needed to elute one 15S Rexeed filter sample.

9.1.7 Ethanol (EtOH 200 proof)

a. Working concentration is 70%-80% vol/vol EtOH to ultrapure water to clean all work surfaces.

9.1.8 Bleach

a. 10% by volume with tap water –200 ml bleach into 2 liters tap water directly into autoclave pan.
a. For cleaning ultra-filtration tubing.

9.1.9 Sodium thiosulfate ($Na_2S_2O_3$)

a. 10% wt/volume with tap water – must be dissolved on a stir plate, adding sodium thiosulfate gradually – 200 g sodium thiosulfate into 1 liter tap water, then bring to 2 liters final volume in autoclave pan.
a. For neutralizing the bleach in first step of cleaning ultra-filtration tubing.

9.2 Media

9.2.1 Single-strength Tryptic Soy Broth (1X TSB)

a. Dissolve 30 g TSB powder (DIFCO 0370-15-5 or equivalent) into 1 L ultrapure water.
b. Autoclave 15 minutes at 121°C and 15 psi. *Some autoclaves have only automatic liquid sterilization settings. In these cases, the automatic liquid setting is used.
c. Label with chemical name, initials, , and date .
d. Store at 4°C for up to 6 months. Various volumes are needed for overnight and log-phase cultures.

9.2.2 Single-strength Tryptic Soy Broth with 1.5% agar (1X TSB with 1.5% agar or 1X TSA)

a. Dissolve 30 g TSB powder (DIFCO 0370-15-5 or equivalent) and 15 g agar into 1 L ultrapure water.
b. Heat to dissolve while stirring.
c. Autoclave *15 minutes at 121°C and 15 psi. *Some autoclaves have only automatic liquid sterilization settings. In these cases, the automatic liquid setting is used.
d. Use for streak plates and bottom layer of double agar layer method.

9.2.3 Double-strength Tryptic Soy Agar (2X TSA)

a. Dissolve 60 g TSB powder and 18 g agar powder in 1 L ultrapure water in a 2L Erlenmeyer flask.

b. Heat to dissolve while stirring.
c. Autoclave *15 minutes at 121°C and 15 psi. *Some autoclaves have only automatic liquid sterilization settings. In these cases, the automatic liquid setting is used.
d. Keep warm in water bath 45°C-48°C.
e. NOTE: 100 ml of 2X TSA needed for one sample/one coliphage type for SAL sample analysis. The volume of 2X TSA to make is determined by number of samples to be analyzed that day. 2X TSA cannot be kept overnight.

9.2.4 2X Tryptic Soy Agar (2X TSA)

a. Dissolve 60 g TSB powder and 18 g agar powder in 1L ultrapure water (in a 2L Erlenmeyer flask).
b. Heat to dissolve while stirring.
c. Autoclave *15 min at 121°C and 15 psi then swirl to mix. *Some autoclaves have only automatic liquid sterilization settings. In these cases, the automatic liquid setting is used.
d. Keep warm in water bath 45°C-48°C.
e. NOTE: 100 ml of 2X TSA is needed for one sample/one coliphage type for SAL sample analysis
f. Volume of 2X TSA to make is determined by number of samples that day. Solution cannot be kept; it must be discarded at day's end.

Double Agar Layer Media

HARD AGAR LAYER

9.2.5 Single-strength Tryptic Soy Broth with 1.5% agar (1X TSB with 1.5% agar)

a. Add 15 g agar into 1 L 1X TSB.
b. Stir while heating to dissolve.
c. Autoclave *15 min at 121°C and 15 psi. *Some autoclaves have only automatic liquid sterilization settings. In these cases, the automatic liquid setting is used.
d. Place medium in water bath and cool to 48°C.

9.2.6 Tryptic Soy Broth with 1.5% agar and nalidixic acid (TSB w 1.5% agar + nalidixic acid (for *E. coli* CN-13))

a. Add 10 ml of stock nalidixic to autoclaved, cooled 1 L 1X TSB w agar (above), mix to evenly distribute.
b. For 150mm plates pour approximately 40 ml/plate. Allow poured plates to solidify under hood.
c. Store at 4°C for up to 2 weeks.

9.2.7 Tryptic Soy Broth with 1.5% agar and strep/amp (TSB w 1.5% agar + strep/amp (for *E. coli* F_{amp}))

a. Add 10 ml of stock strep/amp to autoclaved, 1 L 1X TSB with agar (above), cooled to approximately 50°C, mix to evenly distribute.
b. Pour approximately 40ml/plate into 150 mm plates. Allow to solidify and refrigerate upside down.
c. Store at 4°C for up to 2 weeks.
d. Since a maximum of 4 blank streak plates are needed on hand, only make enough 1.5% TSA to pour four plates/antibiotic. No need to make 1 L for streak plates. If preparing bottom layer for the double agar layer procedure, more would be needed.

SOFT AGAR LAYER

9.2.8 TSB with 0.7% agar: "Soft agar" for top layer of DAL (double agar layer) procedure

a. Prepare 1X TSB as above, add 7 g agar/liter

b. Heat and stir to dissolve.

c. Autoclave 15 min at 121°C and 15 psi. *Some autoclaves have only automatic liquid sterilization settings. In these cases, the automatic liquid setting is used.

d. ***This solution is never used without its antibiotic added – see below.***

e. ***Only 100 ml is ever made at a time. Divide all above quantities by 10.***

9.2.9 TSB with 0.7% agar and nalidixic acid

a. Add 10 ml stock nalidixic to 1 L autoclaved, cooled TSB w 0.7% agar.

b. Mix to distribute uniformly.

c. Keep molten in 45°C -46°C until use.

d. Dispense 5 ml aliquots into sterile 16x125 mm falcon tubes.

e. ***Only 100 ml is ever made at a time. Divide all above quantities by 10.***

9.2.10 TSB with 0.7% agar and strep/amp

a. Add 10 ml stock strep/amp to 1 L autoclaved, cooled TSB w 0.7% agar.

b. Mix to distribute uniformly.

c. Keep molten in 45°C -46°C until use.

d. Dispense 5 ml aliquots into sterile 16x125 mm falcon tubes.

e. ***Only 100 ml is ever made at a time. Divide all above quantities by 10.***

10 SPECIAL APPARATUS AND MATERIALS:

REFERENCE CULTURES

10.1 Reference Cultures

10.1.1 *E.coli* CN-13 (somatic coliphage host [ATCC®#700609™])- nalidixic acid resistant mutant of *E. coli*

10.1.2 CN-13 somatic coliphage (phi-X174 [ATCC®#13706-B1™])

10.1.3 *E.coli* F_{amp} – E. coli HS (pF_{amp}) R (male-specific coliphage host [ATCC®#700891™])

10.1.4 Male-specific stock coliphage (MS2 [ATCC®#15597-B1™])

STREAK PLATES

10.2 Streak plates

10.2.1 1.5% TSA

a. 30g TSB

b. 15g agar/L

10.2.2 TSA with nalidixic acid (for *E. coli* CN-13)

a. Add 10 ml nalidixic acid to 1L of 1.5% TSA.

10.2.3 TSA with strep/amp (for *E. coli* F_{amp})

a. Add 10 ml strep/amp to 1L of 1.5% TSA.

10.2.4 Unstreaked streak plates can be kept at 4°C for up to two weeks. Several unstreaked plates can be made at one time. Make the volume of streak plate and add antibiotic for only as many plates as can be used within 2 weeks. Pour approximately 40 ml/plate.

10.2.5 After streaking, somatic plate can be kept for 1 month at 4°C. MS-2 plate can be kept for only 1 week. New plates should be streaked on a schedule. Once a month a fresh aliquot of stock of both

host bacteria should be taken from the -80°C freezer and new series of streak plates should be made.

10.2.6 2X Tryptic Soy Agar (2X TSA)

a. See 9.2.4.

CULTURES

10.3 Cultures

10.3.1 Overnight Cultures

a. Overnight cultures are made in 10 mL of stock TSB with 100 µL of antibiotic.

10.3.1.a.1 *E. coli* F_{amp} is grown with streptomycin/ampicillin.

10.3.1.a.2 *E. coli* CN-13 is grown with nalidixic acid.

b. Aseptically inoculate the media + antibiotic with a loop full of refrigerated stock culture from streak plate.

c. Incubate overnight at 37°C.

10.3.2 Mid-Log Cultures

a. Add 5 mL overnight culture to 250 mL TSB (amended with the 2.5 mL of appropriate antibiotic solution).

b. Incubate at 37° for 4 hours.

10.3.2.b.1 Note: 10 mL of mid-log culture is needed to analyze 100 mL of sample.

10.3.2.b.2 Note: volume of the mid-log culture can be adjusted based on the number of samples to be analyzed.

OVERNIGHT CULTURES MATERIALS

10.4 Overnight cultures materials

10.4.1 Small test tube rack

10.4.2 Two sterile 15 ml falcon tubes (in cupboard below water bath on right “Lucy”)

10.4.3 Red and green labeling tape

10.4.4 0.5-5 ml pipette set to 5 ml (adjust volume with knob at top)

10.4.5 100-1000 µL pipette set to 100 µL (adjust volume with knob at top)

10.4.6 1x TSB (in 4°C fridge “Tracy”)

10.4.7 Antibiotics strep/amp and nalidixic acid (top left door shelf of 4°C fridge Tracy in falcon tubes)

10.4.8 Streak plates of F-amp (red stripe) and CN-13 (green stripe) E.coli (in 4°C fridge bottom shelf left)

10.4.9 Vortex

10.4.10 Propane tank and nozzle/lighter combination

DEAD-END HOLLOW FIBER ULTRAFILTRATION MATERIALS

10.5 Dead-end hollow fiber ultrafiltration materials

10.5.1 Asahi Kasei Rexeed single use, high flux, wet dialyzer (15S or 25S) from Dial Medical Supply

10.5.2 Masterflex™ L/S™ Variable-Speed Console Drive with Pump Head

10.5.3 Masterflex® L/S® 25 silicone (platinum) tubing

10.5.4 10L carboy

10.5.5 Glassware (beakers, graduated cylinders)

10.5.6 Magnetic stir plate and stir bars

10.5.7 Micropipettes and tips

10.5.8 0.22 µm pore size disposable rapid-flow filtration units

10.5.9 Vacuum pump and tubing
10.5.10 Weigh boats
10.5.11 Balance
10.5.12 10 mL serological pipettes
10.5.13 Drummond Pipet Aid

SINGLE AGAR LAYER PROCEDURE FOR ENUMERATION OF SOMATIC AND F+ COLIPHAGE MATERIALS

10.6 Single agar layer procedure for enumeration of somatic and F+ coliphage materials
10.6.1 Autoclave
10.6.2 Incubator
10.6.3 Water bath
10.6.4 Balance
10.6.5 Vacuum pump and tubing
10.6.6 Weigh boats
10.6.7 Stirring hotplate and stir bars
10.6.8 Glassware (beakers, graduated cylinders, Pyrex media bottles)
10.6.9 Serological pipets
10.6.10 Drummond pipet aid
10.6.11 Micropipettes and sterile tips
10.6.12 0.22 µm pore size disposable rapid-flow filtration units
10.6.13 Large petri plates (150 mm diameter)
10.6.14 Illuminated colony counter
10.6.15 Temperature flask

11 INSTRUMENT OR METHOD CALIBRATION

8.1 Thermometers must be calibrated annually according to MASSTC-SOP-013 – Thermometer Calibration SOP.
8.2 Pipette calibration is done quarterly using an analytical balance at an external laboratory facility.

12 SAMPLE HANDLING AND STORAGE:

12.1 Grab water samples are collected in sterile vessels (e.g., carboys) and shipped overnight on ice (if needed).
12.2 Samples must be refrigerated and/or kept on ice until processing. Maximum holding time is 48 hours.
12.3 Grab water samples need to be collected in sterile vessels (e.g., carboys) and shipped overnight on ice whenever possible (if shipping is necessary).
12.4 If no concentration is necessary, water samples need to be processed within 48 hours of collection.
12.5 If water samples need to be concentrated using Dead-end hollow fiber ultrafiltration, total sample holding time (from sample collection to enumeration using SAL procedure) is 48 hours (including filtration).
12.6 Review MASSTC-SOP-037 – Sample Collection for proper sampling technique.

13 PROCEDURE – INITIAL HOST AND PHAGE PREPARATION

13.1 Complete initial host and phage preparation before any other analysis can begin, or when new stocks are received.

13.2 Establish frozen bacterial stock cultures.

13.2.1 See MASSTC-EXT-MTH-005 – ATCC Bacterial Culture Guide (2015) and MASSTC-EXT-MTH-006 – ATCC Bacteriology Culture Guide (2021) for initial pellet preparation before streaking.

13.2.2 Streak host bacterial reference cultures onto 1.5% TSA plates with their respective antibiotics.

13.2.3 Incubate streaked plates overnight at 36°C ± 1°C.

13.2.4 Pick an individual colony after incubation, inoculate into 1X TSB with its antibiotic, grow to log phase at 36°C ± 1°C.

13.2.5 In a 250 ml media bottle, combine 25ml of TSB containing 0.25 ml appropriate antibiotic, and add 0.1 - 1.0 ml of overnight host culture.

13.2.6 Incubate at 36°C ± 1°C for approximately 4 hours (until solution is visibly cloudy).

13.2.7 Harvest this broth, mix with sterile 100% glycerol in ratio of 1 part glycerol to 4 parts broth – *e.g., for an approximate 1 ml aliquot, mix 250 µL glycerol to 1000 µL log-phase host)* and aliquot into multiple 2 ml cryovials.

13.2.8 Label with bacterial strain and date; freeze at -80°C for up to one year. (Cultures can be frozen at -20°C, but only for up to two months.)

a. Prior to analyses, thaw vials of stock host cultures and store in 4°C fridge for up to one week. These are the working stock cultures.

13.3 Establish frozen coliphage stock cultures.

13.3.1 Take a loopful of bacterial growth from each plated 1.5% TSA host colony (13.2).

13.3.2 Inoculate two 5 ml tubes of 1X TSB without antibiotics (one tube per host bacterium).

13.3.3 Incubate 16-18 hours at 36°C ± 1°C.

13.3.4 Transfer 1.0 ml from each tube to a flask of 25 -30 ml 1X TSB with antibiotic. Use appropriate antibiotic for the specific host strain. Incubate for 4 hours at 36°C ± 1°C to bring cultures to mid-log phase.

13.3.5 Rehydrate phage stocks by adding 1.0 ml 1X TSB without antibiotic to each phage stock.

13.3.6 Add 1.0 ml of each rehydrated phage to its log phase host.

13.3.7 Incubate at 36°C ± 1°C overnight.

13.3.8 Centrifuge suspensions at 3500 g for 10 minutes to remove bacterial cell debris.

13.3.9 Filter supernatant through 0.22 µ filter. There should be 20-25 ml of supernatant.

13.3.10 Aliquot e.g., approximately 1ml into multiple 2 ml cryovials, freeze at -80°C. (*ATCC stores them in 100% glycerol in same ratio as bacterial cryovials*. See 13.2 above.)

13.4 Establish titer of the undiluted phage cultures.

13.4.1 Set up dilution suspensions.

13.4.1.1 Set up the number of falcon tubes (with date and dilution) based on number of dilutions needed – one tube per dilution. (Somatic needs 5-6, MS2 needs up to 10.)

13.4.1.2 Set up falcon tubes with 9 ml each of 1X TSB without antibiotic.

13.4.1.3 Vortex undiluted thawed phage suspension for approximately 5 seconds until thoroughly mixed. (*These are in the 2 ml cryovials, prepared as described above. For enumeration, below, 500 µl of each phage will be needed for the undiluted phage tubes.)*

13.4.1.4 With sterile pipet, transfer 1.0 ml phage to 9 ml sterile TSB without antibiotic, cap, and vortex or mix thoroughly otherwise.

13.4.1.5 This contains 0.1 (10^{-1}) ml of original suspension.

13.4.1.6 Vortex this dilution for 5 seconds as above.

13.4.1.7 With sterile pipet transfer 1.0 ml of the 10^{-1} to another 9 ml sterile TSB without antibiotic, cap, and vortex. This is 0.01 (10^{-2}) ml of original.

13.4.1.8 Vortex this dilution for 5 seconds as above.

13.4.1.9 With sterile pipet transfer 1.0 ml of 10^{-2} to another 9 ml sterile TSB without antibiotic, cap, and vortex. This is 0.001 (10^{-3}) of original.

13.4.1.10 Vortex this dilution for 5 seconds as above.

13.4.1.11 Repeat this dilution series as needed to achieve plates with countable numbers of plaques.

13.4.2 Enumerate/count the suspensions made (Double-Agar Layer method).

13.4.2.1 On the day before plating, set up overnight cultures of host bacteria so that log-phase host can be prepared on day of plate pouring.

13.4.2.1.1 See section 15 for procedure on set up of overnight cultures.

13.4.2.2 Bottom layer plates of 1.5% TSA with the appropriate antibiotics should already be made and at room temperature.

13.4.2.3 Prepare 0.7% TSA (7 g agar/liter 1X TSB). Make no more than 100 ml (0.7 g agar/100 ml 1X TSB) and split into two 50 ml aliquots.

13.4.2.4 Autoclave, cool in a 46°C water bath, and add antibiotic to each – 0.5 ml antibiotic/50 ml of 0.7% TSA. Keep warm and molten in water bath.

13.4.2.5 Prepare top agar tubes in duplicate (15 ml Falcon tubes) with **5 ml aliquots of 0.7% TSA** from above: number depends on number of serial dilutions required, half with nalidixic acid, half with strep/amp, plus one each for a blank. Two falcon tubes /dilution/phage are required.

13.4.2.6 Label bottoms of plates with which antibiotic, host, dilution (or method blank), date, time.

13.4.2.7 Working through **one** antibiotic at a time, aseptically inoculate two top agar tubes with 100 µl each of log-phase host.

13.4.2.8 Immediately add 500 µl undiluted – or dilution of – phage stock to each.

13.4.2.9 Mix inoculum by rolling tubes briefly in palm of hand.

13.4.2.10 Pour contents into the two bottom agar layer plates labeled with dilution and host as appropriate. Gently rock plate to cover the entire plate with top layer agar.

13.4.2.11 Repeat these steps for each dilution (0.1, 0.01. 0.001, etc.), for however many tubes total/phage; for each method blank add 500 µl TSB instead of phage.

13.4.2.12 Repeat steps 13.5.1.1.7 to 13.5.1.1.10 for the second antibiotic.

13.4.2.13 Let agar solidify, then cover and invert plates; incubate at 36°C ± 1°C for 16-24 hours.

13.4.2.14 Plates MUST be dry before inverting to avoid condensation.

13.4.2.15 See page 27 of MASSTC-EXT-MTH-003 – EPA Method 1643 for diagram of the above steps.

13.4.2.16 Note: Titer of undiluted phage (the point of all this) is calculated using all the DAL plates yielding plaque counts within the range of 1 – 100 PFU/plate for somatic phage and 1-300 PFU/plate for male-specific phage (MS2). If the count on a plate exceeds the upper range but can be counted, it can be included; otherwise, result for that plate is recorded as TNTC.

13.5 Calculate phage percent recovery. See Section 19.

14 PROCEDURE – INITIAL PRECISION RECOVERY (IPR)

14.1 At least one laboratory staff member must successfully complete Initial Precision Recovery before processing any other samples (see Section 21).

14.2 If analyst does not successfully complete IPR within acceptable limits, troubleshoot source of issue and restart at beginning. Do not continue.

15 PROCEDURE – SET UP OVERNIGHT CULTURES

15.1 Set up overnight cultures on the day before intended sample collection.

15.1.1 Put on proper PPE.

15.1.2 Spray down hood counter and surfaces with ethanol solution, then wipe with paper towels to ensure workspace is clean.

15.1.3 Put red tape on one falcon tube, green on the other. Do not cover 10 ml mark.

15.1.4 Label the red tape “F-amp” + the date, green tape “CN-13” + the date, with black ultra-fine point marker.

15.1.5 Put both into a small test tube rack.

15.1.6 Put on clean nitrile gloves.

15.1.7 Put 10 ml of 1x TSB into each falcon tube using the 5 ml pipette.

15.1.8 Discard pipette tip in beaker to right of left-hand water bath.

15.1.9 Vortex strep/amp antibiotic, then add 100 µL of antibiotic to the 10 ml tube with RED tape (F-amp).

15.1.10 Discard pipette tip in beaker to right of left-hand water bath.

15.1.11 Vortex nalidixic acid antibiotic, then add 100 µL of antibiotic to the 10 ml tube with GREEN tape (CN-13).

15.1.12 Discard pipette tip in beaker to right of left-hand water bath.

15.1.13 Cap tightly and vortex these mixtures.

15.1.14 Put the small test tube rack with falcon tubes under the hood.

a. Do not turn on fume hood.

15.1.15 Take out the two streak plates from fridge and put them under the hood upside down (lids should be on the bottom).

a. While fridge is open, return 1x TSB and antibiotics.

15.1.16 Start and light the propane torch and turn it down so that about one-third of an inch of blue flame can be seen at the nozzle.

15.1.17 Sterilize the inoculation loop.

a. Hold the loop with the loop end of wire pointing up.

b. Run the wire end of the loop slowly through the blue part of the flame from its connection to the handle to the loop end so that it all begins to glow.

15.1.18 Rest the inoculation loop on the test tube rack, being careful not to let the sterilized portion touch anything.

15.1.19 Loosen the lid to the F-amp (red tape) tube.

15.1.20 Open the F-amp streak plate, and with the inoculation loop, carefully scrape up one small colony of bacteria onto the loop.

a. **Do not double dip** (Do not place the loop onto the colonies more than once without re-sterilization).

b. If unsure about success of picking up enough colony, re-sterilize and begin again at step 9.15.

15.1.21 Immediately return the streak plate to its lid so it is not exposed to the air any longer than necessary.

15.1.22 Place the loop in the falcon tube with red tape and knock it around gently to dislodge the bacteria into the TSB. Once the loop of bacteria has been cleared, remove it and re-sterilize it per 9.17.

15.1.23 Tightly cap the falcon tube.

15.1.24 Sterilize the loop once again per 9.15, rest it on the test tube rack, and loosen the lid of the tube with green tape (CN-13).

15.1.25 Open the CN-13 streak plate, and with the inoculation loop, carefully scrape up one small colony of bacteria onto the loop.

a. **Do not double dip** (Do not place the loop onto the colonies more than once without sterilization).

15.1.26 Immediately return the streak plate to its lid so it is not exposed to the air any longer than necessary.

15.1.27 Place the loop in the falcon tube with green tape and knock it around gently to dislodge the bacteria into the TSB. Once the loop of bacteria has been cleared, remove it and re-sterilize it per 9.17.

15.1.28 Tightly cap the falcon tubes, then vortex both to mix the bacteria into the TSB, replacing in the test tube rack.

15.1.29 Check that both streak plates are closed, the inoculation loop is sterilized, and both test tubes have TSB, their appropriate antibiotic, and bacterial cultures.

15.1.30 Vortex both falcon tubes for about 5 seconds. Loosen the lids of the two tubes a little bit and put the test tube rack holding them into one of the big incubators (either Leo or Winnie).

15.1.31 Return the streak plates to the 4°C fridge, with lids on the bottom.

15.1.32 Take a paper towel, spray with 80% ethanol, and wipe the hood counter and any other used lab bench surfaces with it.

15.1.33 Spray gloves with 80% ethanol and discard them.

15.1.34 Record the date, time, and analyst's full name in the most current laboratory notebook indicating that this process was completed.

15.1.35 *Preferable/optional: put a piece of tape saying "Loose lids" on it on the outside of the incubator holding the cultures to remind the person who will check on them the following day to be cautious.*

16 PROCEDURE – DEAD-END HOLLOW FIBER ULTRAFILTRATION

16.1 Collect samples per MASSTC-SOP-037 – Sample Collection.

16.2 Perform dead end hollow fiber ultrafiltration on necessary samples.

16.2.1 Prepare and aliquot 200 ml elution solution into 400 ml beakers. Label with the appropriate sample designation.

16.2.2 Prepare influent, effluent, and elution tubing

a. One Influent and elution tubing set is needed per sample. Effluent tubing can be re-used and is kept in the waste container under the bench. Influent line: approximately 0.75 m of Masterflex® L/S® 25 tubing is secured to filter inlet port (top port, orange). A 10 mL serological pipette with tip broken off and cotton removed is secured to the other end of influent line.

b. Elution line: approximately 0.3 m of Masterflex® L/S® 25 tubing secured to tubing outlet port.

16.2.3 Complete filtration procedure (see Figure 1).

a. Secure Rexeed 15S filter to a ring stand, with orange capped end up (blue capped end down).

b. Remove the side port cap at the lower blue end of the filter unit.

c. Attach the effluent tubing for waste discharge to the side port and make sure the other end of the waste tubing is in the waste container.
d. Remove the top cap at the orange end of the filter unit and attach the influent line.
e. Run the influent line tubing through the peristaltic pump and lock the tubing in place.
f. Place the other end of the influent line (after attaching 10 ml serological pipette) into 2000 ml beaker holding the sample.
g. Ensure that the pump is set to pump sample in the correct direction (arrow pointing from sample to filter).
h. Turn the pump on high - this produces a flow rate of approximately 850 mL/minute.
i. Throughout the filtration procedure, keep an eye on the influent tubing to make sure it remains in place.
j. Stop filtering when the first large air bubble reaches the filter.
k. Stop the pump and first remove inlet tubing from top of filter (this releases pressure in the filter). Replace it on the top of the filter, then remove the effluent tubing for waste discharge and replace its cap on side port.
l. Leave the influent line in the sample container

16.2.4 Complete elution procedure (see Figure 2).
a. Position beaker with the elution solution near pump.
b. Attach the elution tubing to the outlet at the bottom end of the filter.
c. Place the opposite end of the elution tubing into the beaker with the elution solution.
d. Take the 10 ml serological pipet connected to the inlet tubing out of the sample container and place it into the beaker with elution solution.
e. Recirculate the elution solution through the filter as follows.
15S: pump through three consecutive 1-minute cycles alternating direction (i.e., clockwise, counter-clockwise) with each cycle.
f. After the elution is complete, remove all tubing and place it in designated area for contaminated glassware, etc.
g. Concentrated water sample is ready for the subsequent culture-based and/or molecular analysis (described in SOP section on single agar layer analysis).

16.2.5 Complete decontamination and clean-up. This is typically done the following day so that plates can be poured on the day samples are filtered.
a. Pour 2 liters tap water into a large autoclave bin.
b. Add 200 ml bleach to this bin.
c. Soak all tubing in 10% v/v bleach solution for 10 minutes. Use syringe to pull bleach solution into tubing.
d. Make 10% w/vol solution of sodium thiosulfate (Section 9.1.9.)
e. Flush bleach out of tubing and transfer tubing to sodium thiosulfate solution (10% w/v). Pull sodium sulfate into each length of tubing with syringe and soak for 10 minutes.
f. Flush sodium thiosulfate out of tubing and transfer tubing to filtered tap water, pulling water into each length of tubing, and soak for 10 minutes.
g. Wrap individual tubing setups in autoclave diapers and autoclave 121°C by choosing the scissors icon on autoclave.

Figure 1. D-HFUF filtration set-up

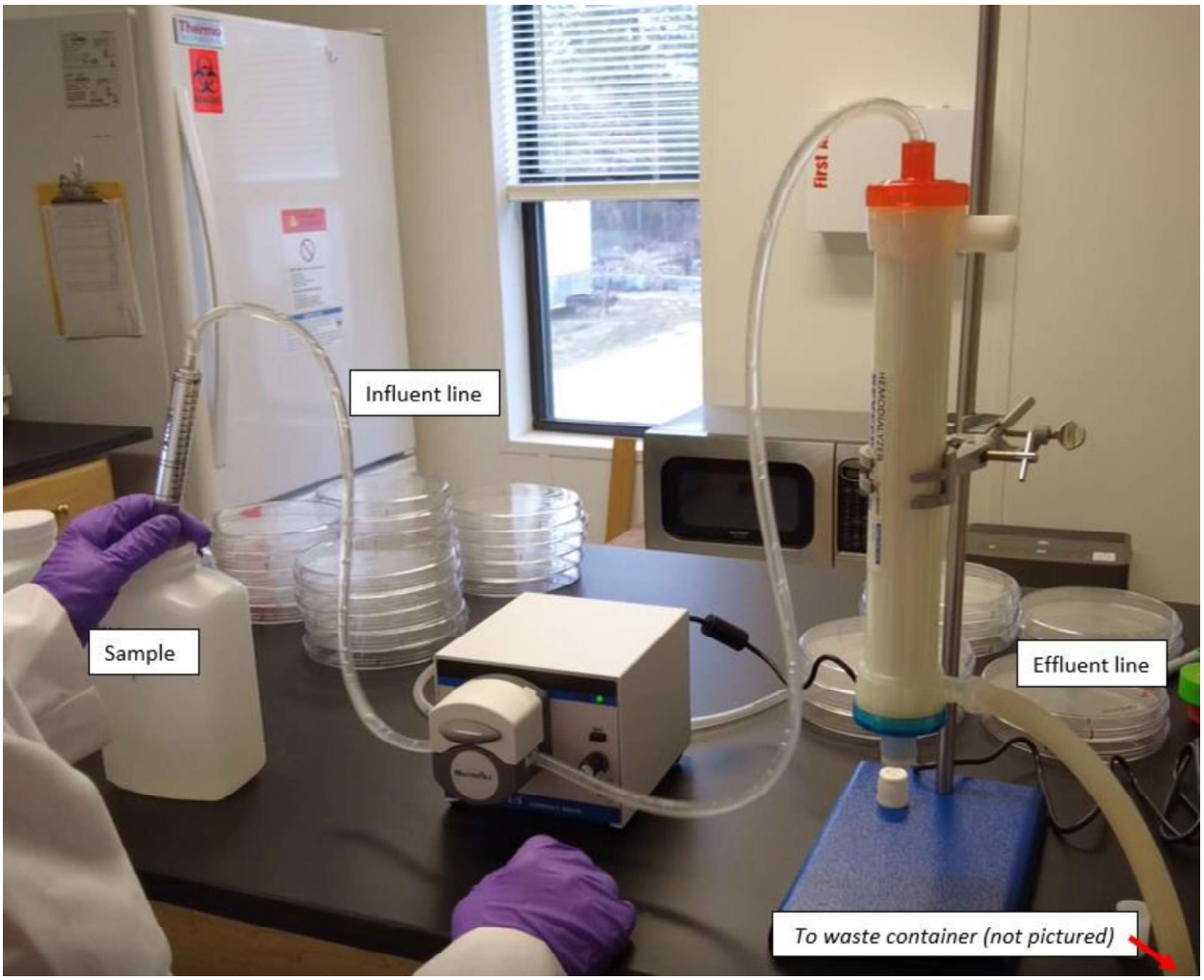
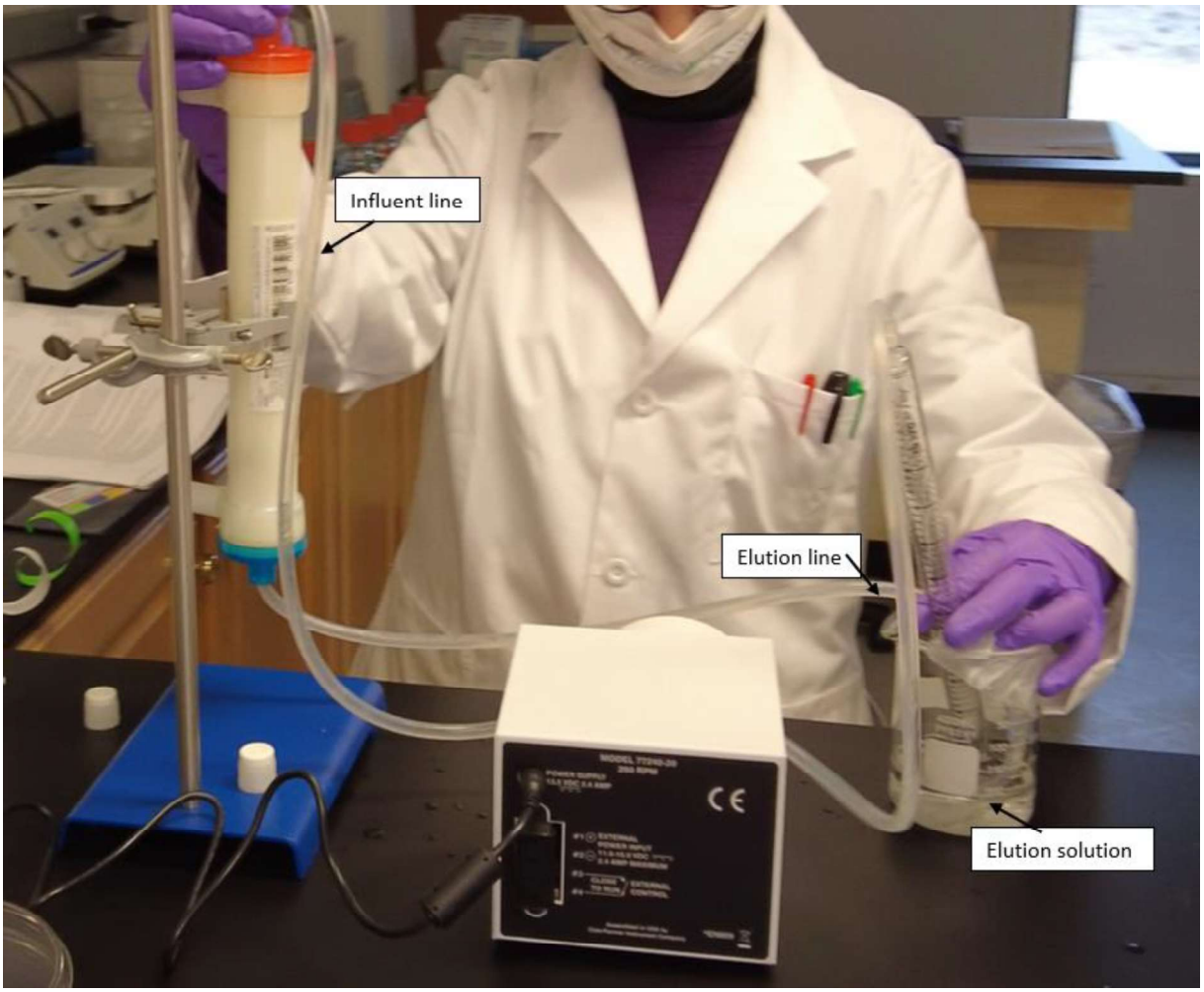


Figure 2. D-HFUF elution set-up



17 PROCEDURE – SINGLE-AGAR LAYER

17.1 Complete single agar layer procedure for enumeration of somatic and F+ coliphage:

17.1.1 See Bacteriophage Plaque Assay (*EPA Method 1602 Section 12)*.

17.1.2 Label all required glassware and plates.

17.1.3 Measure out 100 mL of sample (or as close to this volume as possible after filtration) and pour into 250 mL media bottles. Refrigerate until ready to set up SAL plates. Then proceed as follows.

17.1.4 Place bottle into water bath heated to 45°C to 48°C. Let it warm a bit so that it does not gel the 2X TSA that will be added.

17.1.5 Add 100 mL 2X TSA.

17.1.6 Add 0.5 mL 4M $MgCl_2$.

17.1.7 Add 2 mL appropriate antibiotic (either nalidixic acid or ampicillin/streptomycin, depending on the coliphage type).

17.1.8 Remove sample from water bath.

17.1.9 Add 10 mL mid-log host culture.

17.1.10 Invert the bottle several times to mix.

17.1.11 Pour evenly over 5 plates.

17.1.12 Allow agar to solidify before inverting the plates and incubating overnight at 37°C.

17.2 Complete quality control components per Section 21.

18 PROCEDURE – COUNT PLAQUES

18.1 Count plaques on individual plates, sum all the counts and report as plaque forming units (PFU) per L.

18.2 Note: plaque is a clear zone of lysis in the agar.

18.2.1 Somatic plaques have widely differing morphologies and are typically 2mm-5mm in diameter.

18.2.2 F+ plaques are round and uniform in size, with diameter typically 1mm-3mm.

19 DATA ANALYSIS/CALCULATIONS

19.1 Calculation of phage percent recovery – "Pre-IPR"

19.1.1 Sum the number of PFUs from all dilutions with countable plaques, excluding TNTC and zeros.

19.1.2 Sum the undiluted sample volumes used from all plates with useable counts.

19.1.3 Divide the SUM of PFUs by the SUM of undiluted sample volume (v) to get PFU/ml in the spiking suspension (the original, undiluted).

a. Equation 1: $Phage_{und\ spike} = (PFU_1 + PFU_2 + \ldots PFU_n)/(V_1 + V_2 + \ldots V_n)$

Where:

$Phage_{und\ spike}$ = Phage (PFU/ml) in undiluted spiking suspension

PFU = number of plaque-forming units from plates with counts in ideal range (1-100 for somatic, 1-300 for MS2)

V = volume of undiluted sample on each plate that falls in range of ideal counts

(*from Table 3, page 21 MASSTC-EXT-MTH-002 – EPA Method 1643)*

b. Sample calculation:

Dilution	Number counted on both plates
Undiluted	TNTC, TNTC
0.1	35, 37
0.01	0, 3
0.001	0,0

(35+37+3) / (0.05+0.05+0.005) = 75/0.105 = 714 PFU/ml

Volumes in this example are the 0.5 ml from 0.1 dilution factor = 0.05 of undiluted entered 2x b/c 2 plates had useable counts, and 0.05 is from the 0.01 dilution which was 0.005 of undiluted – here only one volume included b/c only one plate had useable counts. ***These values are examples only.***

19.2 Calculation equation for IPR, OPR, and Matrix Spike

19.2.1 Choose a dilution from the DAL procedure and phage percent recovery that will result in needing a spike volume of between 0.1 and 3.0 ml for a spike concentration target of 100-300 PFU/ml.

a. How to determine spike volume:

Equation 2: $S_{(ml)} = (T \times B)/C$

Where:

S = spike volume (ml)

T = Target number of coliphages per sample (PFU)

B = Number of samples to be spiked (only needed if spiking multiple QC samples)

C = Concentration (PFU/ml) in the dilution used for spiking

b. Example: for IPR (where approximately 100 PFU/100 ml sample is needed)

T = 100 PFU for 100 ml sample
B = four 100 ml samples
C = 714 PFU/ml (from EQ 1 in Initial Host and Phage Preparation)
S = (100 PFU x 4)/714 PFU/ml -= 0.56 ml total

c. Sample Calculation: This is the amount needed for all four 100 ml samples, so each individual sample would be spiked with 0.14 ml of the dilution suspension that will yield target # phages/sample. This calculation must be repeated for each phage. These numbers are only the case for the **EXAMPLE** above.

19.3 Calculation for "True" Spiked Phage (PFU/100 ml) *(See Table 4, p.23 EPA 1643).*

19.3.1 Equation 3: $T_{spiked\ phage} = (Phage_{undiluted\ spike}) \times (V_{spiked\ per\ 100\ ml\ sample})$

Where: $T_{spiked\ phage}$ =Number of spiked phage (PFU/100ml)

$Phage_{undiluted\ spike}$ = Phage PFU/ml in undiluted spiking suspension

$V_{spiked\ per\ 100\ ml\ sample}$ = ml of undiluted spiking suspension per 100 ml sample

19.3.2 Example:

$T_{spiked\ phage}$ = (714 PFU/ml) x 0.14 ml per 100 ml sample = 99.96 PFU/100 ml

19.4 Calculation for Percent Recovery

19.4.1 Equation 4: $R = 100 \times (N_s - N_u)$

Where: R = percent recovery

N_s = Phage (PFU/100 ml) in spiked sample

N_u = Phage (PFU/100 ml in unspiked sample)

$T_{spiked\ phage}$ = True spiked phage (PFU/100 ml) in spiked sample

19.4.2 Example:

N_s = 40, N_u = 3, $T_{spiked\ phage}$ = 100 (99.96)

100 x (40-3)/100 = 37%

19.5 Calculation for PFU count from Single Agar Layer

19.5.1 PFU counts from five plates (comprising one sample) are summed and reported as PFU (somatic or F+ coliphage) per unit volume. For un-concentrated samples, volume is 100 mL; for concentrated samples, unit volume will vary based on the amount of sample filtered.

20 DATA MANAGEMENT/RECORDS MANAGEMENT:

20.1 Ensure that the time and date of cultures, as well as the name of the person who performed the procedure, are recorded promptly, legibly, and in indelible ink in the most current laboratory notebook.

20.2 Record all observations and data according to *MASSTC-SOP-003 – Data and Records Management*.

20.3 Plate counts are recorded in a bound laboratory notebook or project-specific binder. Data is transcribed into project-specific spreadsheets maintained in folder on Sharepoint Cloud storage, available to all team members. The books are kept in a secured location. Only authorized personnel have access to the secured files. Archived data are subject to official retention schedule contained in MASSTC-SOP-003, Records and Archives.

21 QUALITY CONTROL:

INITIAL PRECISION RECOVERY (IPR)

21.1 Complete Initial Precision Recovery. At least one laboratory staff member must successfully complete Initial Precision Recovery before processing any other samples.

21.2 For each phage, four 100 ml ultrapure autoclaved ultrapure water aliquots are needed. These will be spiked (in bulk – so 400 ml spiked at once) with the calculated volumes of the phage dilution determined from the above pre-IPR enumeration calculation. The target spike concentration is 80 PFU per sample.

21.2.1 On Day 1 of IPR:

a. Prepare overnight host cultures - 25 ml of 1X TSB with 0.25 ml of appropriate antibiotic, inoculated with a loop of streak plate host.

b. Incubate at 36 ± 1°C for 16-18 h (then can be held at 4°C until inoculation into log-phase host).

21.2.2 On Day 2 of IPR:

a. Prepare log-phase host.

b. Add 25 ml 1X TSB + 0.25 ml antibiotic + 0.1-1.0 ml overnight host culture to 125 ml media bottle. *For IPR, total 50 ml log-phase host is needed – 10 ml/PBS aliquot plus 10 ml for blank, so two of these bottles/host should be set up.*

c. Incubate approximately 4 hours but be sure to use within 6 hours.

21.3 Prepare and label 5 plates/phage with date, host, phage, antibiotic.

21.4 Set up two water baths, one at approximately 48°C, one at 36±1°C.

21.5 What follows is the description of the IPR procedure, which requires 10 volumetric flasks/phage – four flasks with autoclaved ultrapure water, four flasks with 2X TSA with antibiotic, and two flasks with autoclaved ultrapure water for blanks. Also needed is one temperature flask.

21.5.1 Prepare 500 ml of 2X TSB + agar (30 g TSB + 9 g agar) per phage.

21.5.2 Stir and heat to dissolve (watch carefully), then autoclave.

21.5.3 After cooling to approximately 50°C, add host-specific antibiotic (10 ml/500 ml 2X TSA), mix well, place in approximately 48°C water bath, then transfer 100 ml to each of five 250 -500 ml sterile Erlenmeyer flasks – one set of five per phage, keeping these, with flask weights on, in approximately 48°C water bath to remain molten.

21.5.4 Dispense four 100 ml aliquots of autoclaved ultrapure water + phage dilution (spiked with volume of dilution that was calculated from enumeration above) into four additional 250-500 ml sterile Erlenmeyer flasks.

21.5.5 Dispense 100 ml of autoclaved ultrapure water into a 5^{th} flask as blank.

21.5.6 Dispense 100 ml filtered tap water into a 6^{th} sterile Erlenmeyer (temperature flask). Add thermometer to this flask.

21.5.7 Add 0.5 ml sterile $MgCl_2$ to each autoclaved ultrapure water flask but NOT to temperature flask.

21.5.8 Place autoclaved ultrapure water flasks with phage (and flask weights), blank (no phage added), and temperature flask into 36±1°C water bath for 5 minutes or until the temperature flask just reaches water bath temperature.

21.5.9 Add 10 ml of log-phase host to each autoclaved ultrapure water flask. *(Only one log-phage host set at a time, because host and phage should not be together for more than 10 minutes before plating.)*

21.5.10 Add 10 ml filtered tap water to temperature flask to keep volume of temperature flask the same as volume as the other flasks.

21.5.11 IMMEDIATELY transfer temperature flask and autoclaved ultrapure water flasks to the 48°C water bath.

21.5.12 When temperature flask water reaches approximately 43°C, take sample (one at a time) and IMMEDIATELY add it to a 2X TSA 100 ml Erlenmeyer flask that has its appropriate antibiotic.

21.5.13 Leave this mix together for at least 3 minutes but no more than 10 minutes, then plate.

21.5.14 Pour approximately 40 ml/plate into each of five plates (using 150 mm diameter plates).

21.5.15 Allow to solidify (should only take 10 minutes), allow to cool and harden, cover, invert, and incubate 16-24 hours @ 36 ± 1°C.

21.5.16 Repeat 21.5.8 – 21.5.15 for the second phage/host combination.

21.6 Calculate "True" Spiked Phage (PFU/100 ml) *(See Table 4, page 23 of MASSTC-EXT-MTH-003 EPA Method 1643).*

21.7 Calculate phage percent recovery (see section 19.1).

21.8 Calculate mean percent recovery using the percent recoveries of the four analyses/phage.

21.9 Calculate relative standard deviation (RSD) of the recoveries.

21.10 If analyst's results are not within acceptable limits (Section 19), the analyst must restart at 21.1.

ONGOING PRECISION AND RECOVERY (OPR)

21.11 Complete Ongoing Precision and Recovery (OPR) each time an assay is done - daily.

21.11.1 Prepare one 100 ml reference sample/phage/day. This is the positive control. See MASSTC-EXT-MTH-004 – EPA Method 1602, section 9.2.4.

21.11.2 Spike each with the same volume of phage as was calculated for the IPR (see section 19).

21.11.3 Use the single layer agar method to process each sample.

21.11.4 Count the PFUs from all plates (if too many on a plate = TNTC).

21.11.5 Calculate as: ***Coliphage per 100 ml = Plate1+Plate2+Plate3+Plate4+Plate5***

21.11.6 Compare results with MASSTC-EXT-MTH-003 – EPA Method 1643, Table 1, page 11 for acceptable range of values.

21.12 Lab QA program should compute average percent recovery after five OPRs have been done. Express as percent recovery interval from R-2 standard deviations to R+2 standard deviations /phage.

MATRIX SPIKES

21.13 Complete Matrix Spikes once for every 20 samples.

21.13.1 Prepare two 100 ml samples/phage from the test cell samples.

21.13.2 Spike each with a phage at same concentration as IPR and OPR (previously determined volume).

21.13.3 Prepare two more 100 ml samples (same samples) without a phage spike.

21.14 Calculate percent recovery (R) for the spiked samples using Section 19.4, equation 4.

21.15 Compare results with acceptance criteria in MASSTC-EXT-MTH-003 – EPA Method 1643, Table 2, page 13.

METHOD BLANK

21.16 Complete a Method Blank once daily or each time an assay is done. Include a 100 ml PBS sample with host but NO phage using single layer agar method. *(Not necessary to make 100 ml – only need enough to pour two plates/host. That means 40 ml PBS, 40 ml 2X TSA, 0.2 ml $MgCl_2$, 0.8 ml antibiotic, 4 ml log-phase host.)*

21.17 If method blank has more than 1 PFU, the data shall qualified.

21.18 Method blank should be done after processing samples but before any other OPR.

MEDIA STERILITY CHECK

21.19 Complete a Media Sterility Check on every new batch of medium.

21.19.1 Incubate one plate of each batch of medium at 36°C ± 1 °C for 24 hours.

21.19.2 Observe for growth.

21.19.3 If growth is observed, discard media.

21.20 Negative (*see MASSTC-EXT-MTH-004 – EPA Method 1602, Section 9.2.2 and Section 9.2.6*) and positive (*see MASSTC-EXT-MTH-004 – EPA Method 1602 Section 9.2.4)* controls will be performed each time an assay is run. In case of failed negative or positive control, sample will be reanalyzed if possible and cause of the problem will be determined by trouble-shooting (if needed) and corrected.

MEDIA AND REAGENT STORAGE

21.21 Media and reagents shall be stored as in MASSTC-SOP-041 – Chemical Procurement, Receipt, Storage, and Disposal

22 NONCONFORMANCE AND CORRECTIVE ACTION

22.1 Nonconforming action must be documented as soon as possible after identification.

22.2 Fill out MASSTC-FRM-004 – Nonconforming Work Identification Report.

22.3 Consult MASSTC-SOP-004 – Control of Nonconforming Work for full procedure of control of nonconforming work.

23 INTERNAL AND EXTERNAL REFERENCES:

23.1 MASSTC-SOP-003 – Data and Records Management

23.2 MASSTC-SOP-004 – Control of Nonconforming Work SOP

23.3 MASSTC-SOP-013 – Thermometer Calibration SOP

23.4 MASSTC-SOP-037 – Sample Collection

23.5 MASSTC-EXT-MTH-002 – EPA Method 1642

23.6 MASSTC-EXT-MTH-003 – EPA Method 1643

23.7 MASSTC-EXT-MTH-004 – EPA Method 1602

23.8 MASSTC-EXT-MTH-005 – ATCC Bacterial Culture Guide (2015)

23.9 MASSTC-EXT-MTH-006 – ATCC Bacteriology Culture Guide (2021)

23.10 MASSTC-EXT-MTH-007 – D-EMMD-MEB-009-SOP-01_D-HFUF SOP_final

23.11 MASSTC-EXT-MTH-008 – D-EMMD-MEB-011-SOP-01_SAL 1602_

24 FORMS AND DATA SHEETS:

24.1 MASSTC-FRM-004 – Nonconforming Work Identification Report

24.2 MASSTC-FRM-012 – Point-Check Calibration of Laboratory Thermometers Record Form

Appendix 4

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

LABORATORY QUALITY ASSURANCE PLAN

SOP #: Lab QA Plan **REVISION #: 8.0** **DATE: February 2021**

This and all other DELS-WES SOP and QA documents are available (read/print only) to all DELS-WES employees on the WES server (W:DELS\DELS-QAP\SOPs & QA Docs) or as templates in WinLIMS. It should be noted that the controlled SOP & QA documents are only those viewed on-line on the WES server or in WinLIMS. If this is a printed copy, it is an uncontrolled version and may not be the latest version currently in use.

MassDEP

Massachusetts Department of Environmental Protection
Division of Environmental Laboratory Sciences
Senator William X. Wall Experiment Station
37 Shattuck Street, Lawrence, MA 01843

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LIST OF REVISIONS

Rev. #	Date	Description of Revision	Section #
0	March 1999	None	
1.0	December 2000	Extensive revisions/updates throughout document	
2.0	December 2003	Extensive revisions/updates throughout document	
2.5	August 2004	Extensive revisions/updates throughout document in response to U.S. EPA on-site audit of DEA/WES Laboratories, March 25, 2004	
3.0	December 2005	Extensive revisions/updates throughout document	
3.5	May 2007	Added procedure for monthly testing of germicidal effectiveness of UV lamp used for sanitation. Added clarification regarding the replacement of thermometers that have greater than 1°C correction from the NIST-traceable thermometer. Added maintenance procedures for the Quebec colony counter and dissecting microscope. Added required procedure for autofluorescence testing of each lot of bottles used in enzymatic assays (SM 9223). Added required procedure to check the measured volume accuracy of each lot of disposable pre-sterilized pipettes used in microbiological analyses. Added requirement to label each bottle of media with the date received and date opened. Revised procedure for checking the sterility of dilution/rinse water (i.e., inoculated broth is incubated for 48 hours rather than just 24 hours). Added format used for technical SOPs. Added required procedure for comparison of colony counts between analysts for each bacterial enumerative method performed each month. Added statement indicating that all PTs for all DEA/WES Laboratories must be analyzed in a manner identical to field samples.	7.4.3.2 7.4.5.1 7.4.7.1 & 7.4.7.2 7.4.8.3 7.4.10 7.4.12.2 7.4.14 8.9 10.1 10.2.1
4.0	January 2008	Updated administrative chain-of-command for DEA/WES. Figures 3 through 7 and Table 1 – Added Ann Marie Allen as Acting DEA/WES Deputy Director, and QA Manager. Added labeling information for standard and reagent solutions. Added new procedure for maintaining traceability of standards/reagents to specific analytical QC batches. Updated procedure for analytical records storage and retention. Forms 1 through 11 – Removed from the Laboratory QA Plan and provided links to the separate stand-alone	1.2 & 3.1 3.2.2 & 20.0 7.3.4 7.3.5 18.0 20.0

Rev. #	Date	Description of Revision	Section #
		documents. Other minor revisions/updates throughout document.	
4.1	April 2008	Figures 1 & 2 – Updated organizational charts. Table 8 – Updated List of SOPs	20.0 20.0
5.0	August 2010	Major revisions/updates throughout document Figures 1 & 2 – Updated organizational charts Figures 3 through 7 – Deleted, same information found elsewhere in the document Tables 1 through 6 – Updated Tables 8 through 12 – Updated	 20.0 20.0 20.0 20.0
5.1	October 2012	Figure 1 and 2 – Updated Org Chart Table 1 – Updated Laboratory Equipment List Table 3 – Updated Laboratory Equipment List Table 6 – Changes to equipment calibration procedure Table 7 – Changes to instrument maintenance procedures Table 8 – Added several SOPs Table 9 – Added 2 methods to the Potable Source Water and Not Potable Water Samples Section that were missing in previous versions. Also, sample temperature requirement changed from < 10°C to < 8°C in same section. Other minor revisions/updates throughout document	20.0 20.0 20.0 20.0 20.0 20.0 20.0
5.2	December 2012	Changes throughout document to reflect transition from using EPA Method 524.2 to EPA Method 524.3 Table 5 – Updated for clarity	 20.0
6.0	February 2016	Changes throughout document to reflect staff re-assignments, change of division name from Division of Environmental Analysis (DEA) to Division of Environmental Laboratory Sciences (DELS), and numerous other major changes to update document.	
7.0	February 2019	Numerous changes throughout to update document	
8.0	December 2020	Corrected the acceptance criteria for the balance weight deflection test *Klebsiella pneumoniae* changed to its new taxonomic nomenclature, *Klebsiella variicola* Numerous other changes throughout to update the document related to staff changes, instrumentation changes, and procedural changes related to switching from the previous LIMS (Sample Master) to new LIMS (WinLIMS).	7.4.6.3 7.4.12.7

1.0 INTRODUCTION

1.1 History of the Wall Experiment Station (WES)

The Massachusetts Department of Environmental Protection's (MassDEP's) historic Senator William X. Wall Experiment Station (WES), formerly the Lawrence Experiment Station, was founded in 1887 by the Massachusetts State Board of Health to conduct research leading to the development of practical methods for treating sewage, industrial wastes, and public drinking water supplies. The research conducted at WES laid the foundation for modern methods of wastewater treatment and drinking water purification (1-3). The Station is internationally recognized as one of the first laboratories in the world and the first in North America dedicated to environmental research. In 1975, WES was designated a National Historic Civil Engineering Landmark by the American Society of Civil Engineers.

1.2 Current WES Mission & Programs

WES houses the state environmental reference laboratory for the Commonwealth of Massachusetts. The Station is also designated as the state principal drinking water laboratory as required for primacy under the Safe Drinking Water Act. Massachusetts and other states with federally delegated authority under the Act are required to establish and maintain a state principal laboratory.

A 13,000 square foot laboratory wing, completed in 2011, has been added to the original 22,000-square foot WES facility that was built in 1952 and was completely renovated in 2011. The facility located along the Merrimack River in the heart of Lawrence at 37 Shattuck Street, houses 40 scientists, engineers, and support personnel in two organizational units of the MassDEP – i.e., the Division of Environmental Laboratory Sciences (DELS) under the Bureau of Planning and Evaluation, and the Air Assessment Branch (MassDEP AAB) under the Bureau of Air and Waste. The facility also houses OSHA Consultation Program staff of the Massachusetts Department of Labor Standards (Mass DLS), including the Consultation Program's Industrial Hygiene Laboratory.

The primary mission of WES is to provide technical and laboratory support to all MassDEP programs. WES scientists and engineers analyze water, wastewater, air, soil, hazardous wastes, fish, and other samples for all the important environmental contaminants in support of MassDEP's resource protection, waste prevention, and waste site cleanup programs. Environmental monitoring data generated by WES are used across all MassDEP programs to: 1) Make operational and programmatic decisions; 2) Directly support major criminal and civil enforcement actions; 3) Support investigations which result in the identification of pollution sources which then become the subject of enforcement; and 4) Measure the success and environmental impact of protection efforts.

1.3 Division of Environmental Laboratory Sciences (DELS)

The DELS is organized into three laboratories [i.e., Microbiology (consisting of the Culturable Microbiology & Toxicology Section and the Molecular Microbiology Section), Inorganic Chemistry, and Organic Chemistry (consisting of the GC/LC Section and the MS Section)], the Laboratory Certification Program, and the Laboratory Quality Assurance & Data Program. The approximately 15,000 laboratory analyses performed by DELS annually are largely associated with enforcement cases and special environmental monitoring studies managed by MassDEP regional and program staff. DELS's laboratory and other technical support play a critical role in the Environmental Strike Force's investigation and prosecution of environmental crimes, in water quality assessments conducted by the MassDEP Division of Watershed Management, and in investigations and cleanup of hazardous waste sites and landfills.

DELS is also heavily involved in the development and validation of new analytical methods that better characterize the environment and are more protective of the environment and public health. For example, the Massachusetts Volatile Petroleum Hydrocarbon (VPH) and Extractable Petroleum Hydrocarbon (EPH) Methods were developed by DELS and are now used by Massachusetts and numerous other states and Canadian provinces to more accurately assess petroleum-contaminated water and soil. DELS also conducts basic and applied research dealing with the elucidation of the fate and impacts of contaminants in the ecosystems of Massachusetts and with environmental performance testing of new innovative technologies proposed for use in Massachusetts.

It should be noted that, in Massachusetts, the bulk of environmental samples are collected and analyzed by contractors working for those who are regulated by MassDEP. Unlike state principal laboratories in many other states, MassDEP/DELS-WES does not usually analyze routine compliance monitoring samples from public water supplies and other facilities. Instead, MassDEP/DELS-WES certifies commercial and municipal laboratories to perform the routine compliance analyses and focuses its own analytical capabilities on enforcement and other critical samples. The MassDEP Laboratory Certification Program, which certifies commercial and municipal environmental laboratories, is the largest such program among the New England states. Over 90 laboratories in Massachusetts and other states are certified by DELS-WES for chemical and/or microbiological analyses of potable and/or non-potable water. Through the Laboratory Certification Program, educational outreach, and other activities, DELS-WES plays an important role in ensuring that contractors collecting and analyzing environmental samples are producing high-quality monitoring data.

2.0 PURPOSE OF PLAN

The purpose of this laboratory quality assurance (QA) plan is to document and describe the analytical and QA procedures that the DELS-WES laboratories use to produce scientifically valid and legally defensible data. The QA plan references all the analytical and other standard operating procedures (SOPs) used by the three DELS-WES laboratories, and addresses all elements of the analytical process from sample collection to sample analysis. This QA plan does not apply to the activities of MassDEP AAB or Mass DLS.

3.0 LABORATORY ORGANIZATION AND PERSONNEL RESPONSIBILITIES

3.1 Introduction

This section describes the organization of DELS-WES, and the background and responsibilities of DELS-WES technical personnel. The DELS-WES Director reports to the Director of the MassDEP Office of Research and Standards who, in turn, reports to the Assistant Commissioner for the MassDEP Bureau of Planning and Evaluation (BPE). The Assistant Commissioner for BPE reports to the Deputy Commissioner for Policy and Planning who, in turn, reports directly to the MassDEP Commissioner. The organizational chart for the Division of Environmental Laboratory Sciences (DELS), Wall Experiment Station (WES) is included in Figure 1.

3.2 Personnel

A list of all DELS-WES technical staff with educational background, present specialty, and principal responsibilities is provided in Table 1.

4.0 QUALITY ASSURANCE OBJECTIVES

4.1 Introduction

The DELS-WES QA program has been designed to ensure full compliance with USEPA protocols and procedures. The quality assurance plan for DELS-WES lists the laboratory's standard operating procedures, analytical methodologies and procedures, required sample volumes, and

holding times. The DELS-WES quality assurance plan addresses the required elements in the USEPA *Manual for the Certification of Laboratories Analyzing Drinking Water* (5th edition) as well as those in the USEPA *Quality Assurance Project Plans for Environmental Data Operations* (EPA QA/R-5). The DELS-WES quality assurance plan will be continuously updated to reflect new changes in analytical methodologies that are promulgated by the U.S Environmental Protection Agency.

4.2 Objectives

DELS-WES has developed the following quality assurance objectives as part of its quality assurance program for environmental analytical testing:

4.2.1 Use approved methods for the analysis of drinking water, wastewater, hazardous waste, and air samples, and other matrices.

When requested for health and safety emergency purposes, DELS-WES may run a modified method. In these cases, the modifications are documented in the final report.

When developing new methods or non-standard testing for emerging contaminants, DELS-WES incorporates QC consistent with similar methods which have been approved by the U.S. EPA or other regulatory bodies.

When similar approved methods do not yet exist, DELS-WES incorporates QC consistent with laboratory industry standards or peer-reviewed published literature.

4.2.2 Ensure sample integrity and chain of custody.

4.2.3 Continuously assess the quality of data generated by the laboratory.

4.2.4 Maintain the overall quality of laboratory performance by the use of quality control checks (i.e., spikes, duplicates, blanks, calibration standards, and performance evaluation samples).

4.2.5 Reject and re-evaluate data that fall outside of control limits.

4.2.6 Maintain accurate records of analysis.

4.2.7 Maintain a record of instrument performance as a basis for validating data.

4.2.8 Produce analytical results that can withstand legal scrutiny.

4.2.9 Adhere strictly to the principles of good laboratory practice.

4.2.10 Consistently develop, maintain, update, and use standard operating procedures (SOPs).

4.3 Definitions

4.3.1 Quality Assessment: Procedure for determining the quality of laboratory measurements by use of data from internal and external quality control measures.

4.3.2 Quality Assurance: A definitive plan for laboratory operation that specifies the measures used to produce data of known precision and bias.

4.3.3 Quality Control: Set of measures used within an analytical method to assure that the process is in control.

4.4 Data Qualifications

DELS-WES maintains a wide range of qualifier codes to use when qualifying data. The purpose of the extensive list of codes is to provide data users with sufficient information in a standardized format for data validation decisions.

When a result needs to be qualified, DELS-WES attaches the qualifier code to the result in question no matter if the result is on a field sample or a QC sample. As new technologies are implemented or as new method requirements are published, DELS-WES will add new standardized codes as needed.

A glossary of all DELS-WES active qualifiers is included at the end of every final report. Examples of the Glossary of Qualifier Codes can be found at the end of the final reports included as Report 1 and Report 2.

5.0 SITE SELECTION AND SAMPLING PROCEDURES

Laboratory personnel at the Wall Experiment Station are not directly involved with sampling and site selection. MassDEP personnel working out of the MassDEP four regional offices and the Boston office are directly involved in the selection of sampling sites and analytical parameters. Laboratory analysts are aware of the potential quality control problems that may arise as result of improper selection of sampling and analytical methods. The laboratory encourages communication between laboratory and field personnel to design, develop, and implement correct sampling, preservation, and analysis procedures prior to initiating field activities.

5.1 Sample Collection Procedures

Sampling procedures include charts, maps, sample tables, designated sampling equipment and locations. Procedures also address the frequency of sample collection, the total number of samples to be collected, the number of quality control samples, and site-specific decontamination procedures.

- DELS-WES uses a cloud/web-based LIMS, called WinLIMS, from Quality Systems International Corporation, Ramsey, NJ, to manage samples from prelog through results reporting. When DELS-WES provides a customer with sample containers and coolers prior to collection, a MassDEP sample label is affixed to each sample container.
- For non-prelogged samples, the information required on the sample label are the Client ID, the Client Sample ID, and sufficient information from the Client Sample Description to uniquely identify each sample within the login batch.
- For prelogged samples, the minimum required information is the Client ID, the Client Sample ID, and the WinLIMS generated Login #, Sample ID.
- Other pertinent sample tracking and identification data are recorded by field personnel on the MassDEP Sample Tracking/Chain-of-Custody Form (COC). See Form 1 for an example of a blank COC used for non-prelogged batches, and Form 2 for an example of a COC for a prelogged batch as generated from WinLIMS.

5.2 Sample Containers, Holding Times, and Preservation Procedures

DELS-WES requires the use of the correct, pre-cleaned, single-use sample containers, and preservation reagents as part of a valid sample collection protocol. Samples are collected in the container (plastic or glass) specified in the SOP of the analytical method. The laboratory analyzes samples within the holding times established by the respective U.S. EPA program or by the U.S. EPA-approved analytical method (see Tables 9-12).

5.2.1 Sample Handling Protocol, Wall Experiment Station

The following briefly outlines current procedures for: scheduling sample analysis at DELS-WES; distribution of sample containers and sampling supplies to the regions/programs; and transportation of collected samples back to DELS-WES for analysis. These procedures are updated as necessary as the MassDEP Laboratory Information Management System (LIMS) is more fully integrated into the department's programs.

5.2.2 Scheduling of Sample Analysis

When possible, requests for scheduling of analytical services should be made to DELS-WES well in advance of the sampling event. Requests for analysis should be communicated to the appropriate laboratory supervisor(s). If needed, DELS-WES staff are available to discuss aspects of sampling project plans, but all clients are asked that all formal requests for analyses be made via e-mail. All requests should include: the project name/site where applicable, the number of samples to be collected, the sample analytes, the sample matrix, the projected sampling dates, and if the project is to be charged to any account. DELS-WES laboratory supervisors will confirm via email that their laboratory has the capacity to accept the samples based on the client's projected schedule.

If the projected sampling schedule would result in samples arriving at WES when the sample holding time has elapsed or when the DELS-WES laboratory would not be able to complete the analysis without exceeding the prescribed holding time, DELS-WES will assist the client in transferring the sample(s) to a qualified laboratory for analysis within the holding time.

DELS-WES must identify holding time issues and communicate with its clients as early as possible to allow time for samples to be sent to another laboratory for analysis before the expiration of the sample holding time. Where possible, sample holding time issues are to be identified before sample delivery to DELS-WES so that the samples can be delivered directly to another qualified laboratory. The client has the option to request that DELS-WES continue to process the samples even though the results will be qualified for exceeding holding time. This request needs to be in writing either via email or noted on the COC before the samples are received.

5.2.3 Sampling Supplies

When requested, DELS-WES will provide appropriate pre-cleaned sample containers, disposable sampling equipment, sample preservation reagents, cold-packs, coolers, sample tracking/chain-of-custody forms, and sample labels (see Sample Custody). All necessary supplies will be sent to the regions or other client specified location by MassDEP courier or commercial delivery service. If convenient, supplies can also be picked up at WES by field personnel. Personal protective equipment (PPE) is the responsibility of field personnel.

5.2.4 Sample Custody

DELS-WES is using a Sample Tracking/Chain-of-Custody form (see Form 1 and Form 2 for examples) that has been distributed to the MassDEP Boston and regional offices electronically. The form and the sample labelling procedures described in 5.1, are used to maintain proper sample custody, and must be completed for all sampling events. Careful completion of the form is important.

5.2.5 Sample Transport

Samples can be transported to WES by the sample collector, by courier, or by a commercial overnight delivery service. If overnight delivery service is required, DELS-WES will provide a FEDEX or UPS number and pay for the cost of delivery. Arrangements for sample delivery to DELS-WES should be made during sampling plan development, and confirmed with DELS-WES when requests for analytical services are made. Samples for bacterial analysis, other than for finished drinking water, must be analyzed within 8 hours of sample collection (usually 6-hour field holding time + 2 hours in the laboratory for filtration/preparation and incubation).

5.2.6 Sample Handling Procedures for Environmental Strike Force and Other Enforcement Samples

Environmental Strike Force (ESF) or Other Program/Region - Sample collector needs to:

1. Where the samples are not pre-logged, place labels on bottles and bottle lids, write in the information described in 5.1 on the bottle label and corresponding lid label. Where the samples are prelogged, place preprinted labels on both the bottle and the bottle lid. In both cases, once the bottles are appropriately labeled; place samples in plastic baggie.

2. Place any extra unused labels in a separate baggie in the cooler for return to WES to be reused.

3. Fill the cooler with one large liner size baggie, place the bagged samples, the bagged tags, and plastic packing material to prevent bumping of bottles inside the cooler.

4. If the samples are to be delivered by someone other than the sample collector, use custody seal tape on the outside lid of the cooler to secure the samples inside.

5. Complete the “Relinquished By” section on the Chain-of-custody form.

6. Deliver samples directly to DELS-WES or bring the samples to the MassDEP Boston or regional office mailroom on the day of courier service to Lawrence.

Mail Courier

1. Complete the “Received By” section, including the date and time, on the chain-of-custody form (when samples are received).

2. Deliver the cooler to the Wall Experiment Station (WES).

3. Sign off as “Relinquished By” on the chain-of-custody form, (when samples are turned over to DELS-WES).

4. After DELS-WES removes samples from cooler, take the cooler back to the MassDEP Boston or regional office.

5. Upon return to the MassDEP Boston or regional office, call the Environmental Strike Force or other program to have cooler picked up.

Wall Experiment Station

1. Break the custody seal on the outside lid of cooler if present. If seal has been broken during shipment, please call the ESF or region/program immediately.

2. Measure the temperature of each cooler and record it on the chain-of-custody (COC) form.

   a) A single login batch of samples may arrive in up to 4 different coolers.
   b) At sample receipt, the coolers are arbitrarily designated as cooler 1, 2, 3, or 4.
   c) The temperature of each cooler is taken and recorded on the COC.
   d) Each cooler's designation is recorded on the samples removed from that cooler.

3. Remove the whole baggie full of bagged samples from the cooler.

4. Check samples against the (COC) form; if any discrepancies, call the ESF or region/program immediately. Sign the COC form, including the date and time.

5. Retain the COC form and return the cooler to the courier.

6. Assign a Login # and Samples IDs by entering the batch into WinLIMS. Record the Login # and respective Sample ID's on the COC and complete the COC form.

7. In WinLIMS, record the cooler designation and the cooler temperature for each sample.

8. A representative from each laboratory receiving samples completes the applicable section of the Sample Conditions Review Form (SCRF) which is then filed with the login batch.

9. Scan the completed COC and SCRF, then email the PDF scan to the ESF or region/program.

5.2.7 Sample Containers

When requested, DELS-WES provides new certified pre-cleaned contaminant-free sample containers, either borosilicate glass, high-density polyethylene (HDPE), polypropylene, or sterile HDPE (for microbiological analyses), to all clients. All containers are purchased from vendors and used only once. All clients are instructed to only use new sample containers provided by DELS-WES or directly purchased from vendors specified by DELS-WES. The containers provided are of the appropriate size to obtain enough sample volume for performing the analysis and the associated QC procedures such as duplicates and matrix spikes. As a general rule, the field collector should collect 2.5 times the sample amount needed for the analysis. The collector should adhere to the following field sampling procedures:

1. Some samples must be collected in several containers as only one analysis is performed per container.

2. Samples for volatile organic analysis must be obtained in duplicate glass vials with a Teflon®-faced silicone cap liner and no headspace.

3. Non-volatile organics are collected in amber glass containers with a Teflon-lined cap or polypropylene containers.

4. Microbiological samples must be obtained in sterile containers.

5. Metals are normally collected in HDPE containers as glass can exchange out metals' ions. Mercury in elemental form will pass through the walls of an HDPE container and should be collected in glass.

6. PFAS samples must be collected and shipped under PFAS specific protocols, including being in shipped/transported in a separate PFAS only cooler.

5.2.8 Preservatives

When requested, DELS-WES provides preservatives in separate containers, pre-added to provided sample containers, or single sample ampoules to clients for all sampling events. Preservatives are used to maintain the integrity of target analytes in the sample. A common preservation practice is storage at cold temperature, normally 4 ± 2°C. Storing the samples in a polyethylene ice chest with ice or frozen gel packs provides the necessary cold temperature. Most solid samples require only cooling as a preservative. Water samples are subject to a variety of specific preservation techniques, depending on the target analytes and analytical methods. Preservatives can consist of chemical additives, such as acids or bases added to control pH, ascorbic acid or thiosulfate added to reduce the effect of residual chlorine and other oxidizers, etc.

5.2.9 Holding Times

The holding time allowed until sample analysis is of critical analytical and regulatory importance. Even when samples are correctly preserved and stored, analytes can degrade and be lost if the sample holding time is exceeded. All analytes have required holding times ranging from immediate analysis for dissolved oxygen to up to 6 months for metals (except 28 days for mercury).

6.0 CHAIN-OF-CUSTODY, SAMPLE LOG-IN, SAMPLE CUSTODY, AND SAMPLE DISPOSAL PROCEDURES

6.1 Chain-of-Custody Procedures

6.1.1 For enforcement or other purposes, it is necessary for field personnel to collect samples under the chain-of-custody protocol. MassDEP policy requires that samples be collected under chain of custody if the: 1) Samples are taken as part of an enforcement action; 2) Samples have a potential for litigation; and/or 3) Samples are part of hazardous waste cleanup activities. However, DELS-WES policy requires that ALL samples submitted for analysis to DELS-WES be collected under chain of custody with proper sample transfer sign-off documented on the *WES Sample Tracking/Chain-of-Custody Record* (see Form 1 and Form 2).

6.1.2 MassDEP follows the U.S. EPA's National Enforcement Investigations Center (NEIC) definition of chain of custody. The chain-of-custody documentation procedure begins in the field at the time of collection. A sample is considered under chain of custody if it meets the following criteria: 1) It is in your possession; 2) It is continuously in your view, after being in your possession; 3) It was in your possession and then you locked it up to prevent tampering or it is in a designated secure area; and 4) A chain-of-custody form accompanies the sample from the field to the laboratory.

6.1.3 Each COC is assigned a unique identifier (WinLIMS Login #) where the first 4 digits represent the year the batch record was created. The collection of samples on a given COC are collectively referred to as a Login Batch.

6.2 Sample Log-in Procedures

This section addresses the laboratory's sample receipt, log-in, and custody procedures. Samples collected by field staff are brought to the DELS/WES sample receiving areas for inspection and LIMS log-in. Samples are also delivered by commercial overnight courier services. All incoming samples are evaluated against the following criteria:

6.2.1 Proper sample container

6.2.2 Proper sample volume

6.2.3 Proper sample preservation – i.e., chemical and/or thermal (4 ± 2°C for chemistry samples and 1- 8°C for microbiology samples) preservation.

6.2.4 Correct date and time of sampling

6.2.5 Properly completed sample identification

6.2.6 Properly documented chain-of-custody form

6.2.7 Recording of internal temperature of each sample cooler in the MassDEP Sample Tracking/Chain-of-Custody Form.

6.2.8 Compliance with sample holding times as per EPA methods

6.2.9 If any of the above information is questionable, the DELS-WES staff member logging the samples will contact the respective laboratory supervisor as to whether or not to accept the samples. The supervisor will then immediately notify the DELS-WES Director, Laboratory Quality Assurance (QA) & Data Manager, and the client's project manager and/or point of contact to inform them of any sampling protocol deviations. If the project manager and/or point of contact still wants the samples to be analyzed, they are informed that the data produced will be qualified regarding the sampling deviation in the final analytical report.

Once a sample is in-house (i.e., it has been logged in), if a quality assurance issue is identified regarding that sample, including the potential for a holding time violation, the laboratory supervisor must immediately notify the DELS-WES Director and Laboratory QA & Data Manager. The laboratory supervisor must then telephone the project manager and/or point of contact to inform them of the quality assurance issue. The laboratory supervisor will then follow the telephone call with an email to the project manager and/or point of contact, with read-receipt requested (or equivalent confirmatory documentation), describing the quality assurance issue. The laboratory supervisor must copy the DELS-WES Director and Laboratory QA & Data Manager, and any other appropriate persons on the email and save the email with read-receipt tracking/documentation in the DELS-WES login folder for the sample(s).

For samples from the Watershed Planning Program (WPP) of the Division of Watershed Management (DWM), the laboratory supervisor will send emails regarding any quality assurance issues with read-receipt requested to the WPP QA Officer and copy the DELS-WES Director and Laboratory QA & Data Manager, the Director of the MassDEP Office of Research and Standards, BPE Assistant Commissioner, DWM

Director, and WPP Director. The emails will describe the nature of the QA issue and be saved with read-receipt tracking/documentation in the DELS-WES Login folder for the sample(s).

SAMPLE CONDITIONS THAT CAN RESULT IN SAMPLE REJECTION OR DATA QUALIFICATION

WES Laboratory	Applicable Analytical Method	Conditions where sample rejection applies	Conditions where data qualification applies
Microbiology Laboratory	All	Improper sample container (not sterile) or leaking/broken container	Holding time exceeded Insufficient sample volume or not enough head space
	All drinking water methods	Failure to properly dechlorinate chlorinated finished drinking water samples	
	All non-potable water methods	Failure to dechlorinate chlorinated wastewater effluent samples	
	All source water and non-potable water methods		Failure to properly cool the samples to 1-8°C during shipment.
Inorganic Chemistry Laboratory	All	Improper preservation (e.g., wrong acid, acid when there should not be, cyanide samples not preserved with hydroxide, etc.); insufficient sample quantity.	Holding time exceeded; insufficient sample quantity; containers and/or caps broken or leaking; caps loose; sample collected in improper container.
Organic Chemistry Laboratory GC & HPLC Section	All	Samples collected in plastic containers, except for the collection of tissue samples where HTPE and PP containers free of target are used.	Holding time exceeded
		Container caps are broken or loose	
		Insufficient volume or sample quantity	
Organic Chemistry Laboratory MS Section	Volatiles (VOCs): EPA 524.3, EPA 8260D	Vials not tightly capped	Holding times exceeded.
		Samples not collected in VOA borosilicate glass vials; for EPA 524.3, not collected in amber VOA vials with preservative	
		Air bubble in vial larger than a pea size bubble (6mm in	

SAMPLE CONDITIONS THAT CAN RESULT IN SAMPLE REJECTION OR DATA QUALIFICATION

WES Laboratory	Applicable Analytical Method	Conditions where sample rejection applies	Conditions where data qualification applies
		diameter)	
	THM analysis: EPA 524.3	Sample not preserved with dechlorinating agent (ascorbic acid)	
	Soils/sediments EPA 8260D	Sample not preserved in the field with methanol or frozen at time of collection until delivered to lab	
	Semivolatiles (SVOCs): EPA 525.2, EPA 8270E	Samples received in non-amber containers	Holding times exceeded
		Samples received in plastic containers except for the collection of tissue samples where HTPE and PP containers free of target analytes are used.	
	1,4-Dioxane: EPA 522	Samples not preserved with dechlorinating agent (sodium sulfite) and to pH < 4 with sodium bisulfate; samples received in non-amber containers.	
	EPA 537	Sample collected in a container other than polypropylene.	Not collecting FRB from each site. Sample collected without preservative. Insufficient volume to conduct LFM and/or DUP or LFMDup.
	EPA 537.1 and 533	Sample collected in a container other than polypropylene.	Not collecting FRB from each site. Sample collected without preservative. Insufficient volume to conduct LFM and/or DUP or LFMDup.

6.2.10 After the samples have been evaluated for the correct information, the samples are logged into the MassDEP/WES LIMS. Sample login is not assigned to one person; rather, any available trained laboratory staff member can log in samples. Usually, however, laboratory personnel check the physical conditions of the samples and administrative personnel enter the data into the LIMS. A unique sample identification number (Sample ID) is assigned to each sample in the LIMS. The Login # and the Sample ID is entered on the MassDEP Sample Tracking/Chain-of-Custody Form. The first two digits of the Sample ID represent the year the sample record was created.

The sample is then placed in the sample custody refrigerator and eventually withdrawn for analysis by laboratory staff.

6.3 Sample Custody Protocol

Access to the WES building is strictly limited to authorized personnel. Non-DEP visitors must sign in at the WES front office and be escorted by a WES employee during the entire visit. All samples must be properly identified with a sample label and with the Sample Tracking/Chain-of-Custody Form containing all of the required information regarding the sample. To establish tracking control and documentation of samples in the laboratory, the following procedures have been established:

6.3.1 Incoming samples are received by laboratory personnel who indicate receipt by signing and dating the Sample Tracking/Chain-of-Custody Form. Laboratory personnel receiving the samples are responsible for noting the condition of the samples, including temperature at receipt and any other preservation used and then completing the SCRF. From information on the chain-of-custody form, laboratory personnel mark those samples to be disposed 30 days after the analysis report has been sent out and those to be held until further notice depending on the client's requirements. The 30-day pre-approval notice meets the written permission requirement described in Section 6.3.4 below.

6.3.2 After the samples have been logged into the LIMS, they are either taken directly to the laboratory that will perform the analysis or immediately placed in refrigerators or freezers (for fish tissue samples) in the DELS-WES Sample Storage Laboratory. All refrigerators and freezers used to store environmental field samples are located in laboratory areas that have controlled card access and only selected laboratory employees have access to these areas; the WES security system electronically records the following information each time a WES employee enters a card accessed laboratory/room: Employee ID, date, and time.

6.3.3 Laboratory personnel are responsible for the care and custody of samples handed to them or that they remove from a sample custody refrigerator. The samples shall be handled by a minimum number of laboratory staff members. Within each laboratory, chain-of-custody samples must be stored in a refrigerator within an accessed controlled room except when being tested. All samples are stored at all times under conditions specified in their respective analytical methods, and are isolated from other samples, reagents, and standards that could cross-contaminate them.

6.3.4 Once the analyses have been completed, and sample data verified and reported, the unused portion of the chain-of-custody sample is kept in an accessed controlled refrigerator or freezer in the laboratory that analyzed the sample or in the DELS-WES Sample Storage Laboratory until written permission for disposal is obtained from the MassDEP region or program that submitted the sample(s). At that time, if the sample is hazardous, it will be transferred to the WES Hazardous Waste Storage Room which is kept locked at all times (note: the DELS-WES Director, Laboratory QA & Data Manager, and laboratory supervisors and analysts have card key access to this room). Chain-of-custody samples shall remain locked in this room until they are removed for disposal by a hazardous waste management contractor.

6.4 Laboratory Sample Control and Tracking Procedures

The documentation of a laboratory's sample control and tracking procedure is important in order to:

- Trace a test sample from the field to the final results.
- Describe the sampling and analytical procedures used by the laboratory.
- Support the laboratory's level of detection.
- Support the laboratory's quality assurance practices.

6.4.1 When the samples are delivered to the respective DELS-WES laboratory, laboratory personnel will check the samples for: requested analyses, correct sample volume and collection, proper preservation procedures, proper sample container, sample source, collector, and properly documented sample label. If the samples are deficient in any of the required information, the laboratory supervisor has the final discretion in accepting the samples for analysis.

6.4.2 When the analytical work on the samples has begun, instrument printouts, and/or sample analysis bench sheets become the documentary linkage between analysis and final data reports. All hand entered information in these documents are made using black ink and dated. The analyst initials the top of the first page of each document or report or document/report subsection where applicable. When analyses are completed, any non-instrument reported final analytical data are transferred to the sample bench sheet. Information from the sample bench sheet or from the instrument report are then entered into the LIMS for the generation of the final analytical report directly from the LIMS.

6.4.3 After the analytical results have been reported, the laboratory report becomes part of the WES record system and is stored for 10 years.

6.5 Protocol for Disposal of Hazardous Waste Samples

It is the policy of DELS-WES that all hazardous waste samples be disposed of properly. The laboratory uses the following protocol for the disposal of hazardous waste materials and chain-of-custody hazardous waste samples.

6.5.1 A licensed hazardous waste management contractor is used for the safe, legal, and proper disposal of hazardous materials generated by the laboratory. DELS-WES maintains a hazardous waste facility for the storage of hazardous waste materials. The facility is locked at all times and is accessible only to authorized personnel via card access. The facility has a continuously operating ventilation system that exhausts any vapors to the outside. The types of materials stored in the room include waste oils, caustic and corrosive liquids, waste solvents, PCBs, metals, and laboratory reference standards.

6.5.2 Materials placed in the hazardous waste facility are documented in the Sample and Hazardous Waste Inventory found at W:\WES All\EMS-Sample & Waste Mgt\Sample & Haz-Inf Waste Inventory & Disposal\Disposed\ HW Room Inventory 2020.xlsx.

Information that must be included on the manifest each time samples are deposited in the facility are: the date, container number, size of the container, the sample matrix, contents of the container and its markings, and name of the person depositing the materials.

6.5.3 Hazardous waste samples with high concentrations of toxicants (e.g., PCBs in concentrations greater than 50 ppm) must be placed in metal cans, packed with

vermiculite, and sealed. The outside of the container is then marked with the identified substance and its concentration.

6.5.4 Waste organic solvents are placed in a drum supplied by the hazardous waste management contractor. Waste reference standards are placed in specially marked containers dedicated for analytical standards. Inorganic wastes are placed into a separate 55-gallon drum supplied by the hazardous waste management contractor. The first person to add waste to a drum must write the date on the label to indicate the date when accumulation began. The drums are labeled with the types of waste chemicals contained and placed in the hazardous waste storage room. Great care is taken to avoid mixing reference standards and waste solvents due to current disposal regulations.

6.5.5 Bacteriological plates or broths that contain active cultures are autoclaved for 30 minutes prior to their disposal.

7.0 INSTRUMENT CALIBRATION PROCEDURES

7.1 Instruments

Numerous laboratory instruments are available in the DELS-WES laboratories to perform complex analytical tests required by the MassDEP regions and programs and to maintain USEPA certification as a state principal laboratory under the Safe Drinking Water Act. The laboratory instruments available at DELS-WES are listed in Tables 2 through 5.

7.2 Specialized Glassware

DELS-WES uses specialized glassware for designated analytical tests. DELS-WES uses only "Class A" volumetric flasks, pipettes, and burettes for preparing and delivering standards and reagents as well as for making dilutions in all inorganic and organic chemical analyses. "Class A" glassware (marked "A" or with other equivalent identification) meets National Institute of Standards and Technology specifications. Volumetric syringes are used in preparing dilutions and standards for chromatographic analyses. In order to maintain its calibration, "Class A" volumetric glassware is never placed in a drying oven. If volumetric glassware containing standards is stored in a refrigerator, it is first brought to room temperature for at least 30 minutes prior to being used in preparing calibration standards. All steps in the analyses of organic pollutants are performed using only borosilicate glassware in order to eliminate potential contamination from plastics. Labware used for some steps in inorganic and metals analyses may be of borosilicate glass, or Nalgene® or PMP (Polymethylpentene) or other plastic as long as the material is compatible with the analysis. However, most plastic labware is not “Class A” and must therefore only be used where appropriate.

7.2.1 Glassware Cleaning Procedures. Clean labware is important to laboratory operations as well as an integral part of a quality assurance program. Each DELS-WES laboratory has specific glassware and plastic-ware cleaning procedures for its analytical determinations.

7.3 Calibration Procedures

7.3.1 The laboratory staff calibrates instruments or equipment using certified reference standards traceable to NIST standard reference materials (SRMs) or calibration is performed externally by agencies on service contracts. Calibration procedures are specific for the matrix, analytical method, detection limits, and instrument. Generally, the calibration procedures are specified in the respective EPA, *Standard Methods*, or ASTM methodology.

In all cases, method-specific calibration requirements are followed (see method SOPs). If a method does not specify calibration requirements, a daily initial calibration curve is run with at least a blank and three standards (i.e., assuming that the analytical instrument used accepts three calibration points). The lowest initial calibration standard used is always less than the MCL when analyzing drinking water and is always set at the minimum reporting level or lower. Sample data associated with unacceptable initial calibrations are never reported. Once initial calibration has been completed, adjustment of the calibration curve zero point (i.e., re-zeroing or auto-zeroing) is prohibited.

Initial instrument calibrations are verified using second source standards or using method-specific calibration acceptance criteria. Continuing calibration verifications (CCVs) are used to confirm the validity of initial calibrations according to method-specific criteria. Method-specific initial and continuing calibration criteria are achieved or method-specific corrective action is taken promptly and documented.

For each analysis, a CCV is analyzed immediately after the initial calibration (or calibration check), at the end of each analytical batch, and periodically throughout the batch (e.g., after every 10 samples). For GC/MS methods, the CCV is run at the beginning of each day and prior to any overnight runs. Whenever a CCV fails to meet the method-specific criteria, all analyses are stopped and an initial multipoint method-specific calibration is performed. If the same analyte fails the CCV during GC/MS analysis, the data for that analyte are qualified in the final analysis report.

7.3.2 As part of the calibration protocol for laboratory instrumentation, the method detection limit (MDL) is experimentally determined for each method and sample matrix for which it is required (note: determination of the MDL is no longer required for many new EPA analytical methods). The MDL is referenced in Section 40, Code of Federal Regulations, Part 136, Appendix B. MDLs for laboratory analytical procedures are determined based on the frequency required by the specific analytical methods. If an analytical method does not specify a frequency for determining MDLs, a new MDL is only determined if environmental field samples are received or will be received for testing and the current MDL is more than 1 year old. It should be noted, however, that an MDL-check spiked sample is generally analyzed with PT samples for each drinking water analytical method. If unacceptable results are obtained, the laboratory must stop the analytical process to identify, correct, and document the deficiencies.

7.3.3 DELS-WES utilizes both physical and chemical calibration standards as part of its analytical procedures. Physical standards are used for calibrating analytical balances with ASTM Class 1 weights, and calibrating laboratory ovens and refrigerators with National Institute of Standards and Technology certified thermometers. Chemical calibration standards are used in the preparation of stock, intermediate, and working standards. Chemicals or reagents used in the preparation of calibration standards or analyses are analytical reagent (AR) grade or better in quality. All standard containers are labeled with expiration date. The laboratory uses ultra-high purity grade and lab grade gases for its analytical instrumentation.

7.3.4 Calibration standards used in inorganic, volatile organic, and semi-volatile organic chemical quantitation are prepared and stored as specified in the respective *Standard Method* or EPA analytical method (see method SOPs). All calibration standards and reagent solutions are labeled with the name of the solution, preparation date, expiration date, and initials of preparer.

7.3.5 For all calibration standards and other analytical reagents for a specific analytical method, the following information, as appropriate, is recorded in a standard/reagent preparation form specific to that method (**note:** in order to trace a set of prepared calibration standards/analytical reagents to a specific analytical run for a given method, the LIMS WL batch number for that run is recorded on the standard/reagent preparation form and the form is then also scanned and saved with the electronic raw data file for that WL batch number):

- Name of the standard/reagent or names of analytes contained in a standard/reagent mixture
- Date of standard/reagent preparation
- Date of standard/reagent expiration
- Standard/reagent lot numbers
- Parent solution numbers
- Weight or volume of standard/reagent
- Concentration of parent solution
- Aliquot volume of standard/reagent taken
- Dilution volume of standard/reagent
- Final concentration of standard/reagent
- Name or initials of analyst preparing standard/reagent

7.3.6 Whenever a new set of calibration standards is prepared, a standard calibration curve must be performed. The purpose of preparing a calibration curve is to determine the accuracy and correctness of the concentrations to their assigned values. The procedure for preparing a calibration curve must be performed as follows:

7.3.6.1 Calibration curve is prepared using at least three different concentrations of standards. Some EPA procedures specify that five or more concentrations must be used for calibration. A method blank must also be prepared.

7.3.6.2 Calibration curve is determined within the instrument's working or linear range. Sample concentrations outside of the calibration curve must be diluted and reanalyzed. In some cases, a calibration standard at or near the concentration of the analyte(s) of interest is prepared and run to quantify these analyte(s). For metal analyses, sample results can be reported from projected parts of the curve beyond the highest calibration standard up to 90% of the upper limit of the Linear Dynamic Range (LDR).

7.3.6.3 Calibration curve is checked against a known certified reference standard for accuracy. The reference standard accuracy is method-dependent.

7.3.6.4 Calibration curve must have a linear regression correlation coefficient value, r, of 0.995 (or the method-prescribed value) or greater.

7.3.6.5 Calibration procedure is performed under the same testing, sampling, and instrumental conditions as the actual measurement process.

7.3.6.6 Instrumentation or calibration curves that fail the calibration procedure are removed from the analytical process. Instrumentation that fails calibration must be repaired or re-calibrated prior to being placed back online. Calibration curves that fall out of conformance to EPA known values must be prepared again and re-evaluated. Instruments and other laboratory equipment are calibrated according to instrument specifications or methodology. Factors governing frequency of calibration are the specific analytical methods, instrument and calibration stability, sample matrices, and analyst experience. Consult each respective laboratory standard operating procedure for specific calibration procedures.

7.4 Quality Control for Equipment and Supplies used in the Microbiology Laboratory – Including Equipment Maintenance Procedures

7.4.1 Incubators

7.4.1.1 Temperatures of air incubators are checked twice each day with readings taken at least four hours apart. The date, time, temperature, and initials of the analyst performing each check must be documented. Incubators used at 35°C must maintain temperature to within 0.5°C. The thermometers must be placed in liquid.

7.4.1.2 Temperatures of water baths are checked twice each day when in use with readings taken at least four hours apart. The date, time, temperature, and initials of the analyst performing each check must be recorded. Water baths used at 44.5°C must maintain temperature to within 0.2°C. The thermometer used in the water bath must be graduated in increments of 0.1°C or smaller.

7.4.2 Refrigerators

Temperatures of refrigerators are checked once each day. Refrigerators must maintain a temperature of 1 to 6°C. The date, temperature, and initials of the analyst performing each check must be documented. Thermometers used in the refrigerators must be graduated in increments of 1°C or smaller. Thermometers must be placed in liquid.

7.4.3 UV Lamp (254 nm) Used for Sanitation

7.4.3.1 Lamps used for sanitation of the filtration funnels must be disconnected monthly and cleaned with ethanol.

7.4.3.2 The effectiveness of the germicidal unit must be tested monthly. The date and initials of the analyst performing each check must be documented. The effectiveness can be tested using the HPC spread plate method. The procedure is as follows:

1. Prepare 2 spread plates containing 200-300 colony-forming-units (CFU) of *E. coli* on plate count agar or a non-selective agar.

2. Expose one plate to the UV light for a minimum of 3 minutes and do not expose the second plate.

3. Incubate the plates at 35ºC for 48 hours.

4. Count colonies on both plates and record results in the monthly QC Form.

5. If the exposed plate does not show a 99% reduction in colonies relative to the unexposed plate, replace the UV bulb and repeat the effectiveness test.

7.4.4 Autoclave

7.4.4.1 The temperature of each autoclave cycle must be recorded from a certified maximum-temperature registering thermometer or from a calibrated autoclave internal thermometer.

7.4.4.2 The date, total time, sterilization time, sterilization temperature, contents, and analyst initials must be recorded for each autoclave cycle.

7.4.4.3 Spore ampoules must be analyzed each week the autoclave is used to ensure proper operation of the autoclave. Refer to the standard operating procedure for the autoclave for the use of these ampoules.

7.4.4.4 The autoclave must complete a cycle within 45 minutes when a sterilization time of 15 minutes is used.

7.4.4.5 Sterilization times to be used are as follows:

Membrane Filter Assemblies	15 minutes
Individual Glassware	15 minutes
Buffered Rinse Water	45 minutes
Media	15 minutes

7.4.4.6 The autoclave timer must be checked quarterly against a stopwatch.

7.4.4.7 The most recent autoclave service report must be placed on file and available for inspection.

7.4.5 Thermometers

7.4.5.1 Thermometers used within the laboratory must be calibrated annually at the temperature used against a thermometer traceable to NIST. The NIST-traceable thermometer must be graduated in increments equal to or smaller than the thermometer being checked. If a thermometer differs by more than 1°C from the reference thermometer, it must be replaced except as noted below. The maximum-temperature registering thermometer used in the autoclave must be measured against the NIST-traceable thermometer by

placing both thermometers in a boiling water bath. The maximum-temperature registering thermometer is calibrated by an external vendor every 5 years.

The following exception will be made to the rule that all thermometers that have a correction factor that exceeds 1°C will be discarded/replaced:

1. Built-in digital thermometers on ultra-low temperature freezers (-80 to -100 degrees Celsius) used for daily readings that have a correction factor exceeding 1 degree Celsius will be deemed acceptable. However, the calibration against a NIST-traceable thermometer will now have to be performed on a quarterly basis instead of the traditional annual calibration.

2. Any thermometer used for readings of autoclaves (121 degrees Celsius) that has a correction factor exceeding 1 degree Celsius will be deemed acceptable. The calibration against a NIST-traceable thermometer will remain on a quarterly basis.

7.4.5.2 Each thermometer must have a tag which identifies the thermometer, the date the thermometer was last calibrated against a NIST-traceable thermometer, and the correction factor.

7.4.6 Balances

7.4.6.1 Balances must be kept clean and free of corrosion.

7.4.6.2 The balance must be calibrated monthly with ASTM Class 1 weights. The results of these checks must be recorded.

7.4.6.3 A weight deflection test must be performed on the balance monthly. The balance must have a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg for a load of 10 g or less. The results of these checks must be recorded.

7.4.6.4 The balance must be serviced at least annually by an outside contractor and the most recent service date must be affixed to the balance.

7.4.7 Colony Counter & Microscopes

7.4.7.1 DELS-WES uses a Leica Quebec Colony Counter, model # 3325, for counting Heterotrophic Plate Count (HPC) plates. The maintenance procedures for this instrument are as follow:

1. The counting plate is cleaned prior to each use. To clean the counting plate, remove the screw on the left side of the instrument. Swing the magnification lens aside and open the black front section attached to the lower hinge. The counting plate is held in place with four screws attached to the back of the front panel. Loosen the screws and slip the plate out of the retainer. Clean the plate with a diluted mild detergent. Do not use an abrasive cleaner. Dry the counting plate with a lint-free

cloth. Place the counting plate back into the retainer and tighten the four screws.

2. The bulb is changed as needed, replaced with a standard 40W soft white incandescent bulb. To change the bulb, unplug the instrument, remove the screws on the face of the instrument, and swing the magnification lens aside. Open the black front section attached to the lower hinge. Unscrew the malfunctioning bulb and replace with a standard 40W soft white incandescent bulb.

7.4.7.2 DELS-WES uses a Spencer or the VWR dissecting binocular microscope for counting colonies on membrane filter plates. A fluorescent light is attached to the microscope and must be turned on when the microscope is in use. The fluorescent light on time is recorded in a log sheet. The microscope is cleaned prior to each use. The lenses are wiped with lens papers and the body of the microscope is wiped with a lint-free cloth.

7.4.8 Sample Containers

7.4.8.1 Each lot of sample containers received must be tested for sterility. The following procedure is used:

1. 25 mL of tryptic soy broth must be placed into one sample container.

2. The sample container is rotated to ensure the broth comes into contact with all surfaces.

3. The container is incubated for 48 hours at 35 ± 0.5°C.

4. After incubation, the broth is checked for turbidity indicating growth at both 24 and 48 hours.

5. Batches of containers for which the sterility check indicates growth are to be returned to the supplier.

7.4.8.2 The calibration of the volume measurements on each lot of sample containers received must be verified by taring the sample container, filling it to the 100-mL mark, and ensuring the weight of water in the container is 100 ± 2.5 grams.

An alternative procedure to determine the accuracy of the volume measurements is to fill a 100-mL Class A volumetric flask (or a pre-calibrated 100-mL graduated cylinder) to the 100-mL mark. The water from this flask is then poured into the sample container. The sample container must measure the volume to be 100 ± 2.5 mL.

7.4.8.3 One bottle from each lot used for enzymatic tests (e.g., SM 9223), which generate a fluorescent response, is checked against a 365-366-nm ultraviolet light source for autofluorescence. Results are recorded in the Microbiology Laboratory QC Sample Bottle Sterility/Accuracy/autofluorescence Check Form. Do not use the bottles from the lot if they fluoresce.

7.4.9 Membrane Filters

7.4.9.1 The membrane filters used by the laboratory must be certified by the manufacturer to be suitable for microbiological analyses. Filters are purchased pre-sterilized.

7.4.9.2 Filters must be 47 mm in diameter and 0.45-μm pore size.

7.4.9.3 Filters that display inhibition or promotion of growth along the grid marks must be returned to the manufacturer.

7.4.9.4 The lot numbers and date of receipt must be recorded for each batch of filters.

7.4.9.5 Each lot of filters received must be tested for sterility. The following procedure is used:

1. One filter from the lot is placed in 25 mL of sterile tryptic soy broth in a sterile container.

2. The container is shaken to ensure the broth comes into contact with the entire membrane surface.

3. The container is incubated for 48 hours at 35 ± 0.5ºC.

4. After incubation, the broth is checked for turbidity indicating growth at both 24 and 48 hours.

5. Membrane filter lots for which the sterility check indicates growth are returned to the supplier.

7.4.10 Pipettes

The laboratory uses disposable plastic pipettes that are purchased pre-sterilized from the manufacturer. Open packages of pipettes must be resealed between use periods. Each lot of pipettes must be checked for volume accuracy and the result recorded in the QC Form. The lot is rejected if the difference is greater than 2.5%.

7.4.11 Filtration Funnels

7.4.11.1 The laboratory uses stainless steel filtration funnels for membrane filtration methods. The funnels must be autoclaved at 121°C for 15 minutes prior to initiating a filtration series. Funnels may be wrapped in Kraft paper prior to autoclaving if they are not to be used on the day they are sterilized.

7.4.11.2 A filtration series is considered to be ended if greater than 30 minutes elapses between samples. Filter assemblies must be re-autoclaved prior to initiating a new series.

7.4.11.3 Filter assemblies are exposed to UV light (254 nm) for a minimum of three minutes between sample filtrations in a series.

7.4.11.4 Funnels that become scratched or corroded must be discarded.

7.4.12 Media

7.4.12.1 Whenever possible, media are purchased in powdered form from suppliers.

7.4.12.2 Each bottle of media received must be marked with the date received and date opened. Record date received, date opened, and amount received in media QC Form.

7.4.12.3 Dehydrated media are to be stored in a cool dry location. The desiccator is to be used whenever possible for media storage.

7.4.12.4 Containers of media must be discarded within six months of opening when not stored in the desiccator. If a desiccator is used for storage, open containers of media may be stored for up to -the manufacturer's expiration date.

7.4.12.5 In no case is media to be used beyond the manufacturer's expiration date.

7.4.12.6 Media must be used in the order received. To minimize waste, media must be ordered in quantities that will be used prior to expiration.

7.4.12.7 Each lot of media received must be tested with positive and negative culture controls prior to use to ensure satisfactory performance. The results of the control checks must be recorded in the media preparation log. Recommended control organisms are:

Total coliform positive:	*Escherichia coli* *Enterobacter aerogenes* *Klebsiella variicola*
Total coliform negative:	*Staphylococcus aureus* *Pseudomonas* sp.
Fecal coliform positive:	*Escherichia coli* *Klebsiella variicola*
Fecal coliform negative:	*Enterobacter aerogenes* *Enterococcus faecalis*
E. coli positive:	*Escherichia coli*
E. coli negative:	*Pseudomonas* non-fluorescent sp.
Enterococci positive:	*Enterococcus faecalis*
Enterococci negative:	*Staphylococcus aureus* *Escherichia coli*
Enterobacter positive:	*Enterobacter aerogenes*
Enterobacter negative:	*Escherichia coli*

7.4.12.8 Media preparation logs must include the media type, lot number of the media, date prepared, details of preparation, total volume prepared,

sterilization time and temperatures, final pH and the initials of the analyst preparing the media.

7.4.12.9 Prepared media must be stored in the refrigerator. Broth type media are stored in culture tubes with screw cap tops for three months or less. LTB and EC-MUG screw cap tubes should be refrigerated and placed in a dark area. HPC Agar should be kept in screw cap test tubes and be stored for up to six months while refrigerated. Plated media are stored in a plastic container for two weeks or less. The test tube racks or plastic containers must be clearly marked with the type of media along with the preparation and expiration dates of the media. Any plates or tubes that indicate growth prior to use are discarded. Tubes with gas present in the Durham tube prior to use are discarded.

7.4.13 Reagent-Grade Water

The quality of the reagent water must be tested, recorded, and determined to meet the following requirements:

Parameter	Limits	Frequency
Resistivity	> 10 megohm-cm at 25°C	Each day used (in-line meter)
Conductivity	< 2 μS/cm at 25°C	Monthly
Pb, Cd, Cr, Cu, Ni, Zn	< 0.05 mg/L per metal and < 0.1 mg/L total metals	Annually (by DELS-WES Inorganic Chemistry Laboratory)
Total Chlorine Residual	< 0.1 mg/L	Monthly
HPC	< 500 CFU/mL	Monthly

7.4.14 Dilution/Rinse Water

Each batch of prepared rinse water used by the laboratory must be checked for sterility prior to initial use. The following procedure is used:

7.4.14.1. 50 mL of the water is poured into 50 mL of 2x tryptic soy broth.

7.4.14.2. The broth is incubated for 48 hours at 35°C.

7.4.14.3. During the incubation, the broth is checked at 24 & 48 hours for turbidity or growth. Prepared bottles from each batch must not be used unless satisfactory results (i.e., no turbidity/growth) are obtained from the tested bottle.

7.4.15 pH Meter

7.4.15.1 The pH meter must be standardized each day it is used with a minimum of two buffers. The date and the buffers used must be recorded in the logbook.

7.4.15.2 Aliquots of buffer solution must be used only once.

7.5.15.3 The electrodes must be kept clean and stored in Electrode Storage solution.

7.5 Equipment maintenance procedures for the Inorganic Chemistry Laboratory and Organic Chemistry Laboratory – MS Section

The procedures are described in the analytical method SOPs for these two laboratories.

7.6 Equipment Maintenance Procedures for the Organic Chemistry – GC/LC Section

7.6.1 Liquid Chromatography (LC) Systems (HPLC & UPLC)

7.6.1.1 Annual preventative maintenance under a service contract provides change of pump seals, syringe change, and if needed, UV lamp change. The annual service contract also provides software upgrades.

7.6.1.2 After all HPLC analyses, run 50% solvent and 50% water through system to clean out salts and buffers.

7.6.1.3 Change pre-column frits when high backpressure is observed.

7.6.2 Gas Chromatographs

7.6.2.1 Change liners and septa on a when-needed basis.

7.6.2.2 Biannual ECD wipe tests

7.6.2.3 Change carrier gas tank before empty

7.6.2.4 Call manufacturer if electrically related problem

7.7 Maintenance Procedures for Common Laboratory Equipment in All DELS-WES Laboratories

7.7.1 Analytical Balances

7.7.1.1 Record 1.0000-gram weight and 5.0000-gram weight each day the balance is used. (100, 10, and 0.1 g weights may also be used, as appropriate). The date and analyst's initials must be entered into a record book or log that is kept near the balance.

7.7.1.2 A qualified service contractor checks balance calibration twice a year; external calibration records are maintained in W:\DELS\DELS-QAP\QCDocumentation-General\Analytical Balance Calibration External-All DELS Labs.

7.7.1.3 ASTM Class 1 weights are used for routine calibration. ASTM Class 1 weight sets are checked twice a year, at the time of balance calibration, against the calibrated weights of the outside service contractor. If a DELS-WES Class 1 weight set does not agree with the contractor calibrated weight, if it is corroded or damaged in any way, or if has been 5 years since last certified, then it is sent to another outside contractor for cleaning and recalibration/recertification to Class 1 specifications.

7.7.2 Refrigerators and Freezers

7.7.2.1 Temperatures of all refrigerators and freezers are measured with NIST-traceable thermometers and recorded daily by laboratory staff. All thermometers are calibrated at least annually against a NIST-traceable thermometer and the calibration records are maintained in the shared electronic folder for documentation of general quality control (W:\DELS\DELS-QAP\QC Documentation-General).

7.7.2.2 If the temperature reading is outside of the acceptance limits specified on the refrigerator/freezer door, adjustments are immediately made to bring the temperature reading within the acceptance limits.

7.7.3 Fume Hoods and Biological Safety Cabinets

7.7.3.1 Annual certification is performed by a qualified service representative.

7.7.3.2 Hoods and cabinets are maintained free of "clutter" at all times.

7.7.3.3 If used, mat material is kept clean and promptly replaced when soiled.

7.7.4 Reagent-Grade Water

The quality of the reagent water must be tested, recorded, and determined to meet one of the following requirements as monitored by and read from each specific reagent water system:

Parameter	Limits	Frequency
Resistivity	> 18 megohm-cm at 25°C	Each day used (in-line meter)
Conductivity	< 0.055 μS/cm at 25°C	Each day of use

7.8 Equipment maintenance records, including minor maintenance procedures conducted by laboratory personnel as well as preventative maintenance and major repairs conducted by service contractors, are kept up to date and in chronological order. These records are stored in the laboratory in which the equipment is housed/used.

8.0 SAMPLE PREPARATION, ANALYTICAL PROCEDURES, AND METHOD VALIDATION

8.1 DELS-WES utilizes analytical procedures that were developed/approved by the U.S. EPA, American Public Health Association, USGS, MassDEP, AOAC International, and various

manufacturers. DELS-WES analytical laboratory SOPs follow the format specified for technical SOPs in the U.S. EPA Guidance for Preparing SOPs (42) [note: previously referenced as the Environmental Monitoring Management Council (EMMC) Methods Format]. If a sample is analyzed using a procedure outside of the stated approved analytical method SOP, the respective laboratory supervisor and Laboratory QA & Data Manager are notified of the deviation. The change in sample methodology is noted and approved by the laboratory supervisor in the raw laboratory data, QA Level 1 and 2 review forms, and final analysis report. The analytical methods used by DELS-WES laboratories are listed in Tables 8 through 12.

8.2 Method Validation. Method validation is a critical part of any quality assurance program. DELS-WES uses analytical methods that have been validated for environmental analysis by the U.S. EPA and/or other professional scientific organizations. The laboratory performs further method validation by using the following as appropriate for the method:

- Analysis of spiked matrix samples to determine matrix effects
- Analysis of certified reference materials of known concentration to verify calibration and system accuracy
- Participation in proficiency tests for water supply analyses to evaluate the overall quality control system
- Monitoring the overall analytical system with a series of quality control checks
- Assessment of precision via the analysis of duplicate samples

9.0 DATA REDUCTION, VERIFICATION, AND REPORTING

This section describes the procedures for data reduction, data verification, and data reporting used by DELS-WES as part of its quality control program.

9.1 Data Reduction

Data reduction refers to the conversion of raw analytical data to the final analyte concentration in the sample reported in appropriate units after the analysis has been completed. Most chemistry data reduction at DELS-WES is performed directly by the computer/software that controls the analytical instrument. However, data reduction for some wet chemistry and microbiological methods is performed manually. For these methods, the analyst performing the final sample analysis is responsible for converting the raw data to final sample concentration in units specified by the method (e.g., mg/L, μg/L, CFU/100 mL, etc.), documenting the calculation, and recording the final result. Analytical results for blanks, duplicates, spikes, and other quality control samples are also reported along with the sample result.

9.1.1 The data reduction protocol includes the sample handling process for the laboratory. This process includes sample receipt, sample preparation, sample analysis, data acquisition and reduction, raw data analysis, and analytical quality control review.

9.1.2 The laboratory sample file includes the laboratory identification number; dates of collection, receipt, and analysis; analytical tests performed; name and affiliation of sample collector; analytical method; sample concentrations; and quality control data. The laboratory sample file is generated from the data entered in the respective laboratory logbook or bench worksheet, or found on an instrument computer printout that are then entered into the DELS-WES LIMS to generate the official electronic laboratory report. The analyst completing the sample analysis enters the final

concentration and quality control results on the official laboratory report forms in the LIMS. The laboratory supervisor or their delegate is responsible for generating and communicating the final verified LIMS report to the client.

9.1.3 Where data reduction occurs electronically, either partially or in its entirety, the U.S. EPA Good Automated Laboratory Practices (GALP), 1995 edition, guidelines shall be followed as described below. For this purpose, electronically shall refer to algorithms, formulas, text, scanned copies of hardcopy, etc., kept by or on a tamper-resistant electronic media such as write-once compact disks (CD-R) or a limited access password protected software system or data base.

9.1.3.1 A summary description of each piece of software and/or firmware involved in the calculation(s) and its requirements for proper functioning shall be recorded and kept either electronically or by hardcopy.

9.1.3.2 For each test/analyte, all algorithms, formulas, and applicable sample matrices shall be recorded and kept either electronically or by hardcopy.

9.1.3.3 The accuracy of each set of calculations shall be determined in the following manner:

1. The first use of each software/firmware version + calculation(s) combination shall be manually checked for accuracy by a method that does not employ the software/firmware being checked. Alternatively, a vendor's certification of accuracy may be used.

2. At a minimum, one of each type of Quality Control sample calculation shall be checked – e.g., percent recovery of standard, surrogate, etc.; proper calculation of Acceptance Criteria Range such as Instrument Performance Check or Quality Control Sample pass-fail; Relative Percent Difference (RPD); Matrix Spike; etc.

3. At a minimum, the calculations from seven to ten randomly chosen samples are checked.

4. All calculations must agree within the limits of rounding error.

5. A preliminary check of accuracy shall be performed by the analyst/user, but a person other than the analyst/user must confirm accuracy.

6. The dates, names, and signatures (initials) of the persons who perform this determination must be recorded.

7. The first and last use of a particular test/analyte software/firmware calculation combination shall be recorded to facilitate cross-referencing with the data and to determine the date this information may be disposed of.

8. This information shall be recorded and kept either electronically or by hardcopy.

9.1.3.4 The above shall be kept, at a minimum, with the equipment used to generate the raw data. In the case it is kept electronically in a central location, it shall

be readily available at the location of the equipment used to generate the raw data.

9.1.3.5 Any changes to the software/firmware, algorithms, formulas, etc. used in the data reduction require a new verification as described above. Changes to software/firmware that do not affect calculations, e.g. new auto-sampler interface, do not require accuracy verification.

9.1.3.6 The above information shall be kept for 10 years after the last use of that particular combination.

9.2 Data Verification

Data verification refers to the process used to evaluate the completeness, accuracy, precision, and overall conformance of analytical data produced by DELS-WES Laboratories against method, procedural, and quality assurance/control requirements (44). DELS-WES performs data verification on its analytical data using the following criteria:

9.2.1 Was the sample analyzed within the prescribed holding time? If not, the result is qualified as estimated data in the report ("H" Flag – Holding time violated). At the discretion of the project coordinator or point of contact, a new sample may be submitted for analysis.

9.2.2 Was the instrumentation used in analyzing the sample calibrated according to the analytical method and documented in the respective logbook, bench worksheet, or analytical instrument computer printout? Was the correct analytical method used?

9.2.3 Are the correct units used for determining the final concentrations (e.g., solids in μg/g dry or wet wt. and liquids in mg/L or μg/L)?

9.2.4 Are solids (other than fish/biota tissue) and sediments for semi-volatile organic compounds, metals, and other inorganic chemicals reported on a wet- or dry-weight basis with percent dry weight (solids concentration) where required?

9.2.5 Are solids and sediments for volatile organic compounds reported on a wet-weight basis with percent dry weight (solids concentration)?

9.2.6 Was a blank, spike, and/or duplicate analyzed with each sample batch? Was the blank concentration less than the MDL or minimum reporting level (MRL)? If not, were the data properly qualified in the final analysis report?

9.2.7 Was the correct number of significant figures used in the final result? It should be noted that the DELS-WES LIMS is configured for each analytical method with the correct number of significant figures for the test result.

9.2.8 Where reporting to the MDL is required, are reported concentrations less than the MRL but at or above the MDL, qualified as estimated data ("J" Qualifier), or just reported as < MRL? It should be noted that the DELS-WES LIMS is configured for each analytical method with the correct MDL and MRL to appear in the final analysis report if required.

If the final analyte concentration in the sample is less than the MDL or MRL, is the result correctly reported as < MDL or < MRL, respectively?

If the analytical method does not require reporting to an MDL, and the lowest concentration calibration standard used is at or below the MRL, are all concentrations detected below the MRL reported as < MRL or less than a fraction of the MRL for blanks (e.g., < ½ MRL) where applicable?

9.2.9 Have the precision and accuracy of the respective analytical method been determined?

9.2.10 Have independent quality control reference standards been run to determine laboratory accuracy?

9.2.11 Does the final analysis report list all the correct sample ID information as per the *Sample Tracking/Chain-of-Custody Form* – i.e., Client Sample ID, Client Sample Description, date and time of sample collection, receipt at WES, and analysis; sample matrix; sample collector name or initials; login batch point of contact; name of project with contact where applicable, or and analyses requested? Was the correct analytical method used for the sample matrix and analyses requested? Were the quality control data included in the final analysis report within acceptance limits or were the sample data properly qualified in the report if any QC data were outside acceptance limits? It should be noted that the DELS-WES LIMS is configured for each analytical method with the correct QC acceptance limits to appear in the final analysis report.

9.2.12 Have the data on the final analysis report been verified against the data entered in the respective laboratory logbook, bench worksheet, or analytical instrument computer printout? Are all raw data records written legibly in ink and are all changes neatly lined through, initialed, and dated?

9.2.13 Have the data undergone first-level (peer) and second-level (reporting) QA reviews? The 1st level review is performed by the laboratory supervisor or another analyst in the laboratory (i.e., an analyst that did not perform the analysis) while the 2nd level review is performed by the laboratory supervisor, or in the absence of the laboratory supervisor, by the backup laboratory supervisor. The Level 1 review involves a comprehensive review of all raw data to identify and correct any technical, analyte identification/quantitation, calculation, or transcription error. The Level 2 review is a review of the final analytical report. The Level 1 reviews are documented using Reports 3 through 8. The Level 2 review is documented using Report 9. The disposition of Level 1 review documentation is described in Section 18.2; Level 2 review, in Section 18.3.

9.2.14 On completion of the Level 2 review, supervisors or back-up supervisors must enter their password to electronically sign the approval to release of the results.

9.3 Data Reporting

The final laboratory reports generated by DELS-WES include: The Login #, Sample ID, Client Sample ID, Client Sample Description, date of collection, date of receipt, date of analysis, name of collector, analytical method used, sample concentration results, and supporting quality control data. (See Reports 1 and 2 for examples of DELS-WES Final Reports for chemistry results and microbiology results respectively.) The completed reports are then sent electronically to the client, MassDEP region, or MassDEP program that had submitted the sample(s) for analysis. MassDEP program staff who have been approved and trained in using the client view of WinLIMS have the ability to retrieve copies of reports and extracts of reported data on their own. The analytical sample report includes the following information:

- Project Name and location (where applicable)

- Client Sample ID, Client Sample Description, Sample ID, and the Login #.
- Dates of sample collection, receipt, preparation, and analysis
- Analytical method(s) used and detection limit(s)
- Final analyte(s) concentration(s) and units
- Sample matrix
- Data qualifiers, if any
- Blank results (i.e., method blanks, preparation blanks, trip blanks, and field reagent blanks)
- Spike results (i.e., matrix spikes, post-digestion spikes, method of standard addition spikes, surrogate spikes, and reagent water spikes)
- Duplicate results (i.e., sample duplicates or matrix spike duplicates)
- Quality control acceptance limits for each QC type for each method

The reports are scanned and electronically filed indefinitely. The original copies of the reports are kept on file at DELS-WES for 10 years and then destroyed. After the results have been reported, the actual samples are then discarded or retained as instructed by the MassDEP program/regional project coordinator or point of contact. Samples are kept until official written notification is received from the sample project coordinator allowing for their legal and proper disposal. Occasionally, per request of the sample collector/project coordinator or due to an urgent situation, preliminary analytical results are reported with the acknowledgement that the results are not official until they are validated and approved.

10.0 INTERNAL QUALITY CONTROL CHECKS

10.1 Introduction

This section describes the internal quality control checks that are used by DELS-WES as part of its laboratory quality control activities. The quality control procedures stated below are a function of the quality assurance objectives stated in Section 4.0. The analytical requirements for the quality control procedures are specified in method SOPs, while the specific tests are described in Section 10.0. Single-blind quality assurance check samples (QCS) from a PT provider meeting the criteria set by the National Environmental Laboratory Accreditation Program (NELAP) and implemented by the Proficiency Testing oversight Body/Proficiency Testing Provider Accreditor (PTOB/PTPA) or its successor body for accredited PT providers are used to evaluate laboratory accuracy. Additional quality control elements used include:

- Positive controls and blanks for all bacteriological analyses, and laboratory duplicates for all enumerative bacteriological tests.
- A bacterial colony count comparison between analysts must be performed and recorded for each quantification method performed each month. Quantification assays not performed during the routine client testing of a calendar month are exempt from this requirement, e.g., if there were no requests for EPA 1600 analysis during December, a colony count comparison for this methodology would not have to be performed for this month. The colony counts must be within 10% and documented in the Monthly QC Form.

- All method-specific QC requirements and acceptance limits for IPCs, LFBs, LFMs, etc., are followed.

- A quality control sample (QCS) prepared from a separate source is analyzed at least quarterly for all methods.

- A laboratory reagent blank (LRB) is prepared and analyzed with each batch of samples. If the LRB concentration is greater than the MDL or MRL, corrective action is taken.

- A laboratory-fortified blank (LFB), at 10x the MDL or at the mid-level standard as required by the method, is run with each batch of samples for all methods. Control charts are generated using the most recent 20 to 30 data points.

- A laboratory-fortified matrix sample (LFM) is run at a frequency of 10 or 20% as required by the analytical method being performed for all methods, except for Method 524.3.

- For the analyses for which they are required, MDLs are determined according to method-specific instructions.

- Minimum Reporting Levels (MRLs) for all regulated drinking water analytes are lower than or equal to their respective MCLs.

- For regulated drinking water synthetic organic chemicals (SOCs), required MDLs are achieved for most compounds. Analytical data (i.e., analyte concentrations) are reported only when bracketed by initial calibration standards. If the sample analyte concentration exceeds the highest calibration standard, it is reported qualified (E) or the sample is diluted and rerun. For metal analyses, sample results can be reported from projected parts of the curve beyond the highest calibration standard up to 90% of the upper limit of the Linear Dynamic Range (LDR). If the sample analyte concentration falls below the lowest calibration standard (i.e., below the MRL) but is above the MDL, it is reported as an estimated concentration with an "J" qualifier. If the sample analyte concentration is below the MDL, it is reported as < MDL.

- For GC methods, second-column confirmation is performed when recommended by the method.

- When samples are diluted, MRLs and MDLs are raised accordingly in the LIMS and included in the final analysis report.

The overall objective of the Wall Experiment Station quality control program is to demonstrate that the analytical data generated by this laboratory are scientifically valid and defensible. The laboratory's quality control program is based on the following practices:

- Utilization of *Standard Methods for the Examination of Water and Wastewater*, AOAC, USGS, MassDEP, and United States Environmental Protection Agency approved prescriptive and performance-based analytical methods.

- Utilization of approved laboratory calibration and operation of instrumentation

- Compliance with standard procedures for sample collection, sample management (e.g., meeting EPA holding times), and sample analysis.

- Compliance with sample chain-of-custody procedures

- Continuous monitoring for acceptable laboratory accuracy and precision by the use of certified quality control check samples, method blanks, duplicates, surrogate spikes, matrix spikes, trip blanks, and participation in proficiency testing for water supply and other samples.
- Triennial audits by the USEPA-New England Laboratory Certification Team.
- Participation in laboratory round-robin analyses and programs with the USEPA
- Continuing training of laboratory staff in all aspects of environmental laboratory sciences

10.2 Quality Control Samples and Their Definitions

The integral elements of a sound quality control program include training and use of qualified personnel, dependable and well-maintained instrumentation, proper number of calibration standards and check samples, review and surveillance of the program by the Lab Supervisor and Laboratory QA & Data Manager.

The primary means of assessing laboratory precision and accuracy are with the use of quality control samples. The number and type of quality control samples required by the USEPA are described in the respective analytical methods. All DELS-WES laboratories use quality control samples as part of their routine quality control programs.

DELS-WES utilizes the following laboratory quality control samples as part of its analytical program.

10.2.1 Proficiency Test Sample. Obtained from a source independent of the laboratory, these samples are spiked with the analytes of interest. The laboratory may receive single-blind samples, meaning the laboratory can identify it as a PT sample but it does not know its true value, or double blind, meaning the laboratory cannot identify the PT sample in a batch of environmental samples. PT samples must be in the same relative matrix as the environmental samples. These samples are to verify that the laboratory is capable of producing accurate data. All PTs for DELS-WES Laboratories are run in a manner identical to field samples. Since in an analytical batch one field sample is run as a laboratory duplicate, DELS-WES Laboratories may choose to run a PT sample in duplicate. As is the DELS-WES practice for field samples, assuming the method laboratory duplicate QC criterion is met, the analytical result for the “sample” will be reported to the PT provider rather than the result for the “duplicate” or the mean of the duplicate results. Consistent with the DELS-WES practice for field samples, if the method laboratory duplicate QC criterion is not met, the DELS-WES laboratory may choose to rerun the analysis of the PT sample. Also consistent with the DELS-WES practice for field samples, the analysis of a PT sample may be rerun if there is the need to dilute the sample because the initial result was higher than the highest calibration standard used in the calibration curve

10.2.2 Laboratory Check Standard (Quality Control Standard). Obtained from a source independent of the laboratory, this standard contains known concentrations of target analytes. These standards are used to check the bias in laboratory measurements. Laboratory check standards are used after calibration, after blank analysis and before and after sample analysis.

10.2.3 Calibration Standards. These samples contain known concentrations of the target analytes. Analytical methods dictate the number of calibration standards that must be analyzed and plotted on the calibration curve. In all cases, the lowest standard must be at or below the target quantitation limit (i.e., MRL) and the highest standard must fall

within the calibration range of the instrument. These standards are used to produce a calibration curve from which to calculate contaminant concentrations in environmental samples. The minimum frequency is specified in the method.

10.2.4 Calibration Check Standard. This sample contains a known concentration of the target analyte(s). It should be run at or over the calibration range specified by the method. Where not otherwise specified, the calibration check standard concentration should be in the midrange of the calibration curve. It is used to verify that the instrument is still calibrated to the calibration curve. A calibration check sample is analyzed one per sample batch. The acceptance criteria of the calibration check standard is analyte/method specific. The instrument must be re-calibrated whenever data from the calibration check standard falls outside of the acceptance limits.

10.2.5 Method Blank (Laboratory Reagent Blank). A sample of analyte-free reagent water, for aqueous samples, or analyte-free soil, for soil samples, it is prepared and analyzed by the analytical methods being used for the environmental samples. Method blanks are used to check on the cleanliness of the reagents, instrument systems, and laboratory environment. Method blanks are analyzed one per twelve-hour analysis day, one per twenty samples or one per sample batch, whichever requires the greatest number of blank analyses.

10.2.6 Laboratory Duplicates. Sometimes called laboratory replicates, these samples are two aliquots of a single field sample that are analyzed independently. Duplicates are used to check on precision in laboratory results. Laboratory duplicates are analyzed at the rate which requires the greatest number of duplicate analyses: one per twenty environmental samples; one per sample batch; or as specified by the method.

10.2.7 Matrix Spike (Laboratory Fortified Matrix). An aliquot of an environmental sample that has been spiked with known concentrations of target contaminants prior to extraction/digestion and analysis. After analysis, the percent recovery is calculated. Matrix spikes are used to check on bias and matrix interferences in laboratory measurements for an environmental sample matrix of interest. Matrix spikes are analyzed at the rate which requires the greatest number of spike analyses: one per twenty samples, one per sample batch, or as specified by the method.

10.2.8 Matrix Spike Duplicate. A second aliquot of the environmental sample that was used for the matrix spike is spiked with the same concentration of target contaminants, prior to extraction and analysis. After analysis, the relative percent difference between the matrix spike and its duplicate is calculated. The matrix spike duplicate is used to check on precision in laboratory measurements for an actual environmental sample matrix of interest. A matrix spike duplicate is analyzed at the rate which requires the greatest number of matrix spike duplicate analyses: one per 20 samples, one per sample batch or as specified by the method.

10.2.9 Surrogate Spike. Surrogate spikes are used to assess analytical accuracy (% recoveries) in samples tested. Blanks, standards, field samples, quality control samples, and blank and matrix spike samples are spiked with a known concentration of the surrogate, which is an analyte that is similar to method target analytes in chemical composition, extraction efficiency, chromatographic separation, etc., but which is not normally found in environmental samples.

10.2.10 Environmental Sample. Sometimes called a field sample – it is a sample of any matrix taken from an environmental site. An environmental sample is used to characterize the composition or contamination at that sampling point. The size of the sample is dictated

by the methods used on analysis and the quality control that will be performed on it. The number of samples collected is dependent on the objectives of the sampling program.

10.2.11 Trip Blank. Duplicate vials of analyte-free water produced in the laboratory, transported to the field, and shipped back to the laboratory with the volatile organics samples. The purpose of the trip blank is to check for sample contamination originating from sample transport. One trip blank consisting of duplicate vials is collected per cooler of samples shipped for volatile organics analysis.

10.2.12 Field Reagent Blank (FRB). An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sample site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

10.2.13 Equipment Blank. Sometimes called a field or rinsate blank, these samples are produced in the field by rinsing decontaminated sampling equipment with analyte-free water and collecting the rinsate for analysis. Equipment blanks are used to check on sample cross contamination resulting from inadequate decontamination of sampling equipment. DELS-WES has largely eliminated the need for most equipment decontamination by implementing the use of dedicated disposable sampling equipment. Equipment blanks should be analyzed for the same parameters as the environmental samples. Equipment blanks should be collected at least once at the beginning and once at the end of each sampling day.

10.2.14 Field Duplicate. A second environmental sample drawn from the same sampling location immediately after the original environmental sample was drawn. The two samples must be collected independently. Field duplicates are used to check on consistency in sampling technique. Field duplicates should be collected one per twenty environmental samples.

10.2.15 Split Samples. Two samples produced from the same sample collection event and analyzed by two different analytical laboratories. Non-VOC samples must be homogenized prior to sample collection. VOC samples may not be homogenized prior to sample collection. Instead, collect two aliquots from the same bailer of water or from the same region of a split spoon auger. Split samples are used to check precision between analytical laboratories. The number of split samples collected is dependent on the objectives of the sampling program, but usually one per ten environmental samples.

10.2.16 Quality Control Sample – Standard Reference Material (QCS_{SRM}). A sample of a matrix similar to the sample being analyzed which contains analytes of a known or accepted concentration. The QCS_{SRM} is obtained from a source external to the laboratory and contains the analytes of interest at certified concentrations for the method of interest. This QCS_{SRM} is processed in the same manner as the sample, and is used to check method performance.

11.0 PERFORMANCE AND SYSTEMS AUDITS

11.1 Internal Systems Audit

An internal systems audit involves a thorough review, inspection, and evaluation of all components of a laboratory's quality assurance system, including standard operating procedures, personnel qualifications, and facilities. The DELS-WES Quality Assurance Program conducts internal systems audits of the DELS-WES laboratories annually (note: DELS-WES laboratories are also audited every three years by the U.S. EPA-New England Laboratory Certification Team). A systems audit includes the following components:

11.1.1 Review of sample handling and chain-of-custody procedures

11.1.2 Laboratory Methodology – Analytical methods used by the laboratory must be listed in the DELS-WES Laboratory QA Plan. They must be the correct methods based on analyte and sample matrix.

11.1.3 Initial Demonstration of Capability (IDC) – The laboratory must have documentation that an IDC is performed whenever a new instrument is put on-line, when a new analyst performs the method, when major changes have been made to a method or instrument. Records of these analyses must be maintained by the laboratory. The records should include analyst name, analytical method used, date of analysis, and all the raw data.

11.1.4 Routine Monitoring of Analytical Method Performance – Inorganic and organic chemical analytical methods specify the requirements for method performance. Microbiological methods require the use of duplicates, and positive and negative culture controls to assess method performance. Method performance is to be evaluated by the following:

- Instrumentation calibration, including preparation of analytical standards
- Instrument performance
- Method detection limits
- Method validation procedures
- Results of annual external single-blind proficiency testing (PT) as well as of double-blind PTs submitted by the MassDEP programs/regions.

11.1.5 Inventory of Laboratory Equipment, Condition and Maintenance, Physical Plant – The audit is conducted to determine if the laboratory has the proper and/or required instrumentation to perform the required analyses. The operating condition and age of each piece of major instrumentation will be noted. The laboratory's instrumentation maintenance log will be reviewed to determine if the scheduled maintenance has been performed and to note the date of any other repairs.

11.1.6 Laboratory Personnel – Personnel training documentation will be reviewed to ensure up-to-date training.

11.1.7 Data Handling and Documentation – A review will be performed on all procedures used to calculate final concentration values from raw data. The audit will verify representative calculations to show that appropriate procedures and final report units are being used. Documentation of peer reviews of analytical work will be reviewed during an audit.

11.1.8 General Laboratory Quality Control Procedures – During this phase of the audit, quality control procedures will be evaluated to verify that specific QC procedures are being performed and documented in lab records. It includes the following:

- Review of sample spikes, duplicates, matrix spikes, PT results, and sample blank results.
- Review of lab balance certification and daily calibration checks.
- Review of calibration standards purchase date and date of expiration.
- Review of calibration standards logbook or standard preparation forms for date prepared, concentrations, prep analysts, expiration dates, and source lot numbers
- Review of significant figures used.
- Review of QC limits.
- Review for compliance with Lab QA Plan and SOPs.

11.1.9 Report of Systems Audit Findings to Management – Upon completion of the systems audit for a DELS-WES laboratory, the Laboratory QA & Data Manager documents the findings of the audit in a report submitted to the DEL/WES Director and laboratory supervisor. The report focuses on:

- Deviations from the Laboratory QA Plan and SOPs.
- Areas for improvement in laboratory QC and analytical methods.
- Data quality assessment.
- Determination as to whether the laboratory QA objectives are being met.

11.2 Performance Audit

The objective of a performance audit is to review and evaluate the performance of the analyst and the other components of the analytical measurement system.

11.2.1 The WES/DELS laboratory analyzes laboratory quality assurance check samples that are obtained from an external source.

11.2.2 The WES/DELS laboratory participates in an annual Water Supply PT study. This study is a single-blind performance evaluation of the laboratory's accuracy in detecting/quantitating drinking water contaminants.

11.2.3 Laboratory supervisors and analysts perform Level 1 peer reviews of analytical data that includes the validation of the calibration curve and a review of the analytical run including quality control samples.

11.2.4 Laboratory supervisors and analysts perform Level 2 peer reviews of analytical data that checks sample handling, the reporting of analyses, and the frequency of analysis of and compliance with the acceptance criteria of quality control samples.

11.2.5 Laboratory supervisors review quality control data generated by the laboratory during the previous month. Data are reviewed for trends and for deviations from acceptable quality control criteria. Any necessary corrective action is taken and documented.

11.2.6 If staff availability allows, the Quality Assurance Program performs completeness reviews of analytical data packages to ensure that Level 1 and 2 reviews have been performed and any necessary corrective action has been taken and documented.

12.0 PREVENTIVE MAINTENANCE

12.1 DELS-WES laboratories perform preventive maintenance on analytical instruments such as MS source cleaning, detector cleaning, and lubrication according to the guidelines listed in the instrument manufacturer's manual. Analytical instruments, such as gas chromatographs, high-performance liquid chromatographs, inductively coupled plasma spectrometers, and gas chromatograph/mass spectrometers may be covered by manufacturer's service contracts and are regularly scheduled for routine maintenance and cleaning.

12.2 Each DELS-WES laboratory maintains an instrument maintenance logbook. Information recorded in the instrument logbook includes the date of instrument maintenance, nature of work performed on the instrument, replacement of columns, septa, injector liners, traps, lamps, gas purifier traps, reagent water ion-exchange cartridges, and electronic systems components. Maintenance is performed on the instrument when the analyst detects: poor sample peak resolution, high background noise, decreased instrument sensitivity, or failure to meet USEPA instrument quality control requirements.

13.0 DATA QUALITY INDICATORS

13.1 The purpose of the DELS-WES Quality Assurance & Data Program is to ensure that the laboratory generates legally defensible and scientifically valid analytical data. The parameters that the laboratory uses to assess data quality are precision and accuracy.

13.2 Accuracy

Accuracy is defined as the closeness of agreement between the measured value and an accepted reference value. Accuracy in the laboratory is determined by the analysis of matrix spikes or standard reference material at a frequency of one per 20 samples (or one per 10 samples if required by the method used) or one per sample batch and determining the percent recovery of the spiked analyte.

13.2.1 For measurements where matrix spikes are used, the percent recovery is calculated as follows:

$$\%\ R = 100\ x\ \frac{S - U}{C_{sa}}$$

Where:

% R = Percent Recovery

S = Measured concentration in spiked aliquot.

U = Measured concentration in unspiked aliquot.

C_{sa} = Actual concentration of spike added.

13.2.2 When a standard reference material (SRM) is used, the percent recovery is calculated as follows:

$$\% R = 100 \; x \; \frac{C_m}{C_{srm}}$$

Where:

% R = Percent Recovery

C_m = Measured concentration of SRM

C_{srm} = Actual concentration of SRM.

13.3 Precision

Precision is defined as the agreement among a set of replicate measurements without assumption of knowledge of the true value. Precision in the laboratory is determined by duplicate and replicate measurements at a frequency of at least one per twenty samples or one per sample batch.

13.3.1 If calculated from duplicate measurements (i.e., Sample Duplicate), the relative percent difference is the normal measure of precision.

$$RPD = \frac{(C1 - C2) \; x \; 100}{(C1 + C2)/2}$$

Where:

RPD = Relative percent difference

C1 = Larger of the two observed values

C2 = Smaller of the two observed values

If calculated from three or more replicates, the relative standard deviation is used as the measure of precision.

$$RSD = \frac{S}{Y} \; x \; 100$$

Where:

RSD = Relative standard deviation

S = Standard deviation

Y = Mean of replicate analyses

13.3.2 For microbiological analyses, laboratory precision is evaluated using the range of logs (ROL) of duplicate analyses.

Calculation of Precision QC Criterion – Determine the range of logs for the duplicate colony counts as follows *(Standard Methods for the Examination of Water and Wastewater,* 1998, Page 9-10):

ROL for a Duplicate Set = Log_{10} (Count 1) – Log_{10} (Count 2)

If either result of a duplicate set is < 1, add 1 to both values before calculating the logarithms as follows:

ROL for a Duplicate Set = Log_{10} [(Count 1) + 1] – Log_{10} [(Count 2) + 1]

Precision QC Criterion = 3.27 x (Mean Range of Logs for 15 Most Recent Duplicate Sets)

13.4 Accuracy and Precision Control Limits

The accuracy and precision values for data acceptance are compared with the respective USEPA method quality control limits. If there is no published USEPA guidance for accuracy and precision, the laboratory will evaluate the data by method performance and method detection limit parameters. The Laboratory Supervisor reviews spike and duplicate results for compliance with method acceptance limits.

When an analyst enters quality control data into the DELS-WES LIMS, the LIMS generates precision and accuracy control charts that are specific for the analyzed parameter. Monthly quality control charts are generated after a set of 20 data points have been established. Two types of quality control limits are plotted on each respective chart: warning limits and control limits. The warning limits correspond to ± two standard deviations from the mean. The control limits on the chart correspond to ± three standard deviations from the mean. The control limits for bias are based on the USEPA-derived historical mean recovery ± three standard deviations. The control limits for precision range from zero to the USEPA-derived historical mean relative percent difference plus or minus three standard deviation units. Data that fall outside of the control limits are not valid and the analysis must be repeated or the results qualified accordingly.

13.5 Method Detection Limit (MDL)

MDL is defined as follows for all measurements:

$$MDL = t_{(n-1,\ \alpha = 0.99)} \times S$$

Where:

MDL = Method detection limit

S = Standard deviation of the replicate analyses

$t_{(n-1,\ \alpha = 0.99)}$ = Student's t-value for a one-sided 99% confidence level and a standard deviation estimate with n-1 degrees of freedom

Standard deviation is defined as follows:

$$s = \sqrt{\sum_{i=1}^{n} \frac{(y_i - \bar{y})^2}{n-1}}$$

Where:

S = Standard deviation

y_i = Measured value of the i^{th} replicate

$\bar{y}$ = Mean of replicate measurements

n = number of replicates

14.0 CORRECTIVE ACTION

14.1 DELS-WES continually monitors its analytical data for compliance with its quality control plan and established quality assurance guidelines. The laboratory initiates corrective action procedures when errors, deficiencies, deviations, or laboratory data fall outside of established acceptance criteria. Unacceptable quality assurance data will result in the respective sample batch being labeled as suspect data. The need for corrective action may be identified by system or performance audits, or by standard quality control procedures.

14.2 The corrective action procedures that DELS-WES uses for suspect data are:

14.2.1 Qualify data that fall outside of the quality control limits

14.2.2 Investigate and identify the problem

14.2.3 Determine the appropriate corrective action to be taken. The DELS-WES Laboratory QA & Data Manager shall discuss the proposed remedial action with the Laboratory Supervisor, whose data failed to meet acceptance criteria.

14.2.4 Implement a corrective action plan and evaluate the results. The corrective action plan will consist of examining sample collection practices, methodology, reagents, solvents, sample preparation and analysis. Recommended quality control procedures would consist of the use of reference quality control samples, performing matrix or sample spikes, and solvent blanks.

14.2.5 Document that corrective action has eliminated the problem.

14.2.6 Release data that are in compliance with quality control limits.

14.2.7 All corrective action taken is documented in the DELS-WES Corrective Action Form (see Form 4).

15.0 QUALITY ASSURANCE REPORTS TO MANAGEMENT

15.1 If available manpower allows, the DELS-WES Laboratory QA & Data Manager prepares quality assurance reports to the DELS-WES Director. These reports may include any of the following quality assurance program activities:

15.1.1 Revisions of the DELS-WES Laboratory Quality Assurance Plan and of general and analytical method Standard Operating Procedures

15.1.2 Results of systems and performance audits

15.1.4 Recommended corrective actions and results of corrective actions taken.

15.1.5 Data quality assessment of accuracy, precision, and method detection limit results.

15.1.6 Determination of whether stated quality assurance objectives were attained.

15.1.7 Limitations on the use of generated analytical data.

16.0 TRAINING

16.1 The laboratory supervisors are responsible for the training of all employees under their direct supervision. Bench analysts develop and demonstrate proficiency in new analytical methods by performing initial demonstration of capability (IDC) and method detection limit (MDL) studies for each analytical method. IDC and MDL determinations are documented in Form 5. Bench training of laboratory staff is documented in Form 6. External training of laboratory staff is documented in Form 7, group-sign in sheets, training completion documentation, or email confirmations.

17.0 LABORATORY HEALTH AND SAFETY

17.1 Safety Equipment

WES provides its laboratory personnel with laboratory coats and eye protection as well as other laboratory safety equipment. Laboratory personnel must wear laboratory coats, gloves, and eye protection at all times when working in the laboratory. When special laboratory operations warrant, the analyst shall wear a face shield, heat-resistant gloves, and/or hearing protection. An appropriate cartridge respirator may be worn if the analyst has been properly trained, fitted, and approved to use.

The laboratory has the following equipment for maintaining a safe working environment:

17.1.1 A-B-C type fire extinguishers located throughout the building

17.1.2 Laboratory fume hoods and biological safety cabinets that are inspected and certified annually

17.1.3 Eye wash stations and emergency showers located in each laboratory

17.1.4 Automated External Defibrillator (AED) unit and staff members who are trained in its use

17.1.5 Spill clean-up kits for acids, alkalis, and organic solvents.

17.1.6 Dollies for transportation of compressed gas cylinders

17.1.7 First aid kits

17.1.8 N-95 Masks.

17.1.9 Emergency public address system from each phone in the building.

17.1.10 Central security and fire alarm (sensor and pull-box) system for the facility

17.1.11 Warning and Exit signs located throughout the building.

17.1.12 Safety Data Sheets (SDSs) for chemicals and standards used in the laboratory

17.1.13 Explosion-proof refrigerators for the storage of organic solvents

17.1.14 Flammable storage refrigerator for reagents, standards, and samples

17.1.15 Designated receptacles for the disposal of broken glass and other sharps.

17.1.16 HVAC system with exhaust hoods and snorkels (at the bench) and visible and audible alarms to indicate system imbalance.

17.1.17 Inert gas desiccators/storage cabinets.

17.2 WES Laboratory Safety Procedures

WES has adopted the following laboratory safety procedures to protect its staff:

17.2.1 Food and beverages are not allowed to be stored or consumed within any of the DELS-WES laboratories. Drinking water fountains are located outside of the laboratories.

17.2.2 Smoking is not permitted within the entire WES facility. Smokers outside the building must be at least 25 feet away from the building.

17.2.3 Laboratory coats, gloves, respirators and other laboratory apparel must not be worn outside of the laboratory in a public area or where food is consumed. DELS-WES uses ONLY ready-to-use disposable laboratory coats. When dirty/soiled, the laboratory coats are properly disposed of; lab coats that are especially soiled with hazardous materials are disposed of as hazardous waste. Laboratory coats must never be taken home.

17.2.4 Personal items such as coats, hats, umbrellas, and purses are to be stored outside of the laboratories. Staff are allowed to carry personal cell phones, but must follow decon procedures and exit the lab to initiate or answer personal calls. Personal or office/desk laptops must not be brought into laboratories.

17.2.5 Laboratory benches must be kept free of clutter and properly organized.

17.2.6 Working alone outside of normal work hours requires approval by the laboratory supervisor and the WES Director.

17.2.7 Mouth pipetting is prohibited; the use of pipette filling bulbs/devices is required for all pipette use.

17.2.8 Contact lenses may not be worn in the laboratory.

17.2.9 Protective impact-resistant lenses are required at all times in all laboratories.

17.2.10 Face shields are required when potential spill, splatter, or impact conditions may occur.

17.2.11 All chemical storage containers must be labeled. All unlabeled bottles are automatically discarded.

17.2.12 Separate recycle containers are provided in each laboratory for recycling of paper, non-contaminated disposable laboratory plasticware, and non-contaminated disposable laboratory glassware. Broken non-contaminated glassware is not recyclable and must be placed in specifically located cardboard boxes designed for safe disposal of broken glass. When full, these boxes are discarded in the regular trash.

17.2.13 Hazardous waste solvents and other hazardous waste chemicals are transferred to the WES Hazardous Waste/Chemical Storage facility for storage and to await removal by a licensed hazardous waste management contractor. Items contaminated with hazardous material are disposed of properly and not recycled or placed in the trash.

17.2.14 Used glassware must be emptied of solutions and solvents and rinsed with water before being released for regular cleaning. If special instructions for cleaning are necessary, clean-up personnel must be informed.

17.2.15 Chipped and cracked glassware must be placed in the glass-recycling container after being cleaned and decontaminated.

17.2.16 All laboratory analyses are to be reviewed for possible safety problems.

17.2.17 Safety shields are required around high-vacuum or high-pressure reactions.

17.2.18 Gas cylinders must be properly secured before removing protective caps.

17.2.19 Chemicals must be separated for storage based on chemical reactivity (e.g., oxidizers are stored together in a separate cabinet from other chemicals).

17.2.20 Movement of laboratory visitors within the facility shall be restricted. If visitors are allowed in the laboratory, they must be accompanied by a member of the staff and provided with eye protection, as necessary.

17.2.21 Hand washing is required after removing protective gloves and after returning to the laboratory from the restroom or from other outside areas.

17.2.22 Before use of any new, repaired, or moved equipment, the equipment shall be tested and inspected.

17.2.23 No storage of any type is allowed in the hallways, especially chemical or gas cylinder storage.

17.2.24 All operations that could result in the release of vapors or airborne particles, including pathogens, shall be performed in a suitable fume hood, ventilated bench, or biological safety cabinet.

17.2.25 In order to control and avoid inhalation of powdered microbiological culture media, all such media shall always be weighed out under containment in a vented, negative-pressure biological safety cabinet or weighing hood, or under a negative-pressure snorkel.

17.2.26 All surfaces shall be cleaned immediately if a spill occurs and at the end of the workday.

17.2.27 Testing of samples or chemicals by taste is forbidden, and odors should only be checked with care.

17.2.28 Limit the quantity of solvents and other chemicals in the laboratories; keep large chemical quantities in the WES Hazardous Waste/Chemical Storage facility.

17.2.29 See WES Health and Safety Plan for further details.

18.0 RECORDS MANAGEMENT, RETENTION, AND SECURITY

18.1 All analytical data reports, including sample identification information and QC data, generated by DELS-WES laboratories since February 1998 are stored electronically in the DELS-WES LIMS and in MassDEP secure drives that are backed up every business day. For all samples received through the end of calendar year 2007, the final analytical reports are also printed and the hard copy filed with the original Sample Tracking/COC Form in the WES Main Office for approximately one year. After one year, these reports are stored in the DELS-WES Records Storage Room for an additional 9 years and then destroyed after permission for destruction is granted by the State Records Retention Board. Starting with samples received at the beginning of calendar year 2008, the final analytical reports are no longer printed.

18.2 Starting with samples received at the beginning of calendar year 2008, all raw analytical data for a specific analytical method run (including raw PT data), including associated standard/reagent preparation form(s) and Level 1 QA Review Form are scanned and saved electronically under the specific Sample Master LIMS QC batch number, or WinLIMS Worklist (WL) batch number in a MassDEP secure drive.

The Sample Master Level 1 QA Review Form lists the Sample Master LIMS sample login number(s) associated with a specific QC batch number. The hard copy of the scanned data file is filed by the specific LIMS QC batch number in the laboratory that produced the data; this hard copy file is kept in the laboratory for approximately two years and is then transferred to the WES Records Storage Room for an additional 8 years and then destroyed after permission for destruction is granted by the State Records Retention Board.

18.3 Starting with samples received at the beginning of calendar year 2008, the final analysis report, Sample Tracking/COC Form, Sample Conditions Review Form, are saved electronically under the specific LIMS sample login number in a MassDEP secure drive. Prior to the introduction of WinLIMS, Level 2 Review Forms were saved with the Login Batch. Starting in 2017, for analyses managed in WinLIMS, the Level 2 Review Report is saved with the specific WL Batch. The hard copy of this file (i.e., Sample Tracking/COC Form, Sample Conditions Review Form, and Level 2 QA Review Form where applicable) is filed by the specific LIMS sample login number in the WES Main Office for approximately one year and is then transferred to the WES Records Storage Room for an additional 9 years and then destroyed after permission for destruction is granted by

the State Records Retention Board. It should be noted that for analyses managed in Sample Master, the Level 2 QA Review Form(s) list the LIMS QC batch number(s) associated with all requested analyses for the samples in a given sample login number. Therefore, by combining the data under the specific sample login number and those under the associated QC batch number(s), we can easily generate a complete electronic or printed data package for all the samples submitted by a client in a given Sample Tracking/COC Form.

18.4 All raw IDC/MDL data for all staff are filed for approximately two years in the laboratory that produced the data and then are transferred to the WES Records Storage Room for an additional 8 years and then destroyed after permission for destruction is granted by the State Records Retention Board. Starting at the beginning of calendar year 2008, these data are also scanned and saved electronically under the specific laboratory in a MassDEP secure drive.

18.5 Records of purchased analytical standards, including analytes and concentrations, name of vendors, dates of receipt, and expiration dates are filed for approximately two years in the pertinent laboratory and then are transferred to the WES Records Storage Room for an additional 8 years and then destroyed after permission for destruction is granted by the State Records Retention Board. Starting at the beginning of calendar year 2008, these data are also scanned and saved electronically under the specific laboratory in a MassDEP secure drive.

18.6 Records of prepared analytical standards and reagents, including traceability to purchased stocks, analytes and concentrations, dates of preparation, initials of preparer, and expiration dates are filed for approximately two years in the pertinent laboratory and then are transferred to the WES Records Storage Room for an additional 8 years and then destroyed after permission for destruction is granted by the State Records Retention Board. Starting at the beginning of calendar year 2008, these data are also scanned and saved electronically under the specific QC batch number(s) in a MassDEP secure drive

18.7 Access to all WES electronic records is limited to authorized WES/MassDEP personnel via password protection. All DELS-WES electronic data are backed up daily.

18.8 Hard copy laboratory records filed within the individual DELS-WES laboratories, in the WES Main Office, and in the WES Records Storage facility are considered to be secure as access to the WES building is strictly limited to authorized personnel. Non-MassDEP visitors must sign in at the WES front office and be escorted by a WES employee during the entire visit.

18.9 SOPs and Other Control Documents. Only 3 DELS-WES employees have write-access to final/active control documents and access to the electronic signature files: i.e., the DELS-WES Director, Laboratory QA & Data Manager, and the Document Control Custodian (Program Coordinator). Only these 3 employees make changes and add the electronic signatures to the electronic control documents. All new control documents as well as changes and updates are given to the Document Control Custodian who then makes the changes using “Track Changes” The staff who are responsible to approve the document reviews and then notifies the Custodian that the document has been approved. The custodian then inserts the signature and date of approval.

18.10 DELS-WES laboratories record observations, data, and calculations at the time they are made. Handwritten records are made in ink, not pencil. Mistakes in records are crossed out with a single line such that the original entry is still legible and the correct value is entered. All alterations to records are signed and dated by the person making the correction and a brief explanation for the correction is provided when necessary. In the case of records stored electronically, equivalent measures are taken to avoid loss or change of original data.

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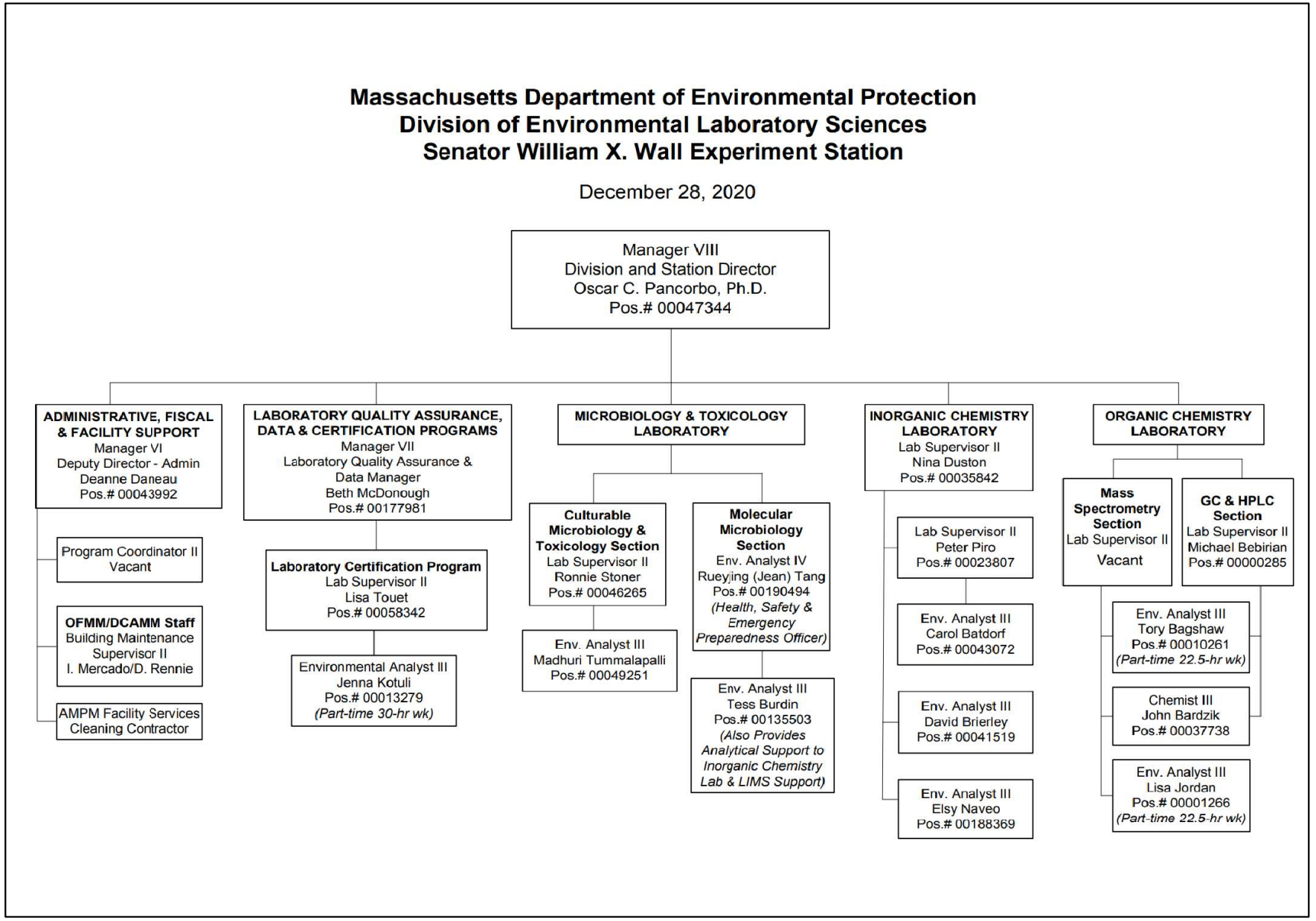
20.0 FIGURES, TABLES, FORMS, AND REPORTS

FIGURE 1. ORGANIZATION CHART

Organizational Chart for the MassDEP Division of Environmental Laboratory Sciences, Senator William X. Wall Experiment Station



TABLES

TABLE 1. DELS-WES TECHNICAL AND SUPPORT PERSONNEL

Position/Title	Name	Educational Degree(s) & Major(s)	Present Specialty	Primary Responsibility (Includes Analytical Methods)	Years of Experience in Field
Administration, Laboratory QA & Data Management, and Support Services					
Division & Station Director	Oscar Pancorbo	Ph.D. & M.S., Environmental Engineering Sciences B.S., Zoology	Environmental Microbiology & Toxicology	DELS-WES Administration	41
Laboratory QA & Data Manager	Beth McDonough	B.S., Chemical Engineering	Env Data Mgmt & Regulatory Processes Analysis	Laboratory QA & Data Management	35
Administrative, Fiscal & Facility Director	Deanne Daneau	A.S., Biology, Registered Nurse	Accounting & Facility Management	DELS-WES Administration, Cost Center Accounting & Budget Management, and Facility Maintenance	31
Program Coordinator	Vacant			WinLIMS Sample Login and Data Entry, WES Front Office Receptionist, Shipping-and-Receiving Clerk, DELS-WES Records Administration & Other Administrative Duties	
Laboratory Certification Program					
Laboratory Certification Program Director & Lab Certification Officer – Chemistry (Inorganic & Organic) & Microbiology	Lisa Touet	B.S., Biochemistry	Environmental Chemistry & Microbiology	Management of the Laboratory Certification Program; Inspection of Chemistry (Inorganic & Organic) & Microbiology Laboratories; Lab Cert Fees, Database, Permit (FMF/PIMS), and Records Administration	33
Lab Certification Officer – Microbiology	Jenna Kotuli	B.A., Env. Science	Environmental Microbiology	Microbiology Lab Inspections & Other Lab Certification Duties and Lab Quality Assurance Duties	21
Microbiology Laboratory					
Culturable Microbiology & Toxicology Section Supervisor	Ronnie Stoner	B.S., Applied Sciences (Medical Lab. Tech.)	Environmental Microbiology & Toxicology	Supervision of and Lead Analyst for all Culturable Microbiology Laboratory Methods and for Cyanotoxin ELISA Analysis, Microtox Acute Toxicity Assay, and BOD_5 assay.	37

TABLE 1. DELS-WES TECHNICAL AND SUPPORT PERSONNEL

Position/Title	Name	Educational Degree(s) & Major(s)	Present Specialty	Primary Responsibility (Includes Analytical Methods)	Years of Experience in Field
Environmental Laboratory Scientist and Backup Supervisor for Culturable Microbiology & Toxicology Section in Absence of Supervisor	Madhuri Tummalapalli	M.S., Env. Science M.S., Plant & Soil Sciences B.S., Agricultural Sci.	Environmental Chemistry & Microbiology	Analytical Support for all Culturable Microbiology and Toxicology Laboratory Work and BOD_5 assay.	22
Molecular Microbiology Section Supervisor; Health, Safety, & Emergency Preparedness Officer; and EMS Support	Jean Tang	Certified Microbiologist (NRCM), Certified Biological Safety Professional (ABSA), Ph.D., Env., Coastal, & Ocean Sciences M.S., Life Sciences B.S., Agricultural Chemistry	Environmental Microbiology & Molecular Microbiology; Biological and Environmental Safety	Direct Supervision of all Molecular Microbiology Laboratory Work; Lead Analyst for all PCR, RT-PCR, and rep-PCR Testing; Analytical Support for all Microbiology Laboratory Work; Management of WES Health, Safety, & Emergency Preparedness Programs; and EMS Support	31
Environmental Laboratory Scientist and Backup Supervisor for Molecular Microbiology Section in Absence of Supervisor; and Analytical Support to the Inorganic Chemistry Laboratory and LIMS Support	Tess Burdin	B.S., Microbiology	Environmental Microbiology & Molecular Microbiology	Analytical Support for Molecular Microbiology Laboratory Work; and Lead Analyst for SM 2130B and other Analytical Support to the Inorganic Chemistry Laboratory.	18

TABLE 1. DELS-WES TECHNICAL AND SUPPORT PERSONNEL

Position/Title	Name	Educational Degree(s) & Major(s)	Present Specialty	Primary Responsibility (Includes Analytical Methods)	Years of Experience in Field
Inorganic Chemistry Laboratory					
Laboratory Supervisor	Nina Duston	Ph.D., Marine Geochemistry M.S., Marine Science B.S., Chemistry-Physics	Environmental Chemistry	Supervision of and Analytical Support for all Inorganic Chemistry Laboratory Work	38
Laboratory Supervisor	Peter Piro	B.A., Chemistry; Biology Minor	Analytical & Environmental Chemistry	Analytical Support for all Inorganic Chemistry Laboratory Work; and Lead Analyst for EPA 200.8, EPA 300.0, EPA 300.1, & EPA 6020.	31
Environmental Laboratory Scientist	Carol Batdorf	M.S., Oceanic Sciences B.S., Aquatic Environments, Biology and Geology Minors	Environmental Chemistry	Lead Analyst for EPA 200.7, EPA 245.1, EPA 6010D, EPA 7471A, EPA 7473, and SM 2340 B; Analytical Support for all Inorganic Chemistry Laboratory Work.	38
Environmental Laboratory Scientist	David Brierley	M.S., Env. Studies B.S., Geology B.S., Chemistry	Environmental Chemistry	Analytical Support for all Inorganic Chemistry Laboratory Work; and Lead Analyst for nutrients and chloride in watershed samples.	26
Environmental Laboratory Scientist	Elsy Naveo	B.S., Chemistry	Environmental Chemistry	Analytical Support for all Inorganic Chemistry Laboratory Work; and Lead Analyst for nutrient analyses using the discrete analyzer.	6
Organic Chemistry Laboratory					
Laboratory Supervisor for GC/HPLC Section	Michael Bebirian	M.S. Eng, Env. Studies B.S., Chemistry	Environmental Chemistry	Supervision of all Laboratory Work; Lead Analyst for EPA 531.2, EPA 552.3, MassDEP 555, EPA 1664, Modified EPA TO-11/CARB 1004, EPA 1010A, and EPA 9071B	36
Environmental Laboratory Scientist	Tory Bagshaw	M.S., Env. Studies B.A., Chemistry	Environmental Chemistry	Analytical Support for all Organic Chemistry Laboratory Work	24
Laboratory Supervisor for MS Section	Vacant			Supervision of all Laboratory Work; Lead Analyst for EPA 524.3, 525.2, 8260D, 8270E, 522, Caffeine, and MA VPH	

TABLE 1. DELS-WES TECHNICAL AND SUPPORT PERSONNEL

Position/Title	Name	Educational Degree(s) & Major(s)	Present Specialty	Primary Responsibility (Includes Analytical Methods)	Years of Experience in Field
Environmental Laboratory Scientist	John Bardzik	M.S., Plastics B.S., Chemistry	Environmental Chemistry	Analytical Support for all Organic Chemistry Laboratory Work	36
Environmental Laboratory Scientist	Lisa Jordan	B.A., Chemistry	Environmental Chemistry	Lead Analyst for all LC/MS/MS Work – EPA 545 (Anatoxin-a & Cylindrospermopsin), EPA 537.1 and 533 (PFAS), and EPA 1694	11

TABLE 2. MICROBIOLOGY LABORATORY EQUIPMENT

TABLE 2. Microbiology Laboratory Equipment

Item	No. of Units	Method	Manufacturer	Model
Analytical Balance 0.1 mg readability Stable base ASTM Class 1 weights (Troemner) Service contracts	1		Mettler-Toledo	MS204S
Magnetic Stirrer Variable speed TFE coated stir bar	3		Thermolyne	Cimarec 2 Nuova
pH Meter Accuracy ± 0.01 units Line Usable with specific ion electrodes	2		Beckman Orion	390 Star A211
Hot Plate Temp Control Temp Control and Magnetic Stirrer	 1 1		 Corning Corning	 PC351 PC620-D
Centrifuge To 3,000 RPM Option of 4 x 50 mL	5		Heraeus Beckman IEC Eppendorf	Megafuge 2.0R Avanti J-25 and TJ-6 Micromax RF 5810R
Refrigerator/Freezer Standard laboratory Capable of maintaining nominal temperature of 4°C Ultra-low temperature (-80 to -85°C) freezers (2)	6		Hotpoint Revco Thermo Scientific	CTX14catgrwh RCF252A14 TSX Series x (2)
Thermometer Alcohol filled and electronic Celsius 1°C or finer subdivision to 180°C NIST certified or traceable **Infrared Thermometer** (1°C Resolution)	Several 1		 Control Company	 4472

TABLE 2. Microbiology Laboratory Equipment

Item	No. of Units	Method	Manufacturer	Model
Glassware Borosilicate Volumetrics should be class A	Several			
Spectrophotometer Range 400 – 700 nm Band width - < 20 nm Use several size & shape cells Pathlength 1 – 5 cm	1		Genesys	Spectronic 20 Genesys
UV Viewing Cabinet	1		UVP Chromato-Vue	C-70G
Water Bath Electric or steam heat Control within 5°C to 100°C	5	Virology Fecal Coliforms & *E. coli*	NB Science VWR Fisher Thermo/Precision VWR	Innova 3000 1187P Isotemp 210 2862 1230
Glassware Filtration funnels	20		Kimble	
Quanti-Tray Sealer	2	Enterolert SM 9223-MPN	IDEXX	Model 2X PLUS
Incubator 35°C – Low Temp Incubator 41.0°C 36.5°C – CO_2 Incubator 36.0°C	 1 1 1 1		 VWR VWR VWR Precision	 2020 1535 2500 30 MR
Reverse osmosis/ultra-filtration reagent water (ASTM Type I) system	1		Millipore	Milli-Q Advantage A10
Autoclave with RO (Watts 200M)	1 1		Gettinge Brinkman	522LS Tuttnauer 3870E
Biological safety cabinets	6		NuAire	

TABLE 2. Microbiology Laboratory Equipment

Item	No. of Units	Method	Manufacturer	Model
Microscopes				
Compound Scope	1		Spencer American Optical	
Dissecting Scope	2		Spencer American Optical	
			VWR	89404
Inverted Scope	2		Nikon	TMS-F
Ice Maker	1		Scotsman	AFE325AS-1B
Glove Box	1		Germfree	Custom-built
Conductivity Meter	1	Method 2510B	Fisher Scientific	09-330/FB61273
Real Time PCR System	1	Molecular Microbiology	Applied Biosystems	7500 Fast
Nano Drop 2000c	1	Molecular Microbiology	Thermo	2000c
Thermocycler	1	Molecular Microbiology	Eppendorf	Mastercycler gradient
Dissolved Oxygen Meter	1	SM5210	YSI	5000
Incubator (low temperature)	1	SM5210	VWR Scientific	3733A

TABLE 3. INORGANIC CHEMISTRY LABORATORY EQUIPMENT

TABLE 3. Inorganic Chemistry Laboratory Equipment

Item	No. of Units	Method	Manufacturer	Model
Analytical Balance 0.1 mg readability Stable base ASTM type 1 (Troemner) Under service contracts	2 1		Mettler Toledo	XS 104 ME54TE/00
Top Loading Balance Under service contract	2		Mettler Mettler	PE 1600 ME4002E
Magnetic Stirrer Variable speed TFE coated stir bar	Several		Fisher Scientific	
pH Meter Accuracy ± 0.1 pH units Line Usable with specific ion electrodes	2 1	SM 4500 HB	Thermo Fisher Orion Beckman Thermo Fisher Orion	VSTAR80 510 Versa Star Pro
Conductivity Meter Readable in ohms or mhos Range of 2 ohms to 2 mhos Line or battery	2	SM 2510B	YSI	35 3200
Hot Plate-temp control	1		Thermolyne	2200
Color Standards To verify wavelength photometers Should cover 200-800 nm	Several			
Refrigerator Standard laboratory, explosion proof for organics Refrigerators capable of maintaining nominal temperature of 4°C	5 Large; 6 Under bench		9 Fisher 1 Thermo Scientific 1 Marvel	

TABLE 3. Inorganic Chemistry Laboratory Equipment

Item	No. of Units	Method	Manufacturer	Model
Freezer Freezers capable of maintaining at least -10°C	5		3 Frigidaire 1 Maytag 1 Woods Frost-Free	
Refrigerator/Freezer	1		Frigidaire	
Drying Oven Gravity or convection Controlled from room temp to 180°C or higher (± 2°C)	1	SM 2540 B, C, D, G	VWR	1370FM
Muffle furnace To 450°C for cleaning organic glassware	1	SM 2540 G	Thermolyne	FA1730
Thermometer Infra-Red 1°C resolution	1		Traceable	4472
Glassware Borosilicate, HDPE, PTFE, PMP Volumetrics and pipettes Class A	Several			
Spectrophotometer, UV-Visible Range 190 - 1100 nm Band width - 1 nm Use several size & shape cells Path length 1 – 10 cm	1	SM 4500 CN E SM 3500 Cr D SM 4500-Cl G SM 5220 D	Thermo Scientific	Evolution 60S
Turbidimeter	1 1	SM 2130 B	HACH HACH	2100 AN TL2300
TCLP Extractor	2	EPA 1311	Millipore	
High pressure filter system	1	EPA 1311	Millipore	
Direct Mercury Analyzer DMA80	1	EPA 7473	Milestone	DMA80
Analytical Balance with DMA80	1	EPA 7473	Presisa	XB220A

TABLE 3. Inorganic Chemistry Laboratory Equipment

Item	No. of Units	Method	Manufacturer	Model
Microwave Digestor	1	EPA 3015A EPA 3051A EPA 3052	Milestone	Ethos EZ
Hot Block 54 positions; 50mL digestion tubes 48 position; 50mL digestion tubes	1 1 1	EPA 200.2 EPA 3010A EPA 3050B	 Environmental Express Perkin Elmer	 SCI54 SPB 50-48
COD Closed Reflux Reactor	1	SM 5220 D	Velp Scientifica	ECO 25 Thermoreactor
Mill Stainless Steel Screens 0.5-mm, 1-mm, 2-mm, & 5-mm mesh sizes	1		Thomas Scientific	Wiley Laboratory Mill, Model 4
Inductively Coupled Plasma – Mass Spectrometer Pulse-Analog Dual Detector Kinetic Energy Discrimination (KED) Dynamic Reaction Cell Computer Controlled, Syngistix V2.5 Background Correction RF Generator Autosampler Chiller Argon, Helium Gas Supply UPS	1	EPA 200.8 EPA 6020	Perkin Elmer Elemental Scientific PowerVar	NexION 1000 SC-4DX FAST ABCDEF800022

TABLE 3. Inorganic Chemistry Laboratory Equipment

Item	No. of Units	Method	Manufacturer	Model
Inductively Coupled Plasma Spectrometer Atomic Emission Spectrophotometer Simultaneous Dual-View Computer Controlled – Syngistix Background Correction RF Generator Autosampler Chiller Argon, Nitrogen, and Compressed Air Gas Supplies UPS	1	Metals EPA 200.7 Minerals EPA 6010D	Perkin-Elmer Elemental Scientific PowerVar	Optima 8300 DV SC-4DX prepFAST ABCDEF520022
Ion Chromatograph Inline Filtration Autodiluter & Intelligent Dilution Computer-controlled, Software	2	EPA 300.0 EPA 300.1 EPA 218.6	Metrohm	930 Compact Flex with 858 autosampler; 930 Compact Flex, 944 UV/Vis Detector, 919 autosampler
Mercury Analyzer Spectrophotometer Dedicated analyzer with Hg lamp Absorption cell: 10-cm quartz cell with end windows Air pump to deliver flow of 1 L/min Drying unit: 6- in. tube with 20 grams magnesium perchlorate or heating device or lamp to prevent condensation on cell	1	EPA 245.1	Perkin Elmer	FIMS 100

TABLE 3. Inorganic Chemistry Laboratory Equipment

Item	No. of Units	Method	Manufacturer	Model
Automated Segmented Flow Analysis System Multi-channel pump Manifold, colorimeter Autosample Standard Predilution Utility	2	Modified SM 4500 NH3-N (B,G) SM 4500 NO3 + NO2-N F SM 4500 Cl E SM 4500-P B(6) F Modified SM 4500-N C w/ SM4500 NO3 + NO2-N F	Skalar	San ++
Discrete Analyzer Autosampler Autodiluter	1	SM4500NO3+NO 2-N F SM 4500 Cl E	Seal Analytical	AQ400
Moisture Analyzer	1	AOAC Int. 950.46B.(b)	Leco	TGM800
Freeze Dryer	1	DEP DELS SOP LabconcoPreeZone20150311	Labconco	FreeZone 2.5 Model 76705
Reagent Water (ASTM Type I) system	1 2		Millipore Thermo Fisher/Barnstead	Milli Q A10 Advantage GenPure (UV-TOC Model)
Fume Hoods Standard fume hoods Metal-free fume hood	 10 1		 Thermo Scientific Nuaire	 Hamilton Safe Aire II
Auto-Titrator	1	SM 2320B	Radiometer	TIM870
Cyanide Distillation	1	SM 4500 CN C EPA 9010C	Environmental Express	SimpleDist

TABLE 3. Inorganic Chemistry Laboratory Equipment

Item	No. of Units	Method	Manufacturer	Model
Desiccator	3		Bel-Art	Secador 120V, Model 4.0, Cat # F420741118
	2		Bel-Art	Inert Gas Purged Model 4.0, Cat #F420741009
Air Displacement Pipettes	Several	General Use	BrandTech	2-20 µL 5-50 µL 10-100 µL 10-1000 µL 20-2000 µL 500-5000 µL 1000-10,000 µL

TABLE 4. ORGANIC CHEMISTRY LABORATORY – GC/LC SECTION EQUIPMENT

TABLE 4. Organic Chemistry Laboratory – GC/LC Section Equipment

Item	No. of Units	Method	Manufacturer	Model
Analytical Balance 0.1 mg readability Stable base ASTM type 1 weights (Troemner)	1		Mettler	AB 204
Refrigerator/Freezer Standard laboratory, explosion proof for organics Refrigerators capable of maintaining nominal temperature of 4°C. Freezer capable of keeping -10°C.	5		2 Fisher Scientific refrigerators (samples)(extracts) Fisher Scientific refrigerator, explosion proof (standards) VWR refrigerator (air samples only) VWR, explosion proof refrigerator (air sample extracts only)	Frost clear 13-986-152 IsoTemp R406GABA R406XABA
Drying Oven Gravity or convection Controlled from room temp to 180°C or higher (± 2°C)	1		Fisher	750G Isotemp
Flashpoint Tester	1	EPA 1010A	PetroTest	PMS-4
Thermometer Methanol filled Celsius submerged in ethylene glycol -5°C to 15°C (0.5°C or finer subdivision) NBS certified or traceable	5		Ertco	Refrigerator thermometers
Infrared Thermometer (1°C Resolution)	1		Control Company	4472

TABLE 4. Organic Chemistry Laboratory – GC/LC Section Equipment

Item	No. of Units	Method	Manufacturer	Model
Glassware Borosilicate Volumetrics, Class A Separatory funnels Kuderna Danish (K-D) concentrators	Several		Kontes, Pyrex, Fisher, & VWR	Various
Steam Bath/Concentrators Electric-generated steam heat with solvent recovery system Control within 0.1°C to 100°C Electric-generated steam heat with nitrogen blowdown.	1		Organomation	S-Evap-KD
	1	MA DEP 555 EPA 3510 EPA 3545A	Thermo-Fisher	Rocket Evaporator
Heat block with nitrogen blowdown	1	Lipid determination TPH	Lab-Line	Multi Block
Digital steel-shot heating bath	1	EPA 552.3	VWR	Digital Heat Block
Extraction Apparatus Accelerated Solvent Extractor	1	EPA 3545A	Dionex	ASE 350
Fume hoods	8		Thermo Scientific	4-, 6- & 8-ft fume hoods
Gas Chromatograph Split/splitless injection Oven temp control ± 0.2°C	1	EPA 552.3	Thermo Scientific	1310 GC-ECD
Oven temp programmer	1	Oil ID & MA EPH	Thermo Scientific	1310 GC-FID
Electron capture detector Linearized	2 on 1 GC	See above	Thermo Scientific	1310 GC-ECD
Flame ionization detector	1	See above	Thermo Scientific	1310 GC-FID

TABLE 4. Organic Chemistry Laboratory – GC/LC Section Equipment

Item	No. of Units	Method	Manufacturer	Model
High Performance Liquid Chromatograph Constant flow Capable of injecting 1 – 200µL Gradient System Post column reactor Fluorescence detector	1	EPA 531.2	Waters	Alliance HPLC
Isocratic System Photoarray/UV detector		MassDEP 555 Carbonyl compounds	Waters	Acquity UPLC H-Class

TABLE 5. ORGANIC CHEMISTRY LABORATORY – MS SECTION EQUIPMENT

TABLE 5. Organic Chemistry Laboratory – MS Section Equipment

Item	No. of Units	Method	Manufacturer	Model
Analytical Balance 0.1 mg readability Stable base ASTM type 1 weights (Troemner)	2		Mettler	AB204 AL104
Fume hood	1			4-ft Fume Hood
Hot Plate-temp control	1		Equatherm	
Refrigerator/Freezer Standard laboratory, explosion proof for organics Capable of maintaining nominal temperature of 4°C for refrigerators and -10°C for freezers	3 Refrig 4 Freezer		Thermo Scientific (2) Fisher Scientific (1) VWR Scientific (1) Fisher Scientific (3)	
Gas Generator Hydrogen Generator	1		Packard	9200
Sample Concentrator	1 1	 EPA 537.1 EPA 533	Thermo Scientific Organomation	Rocket Evaporator N-Evap-111
Drying Oven Gravity or convection Controlled from room temp to 180°C or higher (± 2°C)	2		Fisher Scientific VWR Scientific	#18 1370FM
Muffle furnace To 450°C for cleaning organic glassware and decontaminating sodium sulfate.	1		Thermolyne	

TABLE 5. Organic Chemistry Laboratory – MS Section Equipment

Item	No. of Units	Method	Manufacturer	Model
Thermometer -30°C to 10°C 1°C or finer subdivision NBS certified or traceable	Several			
Glassware Borosilicate Volumetrics should be class A Separatory funnels	Several			
Gas Chromatograph/Mass Spectrometer/Data System (GC/MS/DS)	1	EPA 524.3 EPA 8260D	Thermo Scientific	Thermo Trace 1300 ISQ Quadrupole MS TraceFinder Software
Purge and Trap Concentrator and Auto Sampler (Refrigerated)	1		Teledyne Tekmar	AtomX
Chiller	1		Thermo Scientific	Haake A 28 F
Gas Chromatograph/ Mass Spectrometer/Data System (GC/MS/DS)	1	EPA 525.2 EPA 522 Caffeine by modified EPA 525.2	Thermo Scientific	Thermo Trace 1300 ISQ Quadrupole MS TraceFinder Software
Gas Chromatograph/Tandem Triple-Quad Mass Spectrometer (GC/MS/MS)	1	EPA 8270E	Thermo Scientific	
Ultra Performance Liquid Chromatograph/Tandem Triple-Quad Mass Spectrometer (LC/MS/MS)	1	EPA 533 EPA 537.1 EPA 545	Waters	Acquity I-Class UPLC Xevo TQ-S Micro Detector
	1	Modified EPA 533 EPA 537 EPA 1694	Waters	Acquity H-Class UPLC Xevo TQD Detector

TABLE 6. EQUIPMENT CALIBRATION PROCEDURES

TABLE 6. Equipment Calibration Procedures

INSTRUMENT	FREQUENCY OF CALIBRATION	STANDARD REFERENCE MATERIALS USED	GENERAL PROCEDURES	CALIBRATION ACCEPTANCE LIMITS
Analytical Balances	Each day of use Semi-annually calibrated by qualified service representative	Check with 2 different Class S weights. Calibrated against certified ASTM Class I weights.	Manufacturer's instructions	By weight range, according to manufacturer's specifications
Analytical Balances (Microbiology)	Monthly Semi-annual	NIST-certified weights By vendor	Check accuracy Checked by a qualified service representative	± 0.001g
Top Loading balance	Each day of use	Same as for Analytical Balances	Manufacturer's instructions	By weight range, according to manufacturer's specifications
Auto Analyzer	When used	6 calibration standards + a blank + 2 QC samples	Manufacturer's instructions	± 2-3 standard deviations
Autoclaves	Daily or when in use	Pressure and temperature gauge NIST-certified Stop Watch	Record date, contents, sterilization time and temperature for each cycle. Check cycle time	Reaches temperature of 121°C in 30 minutes. Maintains 121°C during sterilization cycle and completes the entire cycle within 45 minutes when a 12-15 minute sterilization period is used.
Conductivity meter	When used	1 standard + blank	Manufacturer's instructions	± 2% of span plus ± least 1 significant digit at 25°C.
Millipore ASTM Type I Reagent Water System – Microbiology Lab	In-line Annually	Check conductivity pH, heavy metals, and organic contaminants	Manufacturer's instructions & WES SOPs	ASTM Type I reagent water must have a resistivity of > 10 megohm-cm (conductivity of < 0.1 μS/cm) at 25°C
Millipore and ThermoFisher/Barnstead ASTM Type I Reagent Water Systems – Inorganic Chemistry Lab	Daily	Check conductivity & TOC	Manufacturer's instructions	ASTM Type 1 reagent water must have a resistivity of >18 megohm-cm at 25°C. TOC < 10 ppb
Millipore ASTM Type I Reagent Water System – Organic MS Lab	Resistivity readings taken daily. Conductivity readings taken twice monthly.	Resistivity measurements taken directly from the RO Pod display. Conductivity measurements taken from a calibrated conductivity meter.		

TABLE 6. Equipment Calibration Procedures

INSTRUMENT	FREQUENCY OF CALIBRATION	STANDARD REFERENCE MATERIALS USED	GENERAL PROCEDURES	CALIBRATION ACCEPTANCE LIMITS
Millipore ASTM Type I Reagent Water System – Organic GC/LC Lab	Resistivity readings taken daily. Conductivity readings taken twice monthly.	Resistivity measurements taken directly from the RO Pod display. Conductivity measurements taken from a calibrated conductivity meter.		
Gas Chromatographs (ECD, FID) High-Performance Liquid Chromatograph (HPLC)	When used	5 calibration standards + a blank	1. Run calibration curve of 5 standards and calculate r value 2. Compare resolution, sensitivity, and retention times with previous runs 3. Record values in instrument work book	Regression coefficient (r) > 0.99
Gas Chromatograph/Mass Spectrometers (GC/MS)	When used	5 to 7 concentrations depending on method used.	Calibrate as per analytical method	% RSD < 30% of the mean RF for SVOCs. For VOCs, low level standard must be ≤ 150% recovery; medium and high levels must be ≥ 50% recovery.
Hot Air Ovens	Daily or when in use	Thermometer	Check temperature	170° - 180°C for at least two hours ± 10°C
DMA80-Mercury Analyzer	Check calibration when used; put in new curve when needed	8 calibration standards then check curves with 2 QC standards	Manufacturer's instructions	90 - 110%
FIMS – Mercury Analyzer	When used	5 calibration standards QCS samples	Manufacturer's instructions	95 - 105%
Incubators	Twice daily, with readings taken at least 4 hr. apart when in use	Thermometer	Check temperature	Maintain temperature to an accuracy of ± 0.5°C or within a given range as called for in the analytical method.
Inductively-coupled plasma emission spectrometer (ICP)	When used	Run instrument check standard and calibration blank every 10 samples	Manufacturer's instructions. Record values in instrument log book.	Maximum response < 0.006 nm of reference line, ± 10% of original curve. Verify calibration with QC check standard from an external source. If recovery is not 95-105%, recalibrate.
Laboratory Fume Hoods and Biological Safety	Annually	Flow meter	Checked by a qualified service representative	100 LFPM face velocity.

TABLE 6. Equipment Calibration Procedures

INSTRUMENT	FREQUENCY OF CALIBRATION	STANDARD REFERENCE MATERIALS USED	GENERAL PROCEDURES	CALIBRATION ACCEPTANCE LIMITS
Cabinets				
pH Meters	Every 8 hours of operation	3 concentrations of buffers for older pH meters: 4, 7, and 10; for newer meters, perform a 2-point calibration with pH 4 and 7 buffers.	Bracket pH value expected as closely as possible with buffer	± 0.05 pH units
Refrigerators	Calibration Checked Daily	Thermometer	Thermometer bulb immersed in liquid	Temperature maintained at 1° to 6°C. Thermometer graduated in at least 1°C increments.
Freezers	Calibration Checked Daily	Thermometer		≤ -10°C
Thermometers	Annually	Calibrated against NIST-traceable thermometer by qualified technician	Manufacturer's instructions	± 1°C
Turbidimeter	When used	4 calibration standards + a blank.	Manufacturer's instructions	10% turbidity units
Water baths for bacterial analyses	Twice each day when in use with readings taken at least 4 hrs. apart	Thermometer	Check temperature	Maintain temperature at 44.5°C ± 0.2°C
Inductively Coupled Plasma Mass Spectrometer (ICP-MS)	When used	Run instrument check standard and calibration blank every 10 samples, QCS after calibration. MRL(s) after calibration. Run tuning solution and daily solution before calibration	Manufacturer's Instructions and analysis method	Method Specific

TABLE 7. INSTRUMENT MAINTENANCE PROCEDURES

INSTRUMENT	PROCEDURE	FREQUENCY
MICROBIOLOGY LABORATORY		
Autoclave	Flush trap, check seals, clean chamber of any residue.	Each day of operation or as needed.
Water Baths	Drain and fill to level requirements, add sanitizing solution to curb growth.	As needed
Incubators	Check for proper temperature operation.	Daily or when used.
Type 1 Reagent Water System	Check resistivity daily	Change cartridges as needed
INORGANIC CHEMISTRY LABORATORY		
ICP-OES	Check nitrogen and argon flow rates, water circulation system, over flow tank, and change peristaltic pump lines.	Each time instrument is used or service contract guidelines
Auto Analyzer	Replace pump tubes and oil pump	Every 3 months
Discrete Analyzer	Check water baselines and compare current gain values to last gain values. If out of control, replace lamp. If running NO_3+NO_2-N test, perform cadmium coil checks, as needed. Change peristaltic pumps tubing. Periodic maintenance done by the analyst: Replace probe wash assembly, syringe assembly with O-ring, aspiration probe and sampling probe.	Each time instrument is used. Monthly. As needed.
pH Meter	Maintain electrolyte level in probe. Properly store and maintain storage solution	Inspect and replace as needed
Turbidity Meter	Clean filter with lens cleaner	Every 3 months
Conductivity Meter	Store cell in reagent water	Inspect and replace as needed
Autotitrator	Maintain electrolyte level in probe	Inspect and replace as needed
Moisture Analyzer	Self-calibrating	Each time instrument is used
Millipore Reagent Water System	Replace all filters and cartridges	Yearly
Ion Chromatograph	Check system pressure baseline conductivity, pulse damper, guard column, pump lines, regenerant flow rate, Ultra Filtration Cell Membrane (UFC)- where applicable, and detector. .	Each time instrument is used
Mercury Analyzer-FIMS	Check argon gas pressure, peristaltic pump and lines for proper flow, operation, and any cracks. Change filter in gas-liquid separator each time and replace O-rings, if necessary.	Each time instrument is used.
DMA 80	Check for oxygen flow rate, oxygen pressure at tank, water build up in waste line and mercury residue in machine before starting a run. Periodic maintenance done by the analyst :	Each time instrument is used.

INSTRUMENT	PROCEDURE	FREQUENCY
	Change out amalgamator, catalyst tube, silicon O-rings, silicon joints, grease boat O-ring and exposed steel rods of the horizontal and vertical actuators. Should need to be done no sooner than 3000 burns. Condition the catalyst tube and run a stability test. New calibration curve must be done after changing parts or changing out an oxygen tank.	As needed.
ICP-MS	Check Argon/helium pressure. Check air filters and chiller. Check rough pump oil and replace peristaltic pump tubing daily. Replace fluids according to manufacturer's recommendations.	Each time instrument is used or according to manufacturer's specifications.
ORGANIC CHEMISTRY LABORATORY		
GC & HPLC SECTION		
Ni-63 Gas Chromatograph	Replace septa and check glass injector liner for contamination. Check column and detector operation. Perform solvent and column check. Perform 6 month wipe tests on ECDs	Each day of operation. Wipe tests performed every 6 months.
FID Gas Chromatograph	Replace septa and check column and detector operation. Perform solvent and column check for contamination.	Each day of operation or when instrument performance decreases.
HPLC & UPLC	Check connections for pressure leaks. Replace mobile phase carrier solutions. Perform solvent and column check for contamination.	Each day of operation or when instrument performance decreases.
Analytical Balance	Check for accuracy	Every 6 months by metrology service contract
Class 1 Weight Sets	Check for accuracy	Every year by metrology service contract
Laboratory Fume Hoods	Check for proper exhaust flow	Performed yearly by contract ventilation company
MS SECTION		
Gas Chromatograph/Mass Spectrometer	Clean source, check vacuum pressure, replace septum and liner	Each day instrument is used or per service contract.
Purge and Trap	Check and replace fittings and apparatus.	Each day instrument is used or per service contract.

TABLE 8. LABORATORY SOP AND QAP

Document #	Document Title	Latest Revision #	Date
Lab QA Plan	Laboratory Quality Assurance Plan	8.0	February 2021
Overnight Sample Delivery to WES	Operational Procedure for Overnight Sample Delivery to WES	1.0	August 2001
Fish Processing SOP	Processing Fish Samples Intended for Contaminant Analysis	1.0	August 2002
Thermometer Calibration	Standard Operating Procedures for Calibration of Laboratory Thermometers	1.5	December 2007
Lab-Ware Washing SOP	Standard Operating Procedure for Labware Washing in all DELS-WES Laboratories	0	April 2008
PE Samples SOP	Standard Operating Procedures for Performance Evaluation Samples	0	February 2004
QA Review SOP	Level 1 + Level 2 QA Reviews of DELS-WES Analytical Data	1.0	January 2001
Corrective Action Form	Corrective Action Form	1.4	December 2020
IDC Documentation	Initial Demonstration of Capability (IDC) and/or Method Detection Limit (MDL) Determination Form	0	April 2004
QA Rev Form - Chemistry	Quality Assurance (QA) Review Form - Chemistry Laboratories	2.0	December 2005
QA Rev Form - Microbiology	Quality Assurance (QA) Review Form - Microbiology Laboratory	2.1	December 2006
Training-External	Documentation of External Course Work, Training, or Certification	0	May 2004
Training-Laboratory Bench	Documentation of Laboratory Bench Training	1.4	April 2004
Training-External LCO	Documentation of External Course Work or Other Training of LCO Staff	0	February 2007
Training-Internal	Documentation of Internal Course Work, Training, or Certification	0	February 2007
ENHANCED QC MODULE Rev.1	Enhanced Quality Control Module - A Component of the DELS-WES LIMS	1	Projected 2021
Sample Tracking-COC Documentation	Procedure for Completing the WES Sample Tracking & Chain-of-Custody Record	3.4	July 2013
Sample Conditions Review Form	Sample Conditions Review Form (SCRF)	3.0	July 2015
Manual Intg – OCL and IOCL	SOP for Conducting Reintegration of Data Generated by the Organic and Inorganic Chemistry Laboratories	1.0	February 2016
Microbiology Laboratory – Drinking Water Methods			
SM 9215B	SM9215B –Heterotrophic Plate Count - Pour Plate Procedure	1.9	February 2019
SM9222D	SM9222D - Standard Fecal Coliform Membrane Filtration Procedure	2.2	February 2019
SM 9222B	SM9222B – Standard Total Coliform Membrane Filtration Procedure	3.2	February 2019
SM 9222G	SM9222G - Confirmation of E. coli from SM9222B Using Nutrient Agar with MUG	1.1	February 2019
SM 9223B	SM9223 – Enzyme Substrate Coliform Test Presence-Absence Procedure for Analysis of Potable Water	2.4	February 2019
SM 9223B-MPN	SM9223 – MPN Enzyme Substrate Coliform Test Most Probable Number Procedure for Analysis of Potable and Non-Potable Water Samples	1.-2	December 2020

Document #	Document Title	Latest Revision #	Date
EPA 1604	EPA Method 1604 – Membrane Filtration Procedure for the Simultaneous Detection of Total Coliforms and *Escherichia coli* – MI Agar Method	1.6	February 2019
C-perfringens	Membrane Filter Method for *C. perfringens*	0	September 2004
Autoclave SOP	SOP For Getinge 522LS Gravity Steam Sterilizer - DELS-WES Microbiology Laboratory	1.5	February 2010
Reagent-H20-Microb	Reagent Water System - Reverse Osmosis/De-ionization System for the DELS-WES Microbiology Laboratory	2.0	August 2010
SM 9230D	Enzyme Substrate Enterococcus Test Most Probable Number Procedure for Analysis of Non-Potable Water Samples	2.1	February 2019
EPA 1600	USEPA Method 1600 – Standard Enterococci Membrane Filtration Procedure	1.8	February 2019
Microbiology Laboratory – Other Analytical Methods			
BacteroidetesG	Determination of *Bacteroidetes* Group Marker By PCR Assay Based on AEM 66:1587	0	March 2006
BacteroidetesHF	Determination of *Bacteroidetes* Human-Specific Marker - Modified Method of AEM 66:1587	0	Marcy 2006
SM 9213D	SM 9213D - Standard *E. coli* Membrane Filtration Procedure	0	August 2000
SM 9222D	SM 9222D – Standard Fecal Coliform Membrane Filtration Procedure	2.1	February 2016
ENT-esp Marker	Determination of Enterococcal *esp* Gene (Sewage Marker) Based on ES&T 39:283	0	March 2006
Micro-ID	Identification of Enterobacteriaceae	0	November 2009
Microtox Acute 81.9%	Microtox Acute Toxicity Test (81.9% Test)	0	May 2005
Microtox Acute Solids	Microtox Acute Toxicity Test (Solid-Phase Test)	0	May 2005
Inhibitory Residue	Glassware Inhibitory Residue Test	0	May 2007
Rep-PCR Fingerprinting	Rep-PCR	0	April 2013
SM 5210B	SM5210 – Determination of Biochemical Oxygen Demand (BOD)	0	September 2000
Inorganic Chemistry Laboratory – Drinking Water Methods			
EPA 200-2	USEPA Method 200.2 – Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements	1.3	March 2020
EPA 200-7	USEPA Method 200.7 – Determination of Metals & Trace Elements & Hardness in Water & Wastes by ICP-AES	6.0	February 2019
EPA 200-8	USEPA Method 200.8 – Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Mass Spectrometry	4.0	November 2020
EPA 245-1	USEPA Method 245.1 – Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry	3.2	February 2019
EPA 300-0	USEPA Method 300.0 – Determination of Inorganic Ions	3.0	February 2019
EPA 300-1	USEPA Method 300.1 Part B. Inorganic Disinfection By Products – Determination of Inorganic Anions in Drinking Water by Ion Chromatography	3.0	February 2019

Document #	Document Title	Latest Revision #	Date
SM 2130B	SM2130B - Determination of Turbidity, Nephelometric Method	2.1	March 2020
SM 2320B	SM2320B - Determination of Alkalinity by the Titration Method	1.4	October 2020
SM 2510B	SM2510B - Determination of Conductivity (Laboratory Method)	1.2	November 2006
SM 4500-Cl F	SM4500-CLF – Determination of Chlorine Residual DPD Ferrous Titrimetric Method	1.0.	November 2003
SM 4500CN-C E	SM4500 CN-CE - Determination of Total Cyanide by the Distillation/Spectrophotometric Method	2.3	February 2019
SM 4500H-B	SM4500-H+B - Determination of pH by the Electrometric Method	1.3	October 2015
Reagent-H20-Chem	Milli-Q Water System for the Inorganic Chemistry Laboratory - Millipore A10 Advantage with Q-Pod Element	1.0	September 2010
Inorganic Chemistry Laboratory – Other Analytical Methods			
Auto Shredder Waste	Grinding, Sample Mixing and Subsampling of Auto Shredder Waste For Metals and PCBs Analysis	0	January 2012
AOAC Int. 950.46B.(b)	Moisture in Meat (Fish Tissue)	0	May 2020
SM 4500 NH3-N (B, G)	Determination of Ammonia, Colorimetric Automated Phenate	0	Projected 2021
SM4500 Cl E	Determination of Chloride by Automated Ferricyanide	0	Projected 2021
SM 4500-N C, SM 4500 NOx-N F	Determination of Total Nitrogen	0	August 2020
SM 4500 NOx-N F	Determination of Nitrate Nitrite Nitrogen, Colorimetric-Automated, Cadmium Reduction	0	Projected 2021
SM 4500-P B(5), F	Determination of Total Phosphorus	0	August 2020
EPA 420-1	USEPA Method 420.1 – Determination of Phenol Phenolics, Total Recoverable	0	October 2000
EPA 1311	USEPA SW846 Method 1311 – Determination of TCLP Toxicity Characteristic Leaching Procedure	1.0	December 2010
EPA 3015 Draft	USEPA 3015 – Sample Preparation Procedure for Microwave-Assisted Acid Digestion of Aqueous Samples and Extracts	0	February 2000
EPA 3050B	USEPA Method 3050B – Acid Digestion of Sediments, Sludges, and Soils	0	March 2000
EPA 3051 Draft	USEPA 3051 – Sample Preparation Procedure For Microwave-Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils.	0	February 2000
Mod EPA 3052	Modified USEPA Method 3052 – Microwave Assisted Acid Digestion of Organic Matrices	1.0	July 2020
EPA 6010D	Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry	4.0	July 2020
EPA 7196A	USEPA SW846 METHOD 7196A – Determination of Hexavalent Chromium	0	February 2000
EPA 7473	Mercury In Solids And Solutions By Thermal Decomposition, Amalgamation, And Atomic Absorption Spectrophotometry Using The Milestone DMA-80 Mercury Analyzer	2.0	July 2020
EPA 9010B	USEPA SW846 METHOD 9010B – Total and Amenable Cyanide by Distillation	0	August 2002
EPA 9040A	USEPA SW846 METHOD 9040A – Determination of pH in RCRA Samples	0	September 2000

Document #	Document Title	Latest Revision #	Date
EPA 9045A	USEPA SW846 Method 9045A – Determination of Soil pH	0	November 2000
SM 2340 B	SM 2340- Hardness by Calculation	0	July 2020
SM 2540B	SM2540B – Determination of Total Solids Dried at 103-105°C	0	October 2000
SM 2540C	SM2540C – Determination of Total Dissolved Solids Dried at 180°C	1.0	November 2006
SM 2540D	SM2540D – Determination of Total Suspended Solids Dried at 103-105°C	1.0	November 2006
SM 2540G	Determination of Total Fixed & Volatile Solids in Semi-Solid Samples	0	October 2000
SM 3500Cr-B	Determination of Hexavalent Chromium	0	March 2003
SM 4500-0-C	SM4500-OC – Determination of Dissolved Oxygen, Iodometric Method with Azide Modification	0	September 2000
SM 4500-P F; SM 4500-N C	SM 4500-P F & SM 4500-N C – Determination of Total Phosphorus Automated Ascorbic Acid Reduction Method & Determination of Total Nitrogen Persulfate Method	0	August 2020
USGS I 4650 03	Determination of Total Nitrogen and Total Phosphorus	0	April 2006
SM 5220-D	Modified Chemical Oxygen Demand Closed Reflux, Colorimetric Method	1.00	November 2015
Organic Chemistry Laboratory – GC/LC Section – Drinking Water Methods			
EPA 531-2	USEPA Method 531.2– Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Post Column Derivatization	0	Projected 2021 (1st Quarter)
EPA 552-3	USEPA Method 552.3 – Determination of Haloacetic Acids & Dalapon in Drinking Water by Micro Extraction, Derivatization and GC with ECD	0	Projected 2021 (1st Quarter)
MassDEP 555	USEPA Method 555 – Determination of Chlorinated Acids in Water by High Performance Liquid Chromatography with a Photodiode Array Ultraviolet Detector	2.5	June 2020
Organic Chemistry Laboratory – GC/LC Section – Other Analytical Methods			
EPA 1010A	SW846 Method 1010A – Pensky Martens Closed Cup Method for Determining Ignitability	1.0	August 2010
EPA 1664 LIQ-LIQ	USEPA Method 1664 – Determination of N-Hexane Extractable Material (SGT-HEM) by Extraction and Gravimetry	1.0	February 2003
EPA 1664 SPE	USEPA Method 1664 – Determination of N-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Total Petroleum Hydrocarbons) by Solid Phase Extraction (SPE) and Gravimetry	1.0	February 2003
EPA TO11A/CARB 1004	Compendium Method TO-11 and CARB 1004 – Determination of Carbonyl Compounds in Air by Extraction & UPLC Analysis	2.0	March 2020
EPA 9071B	N-Hexane Extractable Material in Soils and Sediments by Accelerated Solvent Extraction and Gravimetric Analysis	1.1	August 2013
MA EPH	MA EPH Method – Determination of Extractable Petroleum Hydrocarbons	0	February 2003

Document #	Document Title	Latest Revision #	Date
Oil ID	Modified EPA Method 3580A, 3510C, 3545 & 8015B – Oil Identification in Waste Oils, Wastewaters, Soils and Sediments	0	Projected 2021
Organic Chemistry Laboratory –MS Section – Drinking Water Methods			
EPA 524-3	USEPA Method 524.3 – Measurement of Purgeable Organic Compounds in Water by Capillary Column GC/MS	1.1	February 2016
EPA 525-2	USEPA Method 525.2 – Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column GC/MS	2.4	February 2016
EPA 537	USEPA Method 537– Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)	0	January 2021
EPA 533	EPA Method 533 – Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry		Projected 2021
EPA 537.1	USEPA Method 537.1 – Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)		Projected 2021
Organic Chemistry Laboratory –MS Section – Other Analytical Methods			
EPA 8260D	USEPA Method 8260D – Determination of Volatile Organic Compounds By Gas Chromatography/Mass Spectrometry (GC/MS)	0	March 2003
EPA 8270E	USEPA Method 8270E – Determination of Semi-Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)	0	April 2003
Caffeine in water	Caffeine In Water by Solid-Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry	0	October 2006
EPA 5035A	USEPA Method 5035A – Sampling Volatile Organic Compounds In Soils and Sediments	1.0	February 2007
MA VPH	MA VPH Method – Determination of Volatile Petroleum Hydrocarbons	0	February 2003
EPA 522	USEPA Method 522 – Determination of 1,4-Dioxane by Solid-Phase Extraction Gas Chromatography/Mass Spectrometry with Selected Ion Monitoring (SIM)	0	September 2014
Modified 533	Modified EPA Method 533 – Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry		Projected 2021
Modified 1694	Modified EPA Method 1694 – Pharmaceuticals and Personal Care Products in Water, Soil, Sediment and Biosolids by HPLC/MS/MS		Projected 2021

Laboratory Certification Program (LCP) SOPs and Applications; and Administrative SOPs

Document #	Document Title	Latest Revision #	Date
LCP Application	SOP for Reviewing Applications for Laboratory Certification	2.4	December 2017
LCP Database Program User Access	SOP for LabCert Database Users with LABCERT_Program_USER Access	2.7	January 2021

Document #	Document Title	Latest Revision #	Date
LCP Database Program View User Access	SOP for LabCert Database for Users with LABCERT_VIEW_USER Access	2.6	January 2021
LCP Onsite Inspections	SOP for Conducting Onsite Inspections of Laboratories	4.2	January 2021
LCP Records	SOP for the Management of Laboratory Certification Records	2.2	December 2017
LCP PT Revocations	SOP for PT Revocation Process for Chemistry and Radiochemistry Analytes/Methods	1.3	January 2021
LCP Validation Codes Screens	SOP for the Use of the LabCert Validation Codes Screens	1.3	January 2021
LES01app LES01instructions	Application for Certification of Laboratory for Microbiological Analysis of Water and Instructions	0	June 2020
LES02app LES02instructions	Application for Initial Certification of Laboratory for Environmental Analysis – Chemistry and Instructions	0	June 2020
LES03app LES03instructions	Application for Modification of Certification of Laboratory for Environmental Analysis – Chemistry and Instructions	0	June 2020
LES04app LES04instructions	Application for Modification of Certification of Laboratory for Environmental Analysis – Microbiology and Instructions	0	June 2020
Renewal Application	Application for Certification of Laboratory for Environmental Analysis	0	January 2020

WEB/Intranet

WES WEB Procedure.doc	Procedure for Operation of the DELS-WES Web & Intranet Sites	D1.0	July 2001

LCP Policies and Regulations

Document #	Document Title	Latest Revision #	Date
MA Env Lab Cert Regs 310 CMR 42	MA Regulations for the Certification and Operation of Environmental Analysis Laboratories (310 CMR 42.00)	--	June 2020
Chem PT Policy 20-001	Laboratory Certification and Quality Assurance Off ice Policy on Chemistry Proficiency Testing, Policy # WES--20.001	WES-20.001	June 2020
Micro PT Policy 20-002	Laboratory Certification and Quality Assurance Office Policy on Microbiology Proficiency Testing Policy # WES--20.002	WES-20.002	June 2020

EMS Documents

Document #	Document Title	Latest Revision #	Date
ISO 14001 4.4.2 A1.0	DEP WES Anthrax Threat Plan	A1.0	October 2001
aspect_list_admin D1.0	Aspects Identification Macro Map – Administration	D1.0	April 2001
aspect_list_bldg_maint D1.0	Aspects Identification Macro Map – Building Maintenance	D1.0	April 2001
aspect_list_inorganic_lab D1.0	Aspects Identification Macro Map – Inorganic Laboratory	D1.0	April 2001
aspect_list_labcert D1	Aspects Identification Macro Map – Laboratory Certification	D1.0	April 2001
aspect_list_organic_lab D1.0	Aspects Identification Macro Map – Organic Laboratories	D1.0	April 2001
Aspect_list_microbiology_lab D1.0	Aspects Identification Macro Map – Microbiology Laboratory	D1.0	April 2001

Document #	Document Title	Latest Revision #	Date
EMP_Air Emissions D1.0	Air Emissions Environmental Management Program	D1.0	September 2001
EMP_Elec+Gas Usage D1.0	Usage of Electricity and Natural Gas Environmental Management Program	D1.0	September 2001
EMP_Gas_Vehicle D1.0	Gasoline Consumption Vehicle Emissions Environmental Management Program	D1.0	October 2001
EMP_HW _Chem Storage D1.0	Hazardous Waste Storage and Chemical Use Environmental Management Program	D1.0	September 2001
EMP Indoor Air Quality D1.0	Indoor Air Quality Environmental Management Program	D1.0	September 2001
EMP_Noise D1.0	Noise Environmental Management Program	D1.0	October 2001
EMS Obj & Targets List D1.0	List of Objectives & Targets	D1.0	April 2001
EMS Policy D3.1	Environmental Policy for the Senator William X. Wall Experiment Station	D3.1	April 2001
EMS Signif Aspects D1.0	List of Significant Environmental Aspects	D1.0	April 2001
EMS Signif Criteria D1.0	List of Significance Criteria	D1.0	April 2001
EMP_Stormwater D1.0	Stormwater Management Environmental Management Program	D1.0	September 2001
EMP_SW Use & Reduction D1.0	Solid Waste/Paper Use and Reduction Environmental Management Program	D1.0	September 2001
EMP_Water WW D1.0	Usage of Water and Generation of Wastewater Environmental Management Program	D1.0	September 2001
SP Document Control D1.0	System Procedure for Document Control	D1.0	September 2001
SP Emerg Prepare D1.0	System Procedure for Emergency Preparedness and Response Plan	D1.0	September /2001
SP Ext-Int Communication D1.0	System Procedure For External/Internal Communication	D1.0	October 2001
SP Internal audits D1.0	System Procedure For Conducting EMS Internal Audits	D1.0	May 2002
SP Legal & Other Reg D1.0	System Procedure for Determining and Monitoring Legal and Other Requirements	D1.0	April 2001
SP Management Review D1.0	System Procedure For Conducting EMS Management Reviews	D1.0	May 2002
SP Measuring and MonitoringD1.0	System Procedure for Monitoring and Measuring Significant Environmental Aspects	D1.0	May 2002
SP Signif_Aspects D1.0	System Procedure for Determining Significant Environmental Aspects	D1.0	April 2001
SP Struc & Resp D1.0	System Procedure for Structure & Responsibility of EMS	D1.0	September 2001
SP Training D1.0	System Procedure For Training, Awareness And Competence of The WES EMS	D1.0	October 2001
OP Air Emissions Reduction D1.0	Operational Procedure Air Emission Reduction	D1.0	October 2001
OP Analytical Request & Sample Handling D1.0	Operational Procedure Analytical Request & Sample Handling	D1.0	November 2001
OP_Haz Waste-Chem Storage D1.0	Operational Procedure for Hazardous Waste/Chemical Storage and Use	D1.0	December 2001
OP for Hearing Conservation D1.0	Operational Procedure for Hearing Conservation	D1.0	February 2002
OP_Vehicle Emissions Reduction D1.0	Operational Procedure for Vehicle Emissions Reduction	D1.0	February 2002
OP_Water UsageWW Generation D1.0	Operational Procedure for Usage of Water and Generation of Wastewater	D1.0	September 2001

Document #	Document Title	Latest Revision #	Date
OP_Stormwater Mgmt D1.0	Operational Procedure for Stormwater Management	D1.0	September 2001
OP_Solid waste D1.0	Operational Procedure for Solid Waste/Paper Use and Reduction D1.0	D1.0	December 2001
OP Indoor Air Quality D1.0	Operational Procedure for Indoor Air Quality Improvements D1.0	D1.0	October 2001
WES EMS INT-EXT Outreach		Pending	

TABLE 9. MICROBIOLOGY LABORATORY - ANALYTICAL METHODS AND SAMPLE MANAGEMENT ELEMENTS

TABLE 9. Microbiology Laboratory – Analytical Methods and Sample Management Elements

Parameter	Method	Preservative[a]	Holding Time	Sample Volume	Container Type[b]
Potable Water Testing Methods					
Total Coliform	SM 9222B	0.1 mL (4 drops) 3% Sodium Thiosulfate in 120 mL of sample (EPA recommends < 10°C; use 1-8°C)	30 hours	100 mL	Sterile HDPE, G
	EPA 1604 (SM 9222)	0.1 mL (4 drops) 3% Sodium Thiosulfate in 120 mL of sample (EPA recommends < 10°C; use 1-8°C)	30 hours	100 mL	Sterile HDPE, G
	SM 9223B	0.1 mL (4 drops) 3% Sodium Thiosulfate in 120 mL of sample (EPA recommends < 10°C; use 1-8°C)	30 hours	100 mL	Sterile HDPE, G
E. coli	SM9222G	0.1 mL (4 drops) 3% Sodium Thiosulfate in 120 mL of sample (EPA recommends < 10°C; use 1-8°C)	30 hours	100 mL	Sterile HDPE, G
	EPA 1604 (SM 9222)	0.1 mL (4 drops) 3% Sodium Thiosulfate in 120 mL of sample (EPA recommends < 10°C; use 1-8°C)	30 hours	100 mL	Sterile HDPE, G
	SM 9223B	0.1 mL (4 drops) 3% Sodium Thiosulfate in 120 mL of sample (EPA recommends < 10°C; use 1-8°C)	30 hours	100 mL	Sterile HDPE, G
Potable Source Water & Non-Potable Water Testing Methods					
E. coli (MI Agar)	EPA 1604 (SM 9222)	1-8°C, 0.1 mL (4 drops) 10% Sodium Thiosulfate and 0.3 mL 15% EDTA solution pH 6.5 in 120 mL of sample	8 hours (usually 6 hours in the field + 2 hours in the laboratory)	100 mL	Sterile HDPE, G
E. coli (Colilert)	SM 9223B	1-8°C, 0.1 mL (4 drops) 10% Sodium Thiosulfate and 0.3 mL 15% EDTA solution pH 6.5 in 120 mL of sample	8 hours (usually 6 hours in the field + 2 hours in the laboratory)	100 mL	Sterile HDPE, G
Enterococci	EPA 1600	1-8°C, 0.1 mL (4 drops) 10% Sodium Thiosulfate and 0.3 mL 15% EDTA solution pH 6.5 in 120 mL of sample	8 hours (usually 6 hours in the field + 2 hours in the laboratory)	100 mL	Sterile HDPE, G

TABLE 9. Microbiology Laboratory – Analytical Methods and Sample Management Elements

Parameter	Method	Preservative[a]	Holding Time	Sample Volume	Container Type[b]
Enterococci	Enterolert (SM 9230D)	1- 8°C, 0.1 mL (4 drops) 10% Sodium Thiosulfate and 0.3 mL 15% EDTA solution pH 6.5 in 120 mL of sample	8 hours (usually 6 hours in the field + 2 hours in the laboratory)	100 mL	Sterile HDPE, G
Fecal Coliforms (MFC)	SM 9222 D	1- 8°C, 0.1 mL (4 drops) 10% Sodium Thiosulfate and 0.3 mL 15% EDTA solution pH 6.5 in 120 mL of sample	8 hours (usually 6 hours in the field + 2 hours in the laboratory)	100 mL	Sterile HDPE, G
Heterotrophic Plate Count	SM 9215 B	1-8°C, 0.1 mL (4 drops) 3% Sodium Thiosulfate in 120 mL of sample	8 hours (usually 6 hours in the field + 2 hours in the laboratory)	100 mL	Sterile HDPE, G
E. coli DNA Fingerprinting	Rep-PCR	1- 8°C, 0.1 mL (4 drops) 10% Sodium Thiosulfate and 0.3 mL 15% EDTA solution pH 6.5 in 120 mL of sample	8 hours (usually 6 hours in the field + 2 hours in the laboratory)	100 mL	Sterile HDPE, G
Bacteroidetes Group & Human DNA Markers	16S PCR	1-8°C, 1 mL (20 drops) 10% Sodium Thiosulfate and 0.3 mL 15% EDTA solution pH 6.5 in 120 mL of sample	8 hours (usually 6 hours in the field + 2 hours in the laboratory)	1 L	Sterile AJ, G
Enterococci	ENF esp Marker	1-8°C, 0.1 mL (4 drops) 10% Sodium Thiosulfate or tablet	8 hours (usually 6 hours in the field + 2 hours in the laboratory)	100 mL	Sterile HDPE, G
Enterococci	EPA 1611	1-8°C, 0.1 mL (4 drops) 10% Sodium Thiosulfate or tablet	8 hours (usually 6 hours in the field + 2 hours in the laboratory)	250 mL	Sterile, HDPE, G
Acute Toxicity	Microtox Acute Toxicity Test (SDI, Newark, DE)	1-8°C	36 hours	120 mL	AJ
BOD 5d	SM 5210 B[2]	Cool 4°C	48 hours	1 Liter	HDPE, G

[a] Non-chlorinated samples do not require the addition of sodium thiosulfate.

[b] HDPE = High Density Polyethylene; G = Glass, PP = Polypropylene, AJ = Amber Jar with Teflon-lined screw cap

TABLE 10. INORGANIC CHEMISTRY LABORATORY - ANALYTICAL METHODS AND SAMPLE MANAGEMENT ELEMENTS

TABLE 10. Inorganic Chemistry Laboratory – Analytical Methods and Sample Management Elements

Parameter	Method	Preservative	Holding Time	Sample Volume	Container Type
Potable Water Testing Methods					
Antimony	EPA 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Arsenic	EPA 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Barium	EPA 200.7 & 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Beryllium	EPA 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Bromate	EPA 300.1	50 mg/L EDA	28 days	100 mL	HDPE, G
Cadmium	EPA 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Calcium	EPA 200.7[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Chloride	EPA 300.0	None required	28 days	200 mL	HDPE, G
Chlorine, Total Residual	SM 4500-Cl F[2]	None required	Analyze Immediately	100 mL	HDPE, G
Chlorite	EPA 300.1	Cool to 4°C, 50 mg/L EDA	14 days	100 mL	Amber HDPE or G
Chromium	EPA 200.7 & 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Conductance	SM 2510 B[2]	Cool 4°C	28 days	100 mL	HDPE, G
Copper	EPA 200.7 & 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Cyanide, Amenable	SM 4500-CN^- G, C, & E[2]	Cool 4°C, NaOH to pH ≥ 12	14 days	500 mL	HDPE, G
Cyanide, Physiologically Available (PAC)	MassDEP PAC Method	Cool 4°C, NaOH to pH ≥ 12, dechlorinate with 0.6-g ascorbic acid/L	14 days	500 mL	HDPE, G
Cyanide, Total	SM 4500-CN^- C & E[2]	Cool 4°C, NaOH to pH ≥ 12, dechlorinate with 0.6-g ascorbic acid/L	14 days	500 mL	HDPE, G
Fluoride	EPA 300.0	None required	28 days	300 mL	HDPE, G
Hardness	SM 2340 B[2]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Lead	EPA 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Magnesium	EPA 200.7[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Mercury	EPA 245.1[1]	Cool 4°C, HNO_3 pH < 2	28 days	200 mL	HDPE, G
Nickel	EPA 200.7 & 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Nitrogen, Nitrate-N	EPA 300.0	Cool 4°C	48 hours	100 mL	HDPE, G
Nitrogen, Nitrite-N	EPA 300.0	Cool 4°C	48 hours	100 mL	HDPE, G

TABLE 10. Inorganic Chemistry Laboratory – Analytical Methods and Sample Management Elements

Parameter	Method	Preservative	Holding Time	Sample Volume	Container Type
pH	SM 4500-H^+ B[2]	None required	Analyze Immediately	100 mL	HDPE, G
Selenium	EPA 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Silver	EPA 200.7[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Sodium	EPA 200.7[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Sulfate	EPA 300.0	Cool 4°C	28 days	50 mL	HDPE, G
Thallium	EPA 200.8[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Turbidity	SM 2130 B[2]	Cool 4°C	48 hours	100 mL	HDPE, G
Non-Potable Water Testing Methods					
Alkalinity, Total	SM 2320 B[2]	Cool 4°C	14 days	500 mL	HDPE, G
Aluminum	EPA 200.7[1] & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Antimony	EPA 200.7[1] & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Arsenic	EPA 200.7[1] & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Barium	EPA 200.7[1] & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Beryllium	EPA 200.7[1] & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Boron	EPA 200.7[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Cadmium	EPA 200.7[1] & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Calcium	EPA 200.7[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Chloride	SM 4500-Cl^- E[2] & EPA 300.0	None required	28 days	100 mL	HDPE, G
Chlorine, Total Residual	SM 4500-Cl F[2]	None required	Analyze Immediately	100 mL	HDPE, G
Chromium	EPA 200.7[1] & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Chromium, Hexavalent (VI)	SM 3500-Cr D[2]	Cool 4°C	24 hours	200 mL	HDPE, G
COD	SM 5220 D[2]	Cool 4°C, H_2SO_4 pH < 2	28 days	50 mL	HDPE, G
Conductance	SM 2510 B[2]	Cool 4°C	28 days	100 mL	HDPE, G
Copper	EPA 200.7[1] & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Cyanide, Amenable	SM 4500-CN^- G, C, & E[2]	Cool 4°C, NaOH to pH ≥ 12	14 days	250 mL	HDPE, G
Cyanide,	MassDEP PAC	Cool 4°C, NaOH to pH ≥ 12,	14 days	250 mL or 5 g	HDPE, G

TABLE 10. Inorganic Chemistry Laboratory – Analytical Methods and Sample Management Elements

Parameter	Method	Preservative	Holding Time	Sample Volume	Container Type
Physiologically Available (PAC)	Method	dechlorinate with 0.6-g ascorbic acid/L			
Cyanide, Total	SM 4500-CN^- C & E[2]	Cool 4°C, NaOH to pH ≥ 12, dechlorinate with 0.6-g ascorbic acid/L	14 days	250 mL	HDPE, G
Hardness	SM 2340 B[2]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Iron	EPA 200.7[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Lead	EPA 200.7[1] & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Magnesium	EPA 200.7[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Manganese	EPA 200.7 & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Mercury	EPA 7473	Cool 4°C, HNO_3 pH < 2	28 days	200 mL	HDPE, G
Nickel	EPA 200.7[1] 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Nitrogen, Ammonia-N	SM 4500-NH3-N (B, G)	Cool 4°C, H_2SO_4 pH < 2	28 days	500 mL	HDPE, G
Nitrogen, Nitrate-N	EPA 300.0	Cool 4°C	48 hours	100 mL	HDPE, G
Nitrogen, Nitrite-N	EPA 300.0	Cool 4°C	48 hours	100 mL	HDPE, G
Nitrogen, Nitrate- + Nitrite-N	SM 4500-NOx-N F	Cool 4°C, H_2SO_4 pH < 2	28 days	100 mL	HDPE, G
Nitrogen, Total (TN)	SM 4500-N C,	Cool 4°C, H_2SO_4 pH < 2	28 days	500 mL	HDPE, G
pH	SM 4500-H^+ B[2]	None required	Analyze Immediately	100 mL	HDPE, G
Phosphorus, Ortho	EPA 300.0	Cool 4°C	48 hours	50 mL	HDPE, G
Phosphorus, Total	SM 4500-P B (5), F	Cool 4°C, H_2SO_4 pH < 2	28 days	100 mL	HDPE, G
Potassium	EPA 200.7[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Sodium	EPA 200.7[1]	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Solids, Fixed Volatile	SM 2540 E[2]	Cool 4°C	7 days	200 mL	HDPE, G
Solids, Settleable	SM 2540 F[2]	Cool 4°C	48 hours	1 Liter	HDPE, G
Solids, Total	SM 2540 B[2]	Cool 4°C	7 days	200 mL	HDPE, G
Solids, Total Dissolved	SM 2540 C[2]	Cool 4°C	7 days	200 mL	HDPE, G
Solids, Total Suspended	SM 2540 D[2]	Cool 4°C	7 days	1000 mL	HDPE, G
Sulfate	EPA 300.0	Cool 4°C	28 days	50 mL	HDPE, G
Turbidity	SM 2130 B[2]	Cool 4°C	48 hours	100 mL	HDPE, G
Vanadium	EPA 200.7[1] &	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G

TABLE 10. Inorganic Chemistry Laboratory – Analytical Methods and Sample Management Elements

Parameter	Method	Preservative	Holding Time	Sample Volume	Container Type
	200.8				
Zinc	EPA 200.7[1] & 200.8	Cool 4°C, HNO_3 pH < 2	6 months	200 mL	HDPE, G
Biological Tissue Testing Methods					
Metals (Usually limited to As, Cd, Pb, and Se)	EPA 6010D & 6020	Cool 4°C prior to resection & then freeze fillet/edible portion at -10 to -20°C	24 hr to resection & 6 months to analyze frozen fillet or edible portion	50 g	HDPE, G
Mercury	EPA 7473	Cool 4°C prior to resection & then freeze fillet/edible portion at -10 to -20°C	24 hr to resection & 28 days to analyze frozen fillet or edible portion	50 g	HDPE, G
Moisture, Total (%)[6]	AOAC Int. 950.46B (b)	Analyze subsample of tissue collected for mercury by EPA 7473 analysis.		10 g	HDPE, G
Soil, Sediment, and Solid and Liquid Waste Testing Methods					
TCLP	EPA 1311[5]	None required	7 days to extract & 40 days to analyze	300 g	G
Solids, Total	SM 2540 G[2]	Cool 4°C	7 days	50 g	HDPE, G
pH	SM 4500-H^+ B[2]	None required	Analyze Immediately	100 g	HDPE, G
Metals/Elements	EPA 6010D & 6020[5]	Cool 4°C	6 months	50 g	HDPE, G
Chromium, Hexavalent (VI)	EPA 7196[5]	Cool 4°C	24 hours	300 g	HDPE, G
Mercury	EPA 7473[5]	Cool 4°C	28 days	20 g	HDPE, G

[1] EPA 600/R4-94/111, May 1994, *Methods for the Determination of Metals in Environmental Samples.*
[2] *Standard Methods for the Examination of Water and Wastewater*,21st, and 23rd editions.
[3] EPA 600/R-93/100, August 1993, *Methods for the Determination of Inorganic Substances in Environmental Samples*, or EPA 600/R4-941111, May 1994, *Methods for Chemical Analysis of Water and Wastes.*
[4] EPA 600/4-81-055, March 1980, *Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue.*
[5] EPA SW-846, Update VI, Revision 6, June 2018
[6] AOAC Official Methods of Analysis, 15th ed., 1990 vol II.

TABLE 11. ORGANIC CHEMISTRY LABORATORY – GC/LC SECTION - ANALYTICAL METHODS AND SAMPLE MANAGEMENT ELEMENTS

TABLE 11. Organic Chemistry Laboratory – GC/LC Section – Analytical Methods and Sample Management Elements

Parameter	Method	Preservative	Holding Time	Sample Volume	Container Type
Potable Water Testing Methods					
N-Methylcarbamoyl-oximes and N-Methylcarbamates in Drinking Water	EPA 531.2[1]	Cool 4°C, adjust pH 3.8 with potassium dihydrogen citrate	28 days	40-60mL	Amber glass with TFE-fluorocarbon-lined screw cap
Haloacetic Acids and Dalapon in Drinking Water	EPA 552.3[2]	NH_4Cl, cool at 4°C	14 days to extract & 21 days to analyze	40 mL	Glass VOA vial with Teflon-lined septum
Herbicides in Drinking Water	MADEP 555	Sodium sulfite added first to dechlorinate, then HCl to pH < 2, cool 4°C	14 days	1 Liter	Amber glass with Teflon-lined screw cap
Non-Potable Water Testing Methods					
N-Hexane Extractable Oil & Grease, and TPH	EPA 1664	H_2SO_4 pH < 2, cool 4°C	28 days to extract & 14 days to analyze	1 Liter	Amber glass with Teflon-lined screw cap
Extractable Petroleum Hydrocarbons (EPH) in Water Matrices	MA EPH	5-mL of 1:1 HCl, cool at 4°C	14 days to extract &40 days to analyze	1 Liter	Amber glass with Teflon-lined screw cap
Soil, Sediment, and Solid and Liquid Waste Testing Methods					
Oil & Grease and Total Petroleum Hydrocarbons in Soil & Sediment	EPA 9071B	Cool 4°C	Samples must be analyzed within 28 days of collection.	50 g	Glass with Teflon-lined screw cap
Extractable Petroleum Hydrocarbons (EPH) in Soil, Sediment, and Solid Waste	MA EPH	Cool at 4°C	7 days to extract & 40 days to analyze	20 g	4-ounce wide-mouth amber glass jar with Teflon-lined screw cap
Ignitability	EPA 1010A	Cool at 4°C	7 days	200 mL	G
Air Sample Testing Methods					
Carbonyl Compounds in Air	EPA TO-11A/ CARB 1004	Cool at 4°C	14 days	1 DNPH cartridge	DNPH cartridge

[1] EPA 815-B-01-002, September 2001
[2] EPA 815-B-03-002, July 2003[3]

TABLE 12. ORGANIC CHEMISTRY LABORATORY – MS SECTION - ANALYTICAL METHODS AND SAMPLE MANAGEMENT ELEMENTS

Parameter	Method	Preservative	Holding Time	Sample Volume	Container Type
Potable Water Testing Methods					
Volatile Organics in Drinking Water	EPA 524.3[1]	Dechlorinate using 25 mg of ascorbic acid per 40 mL of sample added to the sample bottle prior to collection; acidify to pH 2 with 200 mg of maleic acid added to the sample bottle prior to collection; cool to 10°C or lower	14 days at 6°C or lower, protect from light	40 mL	Amber glass vial with Teflon-lined septum
Semi-Volatile Organics in Drinking Water	EPA 525.2[1]	Dechlorinate with 40-50 mg of sodium sulfite; adjust pH < 2 with 6 N HCl after dechlorination; cool at 4°C	14 days to extract & 30 days to analyze	1Liter	Amber-glass with Teflon-lined screw cap
1,4-Dioxane in Drinking Water	EPA 522	Reduce chlorine with 50 mg/L of sodium sulfite added to bottles prior to shipment, add 1 g/L of sodium bisulfate as microbial inhibitor after the sodium bisulfate has been dissolved. Cool to 10 °C or lower	28 days to extract & 28 days to analyze	599 mL	Amber-glass with Teflon=-lined screw cap
Non-Potable Water Testing Methods					
Volatile Organics in Non-Potable Water	EPA 8260D[2]	Cool at 4°C, adjust pH < 2 with HCl	14 days	40 mL	Glass with Teflon-lined septum
Semi-Volatile Organics in Non-Potable Water	EPA 8270E[2]	Cool at 4°C	7 days to extract & 40 days to analyze	1Liter	Amber glass with Teflon-lined screw cap
Volatile Petroleum Hydrocarbons (VPH) in Water Matrices	MA VPH	Cool at 4°C, adjust pH < 2 with HCl	14 days	40 mL	Glass with Teflon-lined septum
Soil, Sediment, and Solid and Liquid Waste Testing Methods					
Volatile Organics in Soil, Sediment, and Solid Waste	EPA 8260D[2]	Cool at 4°C, add 1-mL methanol per 1-g sample	14 days	10 g	Glass with Teflon-lined septum
Semi-Volatile Organics in Soil, Sediment, and Solid Waste	EPA 8270E[2]	Cool at 4°C	14 days to extract & 40 days to analyze	10 g	Amber glass jar with Teflon-lined screw cap
Volatile Petroleum Hydrocarbons (VPH) in Soil, Sediment, and Solid Waste	MA VPH	Cool at 4°C, add 1-mL methanol per 1-g sample	14 days	15 - 25 g	Glass with Teflon-lined septum

Biological Tissue Testing Methods					
PCBs (Aroclors & 28 Congeners) and Organochlorine Pesticides in Biological Tissue	EPA 8270E[2]	Cool 4°C prior to resection & freeze fillet/edible portion at -10 to -20°C	24 hr to resection & 1 year to analyze frozen fillet or edible portion	20 g	Aluminum foil, dull side toward tissue, no plastic

[1] EPA 815-B-09-009, June 2009
[2] SW-846 Update VI, Revision 6, June 2018

FORMS AND REPORTS

FORM 1. SAMPLE TRACKING/CHAIN-OF-CUSTODY RECORD BLANK

See Form 1. COC v 1.3.2 Blank.pdf

FORM 2. SAMPLE TRACKING/CHAIN-OF-CUSTODY RECORD-PRELOG

See Form 2. COC v. 1.3.2 WinLIMS Pre-Logged.pdf

FORM 3. SAMPLE CONDITIONS REVIEW FORM (SCRF)

See Form 3. WinLIMS SCRF Rev 1.2.pdf

FORM 4. CORRECTIVE ACTION FORM

See Form 4. Corrective Action Form Rev 1.4.pdf

FORM 5. INITIAL DEMONSTRATION OF CAPABILITY (IDC) and/or MDL FORM

See Form 5. IDC-MDL-Documentation Rev 1.4.pdf

FORM 6. DOCUMENTATION OF LABORATORY BENCH TRAINING

See Form 6. Training Form-Laboratory Bench Rev 1.5.pdf

FORM 7. DOCUMENTATION OF TRAINING – OTHER

See Form 7. Training Form-Other Rev 1.3.pdf

REPORT 1. DELS-WES CHEMISTRY FINAL REPORT EXAMPLE

See Report 1. DELS-WES Chemistry FR Example.pdf

REPORT 2. DEL-WES MICROBIOLOGY FINAL REPORT EXAMPLE

See Report 2. DELS-WES Micro FR Example.pdf

REPORT 3. QUALITY ASSURANCE (QA) REVIEW LEVEL 1 - INORGANIC CHEMISTRY LABORATORY

See Report 3. QAL1 Inorg.pdf

REPORT 4. QUALITY ASSURANCE (QA) REVIEW LEVEL 1 - CULTURABLE MICROBIOLOGY LABORATORY

See Report 4. QAL1 Micro Cbl.pdf

REPORT 5. QUALITY ASSURANCE (QA) REVIEW LEVEL 1 - MOLECULAR MICROBIOLOGY LABORATORY

See Report 5. QAL1 Micro Molecular.pdf

REPORT 6. QUALITY ASSURANCE (QA) REVIEW LEVEL 1 - MICROBIOLOGY TOXICOLOGY LABORATORY

See Report 6. QAL1 Micro Toxicity ELISA.pdf

REPORT 7. QUALITY ASSURANCE (QA) REVIEW LEVEL 1 - ORGANIC GC/LC LABORATORY

See Report 7. QAL1 Org GCLC.pdf

REPORT 8. QUALITY ASSURANCE (QA) REVIEW LEVEL 1 - ORGANIC MS LABORATORY

See Report 8. QAL1 Org MS.pdf

REPORT 9. QUALITY ASSURANCE (QA) REVIEW LEVEL 2

See Report 9. QAL2.pdf

Appendix 5

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Controlled Documents

Standard Operating Procedures			
Document ID#	**Document Title**	**Revision#**	**Release Date**
MASSTC-SOP-001	Document Control	001	2020-02-06
MASSTC-SOP-002	Internal Audit Procedure		
MASSTC-SOP-003	Data and Records Management	001	2021-07-30
MASSTC-SOP-004	Control of Nonconforming Work	001	2021-11-18
MASSTC-SOP-005	Corrective Action SOP	000	2021-11-18
MASSTC-SOP-006	Training		
MASSTC-SOP-007	Grab Samples	000	2016-10-27
MASSTC-SOP-008	Composite Sample Retrieval	000	2016-10-27
MASSTC-SOP-009	YSI 556 Calibration	000	2016-10-27
MASSTC-SOP-010	YSI 556 Logging Mode	000	2016-10-27
MASSTC-SOP-011	Power Use Measurements with Calibrated Power Meter		
MASSTC-SOP-012	Sound Meter		
MASSTC-SOP-013	Thermometer Calibration	002	2021-07-26
MASSTC-SOP-014	Ozone Measurement	002	2020-07-30
MASSTC-SOP-015	Sample Preparation and Transportation	000	2021-07-15
MASSTC-SOP-016	YSI ProDSS Field Meter	004	2021-10-18
MASSTC-SOP-017	Sample Equipment Maintenance and Sterilization		
MASSTC-SOP-018	System Dosing		
MASSTC-SOP-019	Facility Influent and Effluent Infrastructure		
MASSTC-SOP-020	Site Security		
MASSTC-SOP-021	Daily Checklists		
MASSTC-SOP-022	Leaching Product Ponding Measurement		
MASSTC-SOP-023	Tipper Trays and HOBOWare Software	000	2020-12-21
MASSTC-SOP-024	Moisture Sensors		
MASSTC-SOP-025	Tank Level Sensors		
MASSTC-SOP-026	Lysimeters		
MASSTC-SOP-027	Soil System Underdrains	000	2020-03-23
MASSTC-SOP-028	Infiltrator Epsilon Ponding Measurement SOP	000	2020-03-27
MASSTC-SOP-029	Infiltrator Columns Ponding Measurement SOP	000	2021-07-20
MASSTC-SOP-030	Sampling and Maintenance Plans	000	2021-03-08
MASSTC-SOP-031	Channel Cleaning		
MASSTC-SOP-032	Infiltrator Lambda Ponding Measurement SOP	000	2020-10-29
MASSTC-SOP-033	Accounts Payable SOP - Laboratory Services		
MASSTC-SOP-034	Infiltrator Epsilon-2 Ponding Measurement SOP	000	2020-10-29
MASSTC-SOP-035	Visitor Logging SOP		
MASSTC-SOP-036	Power Outage		
MASSTC-SOP-037	Sample Collection	002	2021-11-17
MASSTC-SOP-038	Overnight Cultures SOP	000	2021-09-07
MASSTC-SOP-039	Virus Analysis SOP	000	2021-11-18
MASSTC-SOP-040	Centrifuged Samples for Oscar DEPWES		
MASSTC-SOP-041	Chemical Procurement, Receipt, Storage, and Disposal		

MASSTC-SOP-042	Operating Procedure for Report Preparation and Distribution		

Forms and Checklists			
Document ID#	**Document Title**	**Revision#**	**Release Date**
MASSTC-FRM-001	Nonconforming Work Report	000	2021-11-22
MASSTC-FRM-002	Corrective Action Report	000	2021-11-18
MASSTC-FRM-003	Internal Audit Form	Retired	
MASSTC-FRM-004	Nonconforming Work Identification Report	Retired	2019-10-02
MASSTC-FRM-005	Request for Services at MASSTC Form		
MASSTC-FRM-006	Complaint Log Form		
MASSTC-FRM-007	Controlled Document Deployment Tracking	000	2021-03-08
MASSTC-FRM-008	Unused		
MASSTC-FRM-009	Unused		
MASSTC-FRM-010	Weekly Quality Assurance Checklist	012	2021-11-22
MASSTC-FRM-011	Training Log	002	2020-02-11
MASSTC-FRM-012	Point Check Calibration of Laboratory Thermometers	002	2021-07-26
MASSTC-FRM-013	pH Meter Calibration Log	Retired	
MASSTC-FRM-014	Chemical Receipt Log	001	2020-08-21
MASSTC-FRM-015	Vendor Visit Log	000	2016-10-10
MASSTC-FRM-016	Visitor Visit Log	000	2016-10-10
MASSTC-FRM-017	Friday Quality Assurance Checklist	002	2020-02-07
MASSTC-FRM-018	Weekend Quality Assurance Checklist	003	2021-01-24
MASSTC-FRM-019	Monthly Audit Report	003	2020-02-07
MASSTC-FRM-020	Daily DC West Sampler Checks	002	2021-06-09
MASSTC-FRM-021	Daily Shed Checks		
MASSTC-FRM-022	Lift Station Maintenance		
MASSTC-FRM-023	Power Use Record	001	2020-07-19
MASSTC-FRM-024	Pump Overrun Timings		
MASSTC-FRM-025	Daily D-Box Leveler Cleanings	001	2021-01-07
MASSTC-FRM-026	EPA Sensor Challenge Field Data Report		
MASSTC-FRM-027	Daily Greenhouse Checks		
MASSTC-FRM-028	Ozone Measurement Form	002	2020-08-04
MASSTC-FRM-029	Pocket Colorimeter II Ozone Quality Control Form	001	2020-02-05
MASSTC-FRM-030	DC West Alkalinity Log	000	2020-07-16
MASSTC-FRM-031	Ozone Standards Reference	000	2020-07-30
MASSTC-FRM-032	Temperature Verification Log	001	2020-08-21
MASSTC-FRM-033	ProDSS Calibration Checklist	001	2021-06-09
MASSTC-FRM-034	ProDSS End of Day Checklist	002	2021-10-18
MASSTC-FRM-035	Request for Laboratory Services	000	
MASSTC-FRM-036	Monthly Grant Financial Report	000	
MASSTC-FRM-037	Power Outage Checklist		
MASSTC-FRM-038	Winter Preparation Checklist		
MASSTC-FRM-039	Spring Preparation Checklist		
MASSTC-FRM-040	Sampling Plan	000	2021-03-02
MASSTC-FRM-041	pH Buffer Temperature Dependence Reference	000	2021-06-09
MASSTC-FRM-042	MASSTC Chain of Custody - Internal Lab	001	2021-10-05
MASSTC-FRM-043	Daily Virus Project Dosing Log	000	2021-11-09

MASSTC-FRM-044	Pathogen Worksheet		

Appendix 6

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Massachusetts Alternative Septic System Test Center
Barnstable, Massachusetts

Standard Operating Procedure

Title: Data and Records Management

Effective Date: 2021-07-30	**Number:** MASSTC-SOP-003	**Revision:** 001

Authors

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Signature: DocuSigned by: Brian Baumgaertel A809A6344B57407... Date: 7/30/2021

REVISION HISTORY

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the MASSTC Director on the MASSTC Sharepoint Site.

History	Effective Date
Revision #001: Added direction about properly voiding blank space. Edits done by Emily Michele Olmsted.	2021-07-30
Revision #000: Original Issue	2021-02-02

Data and Records Management	Document ID#: MASSTC-SOP-003 Revision: 001 Released Date: 2021-07-30 Released By: Brian Baumgaertel

CONTENTS

1. PURPOSE

1.1. This document defines the policies and procedures by which MASSTC shall record and maintain data, notes, and other documentation.

2. SCOPE AND APPLICATION

2.1. This procedure applies to all work conducted by all MASSTC personnel.

3. DEFINITIONS

3.1. Data – Facts about an object [Source: ISO 9000:2015, 3.8.1]

3.2. Document – Information, and the medium on which it is contained. Examples: record, specification, procedure document, drawing, report, standard. [Source: ISO 9000:2015, 3.8.5]

3.3. Record – Document stating results achieved or providing evidence of activities performed. [Source: ISO 9000:2015, 3.8.10]

4. RECORD IDENTIFICATION

4.1. Records are identifiable to the firm, product, person, or event to which they pertain. Records are dated and identify the person who established the record.

4.2. Laboratory records contain sufficient information to maintain an audit trail.

5. DATA FORMATTING

5.1. To maintain consistency in MASSTC records, data shall be recorded in the following formats:

5.1.1. Date – ISO 8601 format: YYYY-MM-DD. Example: 2020-03-27

5.1.2. Time – ISO 8601 format: HH:MM where hours are based on a 24-hour clock. Example: 13:35

5.1.3. Temperature – ISO 80000 format: degrees Celsius or shorthand °C. Example: 5.5°C

5.1.4. Length – SI Units: meters (m), centimeters (cm), millimeters (mm), etc. Example: 15mm

5.1.5. Volume –

5.1.5.1. Gallons (gal) in the context of tank sizes and system flow. Examples: 1,500-gal tank, 7.5 gallons per dose.

5.1.5.2. Liters (L), or milliliters (mL) in every context not defined in 4.6.1.

5.1.6. Mass – SI Units: kilogram (kg), gram (g), milligram (mg), microgram (µg), nanogram (ng).

5.1.7. Concentration – milligrams/liter (mg/L), micrograms/liter (µg/L), nanograms/liter (ng/L).

5.1.8. Electrical Power Use – kilowatt-hours (kWh)

6. HANDWRITTEN DATA

6.1. All data shall be recorded in permanent, indelible ink which cannot be erased.

6.2. Accuracy should always take precedence over expediency. Ensure that a future reader will be able to read what you have written. For example, a hastily-written zero (0) can appear like a six (6) or vice-versa.

6.3. Handwritten data should be converted to electronic format whenever possible. Where an electronic recording method exists, data shall be recorded by the end of the business day.

6.4. Column Headings - column headings shall be carried over between pages.

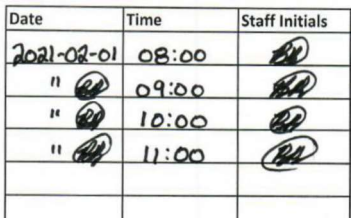
6.5. Skipping lines or pages – the space between handwritten observations or data shall be as minimal as possible to prevent nonchronological additions or observations from being added.

6.5.1. If any space is intentionally left blank, a single diagonal line striking through the space shall be included with the initials of the person recording the strike and the date of striking the blank space, as well as a brief explanation or the word "VOID."

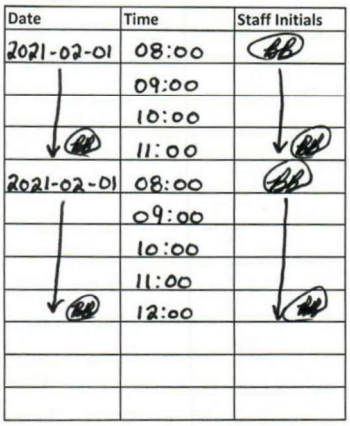
6.6. Errors - shall be struck with a single horizontal line, with the editor's initials placed nearby.

6.7. Repeating rows in tables – two methods are acceptable:

6.7.1. "Ditto" or quotation marks, with initials



6.7.2. Down arrow, with initials



7. MASSTC Electronic Data Management System

7.1. The MASSTC Electronic Data Management System is accessible internally or via VPN at http://10.14.20.130:31983/

7.2. Care should be taken to include as much detail and pertinent events and information in the electronic data management system, as known as the MASSTC Database.

7.3. Care should be taken to ensure that the recording of any event or note is stored in the best place possible with as correct an association as possible.

7.4. The electronic Journal shall function as the general place to record notes and events unless otherwise instructed.

7.4.1. Each entry automatically includes the time, date, and the user. If the event or note being recorded took place at a different time and date, the date and time must be changed by the user to when the event occurred.

7.4.2. In most cases, an entry should be logged in association with an existing project.

7.4.3. In most cases, entries can be logged for a specific asset, infrastructure, and/or equipment if not already associated with a specific project.

7.4.4. The correct category should be chosen for the Journal entry when possible.

7.4.5. Follow any protocol for Journal entry if directed elsewhere (ex. sampling plans, Weekly Quality Assurance tasks).

7.4.6. Journal entries should be used for the following examples:

7.4.6.1. Routine inspections of equipment or assets which do not otherwise have a designated physical location of records (ex. sump checks).

7.4.6.2. Unanticipated system issues with flow, mechanics, or otherwise.

7.4.6.3. Site issues, especially those relevant to the influent channel.

7.4.6.4. Any other circumstances which would be beneficial to have on record.

7.5. Shed checks must be recorded electronically and should include counter information, where applicable. Any notes regarding system issues or other relevant information should be included in the comments section of a shed check.

7.6. Influent pump schedules must be updated online as soon as possible after a pump schedule is changed to ensure that shed checks can remain accurate.

7.7. Laboratory Chains of Custody must be created in the MASSTC Database, per direction of MASSTC-SOP-015 – Sample Preparation and Transportation.

7.8. Projects should be kept as up to date as possible with information such as the start-up, flow, assigned pump, and activity status retained in the digital record in the MASSTC Database.

8. Digital Records Storage

8.1. All MASSTC staff must use Microsoft Office's Sharepoint for work-related documents. Sharepoint exists as a source for storing large amounts of files and eliminates the need for files to be backed up on an external location in the event of local equipment failure.

8.2. Documents that are necessary to one or more MASSTC staff members shall **not** be stored locally, such as through the use of OneDrive or on a computer's desktop, because they are not accessible to all staff.

8.3. Care should be taken when saving a file to Sharepoint to ensure it is stored in the best possible location so that it can be easily found by other staff, including a clear document title and a date formatted as specified in Section 5 whenever possible.

8.4. Consult MASSTC-SOP-001 – Document Control Procedure for Controlled Documents, which must also be stored on Sharepoint.

8.5. The MASSTC-Documents Sharepoint should be used to save all other MASSTC-Documents not falling under the designation of controlled documents.

8.6. The following should be guidance when saving uncontrolled documents:

8.6.1. All documents specific to one project should be saved within the correct Client and Project folder within MASSTC-Documents.

8.6.2. All records pertaining to the upkeep or inspection of MASSTC site equipment or assets should be saved in the Facility folder of MASSTC-Documents.

8.6.3. All lab reports shall be saved in the Labscans folder within the Data folder of MASSTC-Documents. Lab reports shall be retitled in the format of LIMS Client ID_Sample ID_Date of Sample_LIMS ID.

8.6.4. All records and data pertaining to the ProDSS field meter, including scanned Calibration documents, End of Day documents, and direct measurements taken by the meters shall be saved in the ProDSS Downloaded Data folder within the Data folder of MASSTC-Documents.

9. Record Retention Schedule

9.1. Records retention schedules are defined in the Massachusetts Municipal Records Retention Schedule (MASSTC-EXT-PLN-002).

9.2. Records retention schedules not defined in the above are as follows:

Record	Cutoff	Retention
Employee Training Records	End of FY after employee leaves.	5 years
Instrument Calibration Records	End of FY after final action.	3 years
Client Project Data/Notes	End of FY after project completion.	10 years
Grant Project Data/Notes	As defined in grant agreement OR end of FY after project completion.	As defined in grant agreement OR 10 years.
Shed Check Records	End of FY.	5 years
Weekly/Weekend Checklists	End of FY.	3 years
Dosing Pump Schedules	End of FY after project completion.	3 years

APPENDIX A – ELECTRONIC RECORD AND DATA FILE HIERARCHY

Type	Examples	Path
Client project records and data	Tipper data, HOBO Data, pictures	Projects/Client Projects/[*client identifier*]/[*project identifier*]/
Grant project records and data		Projects/Grant Projects/[*grant project identifier*]/
Lab Reports		BCDHE Lab Reports/{ *LIMS Client ID_Sample ID_Date of Sample_LIMS ID*}
Field Meter records and data	Calibration records, end of day records, data exports	Field Meter
General Facility Log Sheets (Including Scans)	Alkalinity logs, sampler check logs	Facility/[*Log Title*]/
Pump Schedules		Facility/Pump Schedules/[Pump Identifier]/
Weather Data		Facility/Weather Data

Appendix 7

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Massachusetts Alternative Septic System Test Center
Barnstable, Massachusetts

Standard Operating Procedure

Title: Ozone Measurement SOP

Effective Date: 2020-07-30	**Number:** MASSTC-SOP-014	**Revision:** 002

Authors

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Ozone Measurement SOP	Document ID#: MASSTC-SOP-014 Revision#: 002 Released Date: 2020-07-30 Released By: Brian Baumgaertel

Revision History

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History	Effective Date
Revision #002: Added further detail to Section 8 – Instrument or Method Calibration including reference to new form. Added more detail to Section 9 – Sample Handling and Storage including information about collection of sample. Added more detail to Section 10 – Procedure including reference to new form. Revisions by Emily Michele Olmsted.	2020-07-30
Revision #001: Fixed references to forms in sections 8.2.6, 8.2.7, and 12.1. Fixed language in section 13.5. Revisions by Brian Baumgaertel.	2020-02-05
Revision #000: Original Issue	2020-01-31

CONTENTS

1. Scope and Application

1.1. This protocol describes the procedure used to test wastewater ozone content.

2. Definitions

2.1. Ozone - 0_3 ; a highly unstable oxidant, which quickly breaks down to diatomic oxygen.

3. Health and Safety Warnings

3.1. Ozone

***3.1.1.* REFER TO *MASSTC-SDS-001 – Ozone SDS* for full details**

3.1.2. Anyone with chronic pulmonary problems, especially asthma, should avoid exposure to ozone.

3.1.3. Oxidizing Gas - May cause fire or intensify fire; oxidizer

3.1.4. Skin Irritant

3.1.5. Eye Irritant

3.1.6. Respiratory Systemic Toxicity

3.1.7. Acute Aquatic Toxicity

3.2. Puncture Hazard – Use of AccuVac Ampuls produces sharp glass

4. Cautions

4.1. Always do tests in sample cells or AccuVac© Ampuls. Do not put the instrument in the sample or pour the sample into the cell holder.

5. Interferences

5.1. Ozone is highly unstable – samples should be collected gently and analyzed immediately.

5.2. Warming the sample or disturbing the sample by stirring or shaking will result in ozone loss.

5.3. Optical faces of test cells (SpecCheck Gels and AccuVac Ampuls) shall be free of dirt, lint, dust, and fingerprint oils.

5.4. Only deionized (DI) water shall be used for blank sample checks.

5.5. The indigo reagent in the AccuVac Ampul is light-sensitive and should be kept in the dark at all times.

5.6. Unless specified by project, do not adjust the range on the Colorimeter. Range should be as mid-range.

6. Personnel Qualifications

6.1. Personnel are required to be knowledgeable of the procedures in this SOP.

6.2. Personnel are required to be knowledgeable of the HACH Ozone Indigo Method, 8311 (MASSTC-EXT-MTH-001)

6.3. Personnel are required to be knowledgeable of the HACH Ozone Pocket Colorimeter II Manual (MASSTC-EXT-MAN-002)

6.4. Personnel are required to be knowledgeable of the Ozone Safety Data Sheet (MASSTC-EXT-SDS-001)

7. Special Apparatus and Materials

7.1. Hach Ozone Pocket Colorimeter II
7.2. Hach SpecCheck Gel Secondary Standard Kit, Ozone, 0-0.75 mg/L set
7.3. Hach Ozone AccuVac Ampuls, 0-0.25 mg/L
7.4. Deionized water

8. Instrument or Method Calibration

8.1. The Hach Pocket Colorimeter II is calibrated at the factory.
8.2. Use the following procedure to verify meter performance using the Hach SpecCheck Gel Secondary Standard Kit, Ozone, 0-0.75 mg/L set. This procedure is also given in MASSTC-EXT-MAN-006 - HACH Ozone Secondary Standards Kit Instructions for Pocket Colorimeter II. Staff should also reference MASSTC-FRM-031 – Ozone Standards Reference for most pertinent information on standards range.
8.3. Ensure that you have a clean workspace. Place the HACH Ozone Test Kit, the Ozone Mid-Range Secondary Standards Kit, and the Ozone Analysis binder in your workspace.
8.4. Turn on the Pocket Colorimeter II by pressing the power/light button.
  8.4.1. From the Ozone (Mid-Range) Secondary Standards Kit, wipe down the **Standard 3** vial with a lint-free cloth and insert into the Colorimeter with the alignment mark facing toward you.
  8.4.2. Place the light-blocking instrument cap over the vial.
  8.4.3. Press the blue ZERO button. It will show 0.00.
  8.4.4. After zeroing is complete, take off the instrument cap and remove Standard 3.
  8.4.5. Wipe down the **BLANK** vial with a lint-free cloth and insert into the Colorimeter with the alignment mark facing toward you. Place the instrument cap on the Colorimeter.
  8.4.6. Press the green checkmark READ button.
  8.4.7. Compare the value on the meter to the value listed on MASSTC-FRM-031 – Ozone Standards Reference and record this value in the Ozone Analysis binder on the MASSTC-FRM-029 – Pocket Colorimeter II Ozone Quality Control Form under the heading for the Blank Reading.
  8.4.8. Repeat 8.4.5 through 8.4.6 for each of Standards 1 and 2. If not using MASSTC-FRM-031 – Ozone Standards Reference and instead are comparing the values of Standards 1 and 2 to the value listed on the Certificate of Analysis, note that Standard 1 and 2 are reversed.
  8.4.9. Record the values on MASSTC-FRM-029 – Pocket Colorimeter II Ozone Quality Control Form. Record additional information (date, time, initials, acceptable range, etc.) as well.

9. Sample Handling and Storage

9.1. Samples should be analyzed immediately after they are taken. Ideally, method calibration verification is completed before taking the sample to minimize the time that the sample waits to be analyzed.
9.2. Samples cannot be preserved for later analysis.
9.3. Sample should be taken in an unused, clean 120 mL sterile vessel with the use of the “fecal stick” tool to allow the sampler to obtain a sample without entering into the riser space.

10. Procedure

10.1. Verify calibration prior to use with the procedure in Section 8. Complete this before collecting sample that is to be analyzed for ozone.

10.2. Refer to Ozone Indigo Method Section 5 (MASSTC-EXT-MTH-001) for additional information.

10.3. Complete reading of laboratory blank.

10.3.1. Prepare the HACH Ozone Test Kit, AccuVac Ampuls, and Ozone Analysis binder on a clean workspace.

10.3.2. Remove the two plastic cups from the HACH Ozone Test Kit and find the one labeled “BLANK.”

10.3.3. Use kit-supplied deionized water (or deionized water in lab if kit-supplied water is out of stock) to pour into the BLANK cup to the fill mark, at least 40 mL.

10.3.4. Place the AccuVac Ampuls (ensuring that they are the correct range, most likely the mid-range) on a clean lab space.

10.3.5. Remove one clean AccuVac Ampul.

10.3.6. Invert the AccuVac Ampul so that the tip is facing downward at a 45° angle into the BLANK cup. Press the AccuVac Ampul forward to simultaneously break the tip and allow liquid to enter the ampule.

10.3.7. Once the AccuVac Ampul has sufficiently sucked in water, place the kit-supplied blue stopper on the tip-end to minimize risk of abrasion or puncture.

10.3.8. Quickly invert the AccuVac Ampul several times to mix.

10.3.9. Clean the AccuVac Ampul with a lint-free wipe.

10.3.10. Remove the instrument cap of the Colorimeter and insert the AccuVac Ampul with deionized water into the cell holder.

10.3.11. Install the instrument cap over the cell holder of the Colorimeter to block light.

10.3.12. Push ZERO button (blue).

10.3.13. Record this reading in the Ozone Analysis binder on MASSTC-FORM-028 – Ozone Measurement Form under the heading Blank Reading (mg/L) O3 along with the AccuVac vial batch number for Blank.

10.3.14. Remove the AccuVac Ampul and place to the side, keeping it accessible.

10.4. Collect sample for ozone measurement.

10.4.1. Using a new sterile vessel (the bottle used to collect bacterial samples) and the bottle-on-a-stick tool (often referred to as the “fecal stick”), obtain a fresh sample from a flowing source. Collect the sample gently and analyze immediately. Do not shake or stir the sample or allow the sample temperature to increase.

10.4.2. Bring the sample to the clean workspace.

10.4.3. Obtain the clean plastic cup from the HACH Ozone Test Kit (not the one labeled “BLANK”) and pour at least 40 mL of sample into the cup.

10.4.4. Remove one clean AccuVac Ampul.

10.4.5. Invert the AccuVac Ampul so that the tip is facing downward at a 45° angle into the cup containing the sample. Press the AccuVac Ampul forward to simultaneously break the tip and allow liquid to enter the ampule.

10.4.6. Once the AccuVac Ampul has sufficiently sucked in water, place the kit-supplied blue stopper on the tip-end to minimize risk of abrasion.

10.4.7. Quickly invert the AccuVac Ampul several times to mix.

10.4.8. Clean the AccuVac Ampul with a lint-free wipe.

10.4.9. Remove the instrument cap of the Colorimeter and insert the AccuVac Ampul into the cell holder.

10.4.10. Install the instrument cap over the cell holder of the Colorimeter to block light.

10.4.11. Push green checkmark READ button.

10.4.12. Record this reading on MASSTC-FORM-028 – Ozone Measurement Form for the correct project under the column heading Ozone Reading (mg/L) O3 along with the AccuVac vial batch number for sample. Fill out additional information on this form (time, date, initials, etc.).

10.4.13. If the sample reading is flashing:

10.4.13.1. Invert the AccuVac Ampul with deionized water used for today's Blank ozone reading several times and clean with a lint-free cloth.

10.4.13.2. Re-insert the AccuVac Ampul with deionized water used for today's Blank ozone reading.

10.4.13.3. Push the blue ZERO button again.

10.4.13.4. Read the Blank again by pushing the green checkmark READ button.

10.4.13.5. If the Blank is not a 0.00 value, repeat steps 10.4.13.1 – 10.4.13.4 again until reading is 0.00. If Blank is a 0.00 value, write a note in the Comments section of MASSTC-FORM-02 – Ozone Measurement Form and accept the sample ozone measurement.

10.5. Clean up the workspace. Place an X on the bottom of any used AccuVac Ampuls and return tip-down into the box. Put ampule box back into upper cabinet. Use tap water to clean the BLANK and sample cups; leave these to air-dry. Turn Colorimeter off. Replace the instrument cap on the Colorimeter and return to kit. Return rubber caps to kit. Dispose of vessel containing sample.

11. Data Analysis/Calculations

11.1. None

12. Data Management/Records Management

12.1. Data will be recorded promptly, legibly and in indelible ink on the Ozone Measurement Forms (MASSTC-FRM-028). Completed forms are scanned and physical copies are archived in the Ozone Measurement Record Binder. The binder is kept in a secured file cabinet in the office of the MASSTC Director. Only authorized personnel have access to the secured files. Archived data are subject to official retention schedule contained in MASSTC-SOP-003, Records and Archives.

13. Quality Control

13.1. Verification of instrument calibration (Section 8) shall be completed prior to using the instrument.

13.2. The Secondary Standards vials shall always be stored in the original case in a cool and dark place.

13.3. A fresh blank using deionized water shall be prepared for each day the instrument is used.

13.4. Samples will be taken directly from the collection point to the site laboratory and be analyzed immediately.

13.5. Samples will be handled with care to avoid excess jostling or shaking of the contents.

14. Internal and External References

14.1. MASSTC-EXT-MTH-001 - HACH Ozone Indigo Method, 8311

14.2. MASSTC-EXT-MAN-002 - HACH Ozone Pocket Colorimeter II Manual

14.3. MASSTC-EXT-MAN-006 - HACH Ozone Secondary Standards Kit Instructions for Pocket Colorimeter II

14.4. MASSTC-EXT-SDS-001 - Ozone Safety Data Sheet

15. Forms and Data Sheets

15.1. MASSTC-FRM-028 - Ozone Measurement Form

15.2. MASSTC-FRM-029 – Pocket Colorimeter II Ozone Quality Control Form

15.3. MASSTC-FRM-031 – Ozone Standards Reference

Massachusetts Alternative Septic System Test Center

Barnstable, Massachusetts

Form

Title: Ozone Measurement Form

Effective Date: 2020-08-04	**Number:** MASSTC-FRM-028	**Revision:** 002

Authors

Name: Brian Baumgaertel
Title: MASSTC Director

Signature: Date:

Name: Emily Michele Olmsted
Title: Environmental Project Assistant

Signature: Date:

Approvals

Name: Brian Baumgaertel
Title: MASSTC Director

Signature: Date:

Ozone Measurement Form

Document ID#: MASSTC-FORM-028
Revision#: 002
Released: 2020-08-04
Released By: Brian Baumgaertel

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Revision #001: Added Column for Blank Reading. Edits by BB.	2020-07-20
Revision #000: Original Issue	2020-01-31

Ozone Measurement Form

Document ID#: MASSTC-FORM-028
Revision#: 002
Released: 2020-08-04
Released By: Brian Baumgaertel

Name of Project: _____

Date	Sample Time	Analysis Time	Accuvac Vial Batch Number for Blank	Blank Reading (mg/L) O_3	Accuvac Vial Batch Number for Sample	Ozone Reading (mg/L) O_3	Sampler Initials	Analyst Initials	Comments

Massachusetts Alternative Septic System Test Center

Barnstable, Massachusetts

Form

Title: Pocket Colorimeter II Ozone Quality Control Form

Effective Date: 2020-07-20	**Number:** MASSTC-FRM-029	**Revision:** 002

Authors

Name: Brian Baumgaertel
Title: MASSTC Director

Signature: Date:

Approvals

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Signature: Date:

Pocket Colorimeter II Ozone Quality Control Form

Document ID#: MASSTC-FORM-029
Revision#: 002
Released: 2020-07-20
Released By: Brian Baumgaertel

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Revision #001: Added columns to record acceptable ranges for each standard. Revisons by Brian Baumgaertel.	2020-02-05
Revision #000: Original Issue	2020-01-31

Pocket Colorimeter II Ozone Quality Control Form

Document ID#: MASSTC-FORM-029
Revision#: 002
Released: 2020-07-20
Released By: Brian Baumgaertel

Date	Time	Standards Set Lot Number	Blank Reading	Blank Acceptable Range	Standard 1 Reading	Standard 1 Acceptable Range	Standard 2 Reading	Standard 2 Acceptable Range	Staff Initials	Comments

Massachusetts Alternative Septic System Test Center

Barnstable, Massachusetts

Form

Title: Ozone Standards Reference

Effective Date: 2020-07-30	**Number:** MASSTC-FRM-031	**Revision:** 000

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Ozone Standards Reference

Document ID#: MASSTC-FORM-031
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Ozone Standards Reference

Document ID#: MASSTC-FORM-031
Revision#: 000
Released: 2020-07-30
Released By: Brian Baumgaertel

Manufacturer: HACH
Product: Medium Range Ozone Secondary Standards Kit
Product Number: 2708000
Lot Number: A8302
Expiration: Oct 2020

Use the following table when verifying meter performance, Section 8 of MASSTC-SOP-014 - OZONE Measurement SOP:

Vial Label:	**Blank**	**Standard 1**	**Standard 2**
Acceptable Range:	0.58 - 0.72	0.39 - 0.51	0.14 - 0.24

Certificate of Analysis Information:

Reference on Certificate of Analysis:	STD 3, A8226	STD 2, A8226	STD 1, A8226
Acceptable Range	0.65 ± 0.07	0.45 ± 0.06	0.19 ± 0.05

Appendix 8

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Massachusetts Alternative Septic System Test Center
Barnstable, Massachusetts

Standard Operating Procedure

Title: Sample Collection

Effective Date: 2021-11-17	**Number:** MASSTC-SOP-037	**Revision:** 002

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Sample Collection	Document ID#: MASSTC-SOP-037 Revision#: 001 Released Date: 2021-11-17 Released By: Brian Baumgaertel

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History	Effective Date
Revision #002: Added field blank to section on virus sample analysis. Reformatted coversheet to include QAM approval. Edits done by EMO.	2021-11-17
Revision #001: Expanded definition of whaler pump to include details about power supply. Added section about sampling lysimeters. Added section about virus samples. Added section about collection from external plants. Edits done by EMO and GH.	2021-09-22
Revision #000: Original Issue	2021-07-20

CONTENTS

1. Scope and Application

1.1. This protocol describes the procedure used to collect water and/or wastewater samples for analysis.

2. Definitions

2.1. Aliquot – a sample taken for chemical analysis or other treatment that represents a portion of the whole; composite samples.

2.2. Aliquoting – the process of dividing a whole sample into aliquots.

2.3. "Bellies" – low spots in sample tubing that can hold liquid. This liquid is susceptible to freezing and can provide conditions for biological growth. See Section 5 – Interferences.

2.4. Composite sample – a mixture of grab samples taken over a period of time, usually 24 hours.

2.5. Composite sample cup – a device fitted to an effluent discharge pipe that maintains a small reservoir of liquid to enable collection by a composite sampler. Cup should have holes to allow drainage and to ensure sampler is accessing new source of non-stagnant liquid.

2.6. Dipper pole – a sampling device composed of a cup attached to a pole. The cup is constructed so that a sterile sample bottle can be slipped in. The pole is of sufficient length to allow the user to avoid entering a confined space or other hard-to-reach area.

2.7. Grab sample – an individual sample taken without the addition of other samples.

2.8. Grabber stick – a device that allows user to close hooks on an object by use of a stick, usually acting as an extension of hands in locations that are difficult to reach.

2.9. Peristaltic sample pump – a type of pump used to "draw up" a sample.

2.10. Project-specific sample tubing – sample tubing which is cut to length for a specific project sampling location to minimize low spots ("bellies") between the sample location and sample equipment.

2.11. Project-specific sample carboy – a sample carboy that is labeled and used for one specific project sampling location. See also "sample carboy".

2.12. Sample bottle – a capped container made of plastic, glass, or other material to contain, prevent contamination of, and securely transport a sample from a sample location to the laboratory where the sample is analyzed.

2.13. Sample carboy – a capped 10-liter Nalgene or equivalent container which is maintained in a clean condition which is used to collect composite samples from an automated sampling device.

2.14. Sample tubing – a section of plastic tubing that is maintained in a clean condition.

2.15. "Whaler" sample pump – a sample pump attached to tubing and a power supply (usually a 12-volt DC battery encased in weather-proof housing unit), often used to sample lysimeters or other hard-to-access locations

3. Health and Safety Warnings

3.1. **Physical Hazards** – use care and good judgement when taking samples. If a sample location is in a place where it cannot be safely collected (e.g. confined space), notify the MASSTC director immediately and do not attempt to retrieve it. Environmental conditions (e.g. rain, snow, etc.) can lead to uneven and/or slippery surfaces so care should be taken to prevent slips and fall. **PPE Required: Closed-toe shoes/boots. Care should be taken to dress appropriately.**

3.2. **Infectious Materials** – even the cleanest wastewater can contain pathogens or toxicants. Proper precautions should be taken to isolate yourself. **PPE Required: gloves, goggles.**

3.3. **Skin Corrosion/Serious Eye Damage** – Some sample bottles contain sulfuric acid (H_2SO_4) as a preservative. **Gloves and safety goggles required.**

4. Cautions

4.1. Whaler sample pumps are hand-made sample retrieval equipment and usually have some unprotected wires. Care should be taken to handle wire connections as gently as possible.

4.2. All sampling bottles must be clearly labelled prior to sampling. Sampler should be familiar with sampling port locations and exercise all safety precautions before accessing areas for wastewater sampling.

4.3. Consult *MASSTC-FRM-040 – Sampling Plan* to ensure the proper samples at the proper location as confirmed by client.

5. Interferences

5.1. Temperature of composite sample should be taken before placing aliquot in refrigeration to ensure the reading is representative of the temperature of the sample in situ.

5.2. Composite samples can become contaminated while transporting from the sampler to the field lab. Aliquot caps should be used.

5.3. Samples can become contaminated while transferring between containers. Care should be taken to sample directly from the source whenever possible.

5.4. Pathogen samples are usually sensitive to ultraviolet light. Keep sterile bottles containing sample in as dark of a condition as possible when transporting from field to longer-term holding (ex. refrigerator).

5.5. Sample retrieval equipment (pumps, tubing, etc.) should be thoroughly cleaned in between sample locations to prevent contamination of samples in between sites.

6. Personnel Qualifications

6.1. Personnel are required to be knowledgeable of the procedures in this SOP.

6.2. Personnel should be trained in the proper use of Personal Protective Equipment (PPE).

7. Special Apparatus and Materials

7.1. Influent channel composite sampler setup (Section 10)

7.1.1. Influent sample carboy and clean carboy cap.

7.2. Project-specific composite sampler setup (Section 11)

7.2.1. Portable or refrigerated sampler.

7.2.2. Project-specific tubing, which attaches to peristaltic pump of composite sampler and draws liquid.

7.2.3. Project-specific composite sample cup.

7.2.4. Power supply – battery or plug in AC adapter.

7.2.5. Ice, if using portable sampler, usually two bags unless below-freezing temperatures expected.

7.2.6. Project-specific sample carboy and clean aliquot cap.

7.2.7. Dipper pole to reach sample locations without confined space entry.

7.2.8. Grabber stick to reach cup or tubing without confined space entry.

7.3. Composite sample aliquoting (Section 12).
  7.3.1. Clean sample bottles.
7.4. Influent channel grab samples (Section 13)
  7.4.1. Clean sample bottles.
  7.4.2. Dipper pole.
7.5. Lysimeter Samples (Section 16)
  7.5.1. Clean sample bottles. Sterile if for biological analysis.
  7.5.2. "Whaler" or peristaltic sample pump and accompanying tubing (depends on location; see definition for more detail).
  7.5.3. YSI ProDSS, depending on volume amount.
7.6. Project-specific grab samples (Sections 14 and 15)
  7.6.1. Clean sample bottles. Sterile if for biological analysis.
  7.6.2. Dipper pole (depends on location).
  7.6.3. "Whaler" or peristaltic sample pump and accompanying tubing (depends on location; see definition for more detail).
7.7. Virus Samples (Section 19)
  7.7.1. Clean, sterile one-gallon HDPE handled containers. See section 19 for instruction on appropriate disinfection.
  7.7.2. PVC cap and tube assembly
  7.7.3. Tape
7.8. Procedure for Wastewater Collection from Locations at External Wastewater Treatment Plants (Section 20)
  7.8.1. Clean, sterile one-gallon HDPE handled containers. See section 20 for instruction on appropriate disinfection.
  7.8.2. "Whaler" or peristaltic sample pump and accompanying tubing (depends on location; see definition for more detail).

8. Instrument or Method Calibration

8.1. Thermometers should be calibrated per *MASSTC-SOP-013 – Thermometer Calibration SOP*.

9. Sample Preparation, Storage, and Transportation

9.1. Consult *MASSTC-SOP-015 – Sample Preparation and Transportation*.

10. Procedure for Influent Channel Composite Sample Setup and Retrieval

10.1. Set up influent channel composite sampler
  10.1.1. The influent channel composite sampler should be set up 24 hours prior to the anticipated sample retrieval time.
  10.1.2. Obtain equipment needed (see section 7.1)
  10.1.3. Bring all equipment to sample location

10.1.4. Inspect sampler tubing for damage, freezing, "bellies", and bio-growth. Tubing should be replaced prior to initiation of sampling if needed.

10.1.5. Place the project-specific sample carboy in the refrigeration compartment.

10.1.6. Pass the sample tubing through the hole in the sample carboy cap.

10.1.7. Place the refrigerator temperature probe in contact with the sample carboy.

10.1.8. Press "On" button on peristaltic sampling pump.

10.1.9. Verify that the first pump cycle runs and that a sample is properly drawn up through the sampler tubing and into the sample carboy.

10.1.10. Fill out the appropriate row on *MASSTC-FRM-020 – Daily DC West Sampler Logs*.

10.2. Retrieve the sample carboy from the sampler after the proscribed period of time (i.e. 24 hours).

10.2.1. Remove the holed sample carboy cap and affix a solid sample carboy cap to prevent contamination during transport from field to pouring station.

10.2.2. Check that sample carboy label is correct for the location being sampled.

10.3. Follow the Procedure for Composite Sample Aliquoting into Sample Bottles (Section 12)

10.4. Consult *MASSTC-SOP-015 Sample Preparation and Transportation*.

11. Procedure for Project-Specific Composite Sample Setup and Retrieval

11.1. Consult *MASSTC-FRM-040 – Sampling Plans* to confirm location of project-specific composite sample.

11.2. Set up composite sampler.

11.2.1. Composite samplers, unless directed differently by client, must be set up 24 hours in advance of the anticipated retrieval time.

11.2.2. Obtain equipment needed (see section 7.2)

11.2.3. Bring all equipment to sample location.

11.2.4. Place project-specific sampling cup/collection container under liquid discharge pipe. Ensure cup is draining to allow for fresh, non-stagnant sample.

11.2.5. Secure tubing in sampling cup/container, usually through a zip tie loop.

11.2.6. Attach tubing to composite sampler, ensuring to place tubing through hole in riser, if applicable, so that riser can be covered without obscuring the tubing.

11.2.7. Place carboy in sampler. Add ice, if not using refrigerated sampler, ensuring that no ice enters the carboy. **Check that carboy label is correct for the project.**

11.2.8. Close up sampler, ensuring that the top fits or that any other internal tubing will allow liquid to enter carboy. Close riser, if applicable.

11.2.9. Attach power supply.

11.2.10. Program sampler to normal conditions – 48 composite samples over 24 hours with tubing rinses – unless directed otherwise by client or MASSTC Director.

11.2.11. **Remain at the sampler to visually confirm the first sample is taken.** This can decrease the loss of any samples due to errors in set up (ex. tubing not submerged in collection cup).

11.2.12. Throughout the rest of the day, check on sampler to ensure that there are no issues and that the ice supply is acceptable. Add a third bag of ice in hot temperatures (usually above 80° F).

11.3. Retrieve the sample carboy from the sampler after the proscribed period of time (i.e. 24 hours).

11.3.1. Affix a solid sample carboy cap to prevent contamination during transport from field to pouring station.

11.3.2. Check that sample carboy label is correct for the location being sampled.

11.4. Follow the Procedure for Composite Sample Aliquoting into Sample Bottles (see section 12)

11.5. Consult *MASSTC-SOP-015 Sample Preparation and Transportation*.

12. Procedure for Composite Sample Aliquoting into Sample Bottles

12.1. Bring composite sample carboy to secure, clean space (usually the kitchen sink in the main office trailer).

12.2. Record temperature and volume of aliquot sample as soon as possible. Aliquot temperature and sample volume should be recorded in the aliquot logbook.

12.3. Inspect sample bottles for improper labels, physical damage, and cleanliness.

12.4. Place opened sample bottles in sink. Be sure to keep preserved and non-preserved caps separate.

12.5. With aliquot cap firmly in place, shake vigorously for 10 seconds.

12.6. Remove aliquot cap and pour non-sterile samples, being careful to maintain a constant stream of liquid from the aliquot. Fill each sample bottle as needed (preserved bottles should not be overfilled), ensuring to leave headspace.

12.7. Recap sample bottles, ensuring that preserved caps are placed on preserved bottles and unpreserved caps are placed on unpreserved bottles.

12.8. Rinse sample bottles in clean tap water, followed by a disinfecting rinse, and one final rinse with tap water.

12.9. Place completed sample bottles in refrigerator. Record time of sample as the last composited sample time on chain of custody, along with initials of sampler.

12.10. If project is following National Sanitation Foundation (NSF) protocol, store leftover aliquot in refrigerator for 24 hours.

12.11. If interrupted during pouring process, return to step 12.4.

13. Procedure for Influent Channel Grab Samples (Sterile)

13.1. Obtain equipment needed (see section 7.4)

13.2. **Double-check that you have all bottles and that they are for the correct location and date.**

13.3. Two methods for sampling are acceptable:

13.3.1. By hand (with proper PPE):

13.3.1.1. Remove the sample bottle cap, being careful not to touch the inside or the cap or bottle with your hand or other object.

13.3.1.2. Dip the sample approximately 5-10 inches into the wastewater at a 45-degree angle.

13.3.1.3. When full, bring the bottle to the surface, and pour off a small amount to leave an air gap at the top of the bottle.

13.3.1.4. Immediately re-cap the bottle.

13.3.2. Using a dipper pole:

13.3.2.1. Place a capped (sterile, if for biological analysis) 100mL sample bottle in the dipper pole cup.

13.3.2.2. Remove the sample bottle cap, being careful not to touch the inside or the cap or bottle with your hand or other object.

13.3.2.3. Dip the sample bottle approximately 5-10 inches into the wastewater.

13.3.2.4. When full, bring the bottle to the surface, and pour off a small amount to leave an air gap at the top of the bottle.

13.3.2.5. Immediately re-cap the bottle.

13.4. To the extent possible, keep the filled sample bottle out of sunlight if doing biological analyses.

14. Procedure For Project-Specific Grab Samples (Non-Sterile)

14.1. Consult *MASSTC-FRM-040 – Sampling Plan* to confirm location of sample and any particular instructions.

14.2. Secure the correct bottles for the particular sampling location to be sampled. **Double-check that you have all bottles and that they are for the correct location and date.**

14.3. Obtain equipment needed (see section 7.5)

14.4. Bring clean water to rinse equipment if sampling more than one site in the field.

14.5. Go to sample location and collect sample. Use the following in the order of preference unless directed otherwise by client (check the Sampling Plan!):

14.5.1. Collect sample by uncapping bottle(s) and placing under free-falling stream of liquid.

14.5.2. If free-falling stream has very slow rate of flow, an alternative, approved, and thoroughly-cleaned container may be used to collect liquid. Take care to place container so that it is not contaminated by another other sources until collection and pouring of bottles.

14.5.3. If it is not possible to either safely or adequately place bottles under free-falling stream, collect sample by using bottle secured to dipper pole and pouring into uncapped sample bottles. Rinse bottle secured to dipper pole at least three times with sample liquid before pouring into sample bottles to minimize any contamination or dilution from bottle secured to pole.

14.5.4. Use whaler pump to collect liquid from a pooled source (such as from a sump or a lysimeter). Ensure that proper purging has been completed per *MASSTC-FRM-040 – Sampling Plan* if necessary for site.

14.6. Recap sample bottles, ensuring that preserved caps are placed on preserved bottles and unpreserved caps are placed on unpreserved bottles. **Double-check that the sample bottles have the correct label.**

14.7. Carefully bring bottles inside as soon as possible.

14.8. Rinse sample bottles in clean tap water, followed by a disinfecting rinse, and one final rinse with tap water.

14.9. Place completed sample bottles in refrigerator. Record time of sample on chain of custody, along with initials of sampler.

14.10. Consult *MASSTC-SOP-015 Sample Preparation and Transportation*.

14.11. Clean any sample retrieval equipment before returning.

15. Procedure For Project-Specific Grab Samples (Sterile)

15.1. Consult *MASSTC-FRM-040 – Sampling Plan* to confirm location of sample and any particular instructions.

15.2. Secure the correct sterile bottles for the particular sampling location to be sampled. **Double-check that all bottles are for the correct location and date.**

15.3. Obtain any sampling equipment needed. **Clean all sampling equipment before use, including disinfection if needed.**

15.4. Commonly used sample retrieval equipment includes:

15.4.1. Dipper pole

15.4.2. Whaler pump and tubing (long or short) attached to battery pack.

15.4.3. Peristaltic pump.

15.5. Bring any clean water needed to rinse equipment if sampling more than one site in the field.

15.6. Go to sample location and collect sample. **Ensure that cap and bottle do not touch anything besides the liquid sample. If bottle or cap is compromised, discard the bottle and obtain a new sample in a new bottle.**

15.7. Use the following in the order of preference unless directed otherwise by client:

15.7.1. Collect sample by uncapping bottle(s) and placing under free-falling stream of liquid.

15.7.2. If it is not possible to either safely or adequately place bottles under free-falling stream, collect sample by using dipper pole.

15.7.3. If free-falling stream has very slow rate of flow, an alternative, approved, and thoroughly-cleaned container may be used to collect liquid. Take care to place container so that it is not contaminated by another other sources until pouring.

15.7.4. Use whaler pump to collect liquid from pooled source (such as from a sump or a lysimeter). Ensure that proper purging has been completed per *MASSTC-FRM-040 – Sampling Plan* if necessary for site.

15.8. Be sure to leave an air gap at the top of the bottle to ensure adequate headspace.

15.9. Recap sample bottles, ensuring that nothing touches the cap or bottle, as this can contaminate the aseptic sample. **Double-check that the sample bottles have the correct label.**

15.10. Carefully bring bottles inside as soon as possible. **Keep bottle in as dark of a condition as possible while still in field, as many pathogen samples are sensitive to ultraviolet light.**

15.11. Rinse sample bottles in clean tap water, followed by a disinfecting rinse, and one final rinse with tap water.

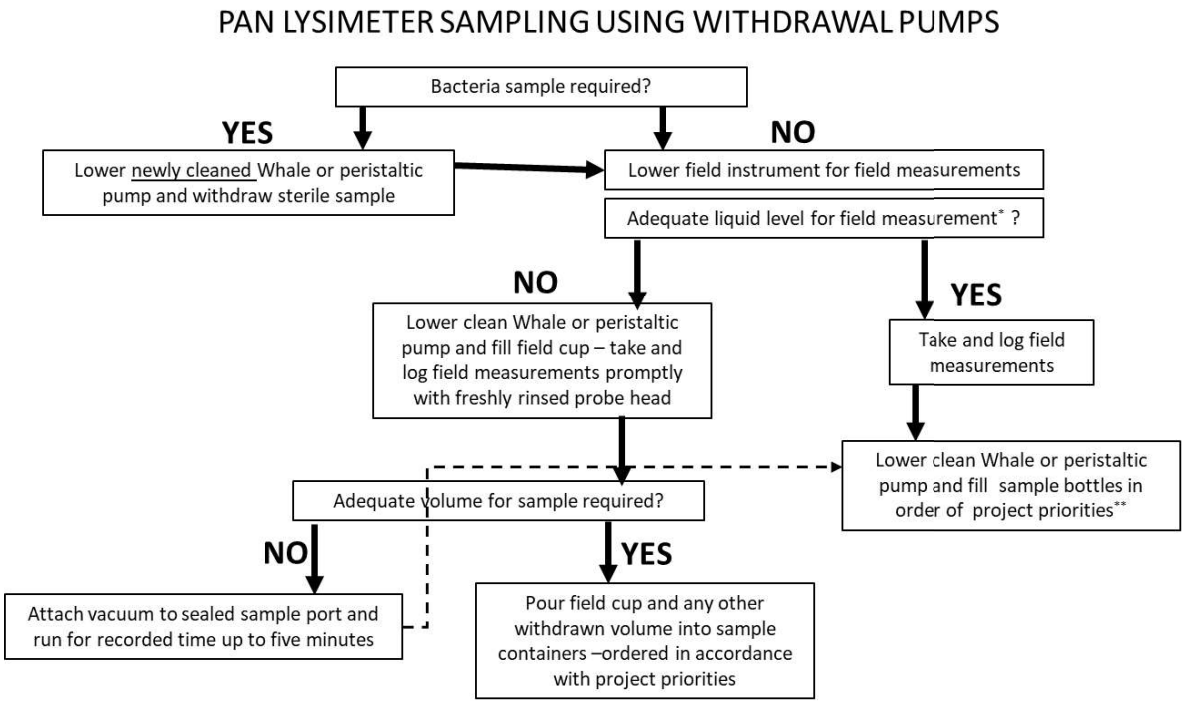
15.12. Place completed sample bottles in refrigerator. Record time of sample on chain of custody, along with initials of sampler.

15.13. Consult *MASSTC-SOP-015 Sample Preparation and Transportation*.

16. Procedure for Samples from Lysimeter

16.1. Obtain any sampling equipment needed. **Clean all sampling equipment before use, including disinfection if needed. See section 7.5.**

16.2. Take samples with whaler pump according to flow chart:



- To determine if there is adequate level for field measurements, use the specific conductance. In most wastewater samples specific conductance should exceed 200 uS. If less, in most instances conclude "inadequate liquid level" or use wetness of probe as indicator.

** In some instances where preserved bottles are required, it is preferable to fill clean one-liter no-preservative bottles and pour into preserved bottles from these. This also allows easier apportionment of samples to priority analyte bottles if necessary.

16.3.

17. Procedure for Ozone Samples

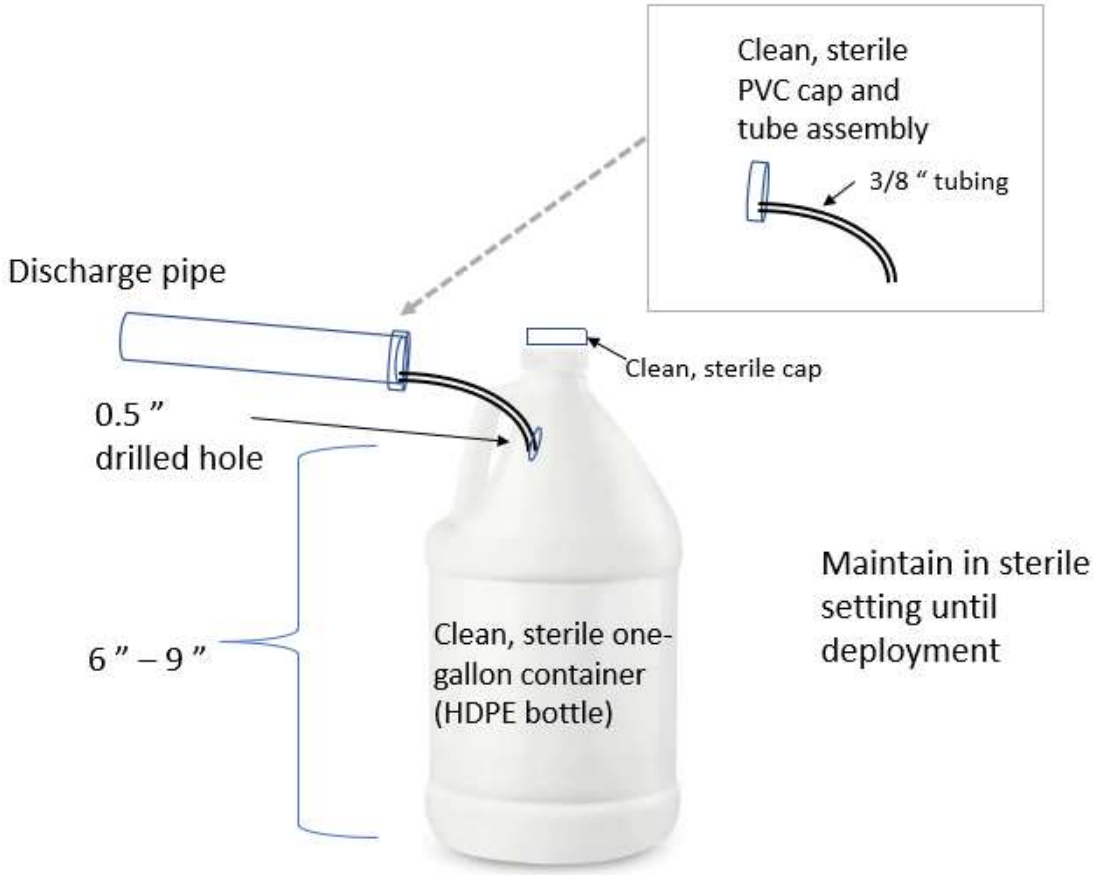
17.1. Consult *MASSTC-SOP-014 – Ozone Measurement.*

18. Procedure for Field samples

18.1. Consult *MASSTC-SOP-016 – YSI ProDSS Field Meter.*

19. Procedure for Virus Samples

19.1. Samples under this section can be retrieved from any location where there is a discharge location comprised of a two-inch PVC pipe below which is a five to six-inch free space or drop. The locations include standard stone-trench systems, shallow-placed soils-based systems, wood-based systems and others where the discharge point is comprised of a two-inch PVC pipe. The completed setup of the sampler is shown here:



19.1.1.

19.2. Ensure that all equipment has been properly disinfected.

19.2.1. Disinfection is performed by adding 1/3 cup of bleach to enough tap water to achieve the full volume of the one-gallon container HDPE bottle and cap.

19.2.2. The solution must remain in the bottle for at least ten minutes and be swirled to contact all surfaces.

19.2.3. Following emptying, the HDPE bottle is then subjected to at least a four-volume full rinse with tap water and stored in a clean, dry location until use.

19.2.4. The cap-and-tube assembly is submersed in a bleach solution (1/3 cup of bleach per gallon) for a minimum of ten minutes followed by a rinse with tap water.

19.2.5. These devices should be stored in a clean, dry location until use.

19.3. Ensure proper sampler deployment.

19.3.1. Prior to deploying the sampler device, the distal end of the two-inch discharge pipe should be cleaned with an alcohol wipe and dried with a paper towel.

19.3.2. The cap-and-tube assembly should be affixed to the discharge pipe with the tube on the bottom round of the pipe. Allow the flow to establish through the 3/8" tube. If you observe any leakage around the cap, tape around the cap to stop the leakage.

19.3.3. Once flow from the tube is observed, direct the tube through the drilled hole in the collection bottle. Make sure the cap is on the bottle to prevent contamination. Make sure that there is no discharge from adjacent pipes near the hole in the collection bottle.

19.3.4. Allow the bottle to fill up to the drilled hole in the bottle. The bottle may be allowed to overfill and spill out the hole in the sample bottle.

19.4. Ensure proper sample collection.

19.4.1. When collecting the sample, be careful not to allow contamination to enter the bottle.

19.4.2. Pull the bottle away from the sample tube and place tape across the hole in the sample bottle.

19.4.3. Use a fresh piece of tape from the roll that has not been exposed prior to removal.

19.4.4. Label the sample with the field location designator and record on chain of custody.

19.4.5. Replace the sample bottle with a container so that field parameters can be measured.

19.4.6. Deliver the sample to the laboratory immediately and place in the refrigerator.

19.5. Collect a field blank each time samples are collected.

19.5.1. Obtain clean, sterile one-gallon container (HDPE bottle) as used for virus analysis.

19.5.2. Label the sample with the field blank designator and record on chain of custody.

19.5.3. Fill with unchlorinated tap water and cap it.

19.5.4. Bring one-gallon container with tap water to sample location and leave among other set ups.

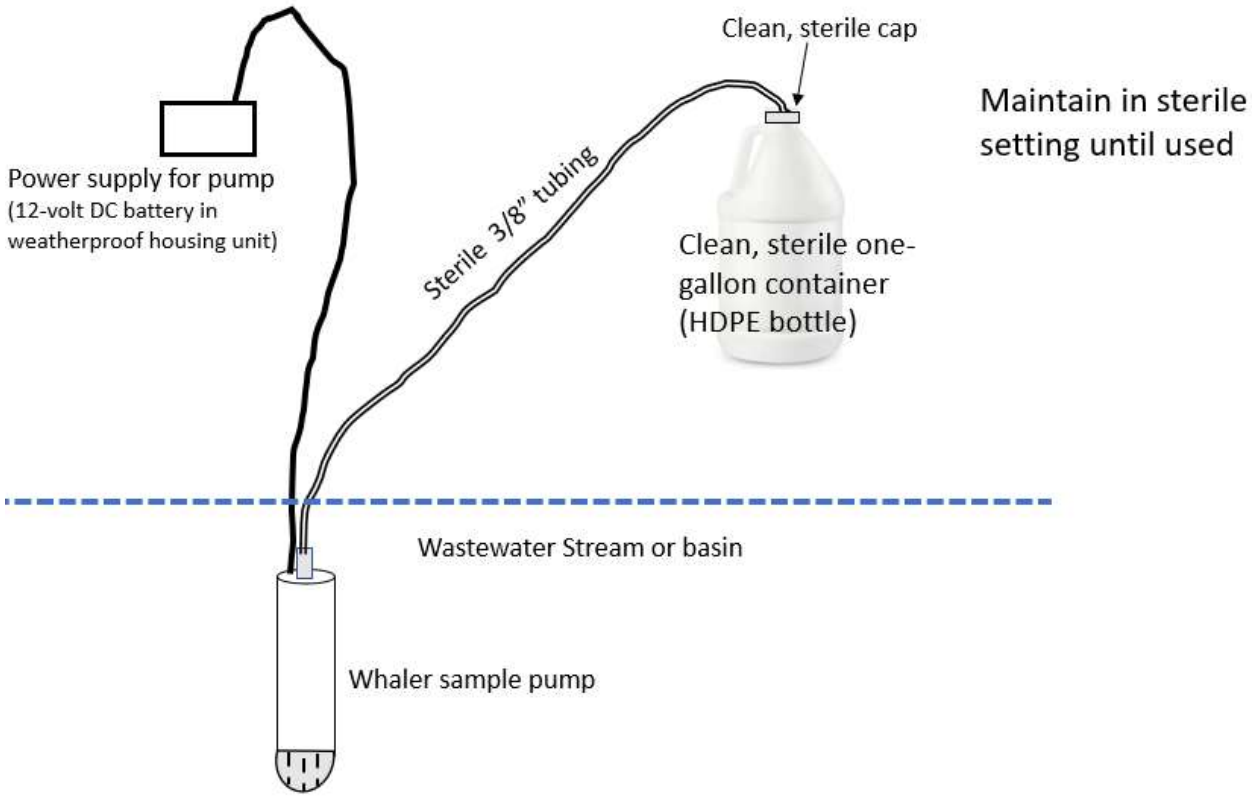
19.5.5. Subject the field sample to the same conditions (travel, temperature, etc) as other samples.

19.5.6. Deliver to laboratory with samples for virus analysis and place in refrigerator.

20. Procedure for Wastewater Collection from Locations at External Wastewater Treatment Plants

20.1. Samples under this section can be taken at any external location that provides a representative sample of the influent or mid process locations at wastewater treatment plants.

20.2. The sampling set up is shown as follows:



20.3. Ensure that all equipment to be used is properly disinfected.

20.3.1. Disinfection is performed by adding 1/3 cup of bleach to enough tap water to achieve the full volume of the one-gallon HDPE container and capping.

20.3.2. The solution must remain in the bottle for at least ten minutes and be swirled to contact all surfaces.

20.3.3. Following emptying, the HDPE bottle is then subjected to at least a four-volume full rinse with tap water and stored in a clean, dry location until use.

20.3.4. The cap-and-tube assembly is submersed in a bleach solution (1/3 cup of bleach per gallon) for a minimum of ten minutes followed by a rinse with tap water.

20.3.5. Pump should be run in a bleach solution for one minute.

20.3.6. Devices should be stored in a clean dry location until use.

20.4. Ensure Proper Sample Collection

20.4.1. Submerse pump with attached 3/8" tubing into the liquid to be sampled.

20.4.2. Run pump for 15 seconds, discarding discharge to area not impacting sampling location (could be discharge container).

20.4.3. Carefully uncap one-gallon HDPE bottle and fill to desired volume (at least 0.75 gallons).

20.4.4. Cap bottle and place immediately on ice and out of direct sunlight.

20.4.5. Label sample and record on chain of custody.

20.4.6. Complete field sampling log and field parameter measurement according to *MASSTC-SOP-016 – YSI ProDSS Field Meter*.

20.4.7. Sample other chemical biological samples as prescribed.

20.4.8. Deliver to laboratory within two hours of collection.

21. Data Management/Records Management

21.1. Aliquot temperature and sample volume should be recorded in the aliquot log book.

21.2. Ensure that the time of sample and initials of sampler are recorded promptly, legibly, and in indelible ink on the chain of custody.

21.3. Record all observations and data according to *MASSTC-SOP-003 – Data and Records Management*.

22. Quality Control

22.1. Always consult *MASSTC-FRM-040 – Sampling Plan*.

23. Internal and External References

23.1. MASSTC-SOP-003 – Data and Records Management.

23.2. MASSTC-SOP-014 – Ozone Measurement.

23.3. MASSTC-SOP-015 – Sample Preparation and Transportation.

23.4. MASSTC-SOP-016 – YSI ProDSS Field Meter.

23.5. MASSTC-SOP-017 – Sample Equipment Maintenance and Sterilization

23.6. Standard Methods (2017) Standard Methods for the Examination of Water and Wastewater, 23rd Edition American Water Works Association (AWWA, WEF and APHA)

24. Forms and Data Sheets

24.1. MASSTC-FRM-020 – Daily DC West Sampler Logs

24.2. MASSTC-FRM-040 – Sampling Plan

Appendix 9

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Massachusetts Alternative Septic System Test Center
Barnstable, Massachusetts

Standard Operating Procedure

Title: Sample Preparation and Transportation

Effective Date: 2021-07-15	**Number:** MASSTC-SOP-015	**Revision:** 000

Authors

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Sample Preparation and Transportation	Document ID#: MASSTC-SOP-015 Revision#: 000 Released Date: 2021-07-15 Released By: Brian Baumgaertel

Revision History

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the MASSTC Director on the MASSTC Sharepoint Site.

History	Effective Date
Revision #000: Original Issue	2021-07-15

CONTENTS

1. Scope and Application

1.1. This protocol describes the procedures used to prepare bottle labels and chains of custody for sampling as well as for the preparation of transportation of samples.

2. Definitions

2.1. None.

3. Health and Safety Warnings

3.1. Biohazard – Contact with wastewater can result in sickness, injury or death. **Wear goggles and gloves**.

3.2. Chemical – Contact with preservatives (such as sulfuric acid) can result in sickness, injury or death. **Wear goggles and gloves and hold bottles upright.**

4. Cautions

4.1. Ensure that the correct bottle size, type, and preservative is being used. Consult MASSTC Director or Water Quality Lab Director if needed.

4.2. Ensure the correctly-labeled bottle is used at the corresponding sample location.

5. Interferences

5.1. Always consult MASSTC-FRM-040 – Sampling Plan to ensure that the correct parameters and frequency of sampling are followed.

5.2. Ensure that the correct lab report turnaround time as requested by client is being followed and is specified on the chain of custody.

5.3. Sample locations must be grouped together on the Chain of Custody to minimize confusion of Barnstable County Water Quality Lab Staff. For example, do not create an order for “Port 1 TSS”, then an order for “Final Effluent nitrate”, then go back to “Port 1 alkalinity.”

5.4. Samples must have unique sample numbers (no duplicates) on the day on which they are received by the Water Quality lab.

5.5. When samples are being packed into a cooler for transportation, a MASSTC Staff Member must initial next to the sample as it is placed in the cooler to minimize the chance of losing a sample.

6. Personnel Qualifications

6.1. Personnel are required to be knowledgeable of the procedures in this SOP.

6.2. Personnel are required to be knowledgeable of the Sampling Plan and Sampling Sketch (MASSTC-SOP-030).

7. Special Apparatus and Materials

7.1. Coolers.

7.2. Ice packs or ice.

8. Instrument or Method Calibration

8.1. None.

9. Procedure for Creation of Lab Orders and Preparation of bottles

9.1. Prepare bottle labels and chains of custody. This should be done no later than Friday afternoon for the week before so that all MASSTC staff have the opportunity to double-check bottles and Chains of Custody.

9.1.1. Consult Sampling Schedule calendar, found electronically on Sharepoint in MASSTC-Documents – Documents – Uncontrolled Documents – Chains, as the Excel sheet "Sample Schedule [*dates*]."

9.1.1.1. Sampling Schedule will state which project and location must be sampled on that day of the week.

9.1.1.2. If any clients have requested non-routine samples, be sure to include these.

9.1.2. Create the Chain of Custody.

9.1.2.1. Open the MASSTC Database, located at http://10.14.20.130:31983/

9.1.2.2. Under the left-hand column sections, click on Sampling, and then click on Chains.

9.1.2.3. Click on Add Chain to create a new Chain of Custody. Alternatively, find the most recently used Chain of Custody, click on the downward facing arrow, and click on Duplicate.

9.1.2.4. Consult MASSTC-FRM-040 – Sampling Plan in the Sampling Plans Binder or the most updated electronic copy and ensure that every sample parameter named in the Sampling Plan appears on the Chain of Custody.

9.1.2.4.1. If MASSTC-FRM-040 – Sampling Plan does not exist for the project in question, consult MASSTC-SOP-030 – Sampling and Maintenance Plans to complete this form.

9.1.2.4.2. Include any Rush Order on lab turnaround. This should be chosen from drop-down menu on electronic Chain of Custody and also should be stamped with the red "Rush" stamp. Any sample parameters that have different rushes must be put on separate Chains of Custody.

9.1.2.4.3. Include any influent samples required by client on Chain of Custody.

9.1.2.5. **Sample locations should appear on the Chain of Custody grouped by sample location.** Do not interchange sample locations out of order on the Chain of Custody. This minimizes errors made by Water Quality Lab Staff. If a sample parameter was missed at one location, delete any sample parameters of other locations and then re-add them.

9.1.2.6. Ensure that the Sample Date is correct. If changed, make sure to click Apply Changes.

9.1.2.7. Ensure that the Starting Chain Sample Number is correct. There should never be duplicate numbers of samples brought to the Water Quality Lab on a single day. If the starting number needs to be changed, key in the correct number and click Apply Changes.

9.1.2.8. **As much as possible, try to start samples at #1 and go chronologically without number gaps.** If known in advance that samples will be delivered on a different day than they are taken, try starting at 100, 200, etc so as to ensure no duplicates.

9.1.2.9. **Separate any BOD_5 or $cBOD_5$ order from other parameters.** This helps the Barnstable County Water Quality Lab staff, and also increases likelihood of timely reports.

9.1.2.10. Make sure the "Send Report To" has the client's actual name (and does not say "Client"). Click Apply Changes if this was changed.

9.1.2.11. The fields for Send Report To and Bill To should be the same unless otherwise confirmed by the MASSTC Director.

9.1.2.12. Add the LIMS client ID into the comments section (preferably, to minimize errors of Barnstable County Water Quality Lab staff).

9.1.3. When the Chain of Custody is completed and you are certain that the Chain of Custody correctly matches the Sampling Plan's requirements, click on Print Chain.

9.1.3.1. Print a physical copy of the Chain of Custody and place it on the table where the sample bottles will be set up.

9.1.3.2. Save a copy of the Chain of Custody electronically in Sharepoint – MASSTC-Documents – Documents – Uncontrolled Documents – Chains. The name should automatically be created by the system but make sure to update the title date to the day that the samples will be taken.

9.1.4. Print the labels.

9.1.4.1. Click on Print Labels and save the labels.

9.1.4.2. Using Dymo Print software, print the labels on the Dymo Label Printer.

9.1.4.3. **When printing a large number of labels in one job (more than 4 labels), you must stand by the label printer and gently pull the labels as they are printed.** If this is not done, the label printer may jam and a label may be skipped without your noticing.

9.1.5. Place the labels on the bottles. If unsure of the correct bottle to use, consult the Chain of Custody which states the bottle size and preservative.

9.1.6. Finish all bottle preparation and Chains of Custody needed for the entire week based on the Sampling Board and Sampling Plans.

9.1.7. Make sure bottles and Chains of Custody are grouped together clearly and separated by day.

9.1.8. Consult another MASSTC Staff member to ensure nothing has been missed. If corrections need to be made, restart at 10.1.

9.1.9. By the end of the last business day of the week (usually Friday), after other MASSTC staff have looked over the bottles and Chains of Custody, e-mail all Chains of Custody to the Water Quality Lab Staff.

9.1.10. If any Chains of Custody need to be created or edited later during the week, restart at 9.1.2.

10. SAMPLE STORAGE

10.1. Samples should be analyzed as soon as possible after they are taken, but at the very least, within holding time.

10.2. Samples cannot be preserved for later analysis.

10.3. Samples must be bottled and stored according to the following table:

Parameter	Container Volume	Container	Processing/Storage	Holding Time
Alkalinity	60-125 ml	Polyethylene	Stored on ice (dark)	14 Days
Ammonium	60-125 ml	Polyethylene (H_2SO_4 Preserved)	Stored on ice (dark)	28 Days

BOD_5 or $CBOD_5$	500 ml	Nalgene	Stored on ice (dark)	48 Hours
COD	60-125 ml	Polyethylene (H_2S0_4 Preserved)	Stored on ice (dark)	28 Days
E. Coli	100 ml	Polyethylene	Collected aseptically by hand at discharge site; stored on ice (dark)	<6 Hours
Fecal Coliform	100 ml	Polyethylene	Collected aseptically by hand at discharge site; stored on ice (dark)	<6 Hours
Nitrate + Nitrite	60-125 ml	Polyethylene	Stored on ice (dark)	48 Hours
Ortho-phosphate	60-125 ml	Polyethylene	Stored on ice (dark)	48 Hours
Total Kjeldahl Nitrogen	60-125 ml	Polyethylene (H_2S0_4 Preserved)	Stored on ice (dark)	28 Days
Total Phosphorus	60-125 ml	Polyethylene (H_2S0_4 Preserved)	Stored on Ice (dark)	28 Days
Total Suspended Solids	1000 ml	Polyethylene	Stored on Ice (dark)	7 Days

10.4. Samples must be rinsed with tap water, disinfected, and rinsed again before placing in fridge.

10.5. See MASSTC-SOP-037 – Sample Collection for procedure on procuring samples.

11. SAMPLE TRANSPORTATION

11.1. On the appropriate day, collect and pour samples according to MASSTC-SOP-037 – Sample Collection.

11.1.1.1. Write the time at which the sample was taken and the initials of the person who took the sample in the Comment field on the Chain of Custody for each sample.

11.1.2. Store sample bottles in refrigerator until delivery.

11.1.3. When sample collection is complete, contact the courier service to confirm that samples are ready, or use schedule already arranged with courier for pick up deadline.

11.1.3.1. If a MASSTC Staff Member is delivering samples, alert the person delivering samples when ready.

11.1.4. Before delivery, MASSTC Staff must pack samples into coolers and add ice to ensure samples remain cold.

11.1.4.1. **The MASSTC Staff member who packing coolers must check off each sample as it is placed into the cooler and write his/her initials next to the number on the Chain of Custody as a written record.**

11.1.4.2. If any samples are missing, that MASSTC Staff Member must attempt to find the missing sample(s), or reprint the label and retake the sample(s).

11.1.5. Before delivery, MASSTC Staff Member must sign at bottom of Chain of Custody for release.

11.1.6. Samples must be delivered to the Barnstable County Water Quality Lab on the same day as taking samples or as soon as possible.

11.2. Staff must be aware of holding times to ensure that no samples are delivered to the Water Quality Lab outside of holding time.

12. Data Analysis/Calculations

12.1. None.

13. Data Management/Records Management

13.1. Save Chains of Custody in PDF format as stated in 10.1.3.2. Archived data are subject to official retention schedule contained in MASSTC-SOP-003, Records and Archives.

14. Quality Control

14.1. Always consult the MASSTC-FRM-040 – Sampling Plan to ensure proper sample order and chains of custody are created.

15. Internal and External References

15.1. MASSTC-SOP-003 – Data and Records Management

15.2. MASSTC-SOP-030 – Sampling and Maintenance Plans

15.3. MASSTC-SOP-037 – Sample Handling

16. Forms and Data Sheets

16.1. MASSTC-FRM-040 – Sampling Plan

Appendix 10

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Quality Assurance Plan

(Revision 027)
Revised
20 September 2021

County of Barnstable
Barnstable County Department of Health and the Environment
Water Quality Laboratory
Superior Court House
3195 Main Street
P.O. Box 427
Barnstable, MA 02630

Table of Contents

1. Introduction

This Quality Assurance (QA) manual has been developed to describe the overall quality assurance program employed by the Barnstable County Department of Health and the Environment Water Quality Laboratory. The County Laboratory performs environmental analyses of volatile organic compounds, metals, and wet chemistry parameters using *Methods and Guidance for the Analysis of Water* (Version 2, June 1999)[1] approved by the United States Environmental Protection Agency, and *Standard Methods for the Examination of Water and Wastewater* (22th Edition, 2012)[2] approved by American Public Health Association, American Water Works Association and Water Environment Federation. The County Lab also performs analyses of total coliform, fecal coliform, e. coli, and heterotrophic plate count in potable and/or non- potable water using the Standard Method[2]. The County laboratory has also carried out its testing and calibration activities in such a way as to meet the requirements of the International Organization for Standardization (ISO) and the International Electrotechnical Commission (IEC) 17025:2005 [3]. The Laboratory specializes in analysis of drinking water and groundwater whose continuous testing and quality maintenance are of great interest to the residents of the County.

Annually, all laboratory employees must complete a conflict of interest law and ethics online training program.
Access to the training is through the Commonwealth's Ethics Website https://www.mass.gov/online-conflict-of-interest-law-training. The Human Resources Department of Barnstable County requires all departments, including the Barnstable County Water Quality Laboratory, to submit and retain the records regarding the completion of this and other employee trainings relating to workplace policies.
In addition, every year all state, county and municipal employees must be provided with the summary of the conflict of interest law. All public employees are required to sign a written acknowledgment that they have been provided with the summary and records of this receipt are kept with the Department of Health and Environment administrative office.

On an annual basis, all personnel participate in the laboratory ethics training program. The objective of the program is to assess and maintain the quality, accuracy, and precision of the generated data and to provide a permanent record of instrument performance and overall data quality and reliability by implementing well defined QA / Quality Control (QC) procedures. The materials covered in our program addresses the proper procedures to ensure data integrity, recognition and prevention of improper laboratory practices, the promotion of objectivity and impartiality in the generation and reporting of analytical data, and procedures for confidential reporting of data integrity concerns to the laboratory director. The program describes our quality assurance organization and responsibilities, our quality assurance objectives for precision and accuracy, the format for generating our standard operating procedures, and the procedure for maintaining our records. The laboratory documents the content of the training and date of participation for each staff member. This documentation is kept available for review during an inspection.

2. Quality Assurance Policy and Quality Assurance Objectives

2.1 Statement of Policy

It is the policy of the Barnstable County Health Laboratory to provide the possible highest quality analysis of drinking water. We are committed to maintaining a strict QA program and adhering to all policies required by regulatory and accrediting agencies and other organizations[3]. To achieve this high standard of quality, we have implemented the following quality assurance plan for our analytical services.

Our laboratory employs modern analytical instrumentation and is fully automated to provide results of high quality in a timely fashion. The analytical professionals and technicians are well trained with many years of work experience in the respective area of analysis. Therefore, this program assures that the results provided by the County Laboratory are as accurate as possible and highly reliable.

2.2 Quality Assurance Objectives

In order to ensure the production of high quality data and clients' full satisfaction, Barnstable County Laboratory has established the following Quality Assurance Objectives:

- Full compliance with certification requirements;
- Full compliance with regulatory agencies;
- Full compliance with contract requirements;
- Full compliance with published methodologies;
- All personnel concerned with analysis and calibration activities in the Laboratory are required to familiarize themselves with the quality documentation and implement the policy as stated above.

2.3 Subcontracted Laboratories

Barnstable County Laboratory also subcontracts some analyses to other laboratories. Barnstable County Laboratory makes sure these subcontracted laboratories also meet Quality Assurance Policy and Quality Assurance Objectives as stated above. Especially Barnstable County Laboratory makes sure that the subcontracted laboratories must comply with ISO/IEC 17025:2005[3] when the samples for National Sanitation Foundation internal projects are analyzed.

3. Accommodation and Environmental Conditions

The Laboratory is in a newly renovated building. Major renovations have been completed at the former County Jail gymnasium which now has two floors: the first floor serves as the County's Water Quality Laboratory and the second floor is for the Human Services Department.

3.1 Laboratory Layout:

Water Quality Laboratory includes the following sections:

- Reception and Sample Receiving;
- Microbiology;
- Organic Analysis;
- Inorganic Analysis;
- Inorganic Instrumentation;
- Wet Chemistry;
- Data Storage and Reporting;
- Offices

Laboratory map is attached for reference.

3.2 Emergency Eyewashes and Showers

There are two emergency showers and three emergency eyewashes in the laboratory. All laboratory staff well know where they are all located and how to use them. Eyewashes are flushed weekly by laboratory staff to ensure they are operating correctly. Safety showers are tested weekly by laboratory staff too. Weekly check for emergency eyewashes and showers are recorded in a log book.

3.3 Fire Safety Equipment

There are six different sizes of ammonia phosphate base dry chemical fire extinguishers in the laboratory. Four of them is in four rooms respectively, and two in the hall.

4.0 Responsibilities and Authorities

Barnstable County does have its Personnel Policies and Procedures[5] for all County employees to follow. Two of major purposes of the Personnel Policies and Procedures are:

- To ensure that its management and personnel are free from any undue internal and external commercial, financial and other pressures and influences that may adversely affect the quality of their work;
- To avoid involvement in any activities that would diminish confidence in its competence, impartiality, judgment or operational integrity.

Barnstable County laboratory employees must follow not only the County's Personnel Policies and Procedures, but also the requirements of the Laboratory's quality assurance and quality control program. The quality assurance and quality control program requires an effective chain of command within the laboratory. The maintenance of this requirement is the responsibility of the laboratory analysts, office staff and the Laboratory Director.

4.1 Laboratory Director

The Laboratory Director supervises all analysts, technicians, and laboratory administrative personnel. Additional responsibilities include supporting the implementation of the quality assurance plan within the laboratory, maintaining and enforcing standard operating procedures, and maintaining good laboratory practices. The Director may substitute for any analyst when questions arise and performs any analysis as required.

The Laboratory Director also acts as the Quality Assurance Officer of the laboratory. All data are finally reviewed by the Laboratory Director prior to release. Data that fall outside of quality control limits can be accepted if in the judgement of the Director there are suitable technical reasons for these to be accepted. However, these cases are well documented and the reasons for acceptance are fully explained.

If the Laboratory Director is not around for a few days, the organic chemist will conduct final review and signing of reports. All other issues will go to Department Director. If there is still anything else that must need the Laboratory Director, the Laboratory Director will be contacted immediately, and the Laboratory Director will respond as soon as possible to make sure that the laboratory is operated smoothly.

4.1.1 Internal Audits:

One of the major responsibilities of the Laboratory Director is to conduct the laboratory audit once a year. The main purpose of the internal audit is to verify that the laboratory operations continue to comply with the requirements of the management system, quality assurance policy and quality assurance objectives. The internal audit covers sampling, sample log-in, sample analysis and data reporting, etc. The checklist of the Laboratory internal audits is attached (Attachment 1).

4.1.1.1 If there is any finding from the internal audit which affect any associated data, the following measures must be taken:

- The root cause of the finding must be found out and recorded in Lab Corrective Action Log Book;
- Any measures to be taken to prevent happening again must be recorded in Lab Corrective Action Log Book;
- All data affected by this finding will be flagged, and the lab reports will be revised with a lab narrative;
- The customers will be notified in writing with the revised Lab Reports.

4.1.2 Management Reviews

Laboratory Director also work with Department Director together to conduct a review of the laboratory's management system and testing and/or calibration activities to ensure their continuing suitability and effectiveness, and to introduce necessary changes or improvements. The review will take account of the following areas:

- 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;
- Customer complaints;
- Recommendations for improvements;
- Other relevant factors, such as quality control activities, resources and staff training.

A period for conducting a management review is once a year.

4.2 Laboratory Analysts

All analysts conduct sample analysis and maintain quality assurance by following the laboratories quality assurance plan. This is achieved by a thorough knowledge of the appropriate standard operating procedure of each method employed by the analyst. Especially analysts must closely track the holding times of all analyses, and it is analysts' primary responsibility to make sure that all analyses are done within their holding times. Additional responsibilities include complete and accurate work records, immediate notification of quality control problems, and the authority to accept or reject data based on defined quality control acceptance criteria.

4.2.1 The bacteriologist is the primary microbiological resource for the Laboratory. The primary duties of the bacteriologist include performing or supervising all bacteriological analyses, all media and buffer preparation, and all QA/QC record keeping for the microbiology laboratory. The bacteriologist also performs and oversees sample container preparation, preservation, sterilization, and distribution. The bacteriologist also generates the final reports. If there is any positive identification of total coliform in drinking water, the bacteriologist will inform customers right away.

4.2.2 The primary duties of the Inorganic Chemist and Inorganic Analyst are performing all metal analyses by flame or graphite furnace AA and Inductively Coupled Plasma Mass Spectrometer (ICP-MS), all inorganic anion analyses by ion chromatography, and all wet chemistry analyses by closely following Standard Operating Procedures (SOP). The Inorganic Chemist and Inorganic Analyst also generate final data reports and conduct data reviews. Secondary duties include supervision of sample container preparation, preservation, and distribution.

4.2.3 The Organic Chemist is the primary resource for questions regarding organic methodology and analyses. The primary duties of the organic chemist are to analyze volatile organic compounds using Gas Chromagraph – Mass Spectrometer (GC/MS) and Total Organic Carbon (TOC) Analyzer. The organic chemist is responsible for maintaining proper documentation for any modified methods and SOP's. The Organic Chemist also generates data reports and conducts data reviews. Secondary duties include performing sample container preparation, preservation, and distribution.

4.2.4 Analytical training is required for all analysts, and training includes new analyst training and cross training. When a new analyst is hired, a systematic training will be conducted by an experienced analyst and/or laboratory Director. New analyst will complete an Initial Demonstration of Capability if the pertinent analytical method requires it. All analysts are required and encouraged to be cross trained for each other to ensure the analysis is done properly when its primary analyst is absent for a period of time. All training processes will be recorded in Lab Staff Training Log Book (Attachment 2).

4.2.5 All analysts need to enter their own sample data to the Laboratory Information Management System (LIMS), and also cross review data entry to ensure data information are correct in sample received date and time, customer ID, lab ID, analytical date and time, units, and analytical method.

4.3 Information Specialist/Billing Clerk

Information specialist/billing clerk has the following three kinds of duties:

- Maintains customer accounts receivable records and processes payments as received.
- Prepare invoices as scheduled and as needed basis.
- Develop and maintain database applications as needed and directed.

4.4 Administrative Staff

The administrative staff is primarily responsible for sample log-in, inquiries of sample status, final report generation, taking and filling bottle order requests, keeping track of inventory and ordering supplies, and typing and calling in purchase order. Some of the staff's secondary responsibilities include sample container preparation and sample pick-up.

4.5 Laboratory Personnel

Name	Initial	Title
Dan White	DW	Laboratory Director/Chemist
Ryan Lucier (Grady)	RG	Microbiologist
Carol Eastman (part-time)	CE	Microbiologist
Chris Long	CL	Chemist
Liping Xun	LX	Chemist
Andrew Barker	AB	Chemist
Leonard Pitts (part-time)	LP	Chemist
Lacey Adams Prior (part-time)	LAP	Chemist
Steven Lee (part-time)	SL	Chemist
Katarina Soldatov	KS	Laboratory Assistant
Laura Notarangelo	LN	Laboratory Assistant
Veronica Tavares (part-time)	VT	Laboratory Assistant

5. Quality Assurance for Precision and Accuracy

Quality assurance is used to establish and maintain confidence in the precision and accuracy of the data generated by the Laboratory. The routine procedures utilized in assessing precision and accuracy are based on established SOPs.

5.1 All data are recorded in the pertinent logbooks, charts, and Laboratory Information Management System (LIMS). These records are periodically boxed and stored for easy retrieval. LIMS data are archived yearly. All the data are maintained for a period of ten years.

5.2 Forms used in reporting results are generated by the LIMS and are designed to convey all pertinent information to the client. Report forms include date of collection, date of receipt by the laboratory, date of analysis, client name, client I.D. name or number, and laboratory sample I.D. number. All analyses required by the Massachusetts Department of Environmental Protection (DEP), Division of Water

Supply (DWS) are reported on forms supplied by DWS. Included in any report, if requested by the client, are the recoveries of all QC samples and matrix spikes.

5.3 If any analyte exceeds its MCL, the local Health Agent is informed by the report and the client is telephoned as soon as possible and advised of the results. All recommended limits exceeded are explained on the report form sent to the client along with the recommended limit. If any MCL is exceeded for an analysis required by a State or Federal Agency, the client is contacted immediately (24 hours) and advised of the result. The client has the primary responsibility to contact the regulatory agency involved; however, the Laboratory will make the contact within 48 hours if based on follow up conversations with the client, there is no indication of the results being reported.

5.4 A few different methods for internal quality control checks are used. The internal controls include daily instrument blanks, daily method standards, matrix spikes, duplicates, and annual Proficiency Testing (PT) samples analyses, and PT samples are ordered from an accredited PT vendor meeting the criteria of the current policies made by Massachusetts Department of Environmental protection, Laboratory Certification office. Please check the following website for the detailed information: http://www.mass.gov/dep/bspt/wes /wespubs.htm.

5.5 Calibration curves must consist of at least three points and are used to calculate analyte concentration. A separate calibration curve is generated for each analyte included in the analytical method. Instrument calibration is performed each day samples are analyzed. The instrument may be completely calibrated with at least three calibration standards or a single calibration standard may be used to check an existing calibration curve. Again, all analytes of interest are included in the calibration.

5.6 Method blanks are analyzed daily and represent all sample preparation procedures excluding any target analytes. The method blank contains all appropriate surrogate and internal standards, diluents, and modifiers.

5.7 Laboratory fortified blanks (LFB) or Laboratory Control samples (LCS) are analyzed to monitor accuracy of the method. The LFB is a spiked method blank sample containing all the analytes of interest. The recoveries of the analytes are charted and used as a diagnostic tool to monitor system performance. When these recoveries approach either the upper or lower limits established by the method, corrective action is taken before the system fails to meet calibration criteria.

5.8 Quality Control (QC) check samples or Continuing Calibration Verification (CCV) are analyzed to monitor the accuracy of the method. QC check sample is from an alternative source containing all the analytes of interest. The recoveries of the analytes are charted and used as a diagnostic tool to monitor system performance. When these recoveries approach either the upper or lower limits established by the

method, corrective action is taken before the system fails to meet calibration criteria.

5.9 Duplicate analyses are performed on each batch of samples analyzed. The frequency is generally 10 % of all samples in the batch, but at least one sample if less than 10 samples are analyzed. The duplicate samples are prepared and analyzed using the same procedures as the original sample. The recovery of the duplicate analysis is used to monitor the reproducibility of the entire procedure.

5.10 Matrix spikes are performed to account for any matrix effects in an environmental sample. The frequency of matrix spike analysis is 10 % of a sample batch. Duplicate matrix spike analyses are used to monitor reproducibility.

5.11 Quality Control for Purchasing Reagents, Standards and any Other Supplies:

5.11.1 The Laboratory Director must make sure all reagents, standards, containers and any other supplies used in each analytical method meet their minimum Quality Control requirements described in the Method. All the names of vendors, Catalog numbers must be included in the Standard Operating Procedure of the Analytical Method.

5.11.2 All purchasing must be approved by the Laboratory Director to make sure the right items such as reagents, standards, containers, etc are ordered.

5.11.3 Once the ordered items are received, lab assistants and analysts will double check if the received items are right or any damages occurred during transportation. If there is any item received which has been damaged or wrong item has been shipped, the laboratory will contact the vendors right away, and the Laboratory Director will be notified too.

5.11.4 Analysts must log in all received reagents and standards into the laboratory Primary Standard Logbook. The Certificate of Analyte is stored in a three-hole binder.

5.11.5 Bottle sterility check for microbiology laboratory: The laboratory checks at least one bottle per lot of commercially prepared sample containers for sterility by adding approximately 25 ml of sterile non-selective broth to each bottle. The bottle is capped and rotated so that the broth comes in contact with all surfaces and is incubated at 35±0.5ºC and checked for growth at 24 and 48 hours and the results are recorded.

5.12 Precision Criterion of Duplicate Analysis:

In order to determine the acceptability of duplicate analysis of Fecal Coliform, E.coli, Enterococci, and Hetertrophic Plate Count (HPC), their Precision Criterion of Duplicates analysis are obtained by calculating the range of logs of most recent 15 samples and their corresponding duplicates as follows:

5.12.1 Collect the most recent 15 sets of the results of the original samples and their duplicates.

5.12.2 Calculate the Logarithms of each set of the results, and record them as *L1* and

L2. If any result is <1, add 1 to both values before calculating the Logarithms.

5.12.3 Range of Logarithms ($R_{\log}$) is calculated using the following equation:

$$R_{\log} = |L1 - L2|$$

5.12.4 The mean ($\overline{R}$) of $R_{\log}$ is calculated as follows:

$$\overline{R} = \frac{\sum R_{\log}}{n}$$

$\sum R_{\log}$ = the sum of the range of Logs;
n = the number of sets of duplicates.

5.12.5 Precision Criterion is calculated as follows:

$$\text{Pr}\,ecisionCriterion = 3.27 \times \overline{R}$$

5.12.6 If any Range of Logarithms is greater than the precision Criterion, there is a greater than 99% probability that the analysis has exceeded variability limits. For any samples that fall outside the acceptable limits, the acceptability of the imprecision will be determined. If the data are not acceptable, all results since the last precision check must be rejected. The analytical problems will be determined and corrective actions will be taken to resolve problem.

Corrective action based on internal quality control samples or external samples such as performance evaluation sample is used to maintain precision and accuracy of the analytical results. The process for corrective action includes a review of the history of the problem by checking standard preparation logs, instrument maintenance logs, and QC charts. Based on the historical information the cause of the problem is narrowed. The next steps are to change a defective part, clean a dirty part, or if necessary call a service engineer for advice or a visit.

6. Control Charts[2]

Two types of control charts are used in the County Laboratory: (1) accuracy (or means) chart; and (2) precision (or range) chart.

6.1 Accuracy Chart:

The accuracy chart for QC samples is constructed from the average and standard deviation of a specified number of measurements of the analyte of interest. The accuracy chart includes upper and lower warning levels (WL) and upper and lower control levels (CL). ±2s and ±3s are used for the WL and CL, respectively, where s represents standard deviation. These values are derived from stated or measured values for reference materials. The number of measurements, n or n-1, used to determine the standard deviation, s, is specified relative to statistical confidence limits of 95% for WLs and 99% for CLs. The County Laboratory is using the Accuracy Chart for Laboratory Control Sample (LCS)/Laboratory Fortified Blank (LFB), Matrix Spike (MS), and sample surrogate recovery. A chart is constructed for each analytical method. The results are entered on the chart each time the QC sample is analyzed. Everything is done in EXCEL Spreadsheet.

6.2 Precision Chart:

The precision chart also is constructed from the average and standard deviation of a specified number of measurements of analyte of interest. Precision chart is used for percent differences of LCS/LCSD, MS/MSD, and sample and sample duplicate. Perfect agreement between replicates or duplicates results in a difference of zero when the values are subtracted, so the baseline on the chart is zero. Therefore, for precision charts, only upper warning limits and upper control limits are meaningful. A chart is constructed for each analytical method. The results are entered on the chart each time the QC sample is analyzed. Everything is done in EXCEL Spreadsheet.

6.3 Updating of Control Charts:

If measurements never or rarely exceed the WL, recalculate the WL and CL using the 20 to 30 most recent data points. Trends in precision can be detected sooner if running averages of 10 to 20 are kept. Trends indicate systematic error; random error is revealed when measurements randomly exceed warning or control limits.

6.4 Application:

- Control Limit – If one measurement exceeds a CL, repeat the analysis immediately. If the repeat measurement is within the CL, continue analyses; if it exceeds the CL, discontinue analyses and correct the problem.
- Warning Limit – If two out of three successive points exceed a WL, analyze another sample. If the next point is within the WL, continue analyses; if the next point exceeds the WL, evaluate potential bias and correct the problem.
- Trending – If seven successive samples are on the same side of the central line, discontinue analyses and correct the problem.
- Barnstable County Laboratory has calculated our own in-house control limits and warning limits for drinking water and wastewater. Each analyst closely reviews his or her in-house control limits for each analysis based on the procedures stated as above. The laboratory director must review control charts monthly.

7. Lab Corrective Actions

Quality control data outside the acceptance limits or exhibiting a trend are evidence of unacceptable error in the analytical process. The County laboratory takes corrective action promptly to determine and eliminate the source of the error. The laboratory does not report data until the cause of the problem is identified and either corrected or qualified. The customers will also be notified.

7.1 The County Laboratory records any problems and issues that occur and affect data integrity, lab safety and lab operation. If there are any unusual things happened in the Lab, the Lab Director is informed right away, and the Originator must describe the detailed information on these problems and issues in the Laboratory Corrective Action Log Book (See Attachment 3). The Originator needs to address the following three things:

(1) Describe what happened;
(2) Describe how and why they happened;
(3) What actions should be taken to prevent or eliminate them happening again?

7.2 The following three categories of problems and issues can be written down in the lab Corrective Action Log Book:

(1) Improper Lab Practices:
Definition: A scientifically unsound or technically unjustified omission, manipulation, or alteration of procedures or data that bypasses the required QC parameters, making the results appear acceptable.

Any alteration of data such that the data are unauthentic or untrue representations of the experiment or test performed.

Peak integrations are not done properly;
Quality Control Samples do not meet criteria;
Initial Calibrations do not meet criteria;
Sample holding times are out;
Method Blank is contaminated;
Loss of sample;
Equipment malfunction;

(2) Standard Operating Procedures (SOP) Modifications:
Incorporate new equipment into the SOP;
Correct the wrong or inappropriate procedures in the SOP;
Etc.

(3) Any other actions affecting data quality and lab operations:

7.3 Corrective Actions:

Corrective actions begin with the analyst, who is responsible for knowing when the analytical process is out of control. The analyst must initiate corrective action when a QC check exceeds the acceptance limits or exhibits trending and must report an out of control event to the supervisor. The corrective actions to be used when QC data are unacceptable are as follows:

- Check data for calculation or transcription error. Correct results if error occurred.
- Check to see if sample(s) was prepared and analyzed according to the approved method and SOP. If it was not, prepare and/or analyze again.
- Check calibration standards against an independent standard or reference material. If calibration standards fail, reprepare calibration standards and/or recalibrate instrument and reanalyze affected sample(s).
- If a LFB fails, reanalyze another laboratory-fortified blank.
- If a second LFB fails, check an independent reference material. If the second source is acceptable, reprepare and reanalyze affected sample(s).
- If a LFM fails, check LFB. If the LFB is acceptable, qualify the data for the LFM sample (Table 1 lists the data qualifiers) or use another method or the method of standard addition.
- If a LFM and the associated LFB fail, reprepare and reanalyze affected samples.
- If reagent blank fails, analyze another reagent blank.
- If second reagent blank fails, reprepare and reanalyze affected sample(s).
- If the surrogate or internal standard known addition fails and there are no calculation or reporting errors, reprepare and reanalyze affected sample(s).

7.4 Customer Complaints:

Barnstable County laboratory is totally customer-focused organization, and it has been understood that customer complaints represent valuable information about recurrent problems. Laboratory secretary and assistant are always front-line staff to handle customer complaints, and they must give customers their full and undivided attention. Laboratory secretary and assistant must resolve complaints promptly if they are able to. Laboratory secretary and assistant must report any customer complaints to laboratory director. If the customer complaints can not be resolved right away, laboratory secretary and/or lab assistant must report to laboratory director right away. The laboratory director must talk to the customers to find out the root causes for complaints if necessary. Laboratory director must take actions to have any mistakes corrected properly and promptly, and call back to customers to explain the final resolutions for the complaints. All these processes must be recorded in the Laboratory Customer Complaint Logbook (Attachment 4).

If there are any customers of Barnstable County Test Center to complaint to the Test Center Staff about laboratory testing, the Test Center Staff will pass the complaints

to the laboratory. Barnstable County Test Center has its own Customer Complaint Logbook. All the resolutions of the Customer Complaints for the Test Center must be reviewed by Department Director.

7.5 Notification of Customers

The Laboratory will notify the customers regarding any issues which trigger the laboratory corrective action, and which affect their sample and data integrity and quality. The final Laboratory Corrective Actions will need to be submitted to the customers for their approval.

8. Analytical Methods

The analytical methods performed by the Barnstable County Laboratory have come from two sources[1, 2]. The first one is *Methods and Guidance for the Analysis of Water* (Version 2) by EPA, and the second one is *Standard Methods for the Examination of Water and Wastewater*, 22th Edition, 2012 by American Public Health Association, American Water Works Association and Water Environment Federation. Barnstable County Laboratory has its own modified standard operation procedures (SOP) based on the methods stated above. These SOP including any modifications are approved and certified by the Laboratory Certification Office (LCO) of Massachusetts Department of Environmental Protection. All non-approved methods used are for informational purposes only and are documented as such. The Massachusetts Laboratory Certification Identification Number of Barnstable County Laboratory is M-MA009.

8.1 A list of certified methodologies used for specific analysis is noted below:

Parameters	**Methodologies [1, 2]**	
Bacteria	**Potable**	**Non-Potable**
Total Coliform	MF-SM9222B	
Total Coliform	ENZ.SUB.SM9223	
Total Coliform	EPA 1604	
Total Coliform	SM 9223B-COLILERT	
Fecal Coliform		SM9223B-COLILERT-18
Fecal Coliform	MF-SM9222D	MF-SM9222D
E. Coli	NA-MUG-SM9222G	EPA 1604
E. Coli	EPA 1604	EPA 1603
E. Coli	ENZ.SUB.SM9223	
E. Coli	SM 9223B-COLILERT	SM 9223B-COLILERT
Heterotrophic Plate Count	SM9215B	
Enterococci	EPA 1600	EPA 1600
Enterococci	ENTEROLERT	ENTEROLERT
Metals	**Potable**	**Non-Potable**
Aluminum	EPA 200.8	EPA 200.8

Antimony	EPA 200.8	EPA 200.8
Arsenic	EPA 200.8	EPA 200.8
Barium	EPA 200.8	
Beryllium	EPA 200.8	EPA 200.8
Cadmium	EPA 200.8	EPA 200.8
Chromium	EPA 200.8	EPA 200.8
Calcium	SM3111B	SM3111B
Cobalt		EPA 200.8
Copper	EPA 200.8, SM 3111B	EPA 200.8, SM 3111B
Iron		SM 3111B
Lead	EPA 200.8	EPA 200.8
Magnesium		SM 3111B
Manganese	EPA 200.8	EPA 200.8, SM 3111B
Mercury	EPA 200.8	
Nickel	EPA 200.8	EPA 200.8
Potassium		SM 3111B
Selenium	EPA 200.8	EPA 200.8
Sodium	SM 3111B	SM 3111B
Thallium	EPA 200.8	EPA 200.8
Vanadium		EPA 200.8
Zinc		EPA 200.8
Inorganics		
Total Alkalinity	SM 2320B	SM 2320B
Specific Conductivity		120.1, SM2510B
Chemical Oxygen Demand		HACH 8000
Total Organic Carbon		SM 5310B
Chloride		EPA 300.0
Fluoride	EPA 300.0	
Nitrate-N	EPA 300.0	EPA 300.0
Nitrite-N	EPA 300.0	
Sulfate	EPA 300.0	EPA 300.0
Ammonia-N		EPA 350.1
Kjeldhal-N		EPA 351.2
Total Phosphorus		EPA 365.4
Orthophosphate		EPA 365.1
pH	SM 4500-H-B	SM 4500-H-B
Non-Filterable Residue (TSS)		SM 2540D
Total Dissolved Solids	SM 2540C	SM 2540C
Turbidity	EPA 180.1	
Total Hardness ($CaCO_3$)		SM 2340B
Perchlorate	EPA314.0	
Organics		
Volatile Organic Compounds	EPA 524.2	
Volatile Halocarbons		EPA 624.1

Volatile Aromatics		EPA 624.1
Trihalomethanes	EPA 524.2	

8.2 In order to clearly distinguish in the analytical reports between those analyses for which it holds Massachusetts Department of Environment Protection Certification and those for which it does not hold Massachusetts Department of Environmental Protection Certification, Barnstable County Laboratory has started attaching a summary of the laboratory certifications as stated in Section 8.1 to each of analytical reports to all customers.

8.3 Notification of Customers for Any Changes of Analytical Methods:
After Barnstable County Laboratory receives samples from customers, Barnstable County Laboratory realizes certain analytical methods must be changed or modified to proceed with the analyses. The Laboratory will notify and discuss with the customers first for the changes of the methods before conducting analyses.

9. Sample Management

9.1 The generation of quality data begins with the collection of the sample. The integrity of the sample collection is therefore of importance to the laboratory. Samples must be collected in such a way so as not to disrupt the integrity of the sample by the introduction of foreign material or the release of any material of interest. The laboratory maintains sample integrity by supplying the appropriate sample containers, ensuring that the sample containers are properly cleaned and contain the appropriate preservative, enforcing sample holding times to allow adequate time for analysis, and ensuring that adequate volumes of the sample are collected.

9.2 Upon receipt of a batch of samples, the Sample Reception Office examines the samples for breakage or damage while checking the accompanying documents for conformance with sampling procedure. The Sample Receiving Person also insures that the type of preservative is noted. Then the samples are logged into the LIMS by the Sample Receiving Person, and a unique laboratory ID is assigned to each sample. The Sample Receiving Person must ensure all information is entered into the LIMS correctly. All information and documentation are relayed to the analyst for his\her review and if any further sample manipulation is required, it is performed in a timely fashion.

9.3 All chain-of-custody samples received by the laboratory are examined for breakage or damage and sample integrity. Once the chain-of-custody form has been reviewed for clarity and accuracy, it is signed and the samples are received into the laboratory. After receipt, the samples are logged into the sample log book by the Sample Receiving Person, given a laboratory identification number, and stored in a secured area. The internal report form follows the sample through the laboratory until all analyses are complete. At the end of each day when the sample was being analyzed, it is returned to the secured area until all analyzes have been completed.

9.4 If the samples are not properly collected, preserved and handled, they will not be accepted by the laboratory. The following are the Laboratory's policies for the sample rejection:

- The samples are stored in wrong containers such as non-sterile bottles for total coliform;
- The samples are out of the holding times;
- There is no clear identification of the sample matrix;
- There are Bubbles in VOC vials;
- There are no enough sample volumes such as less than 100 ml for Total Coliform analysis;
- The samples are preserved with wrong preservatives;
- Perchlorate samples are not filtered with sterile filters and syringes;
- The samples are not kept in coolers when they are received.

NOTE: When the samples are rejected, the samples receiving officers will make notes on the chain of custody for any rejection reasons.

9.4.1 Notification of Customers

- If the customer is dropping off the samples and the customer's samples are not accepted, the Laboratory assistants shall clearly, respectfully and professionally explain to the customer why the samples should not be accepted, and what the customer could do better to make the laboratory accept its samples next time.
- If Once the samples are rejected for acceptance and the Laboratory assistants could not talk to the customer face-to-face, the Laboratory will notify the customer either by phone or by e-mail right away.

9.5 Subcontracting of Samples for Analyses

9.5.1 Prior to subcontracting out samples to other labs for analyses, Barnstable County Laboratory must notify its customers by e-mail, discuss with its customers and obtain preapproval from its customers. All these processes must be documented too.

9.5.2 There are the following circumstances under which the received samples need to be subcontracted to another certified laboratory:

- There are too many samples, and the number of the samples received exceeds the capacity of the laboratory.
- The instruments break down, and they cannot be fixed right away. Then the samples may need to be subcontracted out.

- The samples received contain the uncertified parameters, and they will be subcontracted out.

9.5.3 If there are any contaminants detected which exceed their Maximum Contaminant Levels, Maximum Residual Disinfectant Level or reportable concentration, the subcontracted laboratories are required to notify Barnstable County Laboratory within 24 hours of obtaining valid data. When it prepares the chain of custody to the subcontracted laboratory, Barnstable County Laboratory will stamp on it with "NOTIFY FOR ANY MCL EXCEEDANCES".

9.5.4 If the final analytical reports need to be submitted to Massachusetts Department of Environmental Protection, Barnstable County Laboratory will stamp on the chain of custody to the subcontracted laboratory with "STATE FORM". The subcontracted laboratory will have to report the data with MA DEP required format.

10. Laboratory Equipments and their Calibration Procedures

Calibration of the laboratory's equipment is performed on a regular basis. The calibration is performed in accordance with the manufacturer's instruction or in accordance with the calibration procedures outlined in the appropriate analytical methodology and, as needed.

Each piece of equipment requires preventive maintenance to ensure optimal performance of the instrument. The preventive maintenance schedule is supported by vendor service maintenance contracts and an inventory of spare parts. All major instruments have separate service contracts that include a yearly preventive maintenance visit. The minor instruments and equipment are covered by a general laboratory preventive maintenance contract where a service representative will annually check and calibrate all ovens, incubators, thermometers, refrigerators, autoclaves, UV-VIS spectrophotometers, and fume hoods.

10.1 Flame Atomic Absorption - This instrument is calibrated daily if samples are analyzed with five calibration standards prepared fresh. The calibration is checked with a QC check sample of an alternative source and a LFB sample. Both of these analyses occur before any samples are analyzed and at the end of the analysis. Also, interferences are analyzed for with a method blank.

10.2 Total Organic Carbon Analyzer (TOC-V $_{CPH/CPN}$) - This instrument is calibrated daily with a six point calibration curve and checked for interferences with a method blank. The calibration is checked with QC and LFB samples at a frequency of 10% of the sample load.

10.3 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS):

There are the following few tuning steps must be done before the ICP-MS is

calibrated for analysis:

- Tuning for sensitivity daily by using a 2 wt% nitric acid solution containing 10 ng/ml of each of the elements: Li, Co, Y, Ce, and Tl. Sensitivity after tuning must meet criteria set by ICP-MS manufacturer;
- Tuning for detector daily by setting P/A factor of each target element;
- Checking of mass calibration, mass resolution and instrument stability.

Initial calibration is composed of six different levels of standards, and initial calibration verification standards are run following the initial calibration to verify the accuracy of the initial calibration. Please refer to the SOP for EPA Method 200.8.

10.4 Ion Chromatograph - Each day a five-point calibration curve of the analytes of interest is generated from freshly prepared standards for nitrite-N, o-phosphate, bromide, nitrate-N, sulfate, chloride, and fluoride standards. Each day a one point calibration standard (continuing calibration) is analyzed to check an existing calibration curve or as the daily single point standard. Interferences are checked with reagent water blank. Checks include QC check and LFB samples at a frequency of 20% and matrix spikes to identify any environmental sample matrix effects.

10.5 GC\MS - Each day the mass spectrometer tune settings are checked with 25 ng of p-bromofluorobenzene (method 524.2) or, 50 ng of p-bromofluorobenzene (for method 624). The background subtracted spectra with the highest abundance is compared to and must meet established criteria for analysis to continue. When a successful BFB analysis is achieved, a single point calibration standard (Continuing Calibration) is analyzed to check the validity of the existing initial calibration curve. Whenever the MS is tuned, a new five point calibration curve may need to be generated. A method blank is analyzed daily to check for interferences and matrix spikes are analyzed for matrix effects.

10.6 PC-Titrate – The PC-Titrate is used to analyze pH, conductance, and alkalinity in aqueous samples:

- PH is calibrated daily using standard solutions of pH = 4.0, pH = 7.0, and pH = 10. A beginning and an end QCs (pH = 7.0) are run every ten samples.
- Alkalinity measurement is based on the pH calibration. A beginning and an end QCs (Alk = 25 mg/L) are run every ten samples.
- Conductance probe is calibrated using seven levels of standards. A beginning and an end QCs (Cond = 100 μmohs/cm) are run every ten samples.

10.7 Flow Injection Analysis (FIA) System (HACH QuikChem 8500 Series)--- The Flow Injection Analysis (FIA) System is equipped with XYZ Autosampler, Data Quality Management (DQM) software and Auto-Dilutor. The FIA is used for analysis of Total Kjeldahl Nitrogen (TKN), ammonia, and total cyanide in aqueous samples.

- The instrument is calibrated daily per specified requirements of each analytical method.

10.8 Refrigerators - Each day the refrigerator is used, the temperature is recorded and must be within ± 2°C of the required 4°C. The thermometers used to measure the temperatures are calibrated yearly with a NIST certified thermometer.

10.9 Balances - Each day the balance is used, the calibration of the balance is checked with two class S weights that bracket the expected weight to be measured. The deflection test is performed for the top-loader balances. The analysts make sure the balances are capable of detecting 100 mg at 150 g. The results of the deflection test are recorded daily. The range of weights available is 0.10 mg to 100 g, and they are all NIST traceable. The accuracy of the reference weights are verified annually.

10.10 Incubators (bacteriology) - The temperature for each incubator is recorded twice each day with readings separated by at least four hours. The temperatures must be within ± 0.2°C for the 44.5°C water bath and ± 0.5°C for the 35°C incubator and ± 0.5°C for the 41.5°C incubator. The thermometers used to measure the temperatures are calibrated yearly with a NIST certified thermometer.

10.11 Incubators (BOD, CBOD) - Each day the incubator is used, the temperature is recorded twice per day. The temperatures must be within ± 0.5°C for incubators to be maintained at 20°C. The thermometers used to measure the temperatures are calibrated yearly with a NIST certified thermometer.

10.12 Ovens - During use the temperature is monitored to ensure that the required temperature is reached and maintained for the appropriate length of time.

10.13 Thermometers - All thermometers are made of glass material and are calibrated yearly with a NIST certified thermometer. Any correction factor to be used in reading the temperature of the thermometer is indicated on the record sheet taped to the refrigerator or incubator where the thermometer is located. The reference thermometers are calibrated annually.

10.14 Reagent Grade Water – The distilled water is produced by using EMD Millipore water purification system. There are three water purification systems from EMD Millipore:

- Two Milli-Qs (Model);
- One Direct-Q (Model)

The quality of the purified reagent water used in microbiology laboratory as stated in the following paragraph must be met[1]:

- The specific conductivity of the reagent water is monitored and recorded daily, and the values of specific conductivity must be less than 2 μmhos/cm

at 25°C.

- The resistivity of the reagent water must be ≥ 16.5 megohm-cm (temperature compensated to 25ºC), and the old cartridges will be replaced if the reading is below 16.5 megohm-cm. The daily resistivity is recorded.
- Residual chlorine level is tested monthly. The amount of the chlorine detected must be less than 0.01 mg/L.
- Heterotrophic plate count (HPC) is performed monthly to check if HPC is less than 500 colony forming units (CFU)/ml. If HPC ≥ 500 colony forming units (CFU)/ml, the distilled water system will be checked and maintained.
- Heavy metal content is tested annually, and no single metal (Cd, Cr, Cu, Ni, Pb and Zn) may be present at ≥0.05 mg/L. Total heavy metals must be less than 0.1mg/L.
- Biosuitability is tested annually, and it must have a ratio of 0.8-3.0.

10.15 Reagents and Other Supplies:

- All inorganic reagents shall be ACS Reagent Grade or equivalent unless the analytical procedure specifies a different grade.
- All organic reagents used to prepare standards shall be of the highest quality obtainable. Organic reagents used to prepare general reagent solutions shall be free of detectable interferences as demonstrated by the analysis of acceptable method blanks.
- All organic solvents shall be free of detectable residue as demonstrated by the analysis of acceptable method blanks. For organic analyses, contamination shall not be restricted to target analytes.
- Supplies such as filter paper, glass wool and boiling beads must be free of contamination as demonstrated by the analysis of acceptable method blanks. For organic analyses, contamination shall not be restricted to target analytes.
- All desiccants must contain moisture indicators.

10.16 Autoclaves: There are three autoclaves in the laboratory:

- Market Forge: Model: STM-E;
- Tuttnauer: Model: 3870M;
- Tuttnauer: Model: 3850E-B/L.

The following parameters are recorded in the lab Autoclave Logbook when any autoclaves are used for sterilization:

- Start time;
- Pressure;
- Temperature;
- Items which are placed in the inside of the autoclaves for sterilization;
- Cycle length;
- Stop time;
- Maximum Temperature
- Initial and date

10.17 Pipettors: All pipettors used for quantitative purposes will be calibrated annually, either through a certified professional service or in-house. In-house calibration will

be conducted gravimetrically, with correction made for temperature and pressure. Calibration records will be kept for 10 years.

11. Preventive Maintenance

Each analytical system or piece of equipment is required to be maintained according to the manufacturer's recommendations. Regular maintenance checks ensure that the systems are able to operate properly and efficiently on a consistent basis.

Each major piece of equipment is covered by a service contract offered by the manufacturer (or a similar company). These contracts include an annual preventive maintenance visit. In addition, more frequent maintenance is performed as recommended by the manufacturer on a regular basis by the laboratory staff as needed.

Maintenance logbooks are utilized to document major preventive as well as emergency maintenance procedures as these are performed. These logbooks are also used to document any routine maintenance / repair procedures.

The following outlines the major and minor preventive maintenance routines for each analytical system in operation within the laboratory:

11.1 Gas Chromatograph
Cut the chromatographic column on a regular basis.
Change injection port septum, o-ring and the glass wool in the liner, when necessary.
Clean by baking, and/or solvent rinsing the Electron Capture Detector, when signal is high.
Service the Electron Capture Detector, when signal high after cleaning the detector.
Cycle/Bake out the entire system once a week, when not in use.

11.2 Mass Spectrometer
Check rough pump oil leads, routinely.
Clean the source when the instrument fails to tune.
Change the electron multiplier when the applied voltage is too high.
Routinely verify that adequate calibration fluids are available for automatic instrument tuning.

Change the o-rings and transfer lines when inspection indicates these are degrading
or weakening.

11.3 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) -
See attachment 5 for preventive maintenance of the ICP-MS.

11.4 Purge and Trap Apparatus -
Purge each line prior to analysis.
Bake the trap once all lines are purged.
Check for leaks in the purge and trap system using the pressurization technique when

air leaks are detected.
Change the ferrules when leak check supports a leak; this procedure may be done frequently at most susceptible sites.
Replace the trap when data indicate its degradation.

11.5 Ion Chromatograph

Regularly check the eluent reservoir to see if it needs to be filled.
Daily check the component-mounting panel for leaks or spills. Wipe up spills. Isolate and repair leaks. Rinse off any dried eluent with deionized water.
Weekly check fluid lines for crimping or discoloration. Relocate any pinched lines. Replace damaged lines.
Check the pump and replace the pump piston rinse seals and piston seals if necessary.
Replace the AS40 Automated sampler tip and tubing once a year.

11.6 Flame Atomic Absorption

11.6.1 Burner Head:

Clean and polish the top surface using mild abrasive soap cleaner;
Clean the burner slot with the Burner Cleaning tool supplied or a piece of stiff card. Do not use an abrasive material inside the slot;
Wash the Burner with detergent solution and rinse with deionized water. Dry the Burner carefully before using it again;
To clean the interior surface of the Burner head, dismantle and use an ultrasonic bath with deionized water, dilute detergent solution or 5% (v/v) solution of nitric acid.

11.6.2 Spray Chamber:

Aspirate a solution of 1% (v/v) hydrochloric acid, or a solution of laboratory detergent to clean the Spray Chamber. Aspirate the solution for 5 minutes, then aspirate deionized water.
For more thorough cleaning, dismantle the Spray Chamber, clean the individual components with lab detergent, rinse thoroughly with deionized water, dry and reassemble.

11.6.3 Nebulizer:

Use the cleaning probe and push through the nebulizer to dislodge to remove any blockage in the nebulizer capillary.

11.7 pH Meter

Clean the electrode prior to and after analysis.
Change the electrode, when necessary.
Store the electrode in pH 7 buffer solution between analyses.
Clean and maintain the magnetic stirrer and magnetic stirring bars, whenever used.
Examine the KCl level in the electrode prior to use, add more solution, when necessary.

11.8 PC-Titrate
Clean the electrodes/probes prior to after analysis.
Change the electrodes/probes when necessary.
Replace all tubings once a year.

11.9 Water Purification Unit
Check the conductivity of the finished water daily.
Change the cartridges when resistivity starts to decrease, approximately every 2-4 months.

11.10 Quebec Colony Counter and Microscope
Microscopes and colony counters are maintained in a clean condition and checked for defects on each use. All glass surfaces are periodically cleaned as needed. Moving parts of all microscopes are lubricated on an-needed basis.

11.11 Total Organic Carbon Analyzer (TOC-V $_{CPH/CPN}$)

11.11.1 Daily Inspection:
Check the level of dilution water;
Drain vessel water and humidifier water;

11.11.2 Periodic Inspections:
Catalyst Regeneration;
Washing or replacing catalyst;
Replacing the carrier gas purification tube and catalyst;
Washing/replacing the combustion and carrier gas purification tubes;
Replacing the CO2 absorber;
Replacing the Halogen Scrubber;
Syringe replacement.

11.11.3 Autosampler (ASI-V) Maintenance

Please see the *USER's MANUAL* [5] for the details.

11.12 Flow Injection Analysis (FIA) System (QuickChem 8500 Series)

11.12.1 The FIA is composed of the following parts:
- Autodiluter;
- Autosampler;
- Pump;
- Valves;
- Manifolds;
- Detectors;
- Flow Cells;
- Interference Filters;

- Leak Detector;
- Computer.

10.12.2 Maintenance Guide for these parts as listed above could be found in Attachment 6.

Generally, if any instruments have problems, the analysts or technical director will try fix them first. If the problem still persists, service maintenances will be called.

12. Standard Operating Procedures (SOPs)

Each method utilized by the laboratory has a standard operating procedure (SOP) developed by the laboratory (Attachment 7). The analyst follows this procedure at all times to ensure proper operation accuracy and precision. The SOP's include the analytes to be measured, the detection limits of the method, and the applicable matrices. Each SOP also includes a detailed description of the method procedure.

The safety issues involved in the analysis are discussed to insure the safety of the operator and the laboratory environment. All reagents and standards are described as well as the recipes for their preparation. When applicable, sample holding times, appropriate containers, preservatives, and methods of collection are discussed. A step-by-step procedure is detailed with all sample preparation, instrument calibration, and sample analysis steps described.

After completion of the analysis, the SOP describes the calculation procedure and the reporting procedure for the analysis to ensure accurate reporting of the results. Following this section is a description of the QA/QC requirements of the method. Described within this section are the QC samples to be analyzed, the acceptance criteria for each QC sample, and potential corrective action to be taken if the criteria are not met.

12.1 Review, Revision, and Approval

12.1.1 Each SOP or sections subject to revision, will be opened for comments to those who are familiar with its content and/or those who will use it. All proposed SOP revisions must be submitted to the Laboratory Director for review, approval and subsequent distribution.

12.1.2 The following information trigger the revision of the SOP:

- The original methods are revised and approved by regulatory agencies such as US Environmental Protection Agency and MA Department of Environmental protection;
- A new instrument is purchased and used for the Method;
- Any different supplies such as reagents, standards, gases, etc are changed;

- Initial calibration range is changed due to certain reasons;

12.2 Distribution and Maintenance

12.2.1 Hardcopy SOPs are distributed as controlled documents for project specific external (out of lab) distribution or uncontrolled documents for external review. The distribution of controlled documents must be recorded to ensure that they are updated when new or revised SOPs released.

12.2.2 SOPs for use within Barnstable County Lab are available as uncontrolled hardcopies, controlled hardcopies in binders.

12.2.3 SOPs submitted to regulatory agencies in support of certification or submitted to clients for contract compliance must be controlled for the duration of the certification or contract.

12.2.4 The Laboratory Director shall be responsible for maintaining updated masters for all documents.

12.2.5 All obsolete SOPs are collected and archived and stored away.

13. Data Report, Validation and Review

13.1 Data reporting is performed through the Laboratory Information Management System and a custom created system to report data to Massachusetts Department of Environmental Protection (MA DEP), Drinking Water Program. There are three sections on any lab reports produced by the Lab: (1) Customer Information, (2) Analytical Information, and (3) Signature and Date.

13.1.1 Regarding the Customer Information, the following items must show on any report:

- Reporting mailing address;
- Public Water Supply (PWS) ID# (for reporting to MA DEP);
- PWS Name (for reporting to MA DEP);
- City/Town (for reporting to MA DEP);
- Class: COM, NTNC or TNC (for reporting to MA DEP);
- Multiple or Single (for reporting to MA DEP);
- Raw or Finished (for reporting to MA DEP);
- Date collected;
- Collected by (or Sampler);
- Routine Sample or Special Sample (for reporting to MA DEP);
- Original, Resubmitted or Confirmation (for reporting to MA DEP);

- Reason for resubmission (for reporting to MA DEP);
- Collection Date of Original Sample (for reporting to MA DEP);
- Sample Notes.

13.1.2 Regarding the Analytical Information, the following items must show on any report:

- Primary Laboratory Name;
- Primary Laboratory Massachusetts Certification Number (Barnstable County Health Laboratory: M-MA 009);
- Subcontracted Laboratory Name if any samples are sent out;
- Subcontracted Laboratory Massachusetts Certification Number;
- Sample Matrix;
- Specific Analytes and their Respective Results, Maximum Contaminant Levels (MCL), Maximum Detection Limits (MDL), Analytical Methods, Analytical Dates and time, Analysis Lab MA Certification Numbers, Analysis Lab Name, and Lab Sample ID#;
- Information on the sample composited by the Lab and lab notes;

13.1.3 Raw Data from Any Instruments and Copies from Original Results Recording Log Books:

When analysts have done their analyses, analysts print out the raw data from instruments or get a copy from the original data log book. After analysts enter these raw data into LIMS, analysts submit the raw data with the reports for review.

These raw data will be provided with final laboratory reports to customers if customers request these raw data.

13.2 Data validation is the process by which data are accepted or rejected based on a set of specific criteria. This process is performed to insure possible accuracy of the data and the calculations in data reduction process.

13.2.1 The initial review of all data is performed by the analyst. The analyst checks the raw sample results and compares them to the daily standard(s), daily method blank, and all QC results. If all the QC results meet the criteria established in the method SOP, the completed report sheet for that sample is submitted for validation. All relevant daily QC samples (standards, blanks, check samples, and LFBs) are included with the report sheet. This information is reviewed by the Laboratory Director. Any analysis performed by the Director will be submitted to the Chemist in charge of that area.

13.2.2 The secondary review of all data is preformed by an Analyst or the laboratory director who did not conduct the associated analyses. The review covers the

following areas:

- All raw data supporting the report are included;
- All data in LIMS are correctly entered;
- All criteria of QA/QC are met;
- The subcontract lab name is indicated on the report if any parameters were subcontracted out;
- Any comments on the report are properly addressed.

13.2.3 The final review is conducted by the Laboratory Director, and the content of review is the same as ones described in Section 13.2.2. The reports are then signed and checked off in the LIMS as being completed.

13.2.4 Data reduction includes adjusting reporting limits for sample amount and any dilutions required, rounding of the results occurs after all calculations have been made, all results are reported in two or three significant figures, and no results are reported below the method reporting limit unless requested by customer. Volatile organic results are reported in ug/L or ug/Kg, metal results are reported in mg/L, and bacteriological results are reported in CFU/100 mL (CFU = colony forming units). Inorganic results are generally reported in mg/L except for pH (0.1 pH units), specific conductivity (umhos/cm), alkalinity and total hardness (mg/L $CaCO_3$), and turbidity (NTU or nephelometric turbidity units).

13.2.5 For customers' reference, Barnstable County Laboratory attaches its certified parameter list approved by Massachusetts Department of Environmental Protection to any analytical report.

13.3 Reporting and Recording of Any Non-Conforming Data

13.3.1 Any non-conforming data found during data review and validation will be flagged, explained and recorded in lab narrative. The customer will be notified by phone and through lab narrative.

13.4 Reporting Turn Around Time

13.4.1 Data will be reported to in a timely manner as meets the client's requirements. Turn around times for data reporting will be clearly specified at the time samples are received.

14. Record Keeping, Logbook Review and Standard Traceability

14.1 As part of the QA/QC plan, the procedure for maintaining the records for all aspects of the laboratory operation are well established. Each instrument has a log book in which each analysis is recorded. Also, the printouts of each analysis are filed according to the intra-laboratory identification number. Included in these files are

copies of the appropriate chain-of-custody forms, quality control reports, and all calculations of the data. All this material is available upon request by the client.

14.2 The results of quality control checks such as temperature records for ovens and refrigerators, quality control results for laboratory glassware, distilled water, and microbiological media are stored as permanent records to maintain quality control in the laboratory. Instruments such as pH meters, analytical balances, and thermometers are calibrated daily or prior to use, and the records of these calibrations are also maintained.

14.3 Standard Traceability and Logbook Review:

14.3.1 All standards and reagents received will be called as primary standards that will be recorded in the laboratory primary logbook. Any standards made from the primary standards will be called as either intermediate or working standards which will be recorded in the laboratory working standard logbooks. All primary standards, intermediate and working standards will be assigned unique identification numbers (ID) which will be also recorded. These unique IDs will be recorded on their Certificate of Analyte will be kept in a three-hole binder. The intermediate, working standards and primary standards will be labelled clearly on their containers with the unique IDs.

14.3.2 There are also the following logbooks:

Instrument running logbook
Instrument maintenance logbook
Media preparation logbook
Refrigerator and freezer temperature logbook
Conductance logbook for lab water purification logbook
Buffer preparation logbook
pH meter calibration logbook
Incubator and water bath temperature logbook
Lab corrective action logbook
Customer complaint logbook
Lab staff training logbook

14.3.3 Logbook Review

Laboratory Director or an analyst assigned by the Laboratory Director will review the logbooks quarterly to make sure all recordings are done correctly and properly.

14.4 Document Control: The laboratory maintains the originals and copies of all analytical reports, logs, charts, used SOPs and any other documentation for a minimum of 10 years.

14.5 Document and Record Storage: All originals and copies of analytical reports, logs,

charts, used SOPs, and any other documents are stored on site in laboratory data reporting area. The data in LIMS are stored in a server managed by the County IT department, and the LIMS data are backed up daily and carried off site daily by the County IT Department.

15. Safety

A formal safety program for the Laboratory is issued to each new employee. All personnel are introduced to the safety equipment available in the laboratory and instructed as to its use. All analysts are made aware of the safety consideration of the chemicals they use by Right-to-Know training and their review of the individual material safety data sheets. If some requests additional safety measures, the request is acted on immediately.

15.1 All gas tanks such as Helium, Argon, Oxygen, Hydrogen, Acetylene, and Air must be chained and secured.

16. References

1. Environmental Protection Agency, *Methods and Guidance for the Analysis of Water*, Version 2, June 1999.

2. American Public Health Association, American Water Works Association and Water Environment Federation, Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998, and 22th Edition, 2012.

3. International Organization for Standardization (ISO) and International Electrotechnical Commission (IEC), General Requirements for the Competence of Testing and Calibration Laboratories, ISO/IEC 17025:2005(E), Second Edition, 2005-05-15.

4. Barnstable County, *Personnel Policies and Procedures*, Effective July, 2005.

6. SHIMADZU CORPORATION, *User's manual* for Total Organic Carbon Analyzer (TOC-V CPH/CPN) (For TOC-Control V Ver.2), Part# 638-94536,

Laboratory Map

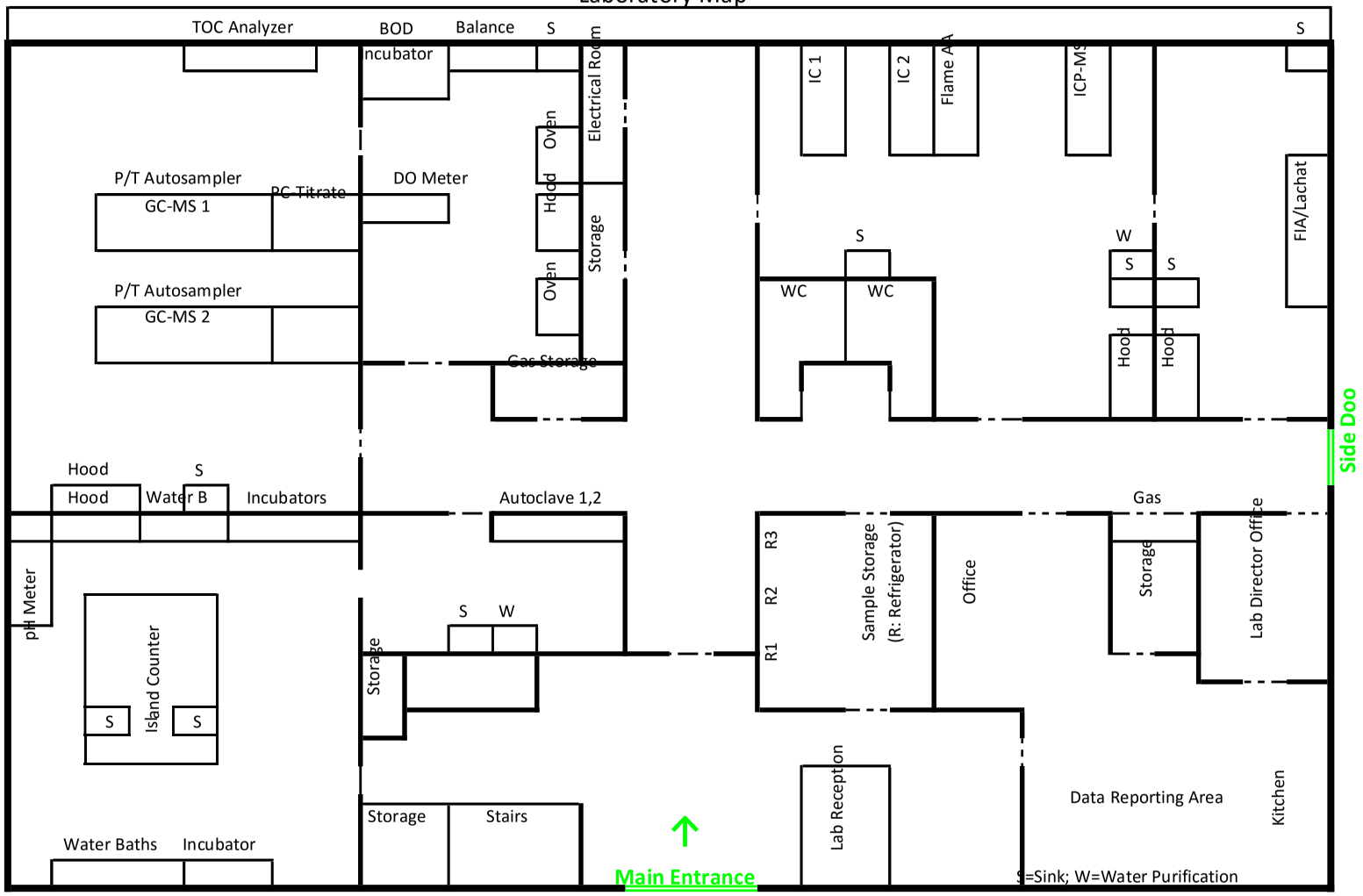

Table 1: QC Data Qualifiers

Symbol	Explanation
B	Analyte found in reagent blank, Indicate possible reagent or background contamination.
E	Reported value exceeded calibration range.
J	Reported value is an estimate because concentration is less than reporting limit and greater than method detection limit or because certain QC criteria were not met.
N	Organic constituents tentatively identified. Confirmation is needed.
PND	Precision not determined.
R	Sample results rejected because of gross deficiencies in QC or method performance. Re-sampling and/or reanalysis is necessary.
RND	Recovery not determined.
U	Compound was analyzed for, but not detected.

ATTACHMENT 1: The Checklist of Laboratory Internal Audits

1. Sample Management

    - Sampling
    - Shipping
    - Receiving
    - Holding times
    - Preservatives
    - Temperature
    - Log-in
    - Storage

2. Analysis

    - Certified methods
    - Standard operating procedures (SOPs)
    - Holding times

3. Quality Assurance and Quality Control (QA/QC)

    - Runlog/Sequence
    - Method blank, Lab Control Sample and Lab Control sample Duplicate (LCS/LCSD), and Matrix Spike and Matrix Spike Duplicate (MS/MSD)
    - Method Detection Limit (MDL) study
    - Accuracy and Precision
    - Control Charts
    - Second Sources
    - Continuing Calibration Verification (CCV)
    - Maintenance logbook
    - Corrective Action Logbook

4. Data Entry, Data Reporting and Data Backup

    - Data entry
    - Report generation
    - Data backup

5. Data Review

    - Analyst level
    - Cross review
    - Final review

6. Report Mailing, Filing and Storage

ATTACHMENT 2: Lab Staff Training Log Book

DATE:	TRAINEE:	TRAINER:
TRAINING START DATE:		
TRAINING ENDING DATE:		
PURPOSES:		
GOALS:		
SPECIFIC TRAINING ACTIONS:		
DOES THE TRAINING MEET THE INITIAL GOALS? EXPLAIN IF NECESSARY.		
COMMENT:		

ATTACHMENT 3: Lab Corrective Action Log Book

Corrective Action Log Book

Date:	Lab:	Originator:

Problem/Issue Description:
Reasons for the Problem/Issue:
Actions Taken to Eliminate The Problem/Issue in the Future:

ATTACHMENT 4: Customer Complaint Log Book

Customer Complaint Log Book

Date:	Name of Customer:	Originator:

Content of the Complaint:
Reasons for the Complaint:
Actions Taken to Satisfy the Customer/Make Things RIGHT:
Comment by Lab Director or Department Director:

ATTACHMENT 5: ICP-MS Routine Maintenance Schedule

Time*	Component	Task	Subsystem
D	Argon gas	Check for argon gas pressure and volume	
D	Peristaltic pump tube	Check for damage	
D	Sampling cone, Skimmer cone	Check orifice	
WN	Sampling cone, Skimmer cone	Clean/replace	Interface
WN	Nebulizer	Clean/replace	Sample introduction
WN	Pelistaltic pump tube	Replace	Sample introduction
WN	Torch	Clean/replace	Torch box
WN	Water filter	Check/replace	Cooling support
1 W	Torch, spray chamber, End cap	Clean	
1 W	Nebulizer	Clean	
1 W	Cooling water	Check water volume and for contaminants	
1 M	Rotary pump	Check oil level and color	Vacuum
1 M	Cooling water filter	Check	
1 M	Extraction Lens (If necessary)	Check	
1 M	Sample tubing	Replace	
6 M	Rotary pump	Change oil	vacuum
1 Y	Oil mist filter of Rotary pump	Check/replace mist filter	vacuum
2 Y	Argon gas filter	Replace (2 years after installation)	

* D = day; WN = when needed; W = week; M = month; Y = year

Attachment 6: Maintenance Guide for Flow Injection Analysis (FIA) System							
Component	**Service**	**Daily**	**Weekly**	**Bi-weekly**	**Monthly**	**6 Months**	**Yearly**
Autodiluter	Clean surfaces	X					
	Prime with DI water	X					
	Replace flared tubing						X
	Replace seals						X
Autosampler	Clean surfaces	X					
	Clean worn gears with dry cloth and alcohol				X		
Pump	Clean rollers with dry cloth and silicone spray				X		
	Replace pump tubes				X		
	Replace pump tubes adapters				X		
Valves	Flush with DI water and air	X					
	Clean ports and O-rings		X				
	Replace valve fitting						X
Manifolds	Flush with DI water and air	X					
	Clean unions and tees			X			
	Replace O-rings					X	
Detectors	Dry and clean surfaces	X					
	Clean tips of fiber optics					X	
Flow cells	Flush with DI water and air	X					
	Replace flares and O-rings					X	
Interference filters	Clean surfaces with cotton swab and isopropanol				X		
System unit	Keep clean	X					
Computer	Clean hard drive					X	
Leak detector	Clean with DI water				X		

Attachment 7: A List of Standard Operating Procedures					
Lab	Method Code	Description	Current Version Number	Current Version Date	Initial
Microbiology	EPA 1604	Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)	002	1/07/2019	RL
Microbiology	SM 9222B, SM9222G	Determination of Total Coliform in Potable and Non Potable Water Using SM 9222B and SM9222G	005	9/30/2019	RL
Microbiology	SM 9223	Determination of Total Coliform Bacteria and *Escherichia coli* (*E.coli*) in Potable Water	003	1/07/2019	RL
Microbiology	SM 9222D	Determination of Fecal Coliform in Potable and Non Potable Water Using SM 9222D	006	9/30/2019	RL
Microbiology	SM 9215B	Heterotrophic Plate Count for Potable Water Using SM 9215B	003	1/07/2019	RL
Microbiology	EPA 1600	Determination of Enterococci in Water using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI)	006	9/30/2019	RL
Microbiology	EPA 1603	Determination of *Escherichia coli* (*E.coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC)	005	9/30/2019	RL
Microbiology	SM 9223B	Determination of Total Coliform and *E.coli* in Water Using the following Method: Idexx Colilert-18, Colilert-24 and Quanti-Tray	001	4/23/2020	RL
Microbiology	SM 9223B	Determination of Fecal Coliform in Waste Water Using the following Method: Idexx Colilert-18, Colilert-24 and Quanti-Tray	001	4/24/2020	RL
Microbiology	SM 9230B	The Detection of Enterococci in Water Using the following Method: Enterolert and Quanti-Tray	001	4/23/2020	RL
Organics	EPA 524.2	Determination of Volatile Organic Compounds in Aqueous Samples Using Gas Chromatography/Mass Spectrometry	014	12/29/2020	LX
Organics	EPA 624.1	Determination of Volatile Organic Compounds in Non-potable Water Using Gas Chromatography/Mass Spectrometry	003	12/29/2020	LX
Organics	SM 5310 B	Determination of Total Organic Carbon (TOC) in Aqueous Samples Using High-Temperature Combustion Method	012	10/28/2020	LX
Organics	HACH 8000	Determination of Chemical Oxygen Demand (COD) in Aqueous Samples	016	11/2020	LP
Inorganics	EPA 200.8	Determination of Trace Elements in Aqueous Samples by Inductively Coupled Plasma – Mass Spectrometry	013	12/31/2020	CL

Inorganics	SM 3111B	Determination of Sodium, Copper, Iron, Manganese, Zinc, Nickel, Potassium, Calcium, Magnesium in Aqueous Samples Using SM 3111B	015	12/29/2020	CL
Inorganics	EPA 314.0	Determination of Perchlorate in Aqueous Samples Using Ion Chromatography	015	10/29/2020	LP
Inorganics	EPA EPA 300.0	Determination of Inorganic Anions in Aqueous Samples Using Ion Chromatography	015	5/11/2018	LP
Inorganics	SM 2320B	Determination of Alkalinity in Aqueous Samples	010	6/16/2020	YN
Inorganics	SM 2510 B EPA 120.1	Determination of Conductance in Aqueous Samples	011	6/17/2020	YN
Inorganics	SM 4500-H-B	Determination of pH in Aqueous Samples	010	6/17/2020	YN
Inorganics	SM 2540 C	Determination of Total Dissolved Solids in Aqueous Samples	010	9/06/2019	LP
Inorganics	SM 2540 D	Determination of Total Suspended Solids in Aqueous Samples	010	9/06/2019	LP
Inorganics	SM 2540B	Determination of Total Solids in Aqueous Samples	010	9/06/2019	LP
Inorganics	EPA 180.1	Determination of Turbidity in Aqueous Samples	008	8/29/2020	AB
Inorganics	SM 2340B	Determination of Hardness in Aqueous Samples (Refer to SM 3111B)	012	12/22/2017	LP
Inorganics	EPA 351.2	Determination of Total Kjeldahl Nitrogen in Aqueous Samples by Semi-Automated Colorimetry	007	12/29/2020	AB
Inorganics	EPA 350.1	Determination of Ammonia Nitrogen in Aqueous Samples by Semi-Automated Colorimetry – Gas Diffusion Method	006	3/05/2019	KK
Inorganics	EPA 365.4	Determination of Total Phosphorous in Aque Samples by Semi-Automated Colorimetry	001	4/07/2021	AB
Inorganics	EPA 365.1	Determination of Orthophosphate in Aqueous Samples by Semi-Automated Colorimetry	001	4/07/2021	AB
Inorganics	SM 2120B	Determination of Color in Aqueous Samples	003	6/15/2018	DB
Inorganics	SM 2150B	Determination of Odor in Aqueous Samples	003	6/15/2018	DB

Appendix 11

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Commonwealth of Massachusetts
Department of Environmental Protection
Senator William X. Wall Experiment Station
37 Shattuck St., Lawrence, MA 01843
Tel: 978-682-5237

Sample Tracking & Chain-of-Custody Record

WES Sample Login #: 20210355

☑ Dispose of samples 30 days after analyses are approved

☐ Seek permission to dispose of samples

Agency-Bureau-Division-Region	
MassDEP Bureau:	BWR-WW
Division/Unit:	DWM-Wastewater
Region:	DEP BOS
Other Agency:	

Project Description	
Project Name:	MASSTC Viral Pathogen WW to GW Sep Study
Coordinator:	Chubb, Marybeth
***Point of Contact (POC):**	Heufelder, George
***POC Phone:**	508-375-6616
***POC Email:**	gheufelder@barnstablecounty.org
	*=Required

Sample Chain-of-Custody from Field Collection Until Delivery to WES

Relinquished By:		Received By:		Date	Time	Secured Storage Location	Samples Involved
Printed name	*Signature*	*Printed name*	*Signature*				
George Heufelder	George Heufelder	Jean Tang	Jean Tang	8/4/2021	11:15 AM	MF21.1	ALL

Comments: 20210628

Sample Chain-of-Custody from WES to Other Entity

Relinquished By WES Staff:		Received By:		Date	Time	Receiving Entity Name, Address and Phone#	Samples Involved
Printed name	*Signature*	*Printed name*	*Signature*				

MassDEP

Commonwealth of Massachusetts
Department of Environmental Protection
Senator William X. Wall Experiment Station
37 Shattuck St., Lawrence, MA 01843
Tel: 978-682-5237

Sample Tracking & Chain-of-Custody Record

Cooler #	Cooler Temp. at Receipt	Custody Seal	
		Intact?	Not Used
1	-7.0 °C	☐ Yes ☐ No	☒
2	°C	☐ Yes ☐ No	☐
3	°C	☐ Yes ☐ No	☐
4	°C	☐ Yes ☐ No	☐

WES Sample Login #: 20210355

Regulated Object Program Name: MASSTC Virus Grant

Regulated Object Program ID: 19-02/319

WinLIMS Sample ID	Client Sample ID	Client Sample Description	Sample Matrix Code	Collector (Last name / First name)	Collection		Cooler #	Disinfectant Residual (mg/L)	Grab (G) or Composite (C)	# Containers	Container Type Code	Preservative Code(s)	To be preserved:	To be filtered:
					Date	Time								
2102795	G-2-2	20210628 G-2-2 Eluate	NPWW	Heufelder, George	6/28/21	09:30:00	1	N/A	G	4	TUBE	17	FLD	FLD
2102796	G-2-4	20210628 G-2-4 Eluate	NPWW	Heufelder, George	6/28/21	09:30:00	1	N/A	G	4	TUBE	17	FLD	FLD
2102797	G-2-5	20210628 G-2-5 Eluate	NPWW	Heufelder, George	6/28/21	09:30:00	1	N/A	G	4	TUBE	17	FLD	FLD
2102798	P-2-3	20210628 P-2-3 Eluate	NPWW	Heufelder, George	6/28/21	09:30:00	1	N/A	G	4	TUBE	17	FLD	FLD
2102799	P-2-4	20210628 P-2-4 Eluate	NPWW	Heufelder, George	6/28/21	09:30:00	1	N/A	G	4	TUBE	17	FLD	FLD
2102800	P-2-5	20210628 P-2-5 Eluate	NPWW	Heufelder, George	6/28/21	09:30:00	1	N/A	G	4	TUBE	17	FLD	FLD
2102801	Raw Influent	20210628 Raw Influent Unciluted	NPWW	Heufelder, George	6/28/21	09:30.00	1	N/A	G	4	TUBE	17	FLD	NA
2102802	PC Pump Cbr	20210628 Pump Chamber Undiluted	NPWW	Heufelder, George	6/28/21	09:30.00	1	N/A	G	4	TUBE	17	FLD	NA

Commonwealth of Massachusetts
Department of Environmental Protection
Senator William X. Wall Experiment Station
37 Shattuck St., Lawrence, MA 01843
Tel: 978-682-5237

Sample Tracking & Chain-of-Custody Record

WES Sample Login #: 20210355

Regulated Object Program Name: MASSTC Virus Grant
Regulated Object Program ID: 19-02/319

WinLIMS Sample ID	Client Sample ID	Client Sample Description	Sample Matrix Code	Analysis Requested: HF183 Marker by ddPCR-Mod 2015 Water Res. 70:337-349
2102795	G-2-2	20210628 G-2-2 Eluate	NPWW	X
2102796	G-2-4	20210628 G-2-4 Eluate	NPWW	X
2102797	G-2-5	20210628 G-2-5 Eluate	NPWW	X
2102798	P-2-3	20210628 P-2-3 Eluate	NPWW	X
2102799	P-2-4	20210628 P-2-4 Eluate	NPWW	X
2102800	P-2-5	20210628 P-2-5 Eluate	NPWW	X
2102801	Raw Influent	20210628 Raw Influent Undiluted	NPWW	X
2102802	PC Pump Cbr	20210628 Pump Chamber Undiluted	NPWW	X

Code Definitions

Matrix Codes

AIR	Air Sample		ME	Marine / Estuarine Water
BIO	Biological Specimen		NPWW	Non-Potable Water or Waste Water
BIO-PLUGS	Biological Specimen-Plug Sample Matrix		RW	ASTM Type I Reagent Water Matrix
DW-FS	Drinking Water - Finished or Source		SLD	Solid
FB-FRB	Field Blank-Field Reagent Blank Matrix		TB	Trip Blank
LW	Liquid Waste			

Sample Preservative Codes

1	Cool 1 - 6° C		7	Filtered (0.45-µm pore size)		13	EDTA, trisodium salt	19	Trizma, pH 7.0
2	pH < 2 with H_2SO_4		8	Sodium thiosulfate ($Na_2S_2O_3$)		14	Methanol	20	Abraxis Diluent (10X)
3	pH < 2 with HNO_3		9	Maleic acid		15	Reagent water (ASTM Type I)	21	Potassium dihydrogen citrate
4	pH < 2 with HCl		10	Sodium sulfite (Na_2O_3S)		16	Monochloroacetic acid (pH = 3)	22	pH < 2 with H_3PO_4
5	pH > 12 with NaOH		11	Ammonium chloride (NH_4Cl)		17	Frozen		
6	L-Ascorbic acid		12	Ethylenediamine		18	Sodium bisulfate ($NaHSO_4$)		

Sample Container Type Codes

DNPH	DNPH-cartridge		500CG	500-mL, clear-glass
VOAA	40-mL, amber-glass VOA vial		500PE	500-mL, HDPE
VOAC	40-mL, clear-glass VOA vial		1LAG	1-L, amber-glass
20AG	20-mL, amber-glass		1LPE	1-L, HDPE
120ST	120-mL, polypropylene, sterile		4LAG	4-L, amber-glass
120AG	120-mL, amber-glass		4LCG	4-L, clear-glass
125PE	125-mL, HDPE		BSCP	Biological Specimen Container, plastic, all sizes
250AG	250-mL, amber-glass		TUBE	Sample Vial or Tube, various sizes
250PE	250-mL, HDPE		PT	PT Sample
250PP	250-mL, polypropylene		OTH	Other
500AG	500-mL, amber-glass		NONE	Field Measurements/Metadata/Cancelled sample

Other Codes

FLD	Field			
NA	Not Applicable			

SAMPLE CONDITIONS REVIEW FORM

Massachusetts Department of Environmental Protection

Division of Environmental Laboratory Sciences – Senator William X. Wall Experiment Station

Login Batch # 20210355 **Date:** 8/4/2021

Role/Lab		Yes	No	NA	Initials
Receiver	Sample Tracking/COC: Were all *Sample Tracking/COC Record* pages checked on both sides?	✓			RM
Receiver	Sample Tracking/COC: Were the Client Sample IDs and Descriptions recorded for all samples?	✓			RM
Receiver	Sample Tracking/COC: Were Collector, Collection Date, and Collection Time recorded for all samples?	✓			RM
Receiver	Sample Tracking/COC: Were Container Types and Grab/Composite recorded for all samples?	✓			RM
Receiver	Sample Tracking/COC: Was the Relinquished/Received Time recorded before any samples were taken for analysis?	✓			RM
Receiver	Sample Tracking/COC: Were all samples (including frozen and WES facility samples) assigned to a real or virtual cooler; were all coolers assigned cooler numbers (1, 2, 3… etc.); and were temperatures recorded for all coolers?	✓			RM
Inorganic Chemistry	Were all sample containers for this laboratory intact, tightly capped, and at acceptable temperatures when received?				
Inorganic Chemistry	Is there any visual indication or other evidence that the samples for this laboratory were not collected according to U.S. EPA or other standard protocol?				
Inorganic Chemistry	Are the number, matrices, and field ID labels of the samples for this laboratory the same as recorded on the *Sample Tracking/COC Record*?				
Inorganic Chemistry	Sample storage location(s):				
Microbiology Culturable	Were all sample containers for this laboratory intact, tightly capped, and at acceptable temperatures when received?				
Microbiology Culturable	Is there any visual indication or other evidence that the samples for this laboratory were not collected according to U.S. EPA or other standard protocol?				
Microbiology Culturable	Are the number, matrices, and field ID labels of the samples for this laboratory the same as recorded on the *Sample Tracking/COC Record*?				
Microbiology Culturable	Sample storage location(s):				
Microbiology Molecular	Were all sample containers for this laboratory intact, tightly capped, and at acceptable temperatures when received?	✓			RM
Microbiology Molecular	Is there any visual indication or other evidence that the samples for this laboratory were not collected according to U.S. EPA or other standard protocol?		✓		RM
Microbiology Molecular	Are the number, matrices, and field ID labels of the samples for this laboratory the same as recorded on the *Sample Tracking/COC Record*?	✓			RM
Microbiology Molecular	Sample storage location(s): MF 21.1				
Org Chem GC & LC	Were all sample containers for this laboratory intact, tightly capped, and at acceptable temperatures when received?				
Org Chem GC & LC	Is there any visual indication or other evidence that the samples for this laboratory were not collected according to U.S. EPA or other standard protocol?				
Org Chem GC & LC	Are the number, matrices, and field ID labels of the samples for this laboratory the same as recorded on the *Sample Tracking/COC Record*?				
Org Chem GC & LC	Sample storage location(s):				
Org Chem GC/MS	Were all sample containers for this laboratory intact, tightly capped, and at acceptable temperatures when received?				
Org Chem GC/MS	Is there any visual indication or other evidence that the samples for this laboratory were not collected according to U.S. EPA or other standard protocol?				
Org Chem GC/MS	Are the number, matrices, and field ID labels of the samples for this laboratory the same as recorded on the *Sample Tracking/COC Record*?				
Org Chem GC/MS	Sample storage location(s):				
LIMS Logger	Were the data from the *Sample Tracking/COC Record* correctly transcribed into the LIMS and have all SCRF questions been answered in the LIMS?	✓			SM

Are there notes (including CARs) recorded on Page 2? Yes____ No X

SAMPLE CONDITIONS REVIEW FORM

Login Batch # 20210355 **Date:** 8/4/2021

Notes (Including CARs):

Appendix 12

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Massachusetts Alternative Septic System Test Center
Barnstable, Massachusetts

Standard Operating Procedure

Title: YSI ProDSS Field Meter

Effective Date: 2021-10-18	**Number:** MASSTC-SOP-016	**Revision:** 004

Authors

Name: Brian Baumgaertel
Title: MASSTC Director

Signature: DocuSigned by: Brian Baumgaertel A809A6344B57407... Date: 10/18/2021

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Approvals

Name: Brian Baumgaertel
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Name: Emily Michele Olmsted
Title: Environmental Project Assistant/ Quality Assurance Manager

Signature: DocuSigned by: E5E55A7B2C05436... Date: 10/18/2021

Revision History

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the MASSTC Director on the MASSTC Sharepoint Site.

History	*Effective Date*
Revision 004 – Updated Added section about turbidity calibration (step 9.10) as well as occasionally references throughout the rest of the document when pertinent. Added definition of Ultrapure Water. Removed not in Scope in Application about nitrate/ammonia probes (no longer relevant). Edited listing of SDS's to one-sentence phrase about where to find all SDS's. Updated by EMO.	2021-10-18
Revision 003 – Updated Section 9 based on new reference point (MASSTC-FRM-033 – ProDSS Calibration Checklist). Updated Section 10 based on new reference point (MASSTC-FRM-034 – ProDSS End of Day Checklist). Added instruction to Section 10 concerning project following NSF protocol. Updated references to Calibration Worksheet to instead reference new form. Added instruction for performing Zero ODO calibration. Added instruction concerning pH calibration mV range (9.7.6.1). Updates done by EMO.	2021-06-09
Revision 002- Removed references to needing to write down CCV for pH and ODO on calibration worksheet. Added instruction of needing to write calibration information and change in standards on white board in lab. Added clarification about reviewing downloaded file to ensure correct site readings were taken at end of day.	2019-12-09
Revision 001- Reformatted several sections for consistency. Removed section on data logging. Made small edits due to changes that have been made by our staff including updates in cleaning procedure. Removed directives to record values in yellow field book which has been since updated to electronic records only. Removed reference to nutrient probes from title as this is stated clearly in the first section of the document.	2019-10-28
Revision 000 - Initial Release	2019-06-07

CONTENTS

1. Scope and Application

1.1. This Standard Operating Procedure (SOP) describes the calibration and use of the YSI ProDSS Handheld Field Meter.

1.2. This SOP DOES NOT apply to the calibration and use of the YSI 556 Handheld Field Meter.

1.3. This SOP is to be adhered to by all MASSTC staff and all others utilizing this field meter.

2. Definitions

2.1 **Meter Body** – the handheld portion of the meter.

2.2 **Sonde** – the component housing individual sensor probes.

2.3 **Sensor Guard** – the black plastic caging that attaches to the outside of the sonde to protect the sensors from being bumped or harmed.

2.4 **Sealing Ring** – the black moldable plastic ring that seals in any air and moisture in the optical dissolved oxygen calibration cup.

2.5 **Calibration cup** – the clear plastic tubing in which liquid for calibrations is placed. If a standard is not specified, this specifically refers to the YSI-manufactured clear plastic tubing with threading in which the sonde sits used for Optical Dissolved Oxygen calibrations.

2.6 **SDS (Safety Data Sheet)** – a document provided by the chemical manufacturer which details the safety precautions and hazards as well as other information on a specific chemical.

2.7 **Small Cleaning Brush** – a hooked wire with black bristles found hanging in the lab.

2.8 **Sonde Weight** – a weight attached to the bottom of the sensor guard.

2.9 **PPE (Personal Protective Equipment) –** equipment worn to minimize exposure to hazards that cause serious workplace injuries and illnesses.

2.10 **Ultrapure water (ASTM Type I Reagent Grade Water) –**water that has been purified to strict chemical and biological specifications, containing, by definition, only H_20, and H+ and OH- ions in equilibrium. Conductivity for ultrapure water is about 0.055 µS/cm at 25°C, also expressed as resistivity of 18.2 MΩ.

3. Health and Safety Warnings

3.1 **Physical Hazards** – use care and good judgement when utilizing field meters. If a sample location is in a place where it cannot be safely analyzed (e.g. confined space), notify the MASSTC director immediately and do not attempt to retrieve it. Environmental conditions (e.g. rain, snow, etc.) can lead to uneven and/or slippery surfaces so care should be taken to prevent slips and fall. **PPE Required: Closed-toe shoes/boots. Care should be taken to dress appropriately.**

3.2 **Infectious Materials** – even the cleanest wastewater can contain pathogens or toxic materials. Proper precautions should be taken to isolate yourself. **PPE Required: gloves**.

3.3 **Fire/Explosive Hazards** - Charge the battery pack in an open area away from flammable materials, liquids, and surfaces. Do not charge or handle a battery pack that is hot to the touch. Failure to follow the safety warnings and precautions can result in personal injury and/or instrument damage. Read **Rechargeable Lithium-Ion battery pack safety warnings and precautions (Section 7.1 of MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F).**

3.4 **Skin Corrosion/Serious Eye Damage** - Some of the chemicals required for these solutions could be hazardous under some conditions; therefore, the standards should only be prepared by qualified chemists in laboratories where proper safety precautions are possible. The user should obtain and read the Safety Data Sheet (SDS) for each chemical and to follow the required instructions with regard to handling and disposal of these chemicals. **PPE Required: Gloves and safety goggles**.

3.4.1 Consult Sharepoint's MASSTC-Safety Data Sheets for hazardous material information.

4. CAUTIONS

4.1 The sensor probes should never be allowed to dry out. Store the sonde in the calibration cup with a small amount of tap water, and ensure the seal is tightened. Store unused probes according to manufacturers' instructions. (Section 4 of MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F)

4.2 When transferring the meter between locations, be sure to keep the sonde and cable off the ground to reduce wear and prevent damage. Be careful not to step on the cable.

4.3 Be careful not to put undue strain on the cable.

4.4 Do not allow the meter body to become submerged in liquid. This can cause irreparable damage to the unit.

4.5 When connecting the meter to the charging cable or computer download cable, be careful not to bend or flex the connector, as this can damage the charging/download port.

5. INTERFERENCES

5.1. Change pH buffer solutions twice per week (Monday and Wednesday unless change in the weeks' work days or significant change in sample loading days) to ensure standards are accurate. When appropriate, standards can be changed more frequently if needed to ensure accuracy of projects following NSF protocol.

5.2. Change conductivity solution once per week to ensure standards are accurate.

5.3. Change turbidity standards once per week to ensure standards are accurate.

5.4. Ensure that probes are kept clean and stored properly to minimize bio-fouling interference.

5.5. Change water used for dissolved oxygen calibration daily to minimize bio-fouling interference.

5.6. Store stock pH buffer solutions in closed area, capped and away from sunlight.

5.7. Always put caps on poured buffer solutions to reduce evaporation loss.

6. Personnel Qualifications

6.1. Personnel are required to be knowledgeable of the procedures in this SOP and all referenced SOP's.

6.2. Personnel are required to receive training on the proper use of the instrument from a qualified member of staff.

6.3. Personnel performing calibrations are required to review relevant Safety Data Sheets specified in Section 3.

7. Special Apparatus and Materials

7.1. US pH buffer solutions (4.00, 7.00, and 10.00)

7.2. Conductivity calibration solution (447 µS/cm)

7.3. Turbidity standards (Ultrapure Water (0 FNU), 124 FNU standard, and 1010 FNU standard)

7.4. YSI ProDSS meter and accompanying probes, cables, and equipment.

8. Sample Handling and Storage

8.1. Measurements of effluent must be taken as close to laboratory analysis sample time as possible unless otherwise specified by the client or director.

8.2. Measurements of effluent should be taken directly from the location as a free-flowing source whenever possible.

8.3. Samples should not be stored for long periods of time before taking measurements, either refrigerated or otherwise, to maximize representativeness of measurements to direct conditions.

8.4. Any measurements of samples not taken by following the above directives should be noted.

9. Opening and Calibration Procedure

9.1 Turn on the ProDSS meter by pressing the power button. Fill out all information on MASSTC-FRM-033 – ProDSS Calibration Checklist.

9.2 Remove the cap to the sensor guard and sealing ring. With a lint-free wipe, gently remove any moisture from the sensors.

9.3 Record the specific conductance reading on MASSTC-FRM-033 – ProDSS Calibration Checklist. If the reading is above 1 µs/cm:

9.3.1 Clean the sensor using the small cleaning brush; dip the brush in clean water and insert it into each hole of the conductivity probe 10-12 times; rinse thoroughly with clean water. Dry the probe using a lint-free wipe and recheck the air reading and record it on MASSTC-FRM-033 – ProDSS Calibration Checklist. If reading is still above 1 µs/cm consult User Manual for cleaning the sensor port.

9.3.2 Use compressed air to blow debris from holes.

9.4 If reading is still above 1 µs/cm see MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F for instruction on cleaning the sensor port.

9.5 (If applicable) complete a conductivity calibration once per week by doing the following:

9.5.1 Use fresh, traceable conductivity calibration solution (solution can be used for one month after being opened). Write the change in standard and the calibration date on the white board in the lab. Pour into the calibration cup to the indicated line.

9.5.2 Place the probe into the calibration cup. The solution must be above the second holes on the conductivity probe.

9.5.3 Gently rotate and/or move the sensor up and down to dislodge any bubbles. Allow for at least 40 seconds of temperature equilibration.

9.5.4 Press the Calibration key and then choose Conductivity. Choose Specific Conductance.

9.5.5 Select Calibration Value and key in the standard for the given temperature; note that temperature compensation values can be found on the side of the bottle.

9.5.6 Observe the actual measurements (the white line on the graph should be flat for 40 seconds).

9.5.7 Select Accept Calibration.

9.5.8 Write the date of the Calibration and meter color as well as the date of the new standards on the white board in the lab.

9.6 Calibrate Optical Dissolved Oxygen (ODO) **daily**:

9.6.1 Make sure the sensor guard is installed on the meters. Make sure there is no water on the sensors; use a moistened lint-free wipe to gently pat them dry. Make sure the threaded black cap and ring are removed – there needs to be ample air exchange.

9.6.2 Put a small amount of tap water into the bottom of the calibration cup; water should be changed every day before doing calibration to reduce bio-fouling. There should be no other debris or fouling of the cup; clean cup with brush as needed.

9.6.3 Insert the probe into the calibration cup, making sure that the top is not sealed for atmospheric venting.

9.6.4 Wait 5-15 minutes so that the air in the cup can be saturated.

9.6.5 Press the Calibration key. Choose ODO, then choose DO%.

9.6.6 Wait for the readings to be stable – the white line on the graph should be flat for about 40 seconds. Record information on MASSTC-FRM-033 – ProDSS Calibration Checklist.

9.6.7 Write the day's ODO post-calibration value on the white board in the lab.

9.7 Complete a 3-point calibration **every day** on pH by doing the following:

9.7.1 Make sure the sensor guard is off. If standards need to be poured:

-Make sure the cups are clean.

-Pour old buffer into the allocated bottles (can be reused for rinse).

-Pour enough buffer of each 4, 7, and 10 so that the liquid level just comes to the bottom white fitting of the cup.

-Write the date of the change in standards on the white board in the lab.

9.7.2 Rinse the sensors with used 7 buffer. Always start the calibration with pH 7 buffer.

9.7.3 Carefully lower the probe into the calibration cup with pH 7 buffer solution. Make sure both the pH sensor and temperature sensor are submerged.

9.7.4 Push the Calibration key then select pH. The Calibration value will automatically be adjusted based on the selected buffer and temperature.

9.7.5 Wait for the pH mV and temperature readings to stabilize; the white line on the graph should be flat for about 40 seconds.

9.7.6 Press the Enter button to accept the calibration. **You must accept the calibration before moving onto the next standard.** The bottom of the screen should say "Ready for cal point 2".

9.7.6.1 **If the pH mV reading for the buffer solution of 7 is NOT within -50 mV to +50 mV, the meter needs further evaluation. Stop the calibration.** Consult the MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F to troubleshoot (including reconditioning probe). Consider using a different meter if the meter cannot be brought back into -50 to +50 mV.

9.7.7 Remove the probe from the 7 standard and rinse it with used buffer of the next standard you're going to calibrate.

9.7.8 Place probe in the next buffer (can be 10 or 4). Wait for the pH mV and temperature readings to stabilize; the white line on the graph should be flat for about 40 seconds. The Calibration value will automatically be adjusted based on the selected buffer and temperature.

9.7.9 Press the Enter button to accept the calibration. **You must accept the calibration before moving onto the next standard.** The bottom of the screen should say "Ready for cal point 3".

9.7.10 Remove the probe from the last standard and rinse it with used buffer of the next standard you're going to calibrate.

9.7.11 Place probe in the last buffer. Wait for the pH mV and temperature readings to stabilize; the white line on the graph should be flat for about 40 seconds. The Calibration value will automatically be adjusted based on the selected buffer and temperature.

9.7.12 **Press the Enter button to accept the calibration.** It will take you back to the calibration screen.

9.7.13 **Change pH standards 2/week (usually Monday and Wednesday).**

9.7.14 If a calibration error message occurs, do not continue calibration. Abort and restart. Pour new pH buffer standards and examine pH bulb on sensor for debris or issues. Consult MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F for further troubleshooting.

9.8 Complete a calibration verification (CCV) on the 7 pH.

9.8.1 Rinse the sensor with used 7 pH buffer and place in the 7 pH standard calibration cup.

9.8.2 Press the Probe button.

9.8.3 Choose the third option (Auto Stable) and then press Enter when highlighted.

9.8.4 Scroll to the very bottom option (Start Auto Stable) and click Enter to begin auto-stabilization. The rest of the settings should remain as is. *Current settings are for 5 samples at 10 second interval; pH stability of 0.2 units, ODO stability of 0.5 units.*

9.8.5 The meter will flash AS lettering when still stabilizing. When stable, the following will occur:

-An audible beep will sound.

-The AS lettering will be green.

-The AS lettering will no longer be flashing.

9.8.6 The choice of Log One Sample should be highlighted; press the Enter button.

9.8.7 If the incorrect site is showing, highlight the second option (Site) and press Enter. Find 1 Check 7 pH and then push Enter; on the next screen, press Enter (the screen should show Select[1Check 7 pH]. The last screen should have "Log Now!" highlighted – press Enter again.

9.8.8 The accuracy of the pH probe is ±0.2 units from the expected pH at the temperature in the solution. If the pH reading in the 7 pH buffer solution is more than ±0.2 units from the expected value, clean and recalibrate the probe. Consider consulting MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F for other troubleshooting issues as to why the reading is inaccurate.

9.9 Complete Optical Dissolved Oxygen (ODO) calibration verification.

9.9.1 Rise off probes and gently pat dry with moistened lint-free wipe.

9.9.2 Reattach sensor guard then place in calibration cup with a small amount of water in the bottom of the cup and wait 5-15 minutes to check ODO.

9.9.3 Press the Probe button.

9.9.4 Choose the third option (Auto Stable) and then press Enter when highlighted.

9.9.5 Scroll to the very bottom option (Start Auto Stable) and click Enter to begin auto-stabilization. The rest of the settings should remain as is. *Current settings are for 5 samples at 10 second interval; pH stability of 0.2 units, ODO stability of 0.5 units.*

9.9.6 The meter will flash AS lettering when still stabilizing. When stable, the following will occur:

-An audible beep will sound.

-The AS lettering will be green.

-The AS lettering will no longer be flashing.

9.9.7 The choice of Log One Sample should be highlighted; press on the Enter button.

9.9.8 If the incorrect site is showing, highlight the second option (Site) and press Enter. Find 2 Check ODO and then push Enter; on the next screen, press Enter (the

screen should show Select[2Check ODO]. The last screen should have "Log Now!" highlighted – press Enter again.

9.9.9 The accuracy of the ODO probe is ±1% from the calibration value (the post-calibration value) of that morning. If the ODO reading has been given 5-15 minutes to be fully saturated in calibration cup and is more than ± 1.0 % from that day's calibration value, clean and recalibrate the probe. Consider consulting the MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F for other troubleshooting issues as to why the reading is inaccurate.

9.10 Complete a Turbidity calibration, if applicable, for field measurements by completing the following:

9.10.1 Clean sensor guard, calibration cup, and sensor weight to ensure no biofouling. A black, unscratched sensor weight must be used to minimize reflection interference.

9.10.2 Clean off probes and volume measuring device with Ultrapure Water (obtain from virus lab). Dry probes using a lint-free cloth. Attach sensor guard and weight.

9.10.3 Pour approximately 150 mL of Ultrapure Water (obtain from virus lab) into clean volume measuring device (flask/graduated cylinder) for first calibration point to measure, then pour into marked calibration cup. **Always pour into container at an angle to minimize bubbles that enter cup.**

9.10.4 Slowly insert probes into solution.

9.10.5 Press the Calibration key, then choose Turbidity. The Calibration value will automatically be adjusted.

9.10.6 Wait for readings to stabilize; the white line on the graph should be flat for about 40 seconds.

9.10.7 Press the Enter button to accept the calibration. **You must accept the calibration before moving onto the next standard.** The bottom of the screen should say "Ready for cal point 2".

9.10.8 Clean off probes, sensor guard, weight, and measuring device with RO Water. Rinse first with RO water, followed by used 124 FNU standard in labeled rinse bottles if available. Dry using a lint-free cloth.

9.10.9 Pour approximately 150 mL of next standard (124 FNU) into clean measuring device (flask/graduated cylinder) to measure, then pour into marked calibration cup. **Always pour into container at an angle to minimize bubbles that enter cup.**

9.10.10 Wait for readings to stabilize; the white line on the graph should be flat for about 40 seconds.

9.10.11 Press the Enter button to accept the calibration. **You must accept the calibration before moving onto the next standard.** The bottom of the screen should say "Ready for cal point 3".

9.10.12 Clean off probes, sensor guard, weight, and measuring device with RO Water. Rinse first with RO water, followed by used 1010 FNU standard in labeled rinse bottles if available. Dry using a lint-free cloth.

9.10.13 Pour approximately 150 mL of next standard (1010 FNU) into clean measuring device (flask/graduated cylinder) to measure, then pour into marked calibration cup. **Always pour into container at an angle to minimize bubbles that enter cup.**

9.10.14 Wait for readings to stabilize; the white line on the graph should be flat for about 40 seconds.

9.10.15 Press Enter to complete the calibration.

9.11 Ensure that MASSTC-FRM-033 – ProDSS Calibration Checklist is completed.

9.12 If needed, perform ODO Zero Point Calibration. This calibration should be used when ODO values at known low-DO situation (such as the influent channel) are showing negative ODO readings.

9.12.1 Create a zero DO solution by mixing 8-10 grams of sodium sulfite into 500 mL of tap water. This should be done in a container that will allow sensor access. **Wait 60 minutes to ensure the solution is oxygen-free.**

9.12.2 Ensure that the ODO and Conductivity/Temperature sensors are installed on the meter, but remove any other probes and store safely.

9.12.3 Submerge the ODO and Conductivity/Temperature probes into the zero ODO solution.

9.12.4 Press the “Cal” key, then choose ODO, then Zero.

9.12.5 Allow the reading to stabilize. The white line on the graph should show no significant change for 40 seconds.

9.12.6 Press Enter to Accept Calibration. You should see “Calibration successful.”

9.12.7 **You must follow up with an ODO % calibration.**

9.12.8 Clean the sensors well to rinse off all zero-ODO solution. If any sensors were taken off (ex. pH), return to place in meter.

9.12.9 Follow step 9.6 of this document to complete the ODO % calibration.

10. TAKING MEASUREMENTS PROCEDURE

10.1 Disconnect meter from power supply and make sure cap to electronic port is closed to prevent debris from entering.

10.2 Make sure the sensor guard (the black caging) is installed and that the sonde weight is attached to the bottom of the probe. Detangle and untwist the cord as needed.

10.3 Bring meter to desired location and gently lower into place. The liquid level should come up to the bottom of the higher cylindrical holes, as indicated by a label.

10.4 Click on the Probe button.

10.5 Choose the third option (Auto Stable) and then press Enter when highlighted.

10.6 Scroll to the very bottom option (Start Auto Stable) and click Enter to begin auto-stabilization. The rest of the settings should remain as is. *Current settings are for 5 samples at 10 second interval; pH stability of 0.2 units, ODO stability of 0.5 units.*

10.7 The meter will flash AS lettering when still stabilizing. When stable, the following will occur:

-An audible beep will sound.

-The AS lettering will be green

-The AS lettering will no longer be flashing.

10.8 The choice of Log One Sample should be highlighted; press on the Enter button.

10.9 If the incorrect site is showing, highlight the second option (Site) and press Enter. Choose from the list of site names. Scroll to and highlight the desired site then push Enter; on the next screen, press Enter (the screen should show Select[sitename]. The last screen should have "Log Now!" highlighted – press Enter again.

10.10 Record any notes in the yellow field book, including initials of the staff member who took the sample and who took the field measurements.

10.11 Bring the meter to the next location and start again at step 10.3.

10.12 Every ten samples must include a continuing calibration verification (CCV) on pH 7 standard and the ODO saturation; bring the meter into the laboratory and do the following:

10.12.1 Rinse meter with tap water and remove sensor guard. Rinse sensors with used 7 pH rinse. Place in 7 pH buffer solution. Perform auto-stable and record electronically under the site name "1 Check 7 pH"

10.12.1.1 The accuracy of the pH probe is ±0.20 units from the expected pH at the temperature in the solution. If the pH reading in the 7 pH buffer solution is more than ±0.2 units from the expected value, clean and recalibrate the probe. **Do not use any pH readings taken since last acceptable calibration verification.** Consider consulting MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F for other troubleshooting issues as to why the reading is out of range.

10.12.2 Rinse off probes with deionized water and pat dry. Reattach sensor guard then place in calibration cup with a small amount of water in the bottom of the cup and wait 5-15 minutes to check ODO. Perform auto-stable and record value electronically under the site name "2 Check ODO".

10.12.2.1 The accuracy of the ODO probe is ±1.0% from the calibration value (the post-calibration value) of that morning. If the ODO reading has been given 5-15 minutes to be fully saturated in calibration cup and is more than ±1 % from that day's calibration value, clean and recalibrate the probe. **Do not use any DO readings taken since last acceptable calibration verification.** Consider consulting MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F for other troubleshooting issues as to why the reading is inaccurate.

10.13 If needed, you can add a sample location in the field. Please note that this is easier to do on the computer if possible.

10.13.1 Go through the steps of auto-stabilization.

10.13.2 Log One Sample should be highlighted; press the Enter key.

10.13.3 Scroll down to Site [] and press the Enter key.

10.13.4 Go to the top of the list and choose Add new…

10.13.5 Site Name [] should be highlighted; press the Enter key.

10.13.6 Key in the desired name and choose the Enter at the bottom of the screen when finished.

10.13.7 Scroll down to Save and push Enter.

10.13.8 Make sure this site is chosen if a sample needs to be logged here.

10.14 If sample was logged incorrectly, there must be an electronic record of this.

10.14.1 The choice of Log One Sample should be highlighted; press on the Enter button.

10.14.2 Highlight the second option (Site) and press Enter. Choose "3 PREVIOUS SITE INCORRECT" from the list of site names. Push Enter. On the next screen, press Enter (the screen should show Select[3 PREVIOUS SITE INCORRECT]. The last screen should have "Log Now!" highlighted – press Enter again.

10.14.3 Log the sample under the correct name.

10.14.4 Make a note in the yellow field book and when downloading data, make sure to be aware of this data going into the database.

10.15 To set the meter on logging mode, do the following:

10.15.1 Follow steps 10.1 to 10.3.

10.15.2 Press the System button on the meter.

10.15.3 Use the down arrows to highlight "Logging [Single]" and press Enter.

10.15.4 Scroll down to the box next to Continuous Mode and press Enter to check the box.

10.15.5 Scroll down to Site[] and make sure the correct site is chosen.

10.15.6 Scroll down to Log Interval and press Enter. Key in the correct interval in the format of HH:MM:SS.

10.15.7 Press Esc.

10.15.8 You should be at the main screen. The green bar at the top should be on "Start Logging".

10.15.9 Press Enter to Start Logging, the screen will show your log interval and site again; double-check that these are correct.

10.15.10 The green bar should be highlighting "Start Now!" Press Enter.

10.15.11 To stop logging, the green bar should be highlighting "Stop Logging" so press Enter to stop logging.

10.15.12 To take out of Logging Mode, press the System Key, go down to Logging Interval, and then uncheck the box next to Continuous Mode.

PROJECTS FOLLOWING NSF PROTOCOL

10.16 Projects following National Sanitation Foundation (NSF) protocol are especially important for documentation of field measurements as this data is reported quickly to NSF and affects the determination of the test.

10.17 Projects following NSF protocol usually have a specified pH range that the effluent sample is required to meet. Any sample that is out of the specified range could constitute a failure of the NSF test and so must be rigorously confirmed and documented.

10.17.1 Typically the acceptable range for pH readings of an NSF test is 6.0 – 9.0 SU (±0.2 SU). Consult the exact Test Plan to confirm for the project in question.

10.18 If any NSF effluent is outside of the specified range (consider only the range given by NSF, i.e. 6.0-9.0 SU, and not 5.8 to 9.2 given the ±0.2 SU), ensure to record the reading.

10.19 Stop taking measurements and complete a CCV per the steps in section 10.12. Be certain to log the values of the CCV for documentation.

10.20 Complete a new calibration of all parameters per section 9. Use new buffers/standards for all parameters to ensure the most accurate readings. Log opening CCV values again after calibration.

10.21 Retake the field measurements for the effluent of the project following NSF protocol.

10.21.1 If NSF effluent is back within range, continue with field measurements as usual.

10.21.2 If NSF effluent is still out of range for pH, repeat steps 10.19 through 10.21.

10.21.2.1 If, after a second recalibration and accompanying CCVs the effluent of the NSF project is still out of range, take final effluent reading and final CCV. Then, alert MASSTC Director so that proper notification can reach NSF personnel.

11. CLOSING PROCEDURE

11.1. Bring meter in and run under sink water to clean off outer debris. Rinse the sensor guard with clean water. Once each week, use a brush and water with dish soap to remove light bio-fouling from the sensor guard and weight.

11.2. Locate and fill out MASSTC-FRM-034 – ProDSS End of Day Checklist for the next steps.

11.3. Detangle the cord and remove any dirt.

11.4. Remove sensor guard and weight.

11.5. Rinse sensors with used 7 pH rinse. Place in 7 pH buffer solution. Record as Close value electronically under the site name “1 Check 7 pH”.

11.5.1. If 7 pH is outside of ±0.2 units from expected value, recalibrate and retake field measurements done since last acceptable check.

11.6. Clean the conductivity sensor at end of each day. Dip the sensor’s small cleaning brush in water, insert the brush at the top of the channels and sweep the channels 15 to 20 times (see MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F page 51 for diagram). Reading should be 0.0; if any higher, reclean using brush.

11.7. Rinse off sensors with deionized water and gently pat dry using a moistened lint-free delicate task wipe. Before placing into calibration cup, brush out calibration cup and replace water with new tap water. Reattach sensor guard then place in calibration cup with a small amount of water in the bottom of the cup and wait 5-15 minutes for cup to be saturated and then check ODO. Record as close value electronically under the site name “2 Check ODO.”

11.7.1. If ODO percent is outside of ±1% from that morning's ODO calibration, recalibrate and retake field measurements done since last acceptable check.

11.8. Reattach black threaded cap and sealing ring and screw onto calibration cup. This will ensure that the sensors are stored in a moist environmental for the short term (less than 4 weeks).

11.9. Make sure to download data and upload onto the MASSTC Sharepoint.

11.10. Review downloaded data to ensure that all recorded sites were recorded under the correct name and that no sites were skipped.

11.10.1. If any sites were missed, reopen meter by performing CCVs and take missing measurements. The meter must then be closed again, starting at 11.1.

11.11. Make sure the meter is connected to the power supply.

11.12. Make sure the cord is hanging off of the ground on the lab bench hook.

11.13. Make sure the cord is still attached to the meter to prevent dust entry into the meter.

11.14. Make sure the meter has been powered off (after downloading data).

11.15. Make sure you have completed MASSTC-FRM-034 – ProDSS End of Day Checklist.

12. DATA ANALYSIS/CALCULATIONS:

12.1. None

13. DATA MANAGEMENT/RECORDS MANAGEMENT

13.1. Measurement data are to be recorded on the meter and downloaded and imported into the MASSTC Data and Facility Management System. Data in CSV (Comma Separated Value) file format are to be downloaded to a backed up and secure file location each day that the meter is used. CSV files are to be imported into the MASSTC Data and Facility Management System as soon as practicable.

13.2. Observations germane to each measurement are to be recorded in indelible ink in a numbered field notebook and will be transcribed into the MASSTC Data and Facility Management System with the appropriate field measurement record as soon as practicable.

13.3. Archived data are subject to official retention schedule contained in MASSTC-SOP-003, Records and Archives.

14. QUALITY CONTROL

14.1. <u>Calibration</u>

14.1.1. pH

14.1.1.1. pH probes are to be calibrated **daily via three-point calibration with 4.00, 7.00, and 10.00 buffers**.

14.1.1.2. pH calibration standards are to be changed twice per week.

14.1.1.3. Rinses between pH calibration standards are to be done using the appropriate buffer solution to eliminate cross-contamination or dilution of buffer solutions.

14.1.2. Dissolved Oxygen

14.1.2.1. Dissolved Oxygen (DO) probes are to be calibrated **daily with fresh tap water**.

14.1.3. Specific Conductance

14.1.3.1. Specific Conductance probes are to be calibrated **weekly**.

14.1.3.2. Specific Conductance standards are to be changed once per week.

14.1.4. Turbidity

14.1.4.1. Turbidity probe is to be calibrated **weekly or on the day of use**.

14.1.4.2. Turbidity standards are to be changed once per week during weeks of use.

14.1.5. Handling of Calibration Standards

14.1.5.1. Upon receipt and subsequent opening, calibration standards are to be logged into the Chemical Receipt Log (MASSTC-FRM-014)

14.1.5.2. When standards are changed, an entry will be made into the Calibration Standards Log (MASSTC-FRM-028)

14.2. Calibration Acceptance Criteria

14.2.1. pH - ± 0.20 pH units

14.2.2. Dissolved Oxygen - ± 1.0%

14.3. Continuing Calibration Verifications (CCV)

14.3.1. pH and Dissolved Oxygen CCV's are to be done following initial calibration, every 10 non-calibration measurements, and as part of the daily meter closeout procedure.

14.3.2. pH CCV's are to use 7.0 buffer.

14.4. Measurements (General)

14.4.1. AutoStable is to be used for all readings to eliminate user bias.

14.4.2. The sonde is to be sufficiently submerged in the liquid to be measured.

14.5. Location of Measurements

14.5.1. Measurement locations are to be defined in writing by the client. Locations will be marked out on a site diagram (MASSTC-FRM-040 – Sampling Plan) and labeled with a printed ¾" label where possible.

14.5.2. Soil-Based Systems Installed at MASSTC (Non-field installations)

14.5.2.1. Final effluent is to be measured within a distribution box prior to final discharge to void.

14.5.2.2. The sonde is to be placed such that flow from the discharge pipe comes into direct contact with the probes (I.E. in very close proximity to the discharge pipe).

14.5.3. Pan Lysimeters

14.5.3.1. Liquid obtained via pan lysimeters can be measured in one of two ways:

14.5.3.1.1. Directly in the lysimeter sump, which is the preferred method.

14.5.3.1.2. By pumping a volume into a separate container.

14.5.3.2. On occasion, it may be necessary to apply a vacuum to the lysimeter port to obtain a sufficient sample. In this case, the Dissolved Oxygen values should be disregarded, and a note stating that a vacuum was used is to be entered into the log for that sample.

14.6. Timing of Measurements

14.6.1. Unless otherwise specified by the client, measurements are to be taken during times of system dosing.

14.6.2. Unless otherwise specified by the client and where practicable, measurements are to be taken within one hour of the time of laboratory sampling, if applicable.

15. Nonconformance and Corrective Action

15.1 Refer to MASSTC-SOP-003 – Control of Nonconforming Work and MASSTC-SOP-004 – Corrective Action for general nonconformance and corrective action procedures.

15.2 Calibration Nonconformance

15.2.1 If CCV is outside of acceptance criteria at any point, the meter must be re-calibrated, and all measurements taken after the last acceptable CCV must be retaken.

15.2.2 If standards are of unknown age, discard and re-pour.

15.2.3 If standards are contaminated, discard and re-pour.

15.3 Measurement Nonconformance

15.3.1 If the sonde impacts a surface with sufficient velocity (e.g. dropped from a height), a physical inspection and a CCV shall be done prior to taking any other measurements.

16. Internal and External References

16.1 MASSTC-SOP-003 – Control of Nonconforming Work

16.2 MASSTC-SOP-004 – Corrective Action SOP

16.3 MASSTC-EXT-MAN-003 – YSI ProDSS User Manual Rev. F

16.4 MASSTC-EXT-MAN-004 – YSI ProDSS Calibration Guide

16.5 MASSTC-EXT-SDS-004 - USA Bluebook pH 10.00 Buffer Solution

16.6 MASSTC-EXT-SDS-005 - USA Bluebook pH 7.00 Buffer Solution

16.7 MASSTC-EXT-SDS-006 - USA Bluebook pH 4.00 Buffer Solution

16.8 MASSTC-EXT-SDS-007 - Conductivity Standard SDS

17. Forms and Data Sheets

17.1. MASSTC-FRM-014 – Chemical Receipt Log

17.2. MASSTC-FRM-028 – Calibration Standards Log

17.3. MASSTC-FRM-030 – Calibration Worksheet

17.4. MASSTC-FRM-033 – ProDSS Calibration Checklist

17.5. MASSTC-FRM-040 – Sampling Plan

Massachusetts Alternative Septic System Test Center

Barnstable, Massachusetts

Form

Title: ProDSS Calibration Checklist

Effective Date: 2021-10-18	**Number:** MASSTC-FRM-033	**Revision:** 002

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ProDSS Calibration Checklist

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Revision History

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the MASSTC Director on the MASSTC Sharepoint Site

History	Effective Date
Revision #002: Added Turbidity calibration section, and reformatted to fit to single page. Edits done by EMO	2021-10-18
Revision #001: Added pH 7 acceptable mV range. Added sections to write pH and ODO CCV. Updated by EMO.	2021-06-09
Revision #000: Original Issue	2020-08-21

ProDSS Calibration Checklist

Document ID#: MASSTC-FORM-033
Revision#: 002
Released: 2021-10-18
Released By: Brian Baumgaertel

Date (mm/dd/yyyy):					
Time:					
Meter color:					
Initials:					
Specific Conductance					
Conductivity reading in dry, room-temperature air:					
Conductivity Calibration - done today?	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
Conductivity Calibration - date last completed on this meter (mm/dd/yyyy):					
Turbidity					
Turbidity Calibration - done today?	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
If calibrated: Date Turbidity standards last changed?(mm/dd/yyyy)					
Optical Dissolved Oxygen					
ODO Calibration - did you successfully complete it today?	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
Today's Post-Cal ODO%					
pH Calibration					
pH Calibration - did you successfully complete it today? (yes or no)	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
pH 7 mV reading was within -50 to +50 mV range? (MUST STOP IF OUT OF RANGE)	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
Date pH standards were last changed:					
Date pH standards must be changed again (2/week):					
Continuing Calibration Verifications (CCV)					
Did you log pH 7 CCV? (yes or no)	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
pH 7 CCV:					
Did you log ODO CCV *(and wait 5-15 minutes in ODO cup for saturation)* ? (yes or no)	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
ODO CCV:					
Notes:					

When completed, please scan and place in: Sharepoint - MASSTC-Documents - Data - ProDSS Downloaded Data - Calibration Logs.
Put the hard copy in the ProDSS Calibration binder in the lab

ProDSS Calibration Checklist

Document ID#: MASSTC-FORM-033
Revision#: 002
Released: 2021-10-18
Released By: Brian Baumgaertel

Date (mm/dd/yyyy):					
Time:					
Meter color:					
Initials:					
Specific Conductance					
Conductivity reading in dry, room-temperature air:					
Conductivity Calibration - done today?	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
Conductivity Calibration - date last completed on this meter (mm/dd/yyyy):					
Turbidity					
Turbidity Calibration - done today?	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
If calibrated: Date Turbidity standards last changed?(mm/dd/yyyy)					
Optical Dissolved Oxygen					
ODO Calibration - did you successfully complete it today?	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
Today's Post-Cal ODO%					
pH Calibration					
pH Calibration - did you successfully complete it today? (yes or no)	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
pH 7 mV reading was within -50 to +50 mV range? (MUST STOP IF OUT OF RANGE)	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
Date pH standards were last changed:					
Date pH standards must be changed again (2/week):					
Continuing Calibration Verifications (CCV)					
Did you log pH 7 CCV? (yes or no)	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
pH 7 CCV:					
Did you log ODO CCV *(and wait 5-15 minutes in ODO cup for saturation)* ? (yes or no)	o Yes o No	o Yes o No	o Yes o No	o Yes o No	o Yes o No
ODO CCV:					
Notes:					

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Massachusetts Alternative Septic System Test Center

Barnstable, Massachusetts

Form

Title: ProDSS End of Day Checklist

Effective Date: 2020-08-21	**Number:** MASSTC-FRM-034	**Revision:** 000

Authors

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ProDSS End of Day Checklist

Document ID#: MASSTC-FORM-034
Revision#: 000
Released: 2020-08-21
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Revision History

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Revision #000: Original Issue	2020-08-21

ProDSS End of Day Checklist

Document ID#: MASSTC-FORM-034
Revision#: 000
Released: 2020-08-21
Released By: Brian Baumgaertel

Date (mm/dd/yyyy):					
Time:					
Meter color:					
Initials:					
Rinse and check 7 pH?					
Cleaned DO cup (including **emptying and scrubbing, then refilling with water**)?					
Emptied and rinsed white tray?					
Emptied and rinsed pH discard/rinse cup?					
Cleaned conductivity probe using brush and clean water?					
Conductivity reading:					
Rise, gently pat dry, and check ODO?					
Wipe down screen and entire meter body?					
Wipe any dirt off of cord?					
Detangle cord?					
Cable hanging and off ground?					
Data has been dowloaded and saved on Sharepoint?					
(If not put on Sharepoint, why not?)					
Connected meter to power and charging?					
Calibration cup is sealed?					
Turned off meter?					
Notes					

When completed, please scan and place in: Sharepoint - MASSTC-Documents - Data - ProDSS Downloaded Data - End of Day Checklists.
Put the hard copy in the ProDSS Calibration binder in the lab

ProDSS End of Day Checklist

Document ID#: MASSTC-FORM-034
Revision#: 000
Released: 2020-08-21
Released By: Brian Baumgaertel

Date (mm/dd/yyyy):					
Time:					
Meter color:					
Initials:					
Rinse and check 7 pH?					
Cleaned DO cup (including **emptying and scrubbing, then refilling with water**)?					
Emptied and rinsed white tray?					
Emptied and rinsed pH discard/rinse cup?					
Cleaned conductivity probe using brush and clean water?					
Conductivity reading:					
Rise, gently pat dry, and check ODO?					
Wipe down screen and entire meter body?					
Wipe any dirt off of cord?					
Detangle cord?					
Cable hanging and off ground?					
Data has been dowloaded and saved on Sharepoint?					
(If not put on Sharepoint, why not?)					
Connected meter to power and charging?					
Calibration cup is sealed?					
Turned off meter?					
Notes					

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Put the hard copy in the ProDSS Calibration binder in the lab

Appendix 13

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Barnstable County Department of Health and the Environment Laboratory

SM 2320B

STANDARD OPERATING PROCEDURE

For

Determination of Alkalinity in Aqueous Samples

(Revision 010)

June 16, 2020

		Signature	Date
Analyst:	Liping Xun	Liping Xun	9/20/21
Laboratory Director:	Dan White		21Sep21

5.1 PC Titrate (Man-Tech), Model PC -1000-1040
5.2 Man-Tech Autosampler, Model PC-1306-475

5.3 pH Electrode, Man-Tech, Model PCE 80-PH1200C

5.4 50 mL plastic vials, Fisherbrand #14-955-240

5.5 Reference filling solution, Mantech 4M KCL, PCE-R001013

5.6 Rinse solution, 200mL of pH 4.00 (SB101-4), Fisher Scientific, fill to 1000mL with tap water.

6. REAGENTS AND STANDARDS

6.1 Reagent water – Deionized water from Milli-Q Direct 8/16 System or Millipore Direct-Q 3 System,

6.2 Buffer Solution pH 4.00 (Red), Fisher Scientific, # SB101-4
Buffer Solution pH 7.00 (Yellow), Fisher Scientific, # SB107-4
Buffer Solution pH 10.0 (Blue), Fisher Scientific, # SB 115-4
Buffer Solution pH 7.00 (Clear), Fisher Scientific, # SB 108-1

6.3 0.02N Sulfuric Acid, Fisher Scientific SA 226-1or make from 98% H2So4(Fisher, #300-212)
6.4 QC standard Sodium Carbonate, Fisher Scientific S495-500.

6 SAMPLE COLLECTION, PRESERVATION AND STORAGE

7.1 Samples are collected in 500 mL plastic bottles. All bottles must be thoroughly cleaned and rinsed with reagent water.

6.2 Samples are stored at 4°C until analysis but must at room temperature when analyzed. Do not open sample bottle before analysis.

6.3 Holding time is 14 days.

7 QUALITY CONTROL

8.1 A duplicate sample is to be included in any set of 10 samples. The agreement between the sample and the duplicate must be ± 20%. If it is outside these limits, a third sample is analyzed. If this is outside the limits, a QC check is run to check

the calibration of the instrument itself. If the instrument is found to be out of calibration, it is to be recalibrated and all analyzes are repeated.

8.2 A Quality Control sample is to be run at the beginning. After every 10 samples, and at the end of the run, a CCV is to be run. Use pH yellow buffer (pH 7). The QC value is 25mg/L. It must fall within 10% of the range. If it is not within the range, a second analysis is performed to verify the measured value. Upon repeat failure, the results from this analysis will be considered unacceptable and the complete procedure will be repeated after the instrument is recalibrated.

8.2 1 To make the QC take 1.0g of Sodium Carbonate dilute to 1000mL with reagent water.

8.2 2 Take 25mL of the stock Sodium Carbonate solution and dilute to 1000mL for the QC 25 mg/L alkalinity Solution.

8.3 A Method Blank is run immediately following every QC sample. For the Blank, take 40mL of reagent grade water and place it in a 50mL plastic vial, and put it on the autosampler.

8.4 Method Detection Limit (MDL)- An MDL is established using reagent water fortified at 10 mg/L alkalinity, seven replicate aliquots of the fortified reagent water are run and processed through the entire analytical method. Then the MDL is calculated as follows:

$$MDL = (t) \times (S_{n-1})$$

Where,
t = student's value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.
S_{n-1} = sample standard deviation (n-1) of the seven replicate analyses.

8.3.1 The laboratory conducts MDL study annually. The MDL's must be run over a period of at least 3 days.

8.5 Accuracy and Precision- 4 replicates of 25 mg/L alkalinity are prepared and analyzed. The Accuracy and Precision study must be run over a period of at least 3 days. The mean measured concentration ($C_{\bar{x}}$) of the replicate values is calculated as follows

$$C_{\bar{x}} = \frac{(C_1 + C_2 + C_3 + C_4)}{n}$$

Where,

$C_{\bar{x}}$ = Mean recovered concentration of the replicate analysis.

$C_1, C_2, \ldots C_n$= Recovered concentrations of the replicate 1,2, …n.

The value derived for $C_{\bar{x}}$ must be within ± 10% of the true value.

The percent relative standard deviation (%RSD) of the replicate analysis as stated above is calculated using the following equation.

$$\%RSD = \frac{(S_{n-1})x100}{(C_{\bar{x}})}$$

Where,

S_{n-1} = sample standard deviation (n-1) of the replicate analysis.
$C_{\bar{x}}$ = mean recovered concentration of the replicate analysis.

9 CALIBRATION

9.1 In a 50 mL sample vial, place 40 mL of each of the 3 buffers. In position #1 on The autosampler place the pH 4.00, in position #2, put in pH 7.00 (yellow), and in position #3, put in pH 10.0.

9.2 A Quality Control Sample (QCS) is analyzed right after the initial calibration for the calibration verification. The acceptance limits for the recovery of the QCS must fall within 90-110%.

9.3 Continuing Calibration Verification (CCV): The QCS is also used as a CCV. The CCV must be analyzed at the beginning of the sequence and at the end of each ten samples as a closing CCV. The acceptance limits for the recovery of the CCV must fall within 90-110%.

10. OPERATIONAL PROCEDURE

10.1 Double click on PC Titration-

10.1 1 Main Menu will show up, Click on RUN Titration. Double Click on line 1, a menu will come up highlight pH CAL 4-7-10 and hit OK.
Under Order Number, highlight and click on Autogenerate.
Under Sample Name, type in pH cal
Under Vial#, type in #1
Hit Save As and type in the date, hit OK

10.1 2 Load the autosampler, #1 position is pH 4.00, # 2 is pH 7.00, and #3

is pH 10.0.

10.1 3 Hit START

10.1 4 The PC Titrate will perform the calibration and will say pH Calibration has passed.

10.2 SAMPLE ANALYSIS

10.2 1 Go to Titrator Main Menu- select RUN TITRATION

10.2 2 Double click on lst line- all the options will appear- select ALK QC-OK

10.2 3 Move the curser to order#, hit AUTO GENERATE, move to sample name type in ID of sample or QC, move to vial # and type that number in.

10.2 4 Go back to lst line-rt click- hit add a row or add rows
Double click on that next line, select pH QC-OK, move to order #, auto Generate, sample name, vial #, Repeat these steps for all samples, Selecting the appropriate analysis –pH-Alk or Cond-pH-Alk options.

10.2 5 After all samples have been typed in and orders hit SAVE, type in current date, OK, then START.

NOTE: *There are two methods set up internally in the Man-Tech instrument: One is for "low" alkalinity lower than 20 mg/L (, and the other one for "regular" alkalinity equal to or above 20 mg/L. In the schedule itself there is a "linked" titration where it will run the method based on the titration, and all samples start in the "low" method and move to regular if they are above the 20 mg/L.*

11. DATA ANALYSIS, CALCULATIONS AND REPORTS

11.1 Calculation:

The PC Titrate automatically calculates the titration.

11.2 Reports

All data, including the date, Lab ID, Client ID, the concentration (mg/L), and analyst initials are placed in the 3ring binder PC Titrate logbook.

12. POLLUTION PREVENTION AND WASTE MANAGEMENT

12.1 The laboratory waste management practices are conducted consistent with all Applicable rules and regulations as stated in the laboratory's Sample and Waste Disposal (Revision 005) on December 15, 2017. Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner

in this SOP.

13. REFERENCES

1. Annual Book of ASTM Standards, Part 31, "Water", Standard D-1067, p13 Method B (1976)

2. American Public Health Association (APHA)
American Water Works Association (AWWA)
Water Environment Federation (WGI)
Standard Methods (SM) for the Examination of Water and Wastewater, 22nd edition SM2320B, 2012.

Barnstable County Department of Health and the
Environment Laboratory

EPA Method 350.1

STANDARD OPERATING PROCEDURE

For

Determination of Ammonia Nitrogen in Aqueous Samples by Semi-Automated Colorimetry Gas Diffusion Separation Method

(Revision 006)

March 5, 2019

		Signature	Date
Analyst:	Andrew Barker	Andrew L Barker	9/28/2021
Laboratory Director:	Dan White		28SEP21

STANDARD OPERATING PROCEDURE (SOP)

For

Determination of Ammonia Nitrogen in Aqueous Samples
by Semi-Automated Colorimetry
Gas Diffusion Separation Method
Salicylate Method

1.0 SCOPE AND APPLICATION

1.1 This SOP covers the determination of ammonia in drinking, ground, and surface waters, domestic and industrial wastes.

1.2 The applicable range is 0.10 – 20 mg/L NH_3 as N. The range may be extended with sample dilution.

2.0 SUMMARY OF METHOD

2.1 The sample containing ammonium is injected into a continuously flowing carrier stream by means of an injection valve, and mixed with a continuously flowing stream of an alkaline solution. The ammonia is separated from the matrix in a diffusion cell across a hydrophobic semi-permeable membrane and absorbed by a flowing acceptor stream. When ammonia in the acceptor is heated with salicylate and hypochlorite in an alkaline phosphate buffer an emerald green color is produced which is proportional to the ammonia concentration. The color is intensified by the addition is sodium nitroprusside. DCIC is used as the hypochlorite source in this method. Heat is used to aid ammonia from the donor in passing into the acceptor, in particular for the low ranges.

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 analyte.

3.2 Calibration Standard (CAL) – A solution prepared from the primary dilution standard or stock standard solutions.

3.3 Instrument Performance Check Solution (IPC) – A Solution of one or more method analytes or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4 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.

3.5 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.

3.6 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, and reagents 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.

3.7 Linear Calibration Range (LCR) – The concentration range over which the instrument response is linear.

3.8 Safety Data Sheets (SDS) [Used to be called as 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.

3.9 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.

3.10 Quality Control Sample (QCS) – A solution of method analytes of known concentrations that 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.

3.11 Stock Standard Solution (SSS) – 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 In alkaline solution, calcium and magnesium will interfere by forming a precipitate. EDTA is added to the Alkaline Donor to prevent this interference

4.2 Lauryl sulfate and detergents can cause low ammonia recoveries, by wetting the membrane.

4.3 Oil and grease will also wet the membrane.

5.0 SAFETY

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

5.2 Barnstable County Health Laboratory maintains a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. Reference files of Safety Data Sheets (SDS) are 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 SDS.

5.3.1 Sulfuric acid.

5.3.2 Sodium nitroprusside.

6.0 EQUIPMENT AND SUPPLIES

6.1 Balance – Analytical, capable of accurately weighing to the nearest 0.0001g (Fisher Scientific,Model ACCU-124D).

6.2 Glassware – Class A volumetric flasks and pipets as required.

6.3 Automated Continuous Flow Analysis Equipment – QuickChem 8500 Series 2 Flow Injection Analysis System (LACHAT Instruments, A Hach Company Brand)

6.3.1 LACHAT XYZ Autosampler.

7.0 REAGENTS, CHEMICALS AND STANDARDS

7.1 Sulfuric Acid (H_2SO_4), Fisher, Cat No. A300-212

7.2 Sodium Thiosulfate Pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$), Fisher, Cat No. RDC50930-500B1

7.3 Sodium Sulfite Anhydrous (Na_2SO_3), Fisher, Cat No. RDC50870-500B1

7.4 Sodium Tetraborate Decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), Fisher, Cat No. AA4011436

7.5 Sodium Hydroxide (NaOH), Fisher, Cat No. S613-3

7.6 Sodium Hypochlorite (NaClO), Fisher, Cat No. 19-546-929

7.7 Disodium EDTA, (Ethylenediamine Tetraacetic Acid Dihydrate) ($Na_2EDTA \cdot H_2O$), Fisher, Cat No. BP120500

7.8 Sodium Nitroprusside (Sodium Nitroferricyanide Dihydrate) [$Na_2Fe(CN)_5NO \cdot H_2O$], Fisher, Cat No. AC21164-1000

7.9 Ammonium Chloride (NH_4Cl), Fisher, Cat No. AC199975000 and A661-3

7.10 Dichloroisocyanuric (DCIC) Acid Sodium Salt ($C_3Cl_2N_3NaO_3$), Fisher, Cat No. AAB2350436

7.11 **Reagent Water**: Ammonia free deionized water produced from Millipore Milli-Q Water Purification System.

7.12 **Degassing with Helium**:

7.12.1 To prevent bubble formation, degas the carrier and buffer with helium. Use He at 140 kPa (20 lb/in2) through a helium degassing tube. Bubble helium through one liter of solution for one minute.

7.12.2 All reagents used in heated chemistry must be degassed.

7.13 **Reagent 1: Alkaline Donor**

In a 1 L volumetric flask, add approximately 800 mL reagent water and 30.0 g ethylenediaminetetraacetic acid, disodium salt (EDTA)
Mix with a magnetic stirrer. Add 12.4 g boric acid. While mixing, add 40 g of sodium hydroxide (NaOH). Dilute to the mark with with reagent water. Degas this solution with helium. The pH of this solution will be approximately 13. This solution is stable for one month.

7.15 **Reagent 2: Buffer**

In a 2 L volumetric flask containing about 1 L reagent water, dissolve 30.0 g sodium hydroxide (NaOH), 25.0 g EDTA, and 67 g sodium phosphate dibasic heptahydrate $Na_2HPO_4 \cdot 7H_2O$ in about 900 mL reagent water. Dilute to the mark with reagent water and invert three times

7.16 Reagent 3: Salicylate Nitroprusside Color Reagent

In a 1 L volumetric flask, dissolve 350 g sodium salicylate $C_6H_4(OH)(COO)Na$ and 3.5 g sodium nitroprusside $Na_2Fe(CN)_5NO \cdot 2H_2O$ in about 800 mL reagent water. Dilute to the mark and invert and mix. Store in a light proof bottle.

7.17 Reagent 4: DCIC Reagent (Hypochlorite generator)

In a 500 mL volumetric flask, dissolve 2.5 g of sodium hydroxide NaOH and 2.5 g sodium dichloroisocyanurate dihydrate, in about 300 mL reagent water stir and bring to volume, this reagent may be degassed.

7.18 Reagent 5: Carrier / Diluent for Preserved Samples (Ammonia)

To a 2 L volumetric flask containing about 1 L reagent water, dilute 4 mL concentrated sulfuric acid H_2SO_4. Dilute to the mark with reagent water. This solution is used as the diluent for standards and over-range samples.

7.19 Calibration Standards

7.19.1 Standard 1 (S1): Stock Standard: 1000 mg/L

In a 1.0 L volumetric flask, dissolve 3.819 ammonia chloride (NH_4Cl) that has been dried for two hours at 110°C in about 800 mL reagent water. Dilute to the mark with reagent water and invert to mix.

7.19.2 Standard 2 (S2): Intermediate Stock Standard: 20.0 mg N/L in 0.04N H_2SO_4

In a 1 Liter volumetric flask, add 20.0 mL of the stock standard (**Standard 1**) to approximately 900 mL reagent water and then 1.099 mL of concentrated sulfuric acid. Dilute the mark with reagent water, and invert to mix.

7.19.3 Calibration Standards: Using Standard 1 (S1) and 2 (S2) (Section 7.20.1, Section 7.20.2) to have the autodilutor prepare the series of standards, as shown below, covering the desired range and a blank

by diluting suitable volumes of standard solution with Reagent 5, all done through the autodilutor (Section 7.18).

Initial Calibration Standard (ICS)	Concentration (mg/L)	Auto Dilution Factor
Level 1	20	1
Level 2	10	2
Level 3	5	4
Level 4	1.0	20
Level 5	0.25	80
Level 6	0	

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples are collected in disposable plastic. Volume collected must be sufficient to insure a representative sample, allow for replicate analysis, and minimize waste disposal.

8.2 Samples must be preserved with H_2SO_4 to a pH<2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

9.0 QUALITY CONTROL

9.1 Barnstable County Health Laboratory operates a formal quality control (QC) program. The QC program for this method consists of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks and other laboratory solutions as a continuing check on performance. The laboratory maintains performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges 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 uses a blank and five calibration standards. If any verification data exceeds the initial values by ±10%, linearity will be reestablished.

9.2.3 Quality Control Sample (QCS) – The QCS is analyzed right after initial calibration (Section 9.2.2) to verify the calibration standards and acceptable instrument performance with preparation and analysis 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 MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) – MDL must be established using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, seven replicate aliquots of the fortified reagent water are taken, processed and analyzed over a period of a minimum of three days. The spiking level is 0.10 mg/L which is the same as Level 6 in Section 7.19.3. The following equation is used to calculate the MDL:

$$MDL = (t) \times (S) \qquad (1)$$

Where

t = Student's value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, and if more replicates are used, use the corresponding t-value].

S = Standard deviation of the replicate analyses.

9.2.4.1 The Standard deviation (S) can be calculated using the following equation:

$$S = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}} \qquad (2)$$

Where, n = number of samples;
x = concentration in each sample.

9.2.4.2 MDLs must be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.2.4.3 One set of MDLs is listed as follows:

Spiking Level =0.10 mg/L							Unit: mg/L			
	MDL01	MDL02	MDL03	MDL04	MDL05	MDL06	MDL07	MEAN	STDEV	MDL
DATE	10/4/2017	10/4/2017	10/4/2017	10/11/2017	10/13/2017	10/13/2017	10/13/2017			
Conc	0.108	0.084	0.102	0.135	0.077	0.087	0.145	0.1054	0.026	0.0818

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 must 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 as follows:

$$R = \frac{C_s - C}{S} \times 100 \qquad (3)$$

Where,
R = percent recovery;
Cs = recovered fortified blank concentration;
C = blank background concentration;
S = concentration equivalent of analyte added to blank.

9.3.2.1 If the recovery of any analyte falls outside the required control limits of 90-110%, the result is judged out of control, and the source of the problem must be identified and resolved before continuing analysis.

9.3.3 The laboratory also uses 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 25 analyses), optional control limits and control charts 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 must be used to establish an on-going precision statement for the level of concentration included in the LFB. These data are kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC) – For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every 10th 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, the IPC solution is reanalyzed. If the second analysis of the IPC solution confirms calibration to be outside the limits, the 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 analysis data of the calibration blank and IPC solution are kept on file with the sample analysis data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM)

9.4.1.1 The laboratory adds a known amount of analyte to a minimum of 20% of the routine samples. In each case the LFM aliquot must be 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.

9.4.1.2 The percent recovery for ammonia is calculated and corrected for concentration measured in the unfortified sample using the following equation:

$$R = \frac{C_s - C}{S} \times 100 \qquad (4)$$

Where, R = percent recovery;
Cs = fortified sample concentration;
C = sample background concentration;

S = concentration equivalent of analyte added to sample.

Acceptable range of R is 90-110%.

9.4.1.3 If the recovery falls outside the designated LFM recovery range (90-110%) and the laboratory performance is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be matrix related, not system related.

9.4.2 Laboratory Duplicate Sample

9.4.2.1 Duplicate samples are analyzed to demonstrate the precision of an analytical system. The duplicate analyses are performed on each batch of samples analyzed at a frequency of 10% of all samples in the batch or at least one sample if less than 10 samples are analyzed.

9.4.2.2 Relative Percent Difference (RPD): The relative percent difference is used to evaluate precision for the duplicate analyses, and RPD is calculated as follows:

$$RPD\ (\%) = \frac{|C_1 - C_2|}{C_{AVG}} \times 100 \qquad (5)$$

Where: C_1 = original sample concentration;
C_2 = duplicate sample concentration;
C_{AVG} = average of the two samples.

9.4.2.3 RPD Acceptable Limits: Acceptable limits of RPD for ammonia as nitrogen are ≤10%. If the recovery falls outside the designated duplicate recovery range and the laboratory performance is shown to be in control (Section 9.3), the recovery problem encountered with the duplicate analysis is judged to be matrix related, not system related.

10 CALIBRATION AND STANDARDIZATION

10.1 Prepare reagent and standards as described in Section 7.

10.2 Set up the Ammonia manifold as shown in Section 17.4 (Ammonia) of the Lachat Instruments Methods Manual (Section 14.2)

10.3 Input data system parameters as shown in Section 17.1 (Ammonia) of the Lachat Instruments Methods Manual (Section 14.2.) Also, see figure 2.

attached here at the end for Data System parameters and figure 3. for the manifold diagram.

10.4 Pump reagent water through all reagent lines and check for leaks and smooth flow. In order to avoid precipitate forming in the manifold tubing: Add the **Buffer Line First** and allow to pump through manifold for at least 5 minutes. Then the Carrier and other reagent lines one by one, ending with the nitroprusside added last. For removal after analysis, reverse this order with the nitroprusside line disconnected first, and the buffer line last. When finished, place all respective reagent lines into water and allow to pump through manifold for ten minutes.

10.5 Place standards in the sampler and sequence the required information in the data system.

10.6 Calibrate the instrument by injecting the standards. The system will then associate the concentrations with the peak area for each standard to determine the calibration curve.

10.7 The initial calibration is deemed acceptable if the following criteria are met:

10.7.1 $R \geq 0.995$

10.7.2 Quality Control Sample (QCS) standard is run right after the initial calibration. The concentration of the QCS is 5.0 mg/L. This standard (Ammonium chloride) is ordered from Fisher Scientific, Acros Organics, ACS reagent grade. The procedure for making the QCS is similar to the one for ICS 2 of the calibration standards described in (Section 7.20.1, 7.20.2), but having a final concentration of 5.0 mg/L The QCS concentration must fall within ± 10% of the stated value.

10.7.3 Instrument Performance Check (IPC) refer to (Section 9.3.4).

10.8 Figure 1. Lists a set of initial calibration peaks and a linear calibration curve

11 PROCEDURE

11.1 FLOW INJECTION SYSTEM START-UP PROCEDURE

11.1.1 Prepare reagents and standards as described in section 7.

11.1.2 Set up manifold as shown in Section 17.4 of the Lachat Instruments Methods Manual.

11.2.3 Input peak timing and integration window parameters as specified in section 17.1 of the Lachat Instruments Methods Manual.

11.2.4 Pump reagent water through all the reagent lines and check for leaks and smooth flow. Switch to reagent lines - add buffer first and pump through the system for 5 minutes, followed by the other reagents, adding salicylate nitroprusside last – and allow the system to equilibrate until a stable baseline is achieved.

11.2.5 Place the standards in the autosampler, and fill the sample tray. Input the information required by the data system, such as concentration, replicates and QC scheme.

11.2.6 Calibrate the instrument by injecting the standards with the autodilutor. The data system will then associate the concentrations with responses for each standard.

11.2.7 After a stable baseline has been obtained, start the sampler and perform the analysis.

11.3 ANALYTICAL SEQUENCE

Please see Table 1 for analytical sequence.

11.4 TROUBLESHOOTING AND SYSTEM NOTES

11.4.1 Allow at least 15 minutes for the heating unit to warm up to 60°C.

11.4.2 If phosphorus is also determined with the Lachat System, a second helium degassing tube should be used and segregated for the individual chemistries.

11.4.3 If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:

11.4.3.1 Place transmission lines in water and pump to clear reagents first.

11.4.3.2 Place reagent lines in 1M HCl and pump for several minutes

11.4.3.3 Place all lines back into water and pump out HCl.

12 POLLUTION PREVENTION

12.1 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 the laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice.

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

13 WASTE MANAGEMENT

13.1 The laboratory waste management practices are conducted consistent with all applicable rules and regulations as stated in the laboratory's Sample and Waste Disposal (Revision 001) on February 25, 2004. Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner in this SOP.

14 REFERENCES

14.1 U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1993, Method 350.1

14.2 Lachat Instruments Methods Manual, QuikChem Method 10-107-06-5-J Rev 2.0, Revision Date, 16 January 2015.

Figure 1.

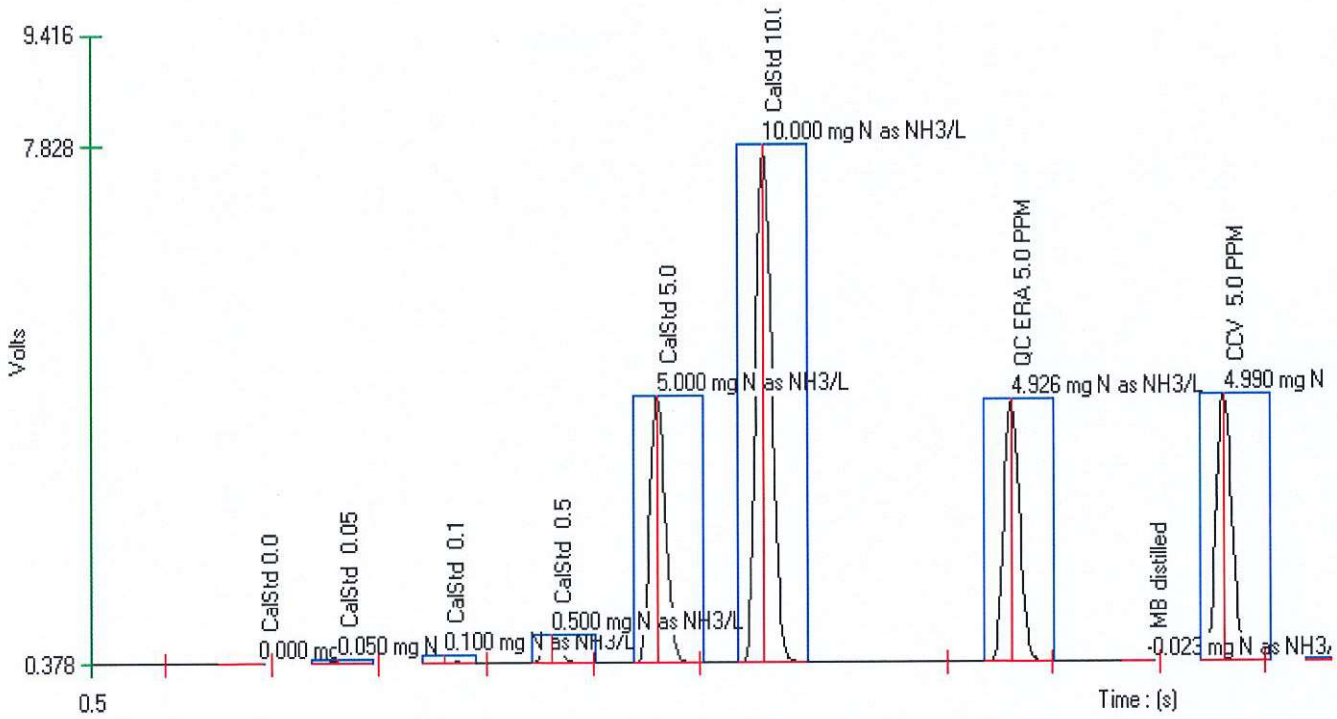
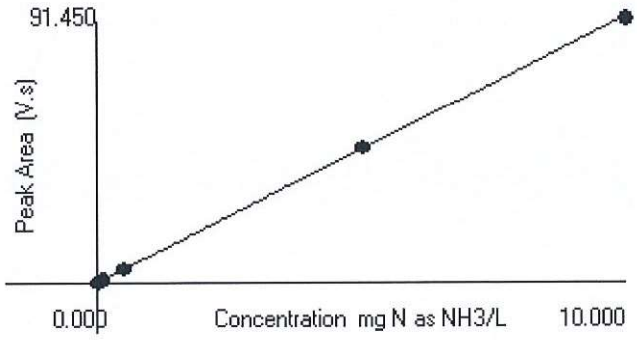


Figure : 1 (Ammonia)



Area = 9.205 * Conc + 0.207
Conc = 0.109 * Area - 0.022
Correlation Coefficient (r) = 0.99998
Weighting : 1/x

Figure 2.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. DATA SYSTEM PARAMETERS FOR THE QUIKCHEM 8000/8500 FOR AMMONIA

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput: (low):	36 samples/h, 110 s/sample
Sample Throughput (high)	45 samples per hour, 80s/sample
Pump Speed:	35
Cycle Period Low:	110s
Cycle Period High:	80s

Analyte Data:

Concentration Units:	mg N/L or µg N/L
Chemistry High Range:	Direct/Bipolar
Peak Base Width:	
Inject to Peak Start:	
Chemistry Low Range:	Brackish
Inject to peak start:	81s
Peak base width:	85s
Brackish shutter offset:	21.4
Brackish shutter width:	30.5

Calibration Data:

Low Range Ammonia

Level	1	2	3	4	5	6	7	8
Concentration µg N/L as NH_3	1000	500	250	100	50	25	10	0.0

High Range Ammonia

Level	1	2	3	4	5	6	7	8	9
Concentration mg N/L as NH_3	20	10	5	2.5	1	0.5	0.25	0.1	0.00

Calibration Rep Handling:	Average
Calibration Fit Type:	2nd order Polynomial
Weighting Method:	1/x
Force through zero:	No

Sampler Timing:

Min. Probe in Wash Period:	5 s
Probe in Sample Period:	39 s

Valve Timing:

Load Period:	32 s
Inject Period:	20 s

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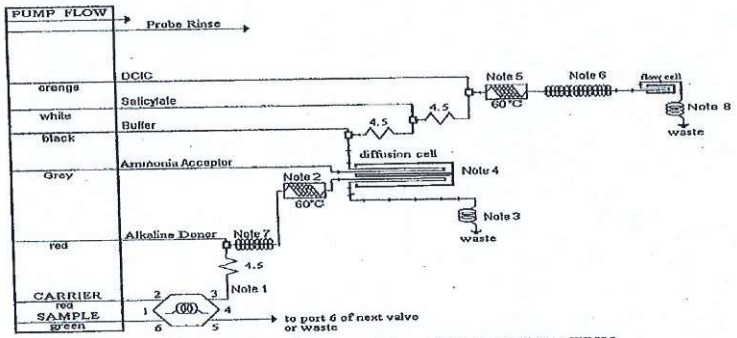
Figure 3.

17.4. AMMONIA MANIFOLD DIAGRAM (REV. 2.0)



Carrier: DI water for ammonia or Reagent 5 for TKN
Acceptor: DI water
Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2 µL/cm.
QC8000/8500 Sample Loop: 350 cm Low Range Ammonia
80 cm High Range Ammonia and TKN
Interference Filter: 660 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The ⊣▨⊢ shows 175 or 650 cm of tubing wrapped around the heater block at the specified temperature.

4.5: 70 cm of tubing on a 4.5 cm coil support
⊣▦⊢ tubing wrapped in a figure 8 around 2, 7 or 22 cm coil supports (see below)
PVC PUMP TUBES MUST BE USED WITH THIS METHOD.

Note 1: 30 cm of manifold tubing is used to connect Port 3 to the first tee.
Note 2: 175 cm of tubing on the heater
Note 3 400 cm of 0.022" i.d. tubing backpressure loop, then to waste. **Waste container for the diffusion block should be on the bench with the instrument.** Placing this on the floor can lead to poor precision due to cavitation/vapor lock.
Note 4: Diffusion block with membrane PN 5033101-10 (Pack of 10 3.5 x 8.6 mm). Flow is **concurrent.** Be sure to place a membrane in the block before beginning. **Run**

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Table 1. A Typical Analytical Sequence with Quality Control Requirements

Injection #	Description of Quality Control Standards and Samples	Acceptance Criteria
1	Level 1 (20 mg/L) of Initial Calibration	R ≥ 0.995
2	Level 2 (10 mg/L) of Initial Calibration	
3	Level 3 (5.0 mg/L) of Initial Calibration	
4	Level 4 (1.0 mg/L) of Initial Calibration	
5	Level 5 (0.25 mg/L) of Initial Calibration	
6	Level 6 (0 mg/L) of Initial Calibration	
7	QCS at 5.0 mg/L	90-110%
8	Blank	≤ 0.10 mg/L
9	CCV at 5.0 mg/L	90-110%
10	MB	≤ 0.10 mg/L
11	LFB at 5.0 mg/L	90-110%
12	Sample 1	
13	Sample 1 – Laboratory Duplicate	≤ 10%
14	Sample 1 - Matrix Spike	90% – 110%
15	Sample 2	
16	Sample 3	
17	Sample 4	
18	Sample 5	
19	Sample 6	
20	Sample 7	
21	Sample 8	
22	Sample 9	
23	Sample 10	
24	Blank	
25	CCV	90-110%
26	MB	≤ 0.10 mg/L
28-35	Sample 11	
36	Sample 11 - Duplicate	≤ 20%
37	Sample 11 - Matrix Spike	90% – 110%
38	Sample 12 to Sample 20	
39	Blank	≤ 0.125 mg/L
40	CCV at 5.0 mg/L	90-110%
41	MB	≤ 0.125 mg/L
42	LFB at 5.0 mg/L	90-110%

Barnstable County Department of Health and the Environment Laboratory

EPA Method 300.0

STANDARD OPERATING PROCEDURE

For

Determination of Inorganic Anions in Aqueous Samples Using Ion Chromatography

Revision 015

May 11, 2018

		Signature	Date
Analysts:	**Chris Long**	Chris Troyer Chris Long	9/21/2021
Laboratory Director:	**Dan White**		21 Sept 21

Standard Operation Procedure for the Determination of Inorganic Anions in Aqueous Samples Using Ion Chromatography

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of the following inorganic anions in reagent waters, mixed domestic and industrial wastewaters, surface water, ground water, solids, leachates (when no acetic acid is used) and finished drinking water using ion chromatography.

Bromide, Chloride, Fluoride, Nitrate-N, Nitrite-N, ortho-Phosphate-P, Sulfate

1.2 This laboratory's Method Detection Limit (Section 8.1.4.) for the above analytes is listed in Table 1.

1.3 Whenever this method is used to analyze unfamiliar samples for any of the above listed anions, anion identification is supported by the use of a fortified sample matrix covering the anions of interest. The fortification procedure is described in Section 8.2.3.2.

2.0 SUMMARY OF METHOD

2.1 A 1.0 or 5.0 mL volume of sample is introduced into an ion chromatograph (IC). The anions of interest are separated and measured, using a system comprised of an ion chromatographic pump, sample injection valve, guard column, suppressor device, and a conductivity detector.

2.2 This method may be modified for limited performance-based attributes provided that they documented and meet the requirements expressed in the Quality Control Section (Section 8.0)

3.0 INTERFERENCES

3.1 Interferences can be caused by substances with retention times that are similar to and overlap those of the anions 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.

3.2 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 ion chromatograms.

3.3 Any anion that is not retained by the column or slightly retained will elute in the area of fluoride and interfere. Known co-elution 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. (Section 8.0, Quality Control.)

3.4 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. This method is not recommended for leachates of solid samples when acetic acid is used for pH adjustment.

3.5 The quantitation of un-retained peaks should be avoided, such as low molecular weight organic acids (formate, acetate, propionate etc.) which are conductive and co-elute with or near fluoride and would bias the fluoride quantitation in some drinking and most waste waters.

3.6 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 a minimum of 5 minutes until no chlorine dioxide remains.

4.0 SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method have 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 specifically listed below in Section 4.3 for hazardous materials.

4.2 The laboratory is 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) is available to all personnel involved in the analysis.

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

4.3.1 Sulfuric Acid (H_2SO_4), if and when used in preparation of the suppressant and when used a preservative. Protective eyeware, clothing and gloves should be worn when handling.

EQUIPMENT AND SUPPLIES

5.1 Ion chromatograph (IC): Dionex (Model ICS-2000; S/N 04020527)

This analytical system is complete with an EluGen II Potassium Hydroxide (KOH) Cartridge, an deionized (DI) water bottle with high purity of DI water (Resistivity >18.0 megohm-cm), an AS40 Automated Sampler, an ion chromatographic pump, injection valves, both guard and analytical separator columns, column heater, chemical suppressor, conductivity detector, and computer based data acquisition and process called CHROMELEON system (Dionex). Ion chromatograph

5.1.1 Anion guard columns – Dionex IonPac AG19, 2×50 mm (P/N 062888**).** These guard columns function as a protector of the separator columns and packed with a substrate identical as that used in the corresponding separator column.

5.1.2 Anion separator columns. - Dionex IonPac AS19 Analytical column, 2×250 mm (P/N 062886). using the conditions outlined in Table 2.

5.1.3 Anion suppressor device – Dionex AERS 500 self-regenerating chemical suppressor (P/N 0082541). This built-in control for electrolytic Auto Suppressor eliminates the need to hand-prepare the acidic regenerant. Adequate baseline stability is attained as measured by a combined baseline drift/noise of no more than 0.5 nS per minute over the background conductivity.

5.1.4 Detector – Dionex Conductivity DS3 Detector.

5.1.5 ASDV Automated Sampler

5.1.6 AutoSampler Sample Vials – 0.5 ml vials equipped with filter caps (Dionex P/N 038010**)** or 5.0 ml vials equipped with filters **(**Dionex P/N 038141**)**

5.2. Data Acquisition System – The Dionex PEAKNET Data Chromatography Software was use to collect and generate all the data.

5.3 Analytical balance (±0.1 mg) – Fisher Scientific (Model ACCU-124D).

5.4 Top loading balance (±10 mg) – OHAUS (Model Scout II).

5.5 Syringes – Glass graduated syringes: 25 µL, 50 µL, 100 µL, 500 µL, 1000 µL.

5.6 Volumetric Pipets; Class A, 2, 4, 5 mL, 10 mL, 25 mL and 50 mL.

5.7 Eppendorf pipettor and tips

5.8 Volumetric Flasks – Class A, various volumes for preparing standards.

5.9 Sampling Containers – Glass or polyethylene, either purchased pre-cleaned or prepared in the laboratory. The containers should be of sufficient volumes to allow replicate analyses of anions of interest.

5.10 Water purification system (E-pure System) – Barnstead International (Model D4641 120 VAC).

5.11 Compressed Nitrogen Gas

5.12 Concentrated HCL – for glassware preparation in the use for oPhosphate-P analysis

6.0 REAGENTS AND STANDARDS

6.1 Reagent water – Deionized water from Direct QUV (Millipore Cat # ZRQSVP030): 18.0 Mohm or better. Or an equivalent Water Purification system.

6.2 Eluant – Dionex Eluent Generator Cartridge – Potassium Hydroxide (EGC III KOH) - Cat# 074532

6.3 Stock Standard Solutions
Stock standard solutions for the preparation of calibration standards, matrix spike solutions, LFB, QCS, are either purchased as certified solutions or prepared from ACS reagent grade materials as listed below.

6.3.1 Primary Source Stock standard solutions
The Primary source stock standard solutions are used for the preparation of calibration standards and are purchased from Inorganic Ventures as listed below:

	ppm	Catalogue #
Bromide (Br^-)	1000	ICBR1-1
Chloride (Cl-)	10,000	ICCL10
Fluoride (F^-)	1000	ICFL-1
Nitrate as Nitrogen (NO_3-N)	1000	ICNNO31-1
Nitrite as Nitrogen (NO_2-N)	1000	ICNNO21-1
Phosphate as Phosphorus ($PO^{=}_4$-P)	1000	ICPPO41-1
Sulfate ($SO_4^{=}$)	10,000	ICSO410

6.3.2 Secondary Source Stock standard solutions
These secondary source stock standard solutions are used for preparing the quality control check solutions (QCS). Any secondary stock solution chosen to be used for the QCS must be from a different manufacturing source or lot number that is being used as a primary source.
These secondary source standards are either purchased as certified solutions or prepared from ACS reagent grade materials as listed below:

6.3.2.1 Purchased Secondary stock standard solutions are used for the preparation of calibration standards and are purchased from UltraScientific as listed below:

	ppm	Catalogue #
Bromide (Br^-)	1000	ICC-001 (100ml)
Chloride (Cl-)	1000	ICC-002 (100ml)
Fluoride (F^-)	1000	ICC-003 (100ml)
Nitrate as Nitrogen (NO_3-N)	1000	ICC-004A (100 ml)
Nitrite as Nitrogen (NO_2-N)	1000	ICC-007A (100ml)
Phosphate as Phosphorus ($PO^{=}_4$-P)	1000	ICC-005A (100ml)
Sulfate ($SO_4^{=}$)	1000	ICC-006 (100ml)

6.4 Once standards are purchased and received, or prepared, they are logged in the Primary Standard Logbook with date of receipt, name of vendor, catalog number, expiration date and a primary standard ID assigned. Purchased chemicals with Certificate of Analyses provided by the vendor will have the laboratory assigned primary standard ID, date and the receiving analyst initials. The bottle will also be identified with primary standard ID and the date received and the analyst initials.

An example of the Logbook is attached (Figure 1).

Primary standard ID is labeled as IP mmddyy X:

Where: IP = Inorganic Primary

mmddyy = the date the standard is received

X = the order that the standard is logged into the logbook on that date in increasing alphabetical order.

6.5 Preparation of Calibration Standards – For each analyte of interest, intermediate calibration standards are prepared by first adding measured volumes of one or more stock standards (Section 6.3.1.) to volumetric flasks and diluting to volume with reagent water. These intermediate calibration standards are then further used to prepare the daily working calibration standards. This laboratory separates the calibrations into the following analytes to be determined within a sample run.

Note : Dilute working standards should be prepared weekly, except those that contain nitrite and phosphate should be prepared daily.

6.5.1 Fluoride, Chloride, NitriteN, NitrateN & Sulfate - Combined

6.5.1.1 Intermediate Calibration Standard –
Fluoride, Chloride, NitriteN, NitrateN & Sulfate - Combined

Combine the following aliquots of stock solutions are combined together in a 500 ml volumetric flask. Stable for 1 month.

a 6.25 mL of 1000 ppm Fluoride stock standard (Section 6.3.1.) to yield 12.5 mg/L Fluoride.

b. 12.5 mL of 10,000 ppm Chloride stock standard (Section 6.3.1.) to yield 250 mg/L Chloride.

c. 6.25 mL of 1000 ppm Nitrite-N stock standard (Section 6.3.1.) to yield 12.5 mg/L Nitrite as Nitrogen.

d. 12.5 mL of 1000 ppm Nitrate-N stock standard (Section 6.3.1.) to yield 25 mg/L Nitrate as Nitrogen.

e. 12.5 mL of 10,000 ppm Sulfate stock standard (Section 6.3.1.) to yield 250 mg/L Chloride.

6.5.1.2 Working Calibration Standards – a minimum of 6 levels are needed for construction a curve. Prepared Daily.

There are six concentration levels for the calibration curve for F, Cl, NO2-N, NO3-N, SO4 as follows :

ppm	Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate
Level 1	0.050	1.00	0.05	0.10	1.00
Level 2	0.10	2.00	0.10	0.20	2.00
Level 3	0.50	10.0	0.50	1.00	10.0
Level 4	1.25	25.0	1.25	2.50	25.0
Level 5	2.50	50.0	2.50	5.00	50.0
Level 6	5.00	100	5.00	10.0	100

The working standards are prepared via dilutions starting with the combined Cl, NO2-N, NO3-N, SO4 Combined Intermediate Standard (Section 6.5.1.1.)

Level 6 – 20.0 ml of Intermediate Calibration Combined Standard to 50 mL

Level 5 – 10 ml of Intermediate Calibration Combined Standard to 50 mL

Level 4 – 5.0 ml of Intermediate Calibration Combined Standard to 50 mL

Level 3 – 2.0 ml of Intermediate Calibration Combined Standard to 50 mL

Level 2 – 10 ml of Level 3 to 50 ml

Level 1 – 5.0 ml of Level 3 to 50 ml

6.5.2 o-PO4-P

6.5.2.1 Intermediate Calibration Standard.
5 mL of 1000 ppm oPO_4-P stock standard (Section 6.3.1) in a 500 ml volumetric flask to yield 10.0 mg/L oPO_4-P.

6.5.2.2 Working Calibration Standards – Prepared Daily
There are six concentration levels for the calibration curve for oPO_4-P as follows :

	oPO_4-P (ppm)
Level 1	0.05
Level 2	0.10
Level 3	0.25
Level 4	0.50
Level 5	1.00
Level 6	2.50

The working standards are prepared via serial dilutions starting with the oPO_4-P Intermediate Standard.

Level 6 - 25 ml of Intermediate Standard to 100 ml
Level 5 – 5.0 ml of Intermediate Standard to 50 ml
Level 4 – 5.0 ml of Intermediate Standard to 100 ml
Level 3 - 5.0 ml of Level 6 to 50 ml
Level 2 – 5.0 ml of Level 5 to 50 ml
Level 1 – 5.0 ml of Level 4 to 50 ml

6.5.3 Bromide

6.5.3.1 Intermediate Calibration Standard -
10 mL of 1000 ppm Bromide stock standard (Section 6.3.1.) in a 100 ml volumetric flask to yield 100 mg/L. Bromide

6.5.3.2 Working Calibration Standards

There are seven concentration levels for the calibration curve for Bromide as follows (with Level 1 as the reporting limit concentration). Prepared Daily.

	Bromide (ppm)
Level 1	1.0
Level 2	2.5
Level 3	5.0
Level 4	10.0
Level 5	25.0
Level 6	50.0
Level 7	100.0 (Intermediate if High Concentrations is expected)

The working standards are prepared via serial dilutions starting Intermediate Standard.

Level 7 – Intermediate Calibration Standard (if high concentration is expected)
Level 6 – 25.0 ml of Intermediate Standard to 50 ml
Level 5 – 25.0 ml of Intermediate Standard to 100 ml
Level 4 – 10.0 ml of Intermediate Standard to 100 ml
Level 3 – 5.0 ml of Intermediate Standard to 100 ml
Level 2 – 2.5 ml of Intermediate Standard to 100 ml
Level 1 – 1.0 ml of Intermediate Standard to 100 ml

6.6 After the working calibration standards are made they are logged in the Working Standard Logbook with date of preparation, initial concentration, amount taken, final volume, final concentration, solvent used, expiration date , analysts initials and assigned an Working Standard ID (see Figure 2).

Working standard ID is labeled as IW mmddyy X:

Where: IW = Inorganic Working

Mmddyy = the date the standard is made.

X = the order that the standard is made on that date in increasing alphabetical order.

6.7 Preparation of Quality Control Check (QCS) solutions
These quality control check solutions are prepared using the secondary source stock standard solutions (Section 6.3.2) to verify new calibration curves and continual verification on a quarterly basis.

6.7.1 For Fluoride, Chloride, NitriteN, NitrateN & Sulfate QCS

6.7.1.1 Fluoride, Chloride, NitriteN, NitrateN & Sulfate - Combined

Using the Secondary Stock Standards, Refer to Section 6.5.1.1. for the preparation of the Combined Intermediate Standard for F, Cl, NO2N, NO3N & SO4

6.7.1.2. Using this Intermediate Combined Standatd, follow the same preparation procedure as outlined in Section 6.5.1.2. for the preparation of the following Level 5 Concentrations :

ppm	Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate
Level 5	2.50	50.0	2.50	5.00	50.0

6.7.2. For o-PO4-P QCS

6.7.2.1. Intermediate oPO_4-P Standard
Using the Secondary Stock Standard, refer to Section 6.5.2.1. for the preparation of the Intermediate Standard for oPO_4-P

6.7.2.2. From this intermediate standard the QCS is prepared by pipetting a 5 mL aliquot into a 50 mL volumetric flask and diluted to the mark with deionized water to yield a 1.0 ppm solution

6.7.3. For Bromide QCS

6.7.3.1. Intermediate Br Standard
Using the Secondary Stock Standard, refer to Section 6.5.3.1. for the preparation of the Intermediate Standard for Bromide

6.7.3.2. From this intermediate standard the QCS is prepared by pipetting 25 mL into in a 100 ml volumetric flask to yield 25 mg/L.

6.8. Preparation of Instrument Performance Check Solution (IPC)
These quality control check solutions are prepared using standards solutions (Section 6.5) at the mid-range concentrations of the calibration curve and is used to verify the curve on an on-going basis during the sample sequence run.

6.8.1. For Fluoride, Chloride, NitriteN, NitrateN & Sulfate IPC

6.8.1.1. Using the Fluoride, Chloride, NitriteN, NitrateN & Sulfate - Combined Intermediate Standard prepared in Section 6.5.1.1. ; Prepare the Level 5 concentration as outlined in Section 6.5.1.2. to yield the following concentrations:

ppm	Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate
Level 5	2.50	50.0	2.50	5.00	50.0

6.8.2. For oPO4-P IPC

6.8.2.1. Using the oPO4-P Intermediate Standard prepared in Section 6.5.2.1. ; Prepare the Level 5 concentration as outlined in Section 6.5.2.2. to yield a 1.0 ppm concentration.

6.8.3. For Bromide IPC

6.8.3.1. Using the Bromide Intermediate Standard prepared in Section 6.5.3.1. ; Prepare the Level 4 concentration as outlined in Section 6.5.3.2. to yield a 25 ppm concentration.

6.9 Preparation of Laboratory Fortified Blanks (LFB)

6.9.1. For Fluoride, Chloride, NitriteN, NitrateN & Sulfate LFB

Using the Fluoride, Chloride, NitriteN, NitrateN & Sulfate - Combined Intermediate Standard prepared in Section 6.5.1.1. ; Prepare the Level 4 concentration as outlined in Section 6.5.1.2. to yield the following concentrations:

ppm	Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate
Level 4	1.25	25.0	1.25	2.50	25.0

6.9.2 For o-PO4-P LFB

Using the Intermediate Calibration Standard (Section 6.5.2.1.) pipette 5 ml into a 200 mL volumetric flask and dilute to the mark with deionized water. This yields a 0.5 ppm LFB solution.

6.9.3 For Bromide LFB
Using the Intermediate Calibration Standard (Section 6.5.3.1.) pipette 25 ml into a 100 mL volumetric flask and dilute to the mark with deionized water. This yields a 25 ppm LFB solution

6.10 Preparation of Matrix Spike (MS) solution used for fortifying samples

6.10.1. Fluoride, Chloride, Nitrite-N, Nitrate-N, Sulfate - Combined MS

6.10.1.1. Using the Fluoride, Chloride, NitriteN, NitrateN & Sulfate - Combined Intermediate Standard prepared in Section 6.5.1.1. ; Prepare the Level 5 concentration as outlined in Section 6.5.1.2. to yield the following concentrations:

ppm	Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate
Level 5	2.50	50	2.5	5.0	50

6.10.2. o-PO4-P
Using the Intermediate Calibration Standard (Section 6.5.2.1.) pipette 1 ml into a 100 mL volumetric flask and dilute to the mark with deionized water. This yields a 1.0 ppm matrix spike solution

6.10.3. Bromide
2.5 mL of 1000 ppm Bromide stock standard (Section 6.3.2.) pipetted into in a 100 ml volumetric flask to yield 25 mg/L.

6.10.4. Sample Fortification is taking equal amounts of sample and MS as prepared in Sections 6,10.1, 6.10.2. & 6.10.3. and running this solution on the IC.

6.11. Preparation of Low Level Check Standard (LLC)

6.11.1 Fluoride, Chloride, Nitrite-N, Nitrate-N, Sulfate - LLC
Use the Level 1 as prepared in the Combined Calibration Standard (Section 6.5.1)

Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate
0.05	1.00	0.05	0.10	1.00 ppm

6.11.2 o-PO4-P - LLC
Use the Level 1 as prepared in Section 6.5.2.2. (0.05 ppm)

6.11.3 Bromide - LLC
Use the Level 1 as prepared in Section 6.5.3.2. (1.0 ppm)

7.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

7.1 Samples are collected in plastic or glass bottles that have been either purchased pre-cleaned or prepared in the laboratory by thoroughly cleaning and rinsing bottles (Section 5.9.) sufficiently with reagent water (Section 6.1.). The volume collected is sufficient to insure a representative sample and allow for replicate analysis and fortification if necessary.

7.2 Samples are shipped iced or stored cold in a cooler at ≤ 4.0 °C. The laboratory will not accept samples whenever the sample bottle has been violated (i.e. loose or broken cap, leaking bottle, improperly labeled), causing concern for contamination.

7.3 Following are the sample preservation and holding times :

Analyte	Preservation	Holding Time
Bromide	None Required	28 days
Chloride	None Required	28 days
Fluoride	None Required	28 days
Nitrate-N	Cool to 4.0 °C	48 hours
Nitrite-N	Cool to 4.0 °C	48 hours
Combined Nitrate/Nitrite*	conc. H_2SO_4 to pH <2	28 days
o-Phosphate-P	Cool to 4.0 °C	48 hours
Sulfate	Cool to 4.0 °C	28 days

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

7.4 Allow any cooled sample to come to room temperature before analysis. In the case of ortho-phosphate it has been observed that degradation occurs in samples that have been held at room temperature for over 16 hrs.

8.0 QUALITY CONTROL

Consists of an initial demonstration of laboratory capability and the on-going assessment of the quality of the data being generated by analysis of laboratory reagent blanks, fortified blanks, quality control samples, and the determination of analyte recoveries. The generated performance records are kept on file and available for review for ten years in accordance this laboratory's QA/QC plan.

8.1 INITIAL DEMONSTRATION OF PERFORMANCE - Refer to Table 4

8.1.1 The instrument's performance and the laboratory's performance is assessed prior to conducting any analyses. The Instrumentation Performance is characterized via the determination of Linear Calibration Range (LCR) and analysis of Quality Control Samples (QCS) The laboratory performance is characterized via the determination of MDL's. (see Table 1).

8.1.2 Linear Calibration Range (LCR) – The LCR is determined initially The verification of linearity uses a blank and a minimum of three standards prepared in the following concentrations listed as below. If the verification data exceeds the initial values by $\pm$ 10%, linearity is re-established. Any non-linear portion of the defined range is nonlinear, then additional standards are used to define the nonlinear portion. Refer to Section 6.5. for the preparation of the Calibration Standards.

8.1.3 Quality Control Sample (QCS) – When first beginning this method, the calibration standards and instrumentation performance is verified by analyzing a QCS from a second source. If the determined concentration are not within $\pm$10% of the expected values, performance of the determinative step of the method is unacceptable. The source of the

problem is identified and corrected before proceeding with the initial determination of MDL's

8.1.4. Method Detection Limit (MDL) – MDL's are established for all analytes using reagent water (blank) fortified at concentrations of two-to-three times the estimated instrumentation detection limit. To determine the MDL values, seven replicate aliquots of the fortified reagent water are analyzed and concentrations determined over a period a minimum of 3 days. The fortified concentrations and preparation procedures used for the analytes are listed as follows:

Analyte	mg/L	Procedure Section
Bromide	1.00	Section 6.5.3. - Level 1
Chloride	1.00	Section 6.5.1. - Level 1
Fluoride	0.05	Section 6.5.1. - Level 1
Nitrate-N	0.10	Section 6.5.1. - Level 1
Nitrite-N	0.05	Section 6.5.1. - Level 1
o-Phosphate-P	0.05	Section 6.5.2. - Level 1
Sulfate	1.00	Section 6.5.1. - Level 1

For each analyte , calculate the MDL as follows:

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

Where:

t = Student's value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.
For 7 replicates **t** =3.14
If more replicates are used, use the corresponding **t**- value

S = standard deviation of the replicate analyses

MDL's are determined every at least every six months or whenever a significant change in the background or instrument response is detected or expected and kept on file for 10 years. (See example in Table 1).

8.2 ASSESSING LABORATORY PERFORMANCE

8.2.1 Accuracy & Precision Studies (A&P)

Annually, the accuracy & precision of each element is determined.

To establish this accuracy & precision for each element, a minimum of seven replicate analyses of a mid-range Calibration Standard is analyzed.

Use the listed Calibration Standards concentrations for the A&P's studies

Anion	ppm	Level Standard	See Section
Bromide	50	Level 5	Section 6.5.3
Chloride	50	Level 5	Section 6.5.1.
Fluoride	2.5	Level 5	Section 6.5.1.
Nitrate-N	5.0	Level 5	Section 6.5.1.
Nitrite-N	2.5	Level 5	Section 6.5.1.
o-Phosphate-P	1.0	Level 5	Section 6.5.2.
Sulfate	50	Level 5	Section 6.5.1.

The accuracy for each analyte is measured by determining the % Recovery of the seven results using the following calculation :

$$\%REC = \frac{(C_s - C)}{S} \times 100$$

Where:

% REC = percent recovery,
C_s = average of the seven determinations
C = concentration of prepared analyte

The Precision for each analyte is expressed as the standard deviation estimate with n-1 degrees of freedom of the seven replicate results and kept on file for 10 years (See example in Table 2)

8.2.2. Method Detection Limits (MDL)
Annually, and every six months or whenever a significant change in the background or instrument response is detected or expected the MDL's are established for all analytes. To determine the MDL values see Section 8.1.4.

8.2.3. Analyte Recovery and Data Quality – Refer to Table 5
On an on-going basis, the laboratory's performance is continually assessed.

8.2.3.1 Laboratory Fortified Blank (LFB) - At least one LFB is analyzed with each batch of 20 samples. The accuracy is calculated as percent recovery (Section 8.2.3.1.1.). If the recovery of any analyte falls outside the required control limits of 90-110%, then that analyte is considered to be out of control and the source of the problem is determined and resolved before continuing analyses. Following are the concentrations of the LFB (preparation procedure : see Section listed below).

Analyte	mg/L	Procedure Section- conc. Level
Bromide	25.0	Section 6.5.3. - Level 5
Chloride	25.0	Section 6.5.1. - Level 5
Fluoride	1.25	Section 6.5.1. - Level 5
Nitrate-N	2.50	Section 6.5.1. - Level 5
Nitrite-N	1.25	Section 6.5.1. - Level 5

o-Phosphate-P	1.00	Section 6.5.2. - Level 5
Sulfate	25.0	Section 6.5.1. - Level 5

8.2.3.1.1. Calculation of Percent Recoveries - calculate the percent recovery for each analyte, corrected for concentration measured in the unfortified sample. These values are compared to the determined LFM recovery range of 90-110-%.

The percent recovery is calculated as follows:

$$\%REC = \frac{Cm}{C} \times 100$$

where,
% REC = percent recovery,
C_m = measured fortified sample concentration,
C = prepared fortified sample concentration,

8.2.3.1.2 The LFB analyses data is used to assess the laboratory's performance against the required control limits of 90-110%. When enough internal performance data is available (minimum of 25 analyses) control limits are established for each analyte. These upper and lower control limits are determined from the percent mean recovery (x) and the standard deviation (S) and are established as follows :

UPPER CONTROL LIMIT = x + 3S

LOWER CONTROL LIMIT = x - 3S

These control limits must be equal to or better than the required control limits of 90-110%. After each 5-10 new recovery measurements, new control limits are calculated on the most recent 25 data points.

In addition, the standard deviation (S) data is used to establish an on-going performance statement for the level of concentrations included in the LFB. These data are kept on file and are available for review.

8.2.3.1.3 These results are incorporated into the on-going Control Charts to document data quality as outlined in Section 8.2.4. and are available for review for 10 years.

8.2.3.2. Laboratory Fortified Sample Matrix (LFM) – The laboratory adds a known amount of the analyte to a minimum of 10% of the collected field samples or at least one with every analysis batch, whichever is greater. This is accomplished by adding equal volumes of the sample to be fortified with an equal amount of the following concentrations then followed by pouring and the necessary portion of such to be analyzed. The concentration of each analyte added is as follows with the preparation procedure section as listed:

Anion	MDL x4*	mg/L MS	Final Conc	Procedure Section
Bromide	0.4	25	12.5	Section 6.9.3
Chloride	4.0	50	25	Section 6.9.1
Fluoride	0.4	5.0	1.25	Section 6.9.1
Nitrate-N	0.4	5.0	2.5	Section 6.9.1
Nitrite-N	0.2	2.5	1.25	Section 6.9.1
o-Phosphate-P	0.2	1.0	0.5	Section 6.9.2
Sulfate	4.0	50	25	Section 6.9.1

In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The added analyte concentration is the same that is used in the laboratory fortified blank (The analyte concentration must be high enough to be detected above the original sample and not less than four times the MDL*). If the concentration of the fortification is less than 25% of the background concentration of the matrix the matrix recovery is not calculated.

8.2.3.2.2. Calculation of Percent Recoveries - calculate the percent recovery for each analyte, corrected for concentration measured in the unfortified sample. These values are compared to the determined LFM recovery range of 90-110- %.

The percent recovery is calculated as follows:

$$\%REC = \frac{(C_s - C)}{S} \times 100$$

Where :

% REC = percent recovery,
C_s = measured in the fortified sample,
C = measured sample concentration,
S = concentration equivalent of analyte added to sample.

Until sufficient becomes available (minimum of 20 analysis) assess the laboratory performance against recovery limits of 80-120%.
When sufficient data becomes available develop control limits from percent mean recovery and the standard deviation of the mean recovery.

8.2.3.2.3. If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that all other QC performance criteria is acceptable, the accuracy problem encountered with the fortified sample is judged to be matrix related, not system related.
Repeated failure to meet suggested recovery criteria indicates potential problems with the procedure and will be investigated.

8.2.3.2.4. These results are incorporated into the on-going Control Charts to document data quality as outlined in Section 8.2.4 and are available for review for 10 years.

8.2.3.3 Laboratory Sample Duplicates – The laboratory analyzes sample duplicate for a minimum of 10% of the collected samples or at least one with every analysis batch, whichever is greater. These results are incorporated to the on-going control charts to document data quality.

Calculate the relative percent difference (RPD) of the initial quantitated concentration (I_c) and duplicate quantitated concentration (D_c) using the following formula

$$RPD = \frac{|(I_C - D_c)|}{([I_C + D_c]/2)} \times 100$$

Where:

RPD = Relative Percent Difference

I_c = initial quantitated concentration

Dc = duplicated quantitated concentration

Duplicate analysis may exhibit matrix dependence. If the RPD for the duplicate measurements falls outside ± 20% and if all other QC performance criteria are met, laboratory precision is out of control for the sample and perhaps the analytical batch. The result for the sample and duplicate will be labeled as suspect/matrix to inform the data user that the result is suspect due to a potential matrix effect, which led to poor precision. This should not be a chronic problem and if it frequently recurs (>20% of duplicate analyses), it indicates a problem with the instrument or individual technique that must be corrected.

8.2.3.4. Laboratory Fortified Blank Duplicates - Quarterly, replicates of the LFB's are analyzed to determine the precision of the laboratory measurements. The RPD is determined as outlined above in Section 8.2.3.3. These results are incorporated to the on-going duplicate (precision range) control charts to document data quality.

8.2.4 QC CONTROL CHARTS

Two types of control charts are used for the continued assessment of the lab's performance :

(1) Accuracy , or Means, Control Chart
(2) Precision , or Range, Control Chart

8.2.4.1 The Accuracy Chart is constructed using the most recent 25 LFB and sample MS %Recovery results. See Section 8.2.3.1.1 for Calculation of %Recoveries for the LFB and Section 8.2.3.2.2 for the MS (see Section 6.10 for MS preparation). The upper and lower warning limits (WL) use $\pm$ 2SD and the upper and lower control limits (CL) use $\pm$ 3 SD .

8.2.4.2 The Precision Chart is constructed using the most recent 25 Sample & Sample Duplicate RPD results. See Section 8.2.3.3 for the calculation of RPD. The warning limits (WL) use $\pm$ 2SD and the control limits (CL) use $\pm$ 3 SD.

8.2.4.3. Application of Control Charts.

8.2.4.3.1. Trending – If seven successive samples are on the same side of the central line of the Accuracy Chart, discontinue analyses, investigate and correct the problem

8.2.4.3.2. Control Limit – If one measurement exceeds a CL, repeat the analysis immediately. If the repeat measurement is within the CL, continue analyses, if it exceeds the CL, discontinue analyses, investigate and correct the problem.

8.2.4.3.3. Warning Limit – If two out three successive points exceed a WL, analyze another sample. If the next point is within WL, continue analyses. If the next point exceeds the WL, evaluate potential bias and correct the problem.

8.2.5 The following items must be included in every sample batch or periodically to continually assess the laboratory's performance. See Table 5. A batch of samples is established as 20 samples:

Calibration Curve – Curve run a minimum of weekly with fresh standards for Chloride, Fluoride, Nitrate-N & Sulfate, fresh standards daily for Nitrite-N and oPO4. New curve is verified with QCS. See Section 6.5. for the preparation of the calibration standards. See Section 6.7 for preparation of QCS.

Instrument Blank (IB) – to verify system clear of residual artifacts & contaminants

Instrument Performance Check Solution (IPC)- a mid-range check standard after calibration, every 10 samples and at end of sample sequence run

Laboratory Reagent Blank (LRB) – after IPC in beginning, every 10 samples after CCS, and at end of sample sequence run.

Laboratory Fortified Blank (LFB) – one per batch of samples (every 20 samples)

Laboratory Fortified Sample Matrix (LFM) – a minimum of 10 % of sample sequence run.

Sample duplicates – a minimum of 10% of sample run

LFB Duplicates - Quarterly

Low Level Check Standard (LLC) - Quarterly

MDL's – every 6 months

8.2.5.1 Instrument Performance Check Solution (IPC) – The laboratory analyzes the IPC of the following concentrations after the Instrument & Calibration blanks are run at the beginning of the day's sample sequence, after every tenth sample and at the end of the sample run. The procedures for preparing the IPC is listed under the listed sections.

Analyte	mg/L	Procedure Section
Bromide	50.0	Section 6.5.3 - Level 5
Chloride	50.0	Section 6.5.1 - Level 5
Fluoride	2.5	Section 6.5.1 - Level 5
Nitrate-N	5.0	Section 6.5.1 - Level 5
Nitrite-N	2.5	Section 6.5.1 - Level 5
o-Phosphate-P	1.0	Section 6.5.2 - Level 5
Sulfate	50	Section 6.5.1 - Level 5

Subsequent analyses of the IPC must verify that the calibration is still within $\pm$10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC. If the second analysis of the IPC confirms the calibration to be outside the limits, sample analysis must be stopped, the cause determined. All samples following the last acceptable IPC must be reanalyzed.

8.2.5.2 Laboratory Reagent Blank (LRB) – An 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 contamination from the laboratory environment.
Values that exceed the MDL indicate a laboratory or reagent contamination is present. The source of the contamination must be determined prior to conducting any sample analysis.
Any sample included in an automated analysis batch which has an invalid LRB, indicated by a quantitated result that exceeds the MDL, must be reanalyzed in a subsequent analysis batch after the contamination problem is resolved.

8.2.5.3 Laboratory Fortified Blank (LFB) – Refer to Section 6.9. for preparation procedure and Section 8.2.3.1 for use in on-going laboratories' QC/QA performance.

8.2.5.4 Laboratory Fortified Sample Matrix (LFM) – Refer to Sections 6.10.1,6.10.2,& 6.10.3, for the preparation of the MS used for fortifying the samples and section 6.10.4. for the Sample Fortification procedure. Refer to Section 8.2.3.2 for use in on-going laboratories' QC/QA performance.

8.2.5.5 Sample Duplicates – Refer to Section 8.2.3.3. and 8.2.3.4.

8.2.5.6 Low Level Check (LLC) – Quarterly, the lowest level standard (MDL) is analyzed to demonstrate the ability to analyze low level samples. Refer to Section 6.11 for preparation procedure.

9.0 CALIBRATION AND STANDARDIZATION

9.1 Establish ion chromatographic operating parameters indicated in Table 2.

9.2 Run the initial calibration using the standards made in Section 6.5. Using injections of 25 microliters (determined by the injection loop volume) of each prepared calibration standard.

9.2.1 The initial calibration is deemed acceptable if the following criteria are met (Table 4):
$R \geq 0.9950$

9.2.2 The calibration curve is verified by analyzing a QCS (Section 6.7) immediately after the initial calibration. The acceptable limit of the QC sample is 90% - 110%.

9.2.3 Once the initial calibration and QCS are done, one blank, one LFB and ten samples could be analyzed. Following the ten samples, a IPC is analyzed as a closing instrument verification (Section 9.3).

9.3 At the beginning of any sequence except for the samples right after initial calibration (Section 9.2.3), IPCs are always analyzed at the beginning of the sequence and the end of every ten samples to confirm the instrument is acceptable.

9.3.1 The concentration of the IPC used for the separate analytes are as follows. The procedure for making these standards are the same as those from making the indicated concentration levels of the initial calibration standards (Sections 6.5) but from a separate (secondary) source as those stock solutions as used in the making of the calibration standards

Analyte	mg/L	Procedure Section
Bromide	50	Section 6.5.3.2
Chloride	50	Section 6.5.1.2
Fluoride	2.5	Section 6.5.1.2
Nitrate-N	5.0	Section 6.5.1.2
Nitrite-N	2.5	Section 6.5.1.2
o-Phosphate-P	1.0	Section 6.5.2.2
Sulfate	50	Section 6.5.1.2

9.3.1.2 The IPC concentration must fall within ± 10% of the stated value. If the response or retention time for any analyte varies from the expected values by more than ±10%, the test is repeated, using fresh IPC standards. If the results are still more than ± 10 %, a new calibration curve must be prepared for that analyte.

9.4 End of Run IPC- at the end of the sample run sequence

9.5 End of Run Blank – at the very end run of the day – an instrumentation blank is run using reagent deionized water

10.0 PROCEDURE

10.1 Samples Preparation

10.1.1 See Section 7.3 for sample storage & handling conditions. Those samples that require refrigeration, ensure the samples have come to room temperature prior to conducting sample analysis.

10.1.2 Samples Pretreatment - The pretreatments prescribed are effective at reducing the chloride and sulfate content of a sample matrix but will not reduce matrix concentrations of other anions such as nitrate or phosphate

10.1.2.1 If the Chloride concentration interferes with the determination of NO2 or NO3 then pre-treat the sample using a Ag pretreatment cartridges to remove the Chloride (Dionex P/N 057089).

10.1.2.2 If the Sulfate concentration interferes with the determination of oPO4 then pre-treat the sample using Ba pretreatment cartridges to remove the sulfate (Dionex P/N 057093).

10.1.2.3 Samples Pretreatment Procedure
Individually and thoroughly rinse each pretreatment cartridge with reagent water in order to insure all residual background contaminants are removed from the cartridge. Filter 3 mL of sample through the series of rinsed cartridges as an initial sample rinse (Ba, Ag) at a flow rate of 1.0 mL/min or less (approximately one drop every 3 to 4 seconds). This flow rate is critical to the pretreatment and must be carefully followed. Discard this fraction and begin collecting the pretreated sample aliquot of collected sample.

10.1.2.4 Pour approximately 0.75 ml sample into 0.5ml autosampler vail (or 6 ml into 5 ml autosampler vail) and place a filter cap into the vial and push down the cap with a special made tool from Dionex to certain position according to instructions provided by the Manufacturer. There is no need to filter the sample since the cap has a filter in it.

10.1.3 Prior to pretreating any field samples, prepare and pretreat both an LRB and an LFB. These pretreated quality control samples are required when an analysis batch contains a matrix that must be pretreated. The pretreated LRB and LFB are used to verify that no background interference or bias is contributed by the pretreatment. If a response is observed in the pretreated LRB, triple or quadruple the volume of reagent water rinse used and repeat until a blank measures no more than ½ the MRL. If this additional rinsing procedure is required, it must be consistently applied to all the cartridges prior to conducting any matrix pretreatment.

10.1.4 Solid Samples - The following extraction should be used for solid materials. Add an amount of reagent water equal to 10 times the weight of dry solid material taken as a sample. This slurry is mixed for 10 minutes using a magnetic stirring device. Filter the resulting slurry using a 0.45u membrane type filter. Ensure that good recovery and peak identification is obtained through the use of fortified samples.

10.2. Sample Analysis

10.2.1. Table 2 summarizes the operating conditions for the ion chromatograph. Included in this table are a representative retention time and MDL results for the analytes that has been achieved by this method

10.2.2 Verify the initial calibration by conducting a QCS. See Section 6.7 for the preparation of the QCS using stock solutions obtained from a secondary source - either purchased or prepared from reagent grade chemicals (Section 6.3.2.).

10.2.3 The injection volume is 25 microliters that is controlled by using 25 microliters sample loop (Dionex P/N: 052682). Use the same size loop for standards and samples. An AS40 Automated Sampler (Dionex P/N: 056830) is used. Data acquisition and processing are done using CHROMELEON CHM-1-IC/Win 2000 Desktop Workstation (Dionex P/N: 060929).

10.2.4 The retention time window used to make identifications in the laboratory is ± 0.2 minutes (determined by ±3 Std Dev of the RT of individual analytes over the course of a day)

10.2.5 If the response of a sample analyte exceeds the calibration range, the sample is diluted with an appropriate amount of reagent water and reanalyzed.

10.2.6 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.

10.2.7 An analytical sequence including initial calibration and other quality control analysis for sample analysis is listed in Table 5.

11.0 DATA ANALYSIS, CALCULATIONS AND REPORTS

11.1 Identify the analytes in the sample chromatogram by comparing the retention time of a suspect peak within the retention time window to the actual retention time of a known analyte peak in a calibration standard. The retention time in the daily calibration check standards (QCS) is used for the identification.

11.2 Compute sample concentration using the initial calibration curve generated in Section 8.1.1.

11.3 Report those values that fall between the MRL and the highest calibration standards without any flagging. Sample analytes with responses that exceeds the highest calibration standard concentration are diluted and reanalyzed.

11.4 A printout of the sample sequence is printed out, dated & initialed, and kept in a notebook (Example of sequence run copy is attached – Table 5). Hard copies of the integrated analyses are printed and kept in filing folder indentified by the sequence number.

11.5 Report results in mg/L. The MRL reported is the lowest Calibration Standard Level used

11.6 Report : NO_2^- as Nitrogen

NO_3^- as Nitrogen
HPO_4 as P

12.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

The laboratory waste management practices are conducted consistent with all applicable rules and regulations as stated in the laboratory's "Sample and Waste Disposal Standard Operating Procedure" , Revision 003 - July 6, 2006). Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner in this SOP.

13.0 REFERENCES

1. U.S. Environmental Protection Agency, "Determination of Inorganic Anions by Ion Chromatography", Method 300.0, Revision 2.1, August 1993

2. Standard Methods for the Examination of Water and Wastewater, Method 4110B, "Anions by Ion Chromatography", 22th Edition of Standard Methods (2012)

Table 1. Method Detection Limits (MDLs)

MDL Study	**Year**	**2017**				
Analyte	**Fluoride**	**Chloride**	**Nitrite-N**	**Nitrate-N**	**Sulfate**	**Bromide**
Spiking Level (ppm)	**0.050**	**1.00**	**0.050**	**0.100**	**1.00**	**1.00**
Run #1	0.058	1.07	0.049	0.108	1.00	0.962
Run #2	0.081	1.15	0.043	0.116	1.02	0.964
Run #3	0.059	1.27	0.046	0.106	1.54	0.962
Run #4	0.059	1.08	0.052	0.107	1.24	0.958
Run #5	0.054	1.13	0.051	0.101	1.10	0.952
Run #6	0.060	0.98	0.025	0.111	1.00	0.966
Run #7	0.061	1.03	0.061	0.105	1.20	0.974
Average	0.062	1.10	0.047	0.108	1.16	0.962
1 Std Dev	**0.009**	**0.096**	**0.011**	**0.005**	**0.195**	**0.007**
MDL	**0.030**	**0.300**	**0.035**	**0.015**	**0.612**	**0.021**
Reporting MDL	**0.050**	**1.0**	**0.050**	**0.10**	**1.0**	**1.0**
Dates Run	07/07/17	07/07/17	07/07/17	07/07/17	07/70/17	09/17/13
	07/08/17	07/08/17	070/8/17	07/08/17	07/08/17	09/19/13
	07/11/17	07/11/17	07/11/17	07/11/17	07/11/17	09/19/13
Analyst	L.Prior	L.Prior	L.Prior	L.Prior	L.Prior	L.Prior

Table 2. Accuracy and Precision (A&P)

A &P Study	**Year**	**2017**				
Analyte	**Fluoride**	**Chloride**	**Nitrite-N**	**Nitrate-N**	**Sulfate**	**Bromide**
Spiking Level (ppm)	**2.5**	**50.0**	**2.50**	**5.00**	**50.00**	**50.0**
Run #1	2.49	49.8	2.51	4.95	49.6	49.69
Run #2	2.51	49.7	2.51	4.95	49.8	49.55
Run #3	2.50	49.9	2.51	4.97	49.0	49.32
Run #4	2.51	49.9	2.52	4.96	49.0	49.42
Run #5	2.54	50.5	2.53	5.02	49.3	49.59
Run #6	2.55	50.3	2.52	5.01	49.4	49.41
Run #7	2.51	50.5	2.55	5.04	49.3	49.57
Average	2.52	50.1	2.52	4.99	49.4	49.51
% RSD	**0.023**	**0.32**	**0.015**	**0.038**	**0.28**	**0.128**
% Mean	**100.6**	**100.2**	**100.8**	**99.7**	**98.7**	**99.0**
Analysis Dates	01/04/17	01/04/17	01/04/17	01/04/17	01/04/17	9/17/13
Analyst	L.Prior	L.Prior	L.Prior	L.Prior	L.Prior	L.Prior

Table 3. Chromatographic Conditions and Equipment of the Ion Chromatographic Instrument

Ion Chromatograph:	Dionex ICS-2000
Sample Loop:	25 µL
Eluent:	EGC III KOH @ 22.0 mM
Eluent Flow:	0.23 mL/min
Columns:	Dionex IonPac AG19 Guard Column 2×50 mm Dionex IonPac AS19 Analytical column, 2×250 mm
Typical System Backpressure:	1900 psi
Suppressor:	Dionex AERS 500 self-regenerating chemical suppressor @ 16 mA current
Detector:	Dionex DS6 - Detection Stabilizer Conductivity at 16 mA held at a temperature of 30°C . Background Conductivity: 0.2 – 1.0 µs
Total Running Time:	15 minutes

Table 4. Initial Demonstration of Capability and Acceptance Requirements

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 8.1.2	Linear Calibration Range (LCR)	Generate calibration curve. At least 5 calibration standards are recommended.	MRL must be no lower than the lowest calibration standard. $R \geq 0.9950$
Section 8.1.3	Quality Control Sample	An external/second source of analyte standard must be run following the initial calibration.	The QCS must be ± 10% of the true value.
Section 8.1.4.	Method Detection Limit (MDL) Determination	Analyze 7 replicate LFBs of the lowest Calibration Standard Level over a period of three days minimum. MDL is determined based on these results.	
Section 8.2.1.	Initial Demonstration of Accuracy and Precision	Analyze 7 replicate LFBs fortified with analyte. Calculate the mean recovered concentration ($C_{\bar{X}}$) and the relative standard deviation (%RSD).	The $C_{\bar{X}}$ must be ± 10% of the true value, and the %RSD must be ≤ 10%.
Section 11.0	Minimum Reporting Level (MRL)	MRL = Chloride, Sulfate = 1.0 mg/L, Nitrate-N,=0.10 mg/L, Nitrite-N= 0.05 mg/L, Fluoride =0.50mg/L, Bromide=1.0 mg/L	The low CAL standard can be lower than the MRL, but the MRL must be no lower than the low CAL standard.

Table 5. Quality Control Requirements

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 8.1.2.	Calibration Curve	At least 5 calibration standards are recommended. New Curves should be established each day for Nitrite & oPO4P and at a maximum weekly for F, Cl, NO3N & SO4.	MRL must be no lower than the lowest calibration standard. $R \geq 0.9950$
Section 8.2.5.1.	Initial IPC	Analyze after Instrument and Method blanks	Recoveries must be between 90-110% of fortified level.
Section 8.2.5.1	Continuing IPC and Ending IPC	Analyze after 10 samples and after the last sample in an analysis batch.	Recoveries must fall between 90-110%.
Section 8.2.5.2	Laboratory Reagent Blank (LRB)	Analyze at the beginning, after 20 samples and after the last sample in an analysis batch	The LRB concentration must be $\leq$ the proposed MDL.
Section 8.2.2.1.	Laboratory Fortified Blank (LFB)	Analyzed with each batch of samples (20 or less).	Recoveries must be between 90-110% of fortified level
Section 8.2.5.6	Low Level Check (LLC)	Analyzed Quarterly	Recoveries must be between 70-130% of fortified level
Section 8.2.3.2.	Laboratory Fortified Sample Matrix (LFM)	Must add known amount of analyte to a minimum of 10% of field samples or at least one within each analysis batch.	Recovery must be 80-120%. If fortified sample fails the recovery criteria, label both as suspect/matrix.
Section 8.2.3.3.	Field or Laboratory Duplicates	Analyze either a field or laboratory duplicate for a minimum of 10% of field samples or at least one within each analysis batch. Calculate the relative percent difference (RPD).	RPD must be ±15%.
Section 8.2.3.4.	Laboratory Fortified Blank (LFB) Duplicates	Quarterly replicates of LFB's are run & included on the on-going charts.	Duplicate Recovery must be 80-120%.
Section 8.1.3.	Quality Control Sample	Analyzed Quarterly – from 2nd source	The QCS must be ± 10% of the true value
Section 8.2.2.	MDL Determination	Every six months or whenever a significant change has occurred	

Table 6. Typical Analytical Sequence with Quality Control Requirements

Injection #	Description of Quality Control Standards and Samples	Chapter 2 Acceptance Criteria
	Calibration Blank	
	Level 1 of Initial Calibration	R ≥ 0.9950 Calibration curve to be done each day for NO2N & oPO4P and maximum of weekly for F, Cl, NO3N & SO4
	Level 2 of Initial Calibration	
	Level 3 of Initial Calibration	
	Level 4 of Initial Calibration	
	Level 5 of Initial Calibration	
1	Instrumentation Blank	≤ ½ MDL
2	QCS (after new calibration curve and quarterly)	90 -110%
3	Initial IPC	90 -110 %
4	LRB	≤ ½ MDL
5	LFB (Duplicates Quarterly)	90 -110 %
6	LLC (Quarterly)	70 -130 %
7	MS (Check Periodically)	80 -120 %
8	Sample 1	
9	Sample 1 – Laboratory Duplicate	
10	Sample 1 – LFM	80-120 %
11- 19	Sample 2 to Sample 10	
20	Continuing IPC	90 -110%
21	Blank	≤ ½ MDL
22	Sample 11	
23	Sample 11 – Laboratory Duplicate	
24	Sample 11 – LFM	
25-34	Sample 12 to Sample 20	
35	Continuing IPC	90 -110%
36	Blank	≤ ½ MDL
37	LFB	90 -110%
38	Sample 21	
39	Sample 21 – Laboratory Duplicate	
40	Sample 21 – LFM	
41	Sample 22…. And so forth	
Last Injections	Ending IPC Calibration Blank LRB	Criteria As Above

Barnstable County Department of Health and the Environment Laboratory

STANDARD OPERATING PROCEDURE

For

Determination of Fecal Coliform in Waste Water Using the following Method:

Idexx Colilert- 18 and Quanti-Tray

ENZ.SUB. SM 9223B and 40 CFR Part 136.3

April 24, 2020

		Signature	Date
Laboratory Director:	**Dan White**		23APR20
Bacteriologist:	**Ryan Lucier**	Ryan Lucier 4.24.20	

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STANDARD OPERATING PROCEDURE

For

Determination of Fecal Coliforms in Waste Water Using IDEXX Colilert®- 18 Test and Quanti-Tray Method

1.0 Scope and Application

1.1 This method is used for the detection and confirmation of fecal coliforms in wastewater. The minimum, non-zero number of bacterial counts detectable with this method is based on the dilution used when processing sample.

1.2 The Colilert-18 method can be applied to test for fecal coliforms in wastewaters with the Quanti-Tray® system (21.1, 21.5).

1.3 Since there is a wide range of fecal coliforms in wastewaters, dilutions may be used to determine and enumerate the actual level.

2.0 Summary of Method

2.1 This method is based on Defined Substrate Technology®. The product utilizes a nutrient indicator (ONPG) that produces a yellow color when metabolized by fecal coliforms at 44.5 ± 0.5°C for 18-22 hours. When the reagent is added to the sample and incubated, it is able to detect fecal coliforms at 1 CFU/100mL.

3.0 Definitions

3.1 In this method, fecal coliform bacteria are those bacteria which produce a yellow color after incubation at 44.5 ± 0.5°C for 18-22 hours.

3.2 Fecal coliform detection is based on the presence of the enzyme β-D-galactosidase which is known to be present in fecal coliform bacteria.

3.3 For the detection of enzyme β-D-galactosidase, Colilert-18 utilizes the chromogenic nutrient indicator ortho-nitrophenyl- β-D-galactopyranoside (OPNG) which produces a distinct yellow color when hydrolyzed by β-D-galactosidase. The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and materials and while operating sterilization equipment.

4.0 Interferences

4.1. Some samples that contain humic material may have an innate color and a control blank of the same water sample may be required for comparison to the inoculated sample or a dilution may be made.

5.0 Safety

5.1 The analyst must know and observe all appropriate safety procedures required in a microbiology laboratory preparing, using, and disposing of samples, reagents and materials, and while operating equipment. Purity of Reagents: Reagent-grade chemicals shall be used in all tests. Whenever possible, use commercial culture media as means of quality control. Use reagent grade water from the Milli-Q system to assure quality of water.

5.2.1 Mouth pipetting is prohibited.

6.0 Equipment and Supplies

6.1 Graduated cylinders, 50mL, 100mL, 250 mL, 500 mL, and 1000mL
6.2 National Institute of Standards and technology (NIST) certified thermometer (Thermometer, Cat. # 1005-3FC, range (-1+101°C, 0, 1°C)
6.3 Thermometers for incubators
6.4 Incubators- temperature at 44.5 ± 0.5° Incubators (Vendor: VWR, Model: 1556, Vendor: VWR, Model: 1545 and/ or water baths temperature at 44.5 ± 0.5° (Vendor: Thermo Scientific, Model: Precision COL35,)
6.5 PPE (Personal Protective Equipment)-safety goggles, gloves, lab coat
6.6 Sterile plastic bottles (Vendor: IDEXX) that hold up to 120mL of sample also if needed, contain sodium thiosulfate to neutralize up to 15mg/L of chlorine.
6.7 Top loading balance (Vendor: Scout, Item #: SC6010
6.8 Sterile pipettes
6.9 Quanti-Tray Sealer
6.10 51 Well Quanti-Tray or Quanti-Tray/2000

7.0 Reagents

7.1 Sterile, non-buffered, oxidant-free water for dilutions
7.2 Colilert® (Cat#:IDEXX WP200) and Colilert®-18(Cat.#: IDEXX WP200I)
7.3 51 Well Quanti-Tray or Quanti-Tray/2000 Comparator
7.4 Antifoam reagent (optional see 12.7)
7.5 Sodium thiosulfate reagent *Standard Methods for the Examination of Water and Wastewater,* (21.3) or sterile vessels containing sodium thiosulfate to neutralize up to 15mg/L chlorine.

8.0 Sample Collection, Preservation and Storage Quality Control

8.1 Sampling procedures as described in detail in the USEPA microbiology methods manual, Section II, A (21.2) and in Standard Methods for the Examination of Water and Wastewater.

8.1.1. Storage temperature and handling conditions: Ice or refrigerate bacteriological samples at a temperature less than 10 °C (2-10 °C) during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Samples shouldn't be completely submerged in water during transit. Don't allow samples to freeze. If sample freezes it can't be thawed. A new sample must be collected.

8.1.2. Holding time Limitations: Examine samples as soon as possible after collection. The required maximum hold time of samples is 8 hours from collection to incubation.

9.0 Quality Control

9.1. Quality control should be conducted on each lot of Colilert®-18 or more often as regulations require. One of the following quality control procedures is recommended for each lot of Colilert®-18 when used for fecal coliform testing:

9.1.1. IDEXX-QC for Fecal Coliform

1. See package insert for instructions.
2. Obtain the mean and range from the website; www.idex.com/water under Quality Certificates.

9.1.2. ATCC: Fill 2 sterile vessels with 100mL of sterile, non-buffered oxidant-free water and inoculate with a sterile loop of, *Escherichia coli* ATCC 11775 (positive control) and *Pseudomonas aeruginos* 10145 or 27853 (negative control).

aseptic TSA ?

9.2. Follow Section 12. Quanti-Tray Enumeration Procedure, Section 1[illegible] and Section 14. Interpretation and Calculations.

vs. Tryptic Soy Broth.

9.3. Sample bottle and Quanti-Tray sterility check per lot

9.3.1. At least one sample bottle/lot and tray/lot are tested [illegible] strength Tryptic Soy Broth (25mL for the bottle and 100mL for th[illegible] incubated at 35 ± 0.5°C. It is recommended that this be performed [illegible] flow hood. Aseptic technique must be maintained. If not available, aseptic technique must be maintained. Do not open bottle for long periods of time nor place the cap on the lab surface facing up. Open cap just enough to add the TSA to the bottle and close immediately. Cap bottle and rotate so that broth comes into contact with all surfaces.

9.3.2. Check samples for growth at 24 and 48 hours. No growth should be observed. Record results in Quality Control Logbook.

9.3.3. If growth is observed, retest, and if still positive, call IDEXX Water Technical Service (1-800-321-0207).

9.4. Monthly Sealer check with food color or dye:

9.4.1. Add 2-3 drops of food coloring dye or equivalent to 100mL of water. Mix well

9.4.2. Add this to the Quanti-Tray and seal the tray.

9.4.3. Observe the tray. There should be no dye observed outside the wells.

9.4.4. If dye is observed outside the well, retest and if it still occurs call IDEXX Water Technical Service (1-800-321-0207).

9.5. Media sterility check using sterile water per lot

9.5.1. Each new lot shall be checked for sterility. Select at least one blister pack and add 100mL of sterile DI water to a vessel. Mix well and add this to a Quanti-Tray and seal. Incubate up to 18 and no longer than 22 hours at 44.50.5°C.

9.5.2. No color should be observed.

9.5.3. If color is observed, retest, and if still positive, call IDEXX Water Technical Service (1-800-321-0207).

9.6. Air Quality- The air quality in the laboratory is monitored monthly. Plates of R2A media are left open in different parts of the laboratory for 15 minutes. A sterile plate is also poured and left closed for the 15 minutes. Incubate plates @ 35°C +/- 0.5 for 48hrs and observe for growth and record in appropriate book.

9.7. Precision of Duplicate Analysis: Duplicates are run on 10% of samples for all analyses requiring enumeration. For each most recent set of 15 samples and its corresponding duplicate (for each type of sample analysis **i.e. fecal**, ***E.coli***, **enterococci**), the range of logs is calculated in order to determine the acceptance criteria of precision. Once established, the criteria of precision will be used to determine if the following duplicate analyses are within acceptable limits. At the end of each week the criteria are updated to include the most recent set of 15 samples run in duplicate. The updated precision is used to determine precision acceptability for the following week.

9.7.1. Obtain the recorded numbers of the most recent 15 samples run in duplicate (for the same type of analysis).

9.7.2. Calculate the logarithm of each result (and if any result is <1 then add 1 to both values before calculating the logarithm) and record in a spreadsheet as *L1* and *L2*.

9.7.3. Range of Logarithms (R_{log}) is calculated using the following equation:

$$R_{log} = \left| L1 - L2 \right| \qquad (1)$$

9.7.4. The mean ($\overline{R}$) of R_{log} is calculated as follows:

$$\overline{R} = \frac{\sum R_{log}}{n} \qquad (2)$$

$\sum R_{log}$ = The sum of the range of logs.

n = The number of sets of transformed duplicates.

9.7.5. Precision Criterion is calculated as follows:

$$Precision\ Criterion = 3.27 \times \overline{R} \qquad (3)$$

9.7.6. Any samples run in duplicate (10% of all samples) have their Range of Logarithms (R_{log}). If the range is greater than the Precision Criterion, there is a greater than 99% probability that the analysis has exceeded variability limits. For any samples that fall outside the acceptable limits, determine if the imprecision is acceptable. If data is not acceptable, all results since the last precision check should be rejected. The analytical problem(s) should be determined and corrective action should be taken to resolve problem.

9.7.7. See **Appendix A** for an example of the precision criterion.

10.0 Calibration and Standardization

10.1. Thermometers and temperature recording equipment- The accuracy of thermometers is checked annually against a certified NIST thermometer at the temperature used. The maximum thermometers and NIST thermometers are checked annually by Alert Scientific. In general, the graduations on the thermometer should be in increments of 0.5 degrees or less. The results of each thermometer check are recorded in the Thermometer Logbook.

10.2. Balances- Balances are checked daily with the provided class S weights. The top loading balance is for anything weighing 3g or more. For this balance the 100.0g, 50.0g, 1.0g, and 100mg weights are used. Each weight is weighed independently, and in addition, a deflection test is performed prior to usage. The results of the check should be recorded in the assigned balance book. The S class weights and balance are checked annually by Alert Scientific.

10.3. Refrigerators- Check and record all temperatures daily. Use a refrigerator maintaining a temperature of 1.0° to 4.4°C to store samples, media, reagents, etc.

10.4. Incubators- Temperature is checked and recorded twice daily with a four-hour separation time between readings and adjusted when needed. Times of measurements shall be recorded along with temperatures.

11.0 Corrective Action

11.1. If an unacceptable result is obtained, then the lab should review the test procedure to determine the cause of the failure and to prevent this from reoccurring again by:

11.2. Defining the problem:

A. Identify corrective action and steps required to correct the problem.

B. Implement corrective action.
C. Document corrective action.

11.3. Repeat testing to ensure that corrective action was successful.

11.4. Examples include:
11.4.1. Procedure followed for preparing the control and or diluent.
11.4.2. Incubation temperature within the required time period.
11.4.3. Call and review problem encountered with IDEXX Water Technical Support (1-800-321-0207).

12.0 Quantification Procedure

12.1. Colilert-18 and the IDEXX Quanti-Tray System work with either the 51 Well Quanti-Tray or the Quanti-Tray/2000.

12.2. Carefully separate one blister pack from the strip taking care not to accidentally open the adjacent pack.

12.3. Ensure the powder is at the bottom of the blister pack.

12.4. Hold the blister pack face down (paper side up)at the top and towards the back and snap back the score line forming a "v" with the opening facing the open vessel.

12.5. Allow the powder to fall into the vessel containing the room temperature sample. Mix the sample well to dissolve the reagent.

12.6. If a dilution is necessary, use sterile deionized or distilled water, not buffered water for making the dilution prior to adding the Colilert®-18. Always add the Colilert®-18 to the final 100mL diluted sample only.

Note: If the sample has some background color, it is recommended that the sample be diluted to at least 1:4 (25mL of a well-mixed sample to 75mL of sterile water) or 1:10 (10mL of a well-mixed sample to 90mL of sterile water) as noted in 12.6. As an alternative, compare the sample with Colilert®-18 to a control blank of the same water sample adding it to the Quanti-Tray (no Colilert-18) and incubate along with the sample with Colilert®-18.

12.7. The use of IDEXX Antifoam reagent may be necessary to reduce foaming and eliminate excess bubbles in the wells of the Quanti-Tray. Alternatively, let the sample sit for 30-60 seconds to allow the foam to dissipate. Note: All the foam doesn't need to dissipate.

12.8. Remove the sterile trays from the plastic bag by opening the bag at the black line (follow the package insert). Reseal the bag with tape or a clip. Label the back of the with a marker to identify the sample. Open the tray via instructions in insert for Quanti-Tray

(Hint: Think "Plastic" facing "Palm"). Pour the reagent mixture from the vessel into the tray avoiding contact with the foil tab. Seal the tray with the Quanti-Tray Sealer.

12.9. For samples with high chlorine a blue flash may be seen when Colilert® or Colilert®-18 is added; if this is seen the sample is invalid. *Note: Per conversation with Idexx representative, when excessive chlorine is in the sample, the color may change to a blue, purple or brownish color and may foam due to excessive chlorine. Sample should be invalidated because such large amounts of chlorine would kill anything in the sample and the reaction cannot continue normally with such a drastic change in water color.*

13.0 Incubation

13.1 Place the sealed tray in a 44.5+/- 0.2 °C incubator for 18 hours and up to 22 hours. For incubation in a waterbath, submerge the Quanti-Tray (do not place in a plastic bag) below the water line using a weighted ring. Note: Do not use a rock or a bottle containing water to hold down the trays. A dry incubator can also be used as long as it can meet the +/- 0.2 °C tolerance throughout the incubator.

14.0 Results Interpretation

14.1. Read results between 18 and 22 hours. Compare each result against the comparator dispensed into an identical tray. Count the number of positive wells and refer to the MPN Table provided with the Quanti-Tray to obtain a Most Probable Number. If the sample was diluted, correct the MPN obtained from the MPN table by multiplying that MPN value with the dilution factor to obtain the final corrected MPN/100mL (if a 1:10 dilution was made, the dilution factor is 10).

14.2. Less yellow than the comparator when incubated at 44.5+/- 0.5 °C is negative for fecal coliforms. NOTE: However, if the results are ambiguous to the analyst based on the initial reading, incubate up to an additional four hours (but not to exceed 22 hours total) to allow the color to intensify.

14.3. Yellow equal to or greater than the comparator when inc positive for fecal coliforms.

MPN
Total Coliforms

14.4. Colilert-18 results are definitive at 18 and up to 22 hour fecal coliforms observed before 18 hours are also valid.

14.5. Positive for fecal coliforms observed before 18 hours ar are also valid.

15.0 Interpretation and Calculations:

15.1. Follow the same interpretation directions from Section 14.0; Count the number of positive wells. Refer to the Quanti-Tray MPN table provided by IDEXX to determine the Most Probable Number (MPN) for total coliforms (yellow wells) in the sample. Correct

the MPN value for any dilution made. The color of positive wells may vary. Use the appropriate Quanti-Tray MPN comparator following the instructions as indicated.

16.0 Method Performance- 40 CFR 136.3 ATP evaluation

16.1. Colilert-18 is equally as sensitive as m-FC

16.2. Variances across the ten sites were similar using the Bartlett's test with Colilert-18 having a p-value (>0.05) of 0.308

16.3. Colilert-18 false positive rate was determined to be –0%; False negatives –7%

16.4 The EPA determined that Colilert-18 is a suitable method for the detection of fecal coliforms in wastewater when incubated at 44.5+/- 0.5 °C.

17.0 Reporting Results

17.1. Report results as MPN/100mL for fecal coliforms

18.0 Verification Procedure

18.1. Not applicable

19.0 Pollution Prevention

19.1. The solutions and reagents used in this method pose no threat to the environment when recycled and managed properly.

19.2. Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

20.0 Waste Management

20.1. Biological wastes shall be autoclaved at 121°C for thirty minutes and disposed of with standard trash. Little chemical waste is generated in association with the microbiological analyses. Those chemical wastes generated shall be stored in a designated area and segregated from non-compatible wastes and removed annually by a licensed Hazardous Materials Disposal Company.

21.0 References

21.1. Colilert-18 Package Insert from IDEXX.

21.2. Standard Methods, "Examination of Water and Wastewater', Revision 20th Edition, Method Enzyme Substrate Test, 9223, 9223 B; 1998.

21.3. Bordner, R., J.A. Winter and P.V. Scarpino (eds.) Microbiological Methods for Monitoring the Environment, Water and Wastes, EPA-600/8-78-017. Office of Research and Development, USEPA. (December 1978)

21.4 Federal Register/ Vol 77, #97/ Friday, May 18th, 2012, pg 29806-29807

21.5 Quanti-Tray Package Insert from IDEXX

21.6 ATP summary report and letter from EPA

21.7. USEPA Manual for Certification of Laboratories Analyzing Drinking Water, Fifth Edition, Section V

22.0 Appendix A – Precision Criterion Table	mTEC– *Escherichia coli*	Duplicate Analyses		Logarithms of Counts		Range of Logarithms (R_{log})
Sample No.	Analytical Date	D1	D2	L1	L2	|L1-L2|
1	50031-11/14/08	4	1	0.60206	0	0.60206
2	50017- 11/13/08	12	10	1.079181	1	0.079181
3	49928- 11/4/08	9	5	0.954243	0.69897	0.255273
4	49886- 10/30/08*	1	1	0	0	0
5	49863-10/29/08**	1	1	0	0	0
6	49814- 10/23/08	20	23	1.30103	1.361728	0.060698
7	49764- 10/21/08	5	5	0.69897	0.69897	0
8	49728- 10/16/08	6	7	0.778151	0.845098	0.066947
9	49666- 10/14/08	1	2	0	0.30103	0.30103
10	49591- 10/7/08	7	6	0.845098	0.778151	0.066947
11	49557- 10/2/08	11	10	1.041393	1	0.041393
12	49512- 9/30/08	10	7	1	0.845098	0.154902
13	49512- 9/30/08	2	2	0.30103	0.30103	0
14	49456- 9/25/08	13	17	1.113943	1.230449	0.116506
15	49402- 9/23/08***	1	1	0	0	0

1) $\Sigma R_{log} =$ 1.744935

2) $\overline{R} = \frac{\sum R_{log}}{n} =$ 0.116329

3) Precision criterion = 0.380396

*= Value of D2 is 0
**=Value of D1 and D2 is 0
***=Value of D1 is 0

Barnstable County Department of Health and the Environment Laboratory

STANDARD OPERATING PROCEDURE

For

Determination of Total Coliform and *E.coli* Water Using the following Method:

Idexx Colilert- 18, Colilert-24 and Quanti-Tray

ENZ.SUB. SM 9223B

April 23, 2020

		Signature	Date
Laboratory Director:	**Dan White**		23APR20
Bacteriologist:	**Ryan Lucier**	Ryan M Lucier	4.24.20

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STANDARD OPERATING PROCEDURE

For

Determination of Total Coliform and *E.coli* Water Using the following Method: Idexx Colilert- 18, Colilert-24 and Quanti-Tray- ENZ.SUB. SM 9223B

1.0 Scope and Application

1.1 This method is used for the simultaneous detection and confirmation of total coliforms and *E.coli* in water. Any positive sample for total coliforms is an indication of contamination. Any positive sample for total coliforms and *E.coli* is an acute violation. he minimum, non-zero number of bacterial counts detectable with this method is based on the dilution used when processing sample.

1.2 The minimum, non-zero number of bacterial counts detectable with this method is a function of the dilution scheme used when processing the sample.

1.3 The Colilert-18/ Colilert-24 method can be applied to fresh waters, drinking waters, ground waters, reuse waters, waste waters and marine waters.

1.4 Since there is a wide range of coliform levels in surface waters and wastewaters, dilutions may be used to determine and enumerate the actual level.

1.5 Colilert-18 can be used for detecting *E.coli* in marine waters (not for total coliforms, unless evaluated by the specific site). Make at least 1:10 dilution using sterile, non-buffered oxidant free water for *E.coli*. In some sub-tropical waters, a 1:20 dilution may be necessary.

2.0 Summary of Method

2.1 This method is based on Defined Substrate Technology®. The product utilizes nutrient indicators that produces a yellow color/ fluorescence when metabolized by total corms and *E.coli*. When the reagent is added to the sample and incubated, it is able to detect these bacteria at 1CFU/100mL at 18-22hours (Colilert 18) and at 24-28hours(Colilert24) with as many as 2 million heterotrophic bacteria/100mL present.

3.0 Definitions

3.1 In this method, coliform bacteria are those bacteria which produce a yellow color and for *E.coli* , also produce a fluorescent signal under a 6 watt, 365-366nm UV light after incubation at 35.0 ± 0.5°C for 18-22 hours(Colilert-18) and 24-28 hours (Colilert 24) with as many as2 million heterotrophic bacteria/100mL present .

4.0 Interferences

4.1 Some samples that contain humic material may have an innate color and a control blank of the same water sample may be required for comparison to the inoculated sample or a dilution may be made.

4.2 Heterotrophic bacteria greater than 2 million/100mL could yield a positive reaction for coliforms.

5.0 Safety

5.1 The analyst must know and observe all appropriate safety procedures required in a microbiology laboratory preparing, using, and disposing of samples, reagents and materials, and while operating equipment.

5.2 Mouth pipetting is prohibited. Purity of Reagents: Reagent-grade chemicals shall be used in all tests. Whenever possible, use commercial culture media as means of quality control. Use reagent grade water from the Milli-Q system to assure quality of water.

6.0 Equipment and Supplies

6.1 Graduated cylinders, 50mL, 100mL, 250 mL, 500 mL, and 1000mL

6.2 National Institute of Standards and technology (NIST) certified thermometer (Thermometer, Cat. # 1005-3FC, range (-1+101°C, 0, 1°C)

6.3 Thermometers for incubators

6.4 Incubators- temperature at 35.0 ± 0.5° Incubators (Vendor: VWR, Model: 1556, Vendor: VWR, Model: 1545 and/ or water baths temperature at 44.5 ± 0.5° (Vendor: Thermo Scientific, Model: Precision COL35,)

6.5 PPE (Personal Protective Equipment)-safety goggles, gloves, lab coat

6.6 Sterile plastic bottles (Vendor:IDEXX) that hold up to 120mL for proper mixing of sample of sample also if needed, contain sodium thiosulfate to neutralize up to 15mg/L of chlorine.

6.7 Top loading balance (Vendor: Scout, Item #: SC6010)

6.8 Sterile pipettes

6.9 Quanti-Tray Sealer

6.10 51 Well Quanti-Tray or Quanti-Tray/2000

6.11 6 Watt 365-366 nm UV light

7.0 Reagents

7.1 Sterile, non-buffered, oxidant-free water for dilutions
7.2 Colilert® (Cat#:IDEXX WP200) and Colilert®-18(Cat.#: IDEXX WP200I)
7.3 51 Well Quanti-Tray or Quanti-Tray/2000 Comparator
7.4 Antifoam reagent (optional see 12.7)
7.5 Sodium thiosulfate reagent *Standard Methods for the Examination of Water and Wastewater,* (21.3) or sterile vessels containing sodium thiosulfate to neutralize up to 15mg/L chlorine.

8.0 Sample Collection, Preservation and Storage

8.1. Sampling procedures as described in detail in the USEPA microbiology methods manual, Section II, A (21.2) and in Standard Methods for the Examination of Water and Wastewater.

8.1.1. Storage temperature and handling conditions: Ice or refrigerate bacteriological samples at a temperature less than 10 °C (2-10 °C) during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Samples shouldn't be completely submerged in water during transit. Don't allow samples to freeze. If sample freezes it can't be thawed. A new sample must be collected.

8.1.2. Holding time Limitations: Examine samples as soon as possible after collection. For drinking waters samples do not exceed 30 hours hold time from collection to incubation. For non-potable water for compliance, the required maximum hold time of samples is 8 hours from collection to incubation.

9.0 Quality Control

9.1. Quality control should be conducted on each lot of Colilert®-18 or more often as regulations require. One of the following quality control procedures is recommended for each lot of Colilert®-18 when used for total coliform and *E.coli* testing:

9.1.1. IDEXX-QC Coliform and *E.coli:* Consists of 3 each of *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*

1. See package insert for instructions.
2. Obtain the mean and range from the website; www.idex.com/water under Quality Certificates.

9.1.2. Quanti-Cult: Consists of 3 each of *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*

1.See the packet insert for instructions.

9.1.3 ATCC: Fill 3 sterile vessels with 100mL of sterile, non-buffered oxidant-free water and inoculate with a sterile loop of ATTC strains, *Escherichia coli* ATCC 11775 or 25922(positive control), *Klebsiella pneumoniae* (positive control) ATCC 31488 and *Pseudomonas aeruginosa* ATCC 10145 or 27853 (negative control).

9.2. Follow Section 12.0 P/A Procedure or Section 13.0 Quanti-Tray Enumeration Procedure and Section 14. Interpretation and Calculations.

9.3. Sample bottle and Quanti-Tray sterility check per lot (20.7; see Section V, 5.4.2)

9.3.1. At least one sample bottle/lot and tray/lot are tested with Tryptic Soy Broth (25mL for the bottle and 100mL for the tray) and incubated at 35 ± 0.5°C. It is recommended that this be performed in a laminar flow hood. Aseptic technique must be maintained. If not available, aseptic technique must be maintained. Do not open bottle for long periods of time nor place the cap on the lab surface facing up. Open cap just enough to add the TSA to the bottle and close immediately. Cap bottle and rotate so that broth comes into contact with all surfaces.

9.3.2. Check samples for growth at 24 and 48 hours. No growth should be observed. Record results in Quality Control Logbook.

9.3.3. If growth is observed, retest, and if still positive, call IDEXX Water Technical Service (1-800-321-0207).

9.4. Monthly Sealer check with food color or dye:

9.4.1. Add 2-3 drops of food coloring dye or equivalent to 100mL of water. Mix well.

9.4.2. Add this to the Quanti-Tray and seal the tray.

9.4.3. Observe the tray. There should be no dye observed outside the wells.

9.4.4. If dye is observed outside the well, retest and if it still occurs call IDEXX Water Technical Service (1-800-321-0207).

9.5. Media sterility check using sterile water per lot

9.5.1. Each new lot shall be checked for sterility. Select at least one blister pack and add 100mL of sterile DI water to a vessel. Mix well and add this to a Quanti-Tray and seal. Incubate up to 18 and no longer than 22 hours at 35°C +/- 0.5°C (for Colilert (24) incubate 24-28hrs at35°C +/- 0.5°C).

9.5.2. No color should be observed.

9.5.3. If color is observed, retest, and if still positive, call IDEXX Water Technical Service (1-800-321-0207).

9.6. Air Quality- The air quality in the laboratory is monitored monthly. Plates of R2A media are left open in different parts of the laboratory for 15 minutes. A sterile plate is also poured and left closed for the 15 minutes. Incubate plates @ 35°C +/- 0.5 °C for 48hrs and observe for growth and record in appropriate book.

9.7.0 Precision of Duplicate Analysis: Duplicates are run on 10% of samples for all analyses requiring enumeration. For each most recent set of 15 samples and its corresponding duplicate (for each type of sample analysis **i.e. fecal**, ***E.coli***, **enterococci**), the range of logs is calculated in order to determine the acceptance criteria of precision. Once established, the criteria of precision will be used to determine if the following duplicate analyses are within acceptable limits. At the end of each week the criteria are updated to include the most recent set of 15 samples run in duplicate. The updated precision is used to determine precision acceptability for the following week.

9.7.1. Obtain the recorded numbers of the most recent 15 samples run in duplicate (for the same type of analysis).

9.7.2. Calculate the logarithm of each result (and if any result is <1 then add 1 to both values before calculating the logarithm) and record in a spreadsheet as *L1* and *L2*.

9.7.3. Range of Logarithms (R_{log}) is calculated using the following equation:

$$R_{log} = | L1 - L2 | \qquad (1)$$

9.7.4. The mean ($\overline{R}$) of R_{log} is calculated as follows:

$$\overline{R} = \frac{\sum R_{log}}{n} \qquad (2)$$

$\sum R_{log}$ = The sum of the range of logs.

n = The number of sets of transformed duplicates.

9.7.5. Precision Criterion is calculated as follows:

$$Precision\ Criterion = 3.27 \times \overline{R} \qquad (3)$$

9.7.6. Any samples run in duplicate (10% of all samples) have their Range of Logarithms (R_{log}). If the range is greater than the Precision Criterion, there is a greater than 99% probability that the analysis has exceeded variability limits. For any samples that fall outside the acceptable limits, determine if the imprecision is acceptable. If data is not acceptable, all results since the last precision check should be rejected. The analytical problem(s) should be determined and corrective action should be taken to resolve problem.

9.7.7. See **Appendix A** for an example of the precision criterion.

10.0 Calibration and Standardization

10.1. Thermometers and temperature recording equipment- The accuracy of thermometers is checked annually against a certified NIST thermometer at the temperature used. The maximum thermometers and NIST thermometers are checked annually by Alert Scientific. In general, the graduations on the thermometer should be in increments of 0.5 degrees or less. The results of each thermometer check are recorded in the Thermometer Logbook.

10.2. Balances- Balances are checked daily with the provided class S weights. The top loading balance is for anything weighing 3g or more. For this balance the 100.0g, 50.0g, 1.0g, and 100mg weights are used. Each weight is weighed independently, and in addition, a deflection test is performed prior to usage. The results of the check should be

recorded in the assigned balance book. The S class weights and balance are checked annually by Alert Scientific.

10.3. Refrigerators- Check and record all temperatures daily. Use a refrigerator maintaining a temperature of 1.0° to 4.4°C to store samples, media, reagents, etc.

10.4. Incubators- Temperature is checked and recorded twice daily with a four-hour separation time between readings and adjusted when needed. Times of measurements shall be recorded along with temperatures.

11.0 Corrective Action

11.1. If an unacceptable result is obtained, then the lab should review the test procedure to determine the cause of the failure and to prevent this from reoccurring again by:

11.2. Defining the problem:

A. Identify corrective action and steps required to correct the problem.
B. Implement corrective action.
C. Document corrective action.

11.3. Repeat testing to ensure that corrective action was successful.

11.4. Examples include:

11.4.1. Procedure followed for preparing the control and or diluent.
11.4.2. Incubation temperature within the required time period.
11.4.3. Call and review problem encountered with IDEXX Water Technical Support (1-800-321-0207).

12.0 Presence-Absence (P/A) Procedure

12.1. Colilert-18 and the IDEXX Quanti-Tray System work with either the 51 Well Quanti-Tray or the Quanti-Tray/2000. For accuracy and counting range, use either tray and follow the Presence/Absence Procedure (Section 13.0-13.6). The use of IDEXX Antifoam may be necessary to reduce foaming and eliminate excess bubbles in the wells of the Quanti-Tray. If not, allow the foam to dissipate for 30-60 seconds prior to adding the sample to the tray.

12.2. Carefully separate one blister pack from the strip taking care not to accidentally open the adjacent pack.

12.3. Ensure the powder is at the bottom of the blister pack.

12.4. Hold the blister pack face down (paper side up) at the top and towards the back and snap back the score line forming a "v" with the opening facing the open vessel.

12.5. Allow the powder to fall into the vessel containing the room temperature sample (with 100mL +/- 2.5mL sample). Aseptically cap and seal the vessel. Mix the sample well to dissolve the reagent.

12.6. If the sample is not already at 33-38°C, then place vessel in a 35°C water bath for 20 minutes or alternatively, at 44.5°C water bath for 7-10minutes(this is part of the 18 hour incubation) If a dilution is necessary, use sterile deionized or distilled water, not buffered water for making the dilution prior to adding the Colilert®-18. Always add the Colilert®-18 to the final 100mL diluted sample only.

Note: If the sample has some background color, it is recommended that the sample be diluted to at least 1:4 (25mL of a well mixed sample to 75mL of sterile water) or 1:10 (10mL of a well mixed sample to 90mL of sterile water) as noted in 12.6. As an alternative, compare the sample with Colilert®-18 to a control blank of the same water sample adding it to the Quanti-Tray (no Colilert-18) and incubate along with the sample with Colilert®-18.

12.7. Incubate Colilert-18 for 18-22 hours at 35+/- .5°C. Incubate Colilert-24 for 24-28hours at 35.0°C +/- 0.5°C. Read results and compare each result against the comparator dispensed into an identical vessel.

12.8. If the sample is less yellow than the comparator, the test is negative. **Note:** *However, if the results are ambiguous to the analyst based on the initial reading, incubate up to an additional four hours (but not to exceed 22 hours total for Colilert-18 and 28 hours for Colilert-24) to allow the color to intensify.*

12.9. If the sample has a yellow color equal to or greater than the comparator, the presence of total coliforms is confirmed. If the color is not uniform, mix by inversion, then recheck.

12.10. If yellow is observed, check vessel for fluorescence by placing a 6 watt, 365-366nm UV light within 5 inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. If the fluorescence is equal to or greater than the comparator, the presence of *E.coli* is confirmed.

12.11. **For Colilert 18, positives for both total coliforms and *E.coli* observed before 18 hours and negatives observed after 22hours are also valid. For Colilert 24, positives for both total coliforms and *E.coli* observed before 24 hours and negatives observed after 28 hours are also valid.**

12.10. For samples with high chlorine a blue flash may be seen when Colilert® or Colilert®-18 is added; if this is seen the sample is invalid.

Note: Per conversation with Idexx representative, when excessive chlorine is in the sample, the color may change to a blue, purple or brownish color

and may foam due to excessive chlorine. Sample should be invalidated because such large amounts of chlorine would kill anything in the sample and the reaction cannot continue normally with such a drastic change in water color.

13.0 Quantification Procedure

13.1 For accuracy and counting range, use the IDEXX Quanti-Tray System with either the 51 Well Quanti-Tray or the Quanti-Tray 2000 and follow the above Presence/Absence procedure (12.1-12.6). The use of IDEXX Antifoam reagent may be necessary to reduce foaming and eliminate excess bubbles in the wells of the Quanti-Tray. Alternatively, let the sample sit for 30-60 seconds to allow the foam to dissipate. Note: All the foam doesn't need to dissipate. It can be used for multiple tube (MPN); 5 tubes X 20mL, 10 tubes X 10mL or 15 tube serial dilutions. Consult Standard Methods for the Examination of Water and Wastewater for the appropriate MPN Tables.

13.2 If a dilution is required, use sterile deionized or distilled water, not buffered water, for making the dilutions. Always add Colilert/ Colilert-18 to the final 100mL diluted sample only.

13.3 Follow the package insert for the Quanti-Tray (20.5) along with the package insert for Colilert-18 (20.1) and/or see 12.1-12.6 above. Remove a sterile tray from the plastic bag (tear open the plastic bag at the bottom which has a black line around the bag) and remove the number of trays required for testing. Close the bag using tape or a clip. Label the back of the with a marker to identify the sample. Open the tray via instructions in insert for Quanti-Tray (20.5). Pour the room temperature reagent mixture from the vessel into the tray avoiding contact with the foil tab. Seal the tray with the Quanti-Tray Sealer.

13.4 Incubate at 35°C+/- 0.5°C for 18-22 hours for Colilert-18 and 24-28 hours for Colilert-24. Pre-warming is not required. See package insert for details.

14.0 Interpretation and Calculations:

14.1. Follow the same interpretation directions from Section 12.9-12.12 to count the number of positive wells. Refer to the Quanti-Tray MPN Table provided by Idexx to determine the Most Probable Number (MPN) for total coliforms (yellow wells) and *E.coli* (yellow and fluorescent wells) in the sample. Read results between 18 and 22 hours (Colilert-18) and/or 24-28 hours (Colilert-24). Count the number of positive wells and refer to the MPN Table provided with the Quanti-Tray to obtain a Most Probable Number. If the sample was diluted, correct the MPN obtained from the MPN table by multiplying that MPN value with the dilution factor to obtain the final corrected MPN/100mL (if a 1:10 dilution was made, the dilution factor is 10). The color and fluorescence of positive wells may vary. Use the appropriate Quanti-Tray MPN comparator following the instructions as indicated.

14.2. For Colilert 18, positives for both total coliforms and *E.coli* observed before 18 hours and negatives observed after 22hours are also valid. For Colilert 24, positives for both total

coliforms and *E.coli* observed before 24 hours and negatives observed after 28 hours are also valid.

15.0 Method Performance

15.1. Colilert-18 is equally as sensitive as compared to LTB, BGBLB and EC+MUG. *E.coli* recovery was not statistically different compared to m- TEC. (20.6)

16.0 Reporting Results

16.1. Quantification, report results as MPN/100mL for total coliforms and *E.coli.*

17.0 Verification Procedure

17.1. Not applicable

18.0 Pollution Prevention

18.1. The solutions and reagents used in this method pose no threat to the environment when recycled and managed properly.

18.2. Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

19.0 Waste Management

19.1. Biological wastes shall be autoclaved at 121°C for thirty minutes and disposed of with standard trash. Little chemical waste is generated in association with the microbiological analyses. Those chemical wastes generated shall be stored in a designated area and segregated from non-compatible wastes and removed annually by a licensed Hazardous Materials Disposal Company.

19.2. It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, particularly the biohazard and hazardous identification rules and land disposal regulations. Compliance with all sewage discharge permits and regulations is also required.

20.0 References

20.1. Colilert-18 and Colilert-24 Package Inserts from IDEXX.

20.2. Standard Methods, "Examination of Water and Wastewater', Revision 20th Edition, Method Enzyme Substrate Test, 9223, 9223 B; 1998.

20.3. Bordner, R., J.A. Winter and P.V. Scarpino (eds.) Microbiological Methods for Monitoring the Environment, Water and Wastes, EPA-600/8-78-017. Office of Research and Development, USEPA. (December 1978)

20.4. Federal Register/ Vol 77, #97/ Friday, May 18th 2012, pg 29806-29807

20.5. Clesceri, L.S., A.E. Greenberg, A.D. Eaton (eds.). 1998 Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association, Washington, DC (also see 21st, 22nd and on-line edition)

20.6. Federal Register/ vol.66, No. 169/ Thursday, August 30th, 2001, page 45818

20.7. USEPA Manual for Certification of Laboratories Analyzing Drinking Water, Fifth Edition, Section V

21.0 Appendix A – Precision Criterion Table	mTEC– *Escherichia coli*	Duplicate Analyses		Logarithms of Counts		Range of Logarithms (R_{log})
Sample No.	Analytical Date	D1	D2	L1	L2	|L1-L2|
1	50031-11/14/08	4	1	0.60206	0	0.60206
2	50017- 11/13/08	12	10	1.079181	1	0.079181
3	49928- 11/4/08	9	5	0.954243	0.69897	0.255273
4	49886- 10/30/08*	1	1	0	0	0
5	49863-10/29/08**	1	1	0	0	0
6	49814- 10/23/08	20	23	1.30103	1.361728	0.060698
7	49764- 10/21/08	5	5	0.69897	0.69897	0
8	49728- 10/16/08	6	7	0.778151	0.845098	0.066947
9	49666- 10/14/08	1	2	0	0.30103	0.30103
10	49591- 10/7/08	7	6	0.845098	0.778151	0.066947
11	49557- 10/2/08	11	10	1.041393	1	0.041393
12	49512- 9/30/08	10	7	1	0.845098	0.154902
13	49512- 9/30/08	2	2	0.30103	0.30103	0
14	49456- 9/25/08	13	17	1.113943	1.230449	0.116506
15	49402- 9/23/08***	1	1	0	0	0

1) $\Sigma R_{log} =$ 1.744935

2) $\overline{R} = \frac{\sum R_{log}}{n} =$ 0.116329

3) Precision criterion = 0.380396

*= Value of D2 is 0
**=Value of D1 and D2 is 0
***=Value of D1 is 0

Barnstable County Department of Health and the Environment Laboratory

STANDARD OPERATING PROCEDURE

For

The Detection of Enterococci in Water Using the following Method:

Enterolert and Quanti-Tray

SM 9230D

April 15, 2021

	Signature	Date
Laboratory Director: Dan White	Ryan L Lucier	6/14/21
Bacteriologist: Ryan Lucier		14 JUN 21

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STANDARD OPERATING PROCEDURE
For
The Detection of Enterococci in Water Using the following Method:

Enterolert and Quanti-Tray
SM 9230

1.0 Scope and Application

1.1 This method is used for the detection and confirmation of enterococci in water. Any positive sample for total coliforms is an indication of contamination. Any positive sample for enterococci is an indication of contamination.

1.2 The minimum, non-zero number of bacterial counts detectable with this method is a function of the dilution scheme used when processing the sample.

1.3 The Enterolert method can be applied to drinking waters, recreational waters (marine and fresh), reuse waters and waste waters. Marine waters must be diluted at least 10-fold with sterile water. It can be used as a Presence/ Absence test or quantification with 5 X 20mL, 10 X 10mL, 15 tube serial dilution (MPN) or with the Quanti-Tray system (19.1).

2.0 Summary of Method

2.1 This method is based on Defined Substrate Technology®. The product utilizes a nutrient indicator that produces a blue fluorescence when metabolized by enterococci. When the reagent is added to the sample and incubated, it is able to detect these bacteria at 1 CFU/100mL at 24-28hours.

2.2 Enterolert is in *Standard Methods for the Examination of Water and Wastewater*, On-line and in the 22nd Edition, AWWA, APHA, WEF; section 9230 (19.3) and in the ASTM Method 6503 (19.10).

3.0 Definitions

3.1 In this method, enterococci bacteria are those bacteria which produce a blue fluorescent signal under a 6 watt, 365-366nm UV light after incubation at 41.0 ± 0.5°C for 24-28 hours.

4.0 Safety

4.1. The analyst must know and observe all appropriate safety procedures required in a microbiology laboratory preparing, using, and disposing of samples, reagents and materials, and while operating equipment.

4.2 Mouth pipetting is prohibited. Purity of Reagents: Reagent-grade chemicals shall be used in all tests. Whenever possible, use commercial culture media as means of quality control. Use reagent grade water from the Milli-Q system to assure quality of water.

5.0 Equipment and Supplies

5.1 Graduated cylinders, 50mL, 100mL, 250 mL, 500 mL, and 1000mL
5.2 National Institute of Standards and technology (NIST) certified thermometer (Thermometer, Cat. # 1005-3FC, range (-1+101°C, 0, 1°C)
5.3 Thermometers for incubators
5.4 Incubators- temperature at 35.0 ± 0.5° Incubators (Vendor: VWR, Model: 1556,Vendor: VWR, Model: 1545 and/ or water baths temperature at 44.5 ± 0.5°) (Vendor: Thermo Scientific, Model:Precision COL35,)
5.5 PPE (Personal Protective Equipment)-safety goggles, gloves, lab coat
5.6 Sterile plastic bottles (Vendor:IDEXX) that hold up to 120mL for proper mixing of sample also if needed, contain sodium thiosulfate to neutralize up to 5 mg/L of chlorine for drinking water samples and up to 15mg/L of chlorine for waste water effluents..
5.7 Top loading balance (Vendor: Scout, Item #: SC6010)
5.8 Sterile pipettes
5.9 Quanti-Tray Sealer
5.10 51 Well Quanti-Tray or Quanti-Tray/2000
5.11 6 Watt 365-366 nm UV light

6.0 Reagents

6.1 Sterile, non-buffered, oxidant-free water for dilutions.
6.2 Store Enterolert at 2-30°C away from the light. The expiration date is indicated on the package (12 months from the date of manufacture).
6.3 Sodium thiosulfate reagent *Standard Methods for the Examination of Water and Wastewater,* (21.3) or sterile vessels containing sodium thiosulfate to neutralize up to 15mg/L chlorine.

7.0 Sample Collection, Preservation and Storage

7.1 Sampling procedures as described in detail in the USEPA microbiology methods manual, Section II, A (19.2) and in Standard Methods for the Examination of Water and Wastewater(19.3).
7.1.1. Storage temperature and handling conditions: Ice or refrigerate bacteriological samples at a temperature less than 10 °C (2-10 °C) during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Samples shouldn't be completely submerged in water during transit. Don't allow samples to freeze. If sample freezes it can't be thawed. A new sample must be collected.
7.1.2. Holding time Limitations: Examine samples as soon as possible after collection. For drinking waters samples do not exceed 30 hours hold time from collection to incubation. For non-potable water for compliance, The required maximum hold time of samples is 8 hours from collection to incubation (19.4).

8.0 Quality Control

8.1. Quality control should be conducted on each lot of Enterolert or more often as regulations require. One of the following quality control procedures is recommended for each lot of Enterolert when used for enterococci testing at 41.0°C +/- 0.5°C:

8.1.1. IDEXX-QC Enterococci: Consists of 3 each of *Enterococcus faecium, Escherichia coli and Streptococcus bovis.*

1. See package insert for instructions.
2. Obtain the mean and range from the website; www.idex.com/water under Quality Certificates.

8.1.2. **ATCC**

A. For each of the American Type Culture Collection (ATCC) bacterial strains Quanti-Cult : Consists of 3 each of *Enterococcus faecium ATCC 35667, Serratia marcescens* ATCC 43862 and *Aerococcus veridans* ATCC 10400), streak the culture onto labeled TSA or Blood Agar plates and incubate at 35°± *2°C* for 18-24 hours.

B. For each bacterial strain, touch a 1µL inoculating loop to a colony and use it to inoculate a labeled test tube containing 5mL of sterile deionized water. Close cap and shake thoroughly.

C. For each bacterial strain, take a 1µL loop from the test tube and use it to inoculate a labeled vessel containing 100mL of sterile deionized water.

8.2. Follow Section 11. P/A Procedure or Section 12. Quanti-Tray Enumeration Procedure and Section 13.0 Interpretation and Calculations

8.3. Sample bottle and Quanti-Tray sterility check per lot (19.9; see section V, 5.4.2)

8.3.1. At least one sample bottle/lot and tray/lot are tested with Tryptic Soy Broth (25mL for the bottle and 100mL for the tray) and incubated at 35 ± 0.5°C. It is recommended that this be performed in a laminar flow hood. Aseptic technique must be maintained. If not available, aseptic technique must be maintained. Do not open bottle for long periods of time nor place the cap on the lab surface facing up. Open cap just enough to add the TSA to the bottle and close immediately. Cap bottle and rotate so that broth comes into contact with all surfaces.

8.3.2. Check samples for growth at 24 and 48 hours. No growth should be observed. Record results in Quality Control Logbook.

8.3.3. No growth should be observed.

8.3.4. If growth is observed, retest, and if still positive, call IDEXX Water Technical Service (1-800-321-0207).

8.4. Monthly Sealer check with food color or dye: (19.9; see Section V; 5.3.2.1.2)

8.4.1. Add 2-3 drops of food coloring dye or equivalent to 100mL of water. Mix well.

8.4.2. Add this to the Quanti-Tray and seal the tray.

8.4.3. Observe the tray. There should be no dye observed outside the wells.

8.4.4. If dye is observed outside the well, retest and if it still occurs call IDEXX Water Technical Service (1-800-321-0207).

8.5. Media sterility check using sterile water per lot

8.5.1. Each new lot shall be checked for sterility. Select at least one blister pack and add 100mL of sterile DI water to a vessel. Mix well and add this to a Quanti-Tray and seal. Incubate up to 24 and no longer than 28 hours at 41.0°C ±.5°C.

8.5.2. No fluorescence should be observed.

8.5.3. If fluorescence is observed, retest, and if still positive, call IDEXX Water Technical Service (1-800-321-0207).

8.6. Air Quality- The air quality in the laboratory is monitored monthly. Plates of R2A media are left open in different parts of the laboratory for 15 minutes. A sterile plate is also poured and left closed for the 15 minutes. Incubate plates @ 35°C +/- 0.5 for 48hrs and observe for growth and record in appropriate book.

8.7. Precision of Duplicate Analysis: Duplicates are run on 10% of samples for all analyses requiring enumeration. For each most recent set of 15 samples and its corresponding duplicate (for each type of sample analysis **i.e. fecal**, ***E.coli***, **enterococci**), the range of logs is calculated in order to determine the acceptance criteria of precision. Once established, the criteria of precision will be used to determine if the following duplicate analyses are within acceptable limits. At the end of each week the criteria are updated to include the most recent set of 15 samples run in duplicate. The updated precision is used to determine precision acceptability for the following week.

8.7.1. Obtain the recorded numbers of the most recent 15 samples run in duplicate (for the same type of analysis).

8.7.2. Calculate the logarithm of each result (and if any result is <1 then add 1 to both values before calculating the logarithm) and record in a spreadsheet as *L1* and *L2*.

8.7.3. Range of Logarithms (R_{log}) is calculated using the following equation:

$$R_{log} = | L1 - L2 | \qquad (1)$$

8.7.4. The mean ($\overline{R}$) of R_{log} is calculated as follows:

$$\overline{R} = \frac{\sum R_{log}}{n} \qquad (2)$$

$\sum R_{log}$ = The sum of the range of logs.

n = The number of sets of transformed duplicates.

8.7.5. Precision Criterion is calculated as follows:

Precision Criterion = 3.27 x R (3)

8.7.6. Any samples run in duplicate (10% of all samples) have their Range of Logarithms (R_{log}). If the range is greater than the Precision Criterion, there is a greater than 99% probability that the analysis has exceeded variability limits. For any samples that fall outside the acceptable limits, determine if the imprecision is acceptable. If data is not acceptable, all results since the last precision check should be rejected. The analytical problem(s) should be determined, and corrective action should be taken to resolve problem.

8.7.8. See **Appendix A** for an example of the precision criterion.

9.0 Calibration and Standardization

9.1. Thermometers and temperature recording equipment- The accuracy of thermometers is checked annually against a certified NIST thermometer at the temperature used. The maximum thermometers and NIST thermometers are checked annually by Alert Scientific. In general, the graduations on the thermometer should be in increments of 0.5 degrees or less. The results of each thermometer check are recorded in the Thermometer Logbook. The temperature of the incubator is checked twice a day (when in use) separated by at least 4 hours to insure it is within the stated limits. Record the date, temperature, time of reading and initials.

9.2. Balances- Balances are checked daily with the provided class S weights. The top loading balance is for anything weighing 3g or more. For this balance the 100.0g, 50.0g, 1.0g, and 100mg weights are used. Each weight is weighed independently, and in addition, a deflection test is performed prior to usage. The results of the check should be recorded in the assigned balance book. The S class weights and balance are checked annually by Alert Scientific.

9.3. Refrigerators- Check and record all temperatures daily. Use a refrigerator maintaining a temperature of 1.0° to 4.4°C to store samples, media, reagents, etc.

10.0 Corrective Action

10.1. If an unacceptable result is obtained, then the lab should review the test procedure to determine the cause of the failure and to prevent this from reoccurring again by:

10.2. Defining the problem:

A. Identify corrective action and steps required to correct the problem.
B. Implement corrective action.
C. Document corrective action.

10.3. Repeat testing to ensure that corrective action was successful.

10.4. Examples include:

10.4.1. Procedure followed for preparing the control and or diluent.
10.4.2. Incubation temperature within the required time period.
10.4.3. Call and review problem encountered with IDEXX Water Technical Support (1-800-321-0207).

11.0 Presence-Absence (P/A) Procedure

11.1. Carefully separate one blister pack from the strip taking care not to accidentally open the adjacent pack.

11.2. Ensure the powder is at the bottom of the blister pack.

11.3. Hold the blister pack face down (paper side up)at the top and towards the back and snap back the scoreline forming a "v" with the opening facing the open vessel.

11.4. Allow the powder to fall into the vessel containing the room temperature sample (with 100mL +/- 2.5mL sample). Aseptically cap and seal the vessel. Mix the sample well to dissolve the reagent.

11.5. Incubate for 24 hours and up to 28 hours at 41.0 °C +/- .5°C.

11.6. Read results at 24 hours or up to 28 hours. Check the vessel for blue fluoresence by placing a 6-watt, 365-366nm UV light within 5 inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. Alternatively, use a UV viewing cabinet.

11.7. If no blue fluoresence is observed, the test is negative.

11.8. If blue fluoresence is observed, the sample is positive for enterococci.

11.9. However, *if the results are ambiguous to the analyst based on the initial reading, incubate up to an additional four hours (but not to exceed 28 hours total) to allow for the fluorescence to intensify.*

12.0 Procedure Quantification

12.1 For accuracy and counting range, use the IDEXX Quanti-Tray System with either the 51 Well Quanti-Tray or the Quanti-Tray-2000 and follow the Presence-Absence procedure above(11.1-11.9) for adding the powder to the sample and incubation. Marine water samples require at least a 1:10 dilution. Enterolert can be used with multiple tubes to yield a MPN; 5 tubes X 20 mL, 10 tubes X 10mL, or 15 tube serial dilution. Consult Standard Methods for the Examination of Water and Wastewater for the appropriate MPN Tables.

12.2 If a dilution is required, use sterile deionized or distilled water, not buffered water, for making the dilutions. Always add Enterolert to the final 100mL diluted sample only.

12.3 Follow the package insert for the Quanti-Tray (20.5) along with the package insert for Enterolert (19.1) and/or see 11.1-11.9 above. Remove a sterile tray from the plastic bag (tear open the plastic bag at the bottom which has a black line around the bag) and remove the number of trays required for testing. Close the bag using tape or a clip. Label the back of the with a marker to identify the sample. Open the tray via instructions in insert for Quanti-Tray(19.4). Pour the sample reagent mixture from the vessel into the tray avoiding contact with the foil tab. Seal the tray with the Quanti-Tray Sealer.

12.4. Incubate at 41°C+/- 0.5°C for 24 hours and up to 28 hours.

13.0 Interpretation and Calculations:

13.1. Follow the same interpretation directions from Section 11.9-11.12 and count the number of positive wells. Refer to the Quanti-Tray MPN Table provided by Idexx to determine the Most Probable Number (MPN) for fluorescent wells in the sample. If the sample was diluted, correct the MPN obtained from the MPN table by multiplying that MPN value with the dilution factor to obtain the final corrected MPN/100mL (if a 1:10 dilution was made, the dilution factor is 10). The fluorescence of positive wells may vary. Use the appropriate Quanti-Tray MPN comparator following the instructions as indicated. Any blue fluorescence is a positive well.

14.0 Method Performance

14.1. Enterolert was found to be equivalent to the MF method. Correlation of 0.97 was found between Enterolert and MF enterococci method. A false positive and false negative rate of 5.1% and 0.4% was found (19.6)

15.0 Reporting Results

15.1. Report results as Presence or Absence for enterococci. For Quantification, report results as MPN/100mL, refer to the Quanti-Tray MPN Table provided by Idexx to determine the MPN. Correct the MPN value for any dilution made. The fluorescence of the positive well may vary in intensity.

15.2. Enterolert is approved by the US EPA for Recreational (19.7) and Waste Water (19.8).

16.0 Verification Procedure

16.1. Not applicable

17.0 Pollution Prevention

17.1. The solutions and reagents used in this method pose no threat to the environment when recycled and managed properly.

17.2. Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

18.0 Waste Management

18.1. Biological wastes shall be autoclaved at 121°C for thirty minutes and disposed of with standard trash. Little chemical waste is generated in association with the microbiological analyses. Those chemical wastes generated shall be stored in a designated area and segregated from non-compatible wastes and removed annually by a licensed Hazardous Materials Disposal Company.
18.2. It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, particularly the biohazard and hazardous identification rules and land disposal regulations. Compliance with all sewage discharge permits and regulations is also required.

19.0 References

19.1. Enterolert Package Insert from IDEXX.
19.2. Bordner, R., J.A. Winter and P.V. Scarpino (eds.) Microbiological Methods for Monitoring the Environment, Water and Wastes, EPA-600/8-78-017. Office of Research and Development, USEPA. (December 1978)
19.3. Federal Register/ Vol 77, #97/ Friday, May 18th 2012, pg 29806-29807
19.4. Clesceri, L.S., A.E. Greenberg, A.D. Eaton (eds.). 1998 Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association, Washington, DC (also see 21st, 22nd and on-line edition)
19.5. Quanti-Tray Package Insert from IDEXX
19.6. Federal Register/ vol.68, No. 139/ Monday, July 21st, 2003/ Rules and Regulations (Recreational Waters)
19.7. Federal Register/ vol.72, No.47/ Monday, March 12, 2007/ Rules and Regulations (Wastewater),
19.8. USEPA Manual for Certification of Laboratories Analyzing Drinking Water, Fifth Edition, Section V
19.9. Budnick, G et al; Evaluation of Enterolert for Enumeration of *E.coli* in Recreational Waters, AEM, Vol62, No. 10, Oct 1966, p3881-3884
19.10. ASTM D6503 Standard Test Method for Enterococci in Water Using Enterolert

20.0 Appendix B - Precision Criterion Table	mTEC- *Escherichia coli*	Duplicate Analyses		Logarithms of Counts		Range of Logarithms (R_{log})
Sample No.	Analytical Date	D1	D2	L1	L2	|L1-L2|
1	50031-11/14/08	4	1	0.60206	0	0.60206
2	50017- 11/13/08	12	10	1.079181	1	0.079181
3	49928- 11/4/08	9	5	0.954243	0.69897	0.255273
4	49886- 10/30/08*	1	1	0	0	0
5	49863-10/29/08**	1	1	0	0	0
6	49814- 10/23/08	20	23	1.30103	1.361728	0.060698
7	49764- 10/21/08	5	5	0.69897	0.69897	0
8	49728- 10/16/08	6	7	0.778151	0.845098	0.066947
9	49666- 10/14/08	1	2	0	0.30103	0.30103
10	49591- 10/7/08	7	6	0.845098	0.778151	0.066947
11	49557- 10/2/08	11	10	1.041393	1	0.041393
12	49512- 9/30/08	10	7	1	0.845098	0.154902
13	49512- 9/30/08	2	2	0.30103	0.30103	0
14	49456- 9/25/08	13	17	1.113943	1.230449	0.116506
15	49402- 9/23/08***	1	1	0	0	0

1) ΣR_{log}= 1.744935

2) $\overline{R} = \frac{\sum R_{log}}{n} =$ 0.116329

3) Precision criterion =0.380396

*= Value of D2 is 0
**=Value of D1 and D2 is 0
***=Value of D1 is 0

Barnstable County Department of Health and the
Environment Laboratory

SM 5310 B

STANDARD OPERATING PROCEDURE

For

Determination of Total Organic Carbon (TOC) in Aqueous Samples Using High-Temperature Combustion Method

(Revision 12)

October 28, 2020

		Signature	Date
Analyst:	Liping Xun	Liping Xun	28/10/20
Laboratory Director:	Dan White		28/10/20

Barnstable County Laboratory

STANDARD OPERATING PROCEDURE

For

Determination of Total Organic Carbon (TOC) in Aqueous Samples Using High-Temperature Combustion Method

1.0 SCOPE AND APPLICATION

1.1 This SOP provides procedures for determination of Total Organic Carbon (TOC) in aqueous samples using High-Temperature Combustion Method (Ref 14.1).

2.0 SUMMARY OF METHOD[1]

2.1 The sample is homogenized and diluted as necessary and an aliquot of sample is injected into a heated reaction chamber packed with an oxidative catalyst such as cobalt oxide and platinum group metals. The water is vaporized and the organic carbon is oxidized to CO_2 and H_2O. The CO2 from oxidation of organic and inorganic carbon is transported in the carrier-gas streams and is measured by means of a non-dispersive infrared analyzer.

3.0 INTERFERENCES

3.1 Removal of carbonate and bicarbonate by acidification and purging with purified gas results in the loss of volatile organic substances. The volatiles also can be lost during sample blending, particularly if the temperature is allowed to rise.

3.2 Filtration, although necessary to eliminate particulate organic matter when only Dissolved Organic Carbon (DOC) is to be determined, can result in loss or gain of DOC, depending on the physical properties of the carbon-containing compounds and the adsorption or desorption of carbonaceous material on the filter.

3.3 Any contact with organic material may contaminate a sample.

4.0 SAFETY

4.1 Do not touch the electric furnace while it is heating. The center of the electric furnace (near the combustion tube insertion opening) reaches very high temperatures, and burns may result.

4.2 Allow the electric furnace to cool to room temperature before removing or exchanging the combustion tube. Burns may result if this procedure is attempted when the furnace is at a high temperature.

5.0 EQUIPMENT AND SUPPLIES

5.1 Total Organic Carbon Analyzer: TOC-$V_{CPH/CPN}$ (SHIMADZU CORPORATION)

5.2 Autosampler: ASI-V (SHIMADZU CORPORATION)

5.3 Supplies:

5.3.1 TOC/TN Catalysts;

5.3.2 40 ml clear and amber vials;

5.3.3 100 ml, and 500 ml volumetric flasks;

5.3.4 Ultra pure compressed air.

5.3.5 Ultra pure Helium.

5.4 Homogenizer: IKA Ultra-Turrax Homogenizer, and the Model: T10.

6.0 REAGENTS AND STANDARDS

6.1 Reagent Water – Deionized water is obtained from MILLIPORE Direct-Q 3 System.

6.2 2M HCL solution.

6.3 Potassium Hydrogen Phthalate, Stock Standard Solution:

6.3.1 Primary Standard: 1000 mg/L (ERA; Catalog# 978) is used for initial calibration. Once the primary standards are received, they will be logged in Primary Standard Logbook. The date of receipt,

name of vendor, catalog number, expiration date and primary standard ID will be recorded in the book. An example of the Logbook is attached (Figure 1).

Primary standard ID is labeled as TOCPmmddyyX:
where: TOCP = TOC Primary
Mmddyy = the date the standard is received
X = the order that the standard is logged into the logbook on that date in increasing alphabetical order.

6.3.2 Working Standards – There are six concentration levels for TOC initial calibration curve, and they are 0.0, 1.0, 5.0, 10, 50, 100 mg/L. The following Table lists the procedures for making these standards using reagent water:

Level	Standard Used to Make Calibration Standard		Final Volume (mL)	**Final Conc (mg/L)**
	Conc (mg/L)	Volume Taken from This Standard (mL)		
L6	1000 (Section 6.3.1)	10	100	**100**
L5	1000 (Section 6.3.1)	5	100	**50**
L4	100 (L6)	10	100	**10**
L3	50 (L5)	10	100	**5.0**
L2	10 (L4)	10	100	**1.0**
L1	0	0	100	**0**

6.3.3 After the working standards are made, they are logged into a Working Standard Logbook (Figure 2). The primary standard ID used for making the working standard, initial concentration, amount taken, final volume, final concentration, solvent used, expiration date and working standard ID are recorded in the Logbook as follows:

Working standard ID is labelled as IwmmddyyX:
where: TOCW = Inorganic working
Mmddyy = the date the standard is made
X = the order the standard is made on that date in increasing alphabetical order.

6.3.4 Matrix Spiking Standard: The primary standard (1000 mg/L) in Section 6.3.1 is also used as Matrix Spiking Standard.

0.50 mL of the primary standard (1000 mg/L) is used to spike into 50 mL of one of the field samples, and the final concentration of TOC in the spiked field sample is 10 mg/L.

6.3.5 Quality Control Standard (ICV): 1000 mg/L (Ultra Sci; Cat# IQC-106):

Take 1.0 ml Quality Control Standard (1000 mg/L) in to 100 mL volumetric flask, make 10 mg/L solution of Quality Standard.

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Aqueous samples are collected in 40 ml clear or amber glass VOA vials. The samples must be kept cool (4°C) and protected from sunlight and atmospheric oxygen.

7.2 40 mL of the sample is acidified with 0.4 mL of 4.5 N of H_2SO_4 to make sure pH $\leq$ 2.

8.0 QUALITY CONTROL

8.1 Initial Demonstration of Performance

8.1.1 Linear Dynamic Ranges (LDR): Linear calibration ranges are primarily detector limited. The upper limit of the linear calibration range must be established by analyzing a few of high level of standards, and one of which is close to the upper limit of the linear range. The upper LDR limit must be an observed signal no more than 10% below the level extrapolated from lower standards. The upper limit of the LDR is 50 mg/L for the study conducted on 5/28/2009.

8.1.2 Method Detection Limit (MDL): MDL is established by analyzing a TOC standard of the concentration of 1.0 mg/L in reagent water which is two-to-three times the estimated instrument detection limit. To determine MDL values, seven replicate aliquots of the fortified reagent water are analyzed and concentrations determined over a period of a minimum of three days. The fortified

concentration is 1.0 mg/L, and Section 6.3.2 lists the procedure for making this fortified concentration. Perform the calculations as follows and report the concentration values in mg/L:

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

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. If more replicates are used, use the corresponding t-value].

S = Standard deviation of the replicate analyses.

The MDL is determined once a year or whenever a significant change in the background or instrument response is detected or expected.

Table1 lists one set of MDL study results.

8.1.3 Quality Control Sample (QCS): A QCS is always run following the initial calibration curve. The analysis of the QCS must be within ± 10% of the true value. If the QCS is not within the required limits, an immediate second analysis of the QCS is analyzed to confirm unacceptable performance. If the second run of the QCS still fails, the source of the problem must be identified and corrected before either proceeding on with the initial determination of method detection limits or continuing with on-going analyses.

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 instrument performance of a blank sample and evaluate contamination from the laboratory environment. The values that exceed ½ the Method Reporting Limit (MRL) 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 laboratory analyzes a LFB with each analysis batch immediately following the LRB. The LFB is spiked at a concentration of 10 mg/L. The recovery of the spiked

standard must fall in the range of 80 -120% 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.

8.3 Assessing Analyte Recovery – The following must be included in every analytical batch:

8.3.1 Laboratory Fortified Sample Matrix (LFM) – The laboratory adds a known amount of the standard at the concentration of 10 mg/L to a minimum of 5% of the collected field samples or at least one with every analysis batch, whichever is greater.

8.3.1.1 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.

8.3.1.2 If the recovery falls the outside of 70-130%, 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.

8.3.2 Sample Duplicate Analysis

8.3.2.1 Sample duplicates are analyzed to demonstrate the precision of an analytical system. The duplicate analyses are performed on each batch of samples analyzed at a frequency of 10% of all samples in the batch or at least one sample if less than 10 samples are analyzed.

8.3.2.2 Relative Percent Difference (RPD): The relative percent difference is used to evaluate precision for the duplicate analyses, and RPD is calculated as follows:

$$RPD\ (\%) = \frac{|C_1 - C_2|}{C_{AVG}} \times 100 \qquad (2)$$

Where: C_1 = original sample concentration;
C_2 = duplicate sample concentration;
C_{AVG} = average of the two samples.

8.3.2.3 Acceptable Limits of the RPD: Acceptable limits of RPD for TOC are ≤20%.
If RPD falls outside of the limits and all of the other quality control and quality assurance parameters are acceptable, the data will be flagged as "Matrix Effect".

9.0 INSTRUMENT OPERATING CONDITIONS, DATA ACQUISITION PARAMETERS, AND ROUNTINE MAINTENANCE

NOTE: Refer to the instrument manual provided by SHIMADU (Ref: 14.2).

10 CALIBRATION AND STANDARDIZATION

10.1 External Standardization: Initial Calibration is conducted using External Method.

10.2 Initial Calibration: Initial Calibration is performed prior to any sample analyses using all standards as stated in Section 6.3.2

10.2.1 Relative Standard Deviation (RSD%) must be less than 20%.

10.3 Initial Calibration Verification (ICV): QCS is used for Initial Calibration Verification. The ICV is analyzed right after the initial calibration. The percent difference of the ICV must be less than 10%.

10.4 Continuing Calibration Verification (CCV): Every ten samples are analyzed between the beginning and closing CCVs. LRB always follows the beginning CCV. The percent difference of CCV must be less than 10%.

11 PROCEDURE

11.1 Follows instructions provided in the Manual (ref: 14.2) to start the instrument, and make sure that the pressure of Ultra pure Air is 200 kpa, and carrier gas flow is 150 ml/min.

11.2 Use TOC-Control V software to set up analytical method and sequence.

11.3 If the sample contains gross solids or insoluble matter, the sample will be homogenized using an IKA Ultra-Turrax Homogenizer to homogenize samples.
Analyze a homogenizing blank consisting of reagent water carried through the homogenizing treatment.

11.4 If inorganic carbon is to be removed before analysis, transfer a representative portion (10 to 15 mL to a 30 mL beaker, add acid to lower pH to 2 or less, and purge with helium for 10 minutes.
Because volatile organic carbon will be lost during purging of the acidified sample, report organic carbon as Total Non-purgeable Organic Carbon.
Check efficiency of inorganic carbon removal for each sample matrix by splitting a sample into two equal portions and adding to one portion an inorganic carbon level similar to that of the sample. The TOC values should agree; if they do not, adjust sample container, sample values, pH purge flow rate, purge time to obtain complete removal of inorganic carbon.

11.5 Transfer 20 ml of acidified samples to a clean 40 ml vial, and purge for about 10 minutes using Helium. After purging, transfer the sample to a 40 ml VOC vial for analysis.

11.6 Injection volume is 50 µl. Each sample is injected three times, and final concentration is the mean value of three readings.

11.7 Injections should be repeated until consecutive measurements are obtained that are reproducible within ±10%.

12 DATA ANALYSIS, CALCULATION AND REPORT

12.1 Data analysis, calculation and report are processed through TOC-Control V software.

13 POLLUTION PREVENTION AND WASTE MANAGEMENT

The laboratory waste management practices are conducted consistent with all applicable rules and regulations as stated in the laboratory's *Sample and Waste Disposal* (Revision 005) on November 7, 2017. Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner in this SOP.

14 REFERENCE

14.1 American Public Health Association, American Water Works Association, and Water Environment Federation, "Standard Methods for the Examination of Water and Wastewater", 22nd Edition, 2012.

14.2 SHIMADZU Corporation, "User's Manual for TOC-VCPH/CPN Total Organic Carbon Analyzer (For TOC-Control V Ver.2)", 638-94536.

Table 1: TOC Method Detection Limit Study										
							Unit: mg/L			
Spiking Level: 1.0 mg/L					Analyst: Ken Ni					
Date	1/4/2017	1/4/2017	1/4/2017	1/5/2017	1/5/2017	1/5/2017	1/6/2017	AVG	STDEV	MDL
	MDL01	MDL02	MDL03	MDL04	MDL05	MDL06	MDL07			
TOC	1.38	1.32	1.22	1.10	1.11	1.12	1.05	1.18	0.124	0.373

Figure 1. TOC Primary Standard Logbook

Barnstable County Laboratory

TOC Primary Standard Logbook

Date	Analyte	Vendor	Catalog No	Lot No	Concentration	Primary Standard ID	Exp Date	Initial

Logbook ID: BCDHE Log 001TOCP Reviewed By_____ Page_____

Figure 2. TOC Working Standard Logbook

Barnstable County Laboratory

TOC Working Standard Logbook

Date	Analyte	Primary Standard ID	Initial Conc	Amt Taken	Final Vol	Final Conc	Solvent	Working Standard ID	Exp Date	Initial

Logbook ID: BCDHE Log 001TOCW Reviewed By_____ Page_____

Barnstable County Department of Health and the Environment Laboratory

EPA Method 180.1

STANDARD OPERATING PROCEDURE

For

Determination of Turbidity in Aqueous Samples

Revision 008

October 29, 2020

		Signature	Date
Analyst:	Andrew Barker	Andrew T Barker	29 OCT 2020
Laboratory Director:	Dan White		29OCT20

Barnstable County Laboratory

STANDARD OPERATING PROCEDURE (SOP)
For

Determination of Turbidity in Aqueous Samples

1. SCOPE AND APPLICATION

1.1 This method covers the determination of Turbidity in drinking water, surface water, domestic water, industrial wastes, and other aqueous samples, by light scattering using a Nephelometer.

2. SUMMARY OF METHOD

2.1 The method is based upon a comparison of the intensity of light scattered by the sample under defined conditions with 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 (nephleometric turbidity units), are made in a nephelometer. A standard suspension of stabilized Formazin (StablCal^{TM})is used to verify the analytical system's on-going accuracy and stability of measurement.

2.1.1 Formazin 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. INTERFERENCES

3.1 Condensation on sample vials and light path materials may distort readings.

3.2 The presence of floating debris and coarse sediments which settle out rapidly will cause variable readings that quickly decline over time.

3.3 Finely divided air bubbles will affect the results in a positive manner.

3.4 The presence of true color (color due to dissolved substances such as humates, which absorb light), will cause turbidity readings to be low, although this effect is generally not significant with finished waters or treated wastewater effluent.

3.5 Large concentrations of light-absorbing materials, such as activated carbon, may interfere with turbidity readings.

3.6 Scratches on sample or standard vials, as derived from improper handling or cleaning, may contribute interference.

4. SAFETY

4.1 Follow general laboratory safety guidelines, such as wearing safety glasses, gloves and a lab coat.

4.2 The laboratory maintains a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of the Material Safety Data Sheets (MSDS) is available to all personnel involved in the analysis.

4.3 Hydrazine sulfate is highly toxic and may be fatal if inhaled, swallowed, or absorbed through the skin. Formazin can contain residual hydrazine sulfate.

5. EQUIPMENT AND SUPPLIES

5.1 Turbidimeter: Hach TU5200

5.2 Sample Vials: HACH Catalog # LZV946

5.3 Autopipette and corresponding disposable tips (optional)

5.4 Kimwipes™ or soft, lint/residue-free cloth

6. REAGENTS AND STANDARDS

6.1 Reagent water – Deionized water from Milli-Q Direct 8/16 System, Millipore Direct-Q 3 System.

6.2 StablCal Calibration Set (0-700NTU) with 10 NTU CCV standard, HACH Catalog # LZY835

6.3 StablCal 1.0 NTU HACH Catalog # 26598-49 (QC)

6.4 StablCal 0.10 NTU HACH Catalog # 27233-42 (MDL)

6.5 Once primary standards are received, log them into the Primary Standard Logbook, recording the date of receipt, name of vendor, catalog number, expiration date, and their primary standard ID.

6.5.1 Primary standard ID is labeled as IpmmddyyX:

Where: IP= Inorganic Primary
Mmddyy = the date standard is received
X = the order that the standard is logged into the logbook on that date in increasing alphabetical order.

7. SAMPLE COLLECTION, PRESERVATION AND STORAGE

7.1 Samples are collected in 250mL plastic bottles with no preservative.

7.2 The samples are stored at 4°C.

7.3 The holding time is 48 hours.

8. QUALITY CONTROL

8.1 Following instrument boot up and self-check, run a CCV standard (10 NTU) to ensure ongoing calibration. The observed value should fall within +/- 10% of the true value. If out of range, ensure there are no sources of interference and calibrate the instrument using the calibration standards, verifying again using the CCV standard.

8.2 After successful calibration verification, run a QC (1.0 NTU), MDL (0.1 NTU), and blank (DI water) sample. The QC sample should fall within +/- 10% of the true value. The MDL sample should fall within +/-20%, and the blank should yield a value of less than 0.05 NTU.

8.3 One duplicate sample should be run, at minimum, for every batch of 10 or fewer samples. The agreement between the sample and duplicate must be +/- 20%. If greater than 20%, run a QC check, recalibrate if needed, and re-run the samples from scratch.

8.4 After each batch of 10 samples or less, run a CCV standard and a blank. If CCV value falls outside +/- 10%, and/or if the blank is greater than 0.05, the analysis must be considered faulty and should be repeated from scratch.

8.5 Record duplicate values and QC values on control charts.

9.0 CALIBRATION AND STANDARDIZATION

9.1 Calibration may be performed as often as needed, but no less than once per month

9.2 For calibration, run Blank (optional for the TU5200), 20 NTU, and 600 NTU calibration standards following the instrument's onscreen prompts.

9.3 Following the prompts, run the 10 NTU CCV standard (the instrument will automatically determine success/failure of calibration using the standard of +/- 10% of true value).

10. PROCEDURE

10.1 Sample Preparation

10.1.1 Samples are refrigerated. In order to prevent condensation, allow samples to warm immediately prior to analysis.

10.1 2 Gently mix the sample to thoroughly disperse solids. Wait until air bubbles dissipate, then pour or pipette the sample into the turbidimeter sample vial, sealing the sample vial lid afterwards.

10.1 3 Wipe any spillage, condensate or dust off the sample vial using a Kimwipe™ or lint-free cloth (as needed), place the vial in the sample chamber, close the lid, and hit the 'read' button. Record the reported value in the turbidity data log.

11. DATA ANALYSIS, CALCULATIONS AND REPORT

11.1 Reports:
Record all data, including the date, Lab ID, Client ID, dilution if needed, the concentration (NTU), and analyst initials in the Turbidity run logbook.

12. POLLUTION PREVENTION AND WASTE MANAGEMENT

12.1 The laboratory waste management practices are conducted consistent with all Applicable rules and regulations as stated in the laboratory's Sample and Waste Disposal (Revision 003) on October 15, 2009. Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner in this SOP.

13. REFERENCES

1. Annual Book of ASTM Standards, Part 31,"Water", Standard D1889-71, 1976.

2. American Public Health Association (APHA)
American Water Works Association (AWWA)
Water Environment Federation (WGI)

3. Standard Methods (SM) for the Examination of Water and Wastewater, 22nd edition SM2130, 2012.

4. Hach Method 10258, Revision 2.0. March, 2018.

Appendix 14

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Massachusetts Alternative Septic System Test Center

Barnstable, Massachusetts

Form

Title: Weekly Quality Assurance Checklist

Effective Date: 2021-11-22	**Number:** MASSTC-FRM-010	**Revision:** 012

Authors

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Approvals

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Title: MASSTC Director
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Weekly Quality Assurance Checklist

Document ID#: MASSTC-FORM-010
Revision#: 012
Released: 2021-11-22
Released By: Brian Baumgaertel

Revision History

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the MASSTC Director on the MASSTC Sharepoint Site

History	Effective Date
Revision #012: Added daily virus dosing check. Edits by EMO.	2021-11-22
Revision #011: Removed C-Slope ponding from Thursdays. Changed last task to encompass cleaning entire work trailer - Edits by BH. Updated formatting (updated EMO).	2021-10-05
Revision #010: Added Zeta Ponding. Changed Daily Tasks fro a checklist into a review sheet. Removed daily reminders to check back of sheet. Removed "Lab counter cleaned, books stowed" and "Lab floor swept/vacuumed" from Friday and moved to Daily/Any Day section. Most important/forgettable daily tasks (shed cleanout, visitor log, and thermometer check) moved to Any Day/Other section. Updated 11:00 shed check to say "as needed." Edits by BH and EMO.	2021-08-26
Revision #009: Removed NitROE and SanTOE tasks from specific days, added to Any Day section. Removed UV water level check form daily tasks and put on Any Day section. Removed Check Operation of all systems. Added Oakson Field Meter Check to Tuesday tasks.	2021-05-14
Revision #008: Removed Oakson tasks. Removed BioOrg task. Removed "check consumables and lab inventory". Moved "check dosing buckets" from Any Day to Friday. Moved SanTOE and NitROE air pressure observations to Monday and Wednesday. Added Cen5 UV water level check to daily. Added NitROE recycle line check to daily. All by BH on 3/8/2021.	2021-03-08
Revision #007: Added SanTOE/NitROE check to "Any Day/Other" list. Removed EljenHomer bubbles check (BH confirmed no flow). Add check CEN5 UV tank water level to daily tasks. EMO	2020-11-13
Revision #006: Added House That Is Green ponding (it accidentally was deleted last revision). Changed heading from "Any Day" to "Any Day/Other." Added Oakson Columns Volume Measurement to Any Day/Other tasks. EMO	2020-10-29
Revision #005: Added Lambda Ponding to checklist. Removed EMO-only designation of tasks. Added Sample bottle and chain creation to Friday. Added Epsilon Ponding to checklist. Updated wording of "Hot List" to Microsoft Planner. Removed sweep/vacuum from "Any Day" tasks (for space). Added Woodchip Columns check to Tuesday. Add photo of BioOrg "juice" to checklist.	2020-08-18
Revision #004: Added checklist of daily tasks to back of list, and reference to completing these	2020-07-08
Revision #003: Added notations regarding recording data on shed checksheets. Edits by EMO.	2020-06-02
Revision #002: Removed Delta Alkalinity Additions, Added Homer checks. Edits by EMO	2020-04-24
Revision #001: Broke week down by day. Combined with Friday list. Edits by BB	2020-02-11
Revision #000: Original Issue	

Weekly Quality Assurance Checklist

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Released By: Brian Baumgaertel

Week of: _____

*****See back of sheet to review workflow each day*****

Task	Date	Initial
Monday		
By 7:55am - House That Is Green: Put dosing tubing into calibration cups		
9:00am *(or so)* - House That Is Green: Calibrate and write in log book		
GST/Stone ponding; put into electronic Sharepoint spreadsheet		
Tuesday		
By 7:55am - House That Is Green: For one cell, put dosing tubing into calibration cup to confirm cells dosing		
by 8:30am - House That Is Green: Confirm dose and pour dose		
10am - House That Is Green ponding; put into electronic spreadsheet		
10:15 - Zeta Ponding; put into electronic spreadsheet		
10:45 - Oakson Field Meter Check (save photo, record in database, record on sheet)		
Wednesday		
Check effluent sumps, note in electronic journal for each sump		
Record power meter readings; put in electronic Sharepoint spreadsheet		
Record pump chamber meter readings; put in electronic Sharept spreadsheet		
Crumpler Ponding; put into electronic Sharepoint spreadsheet		
Thursday		
By 7:55am - House That Is Green: For one cell, put dosing tubing into calibration cup to confirm cells dosing		
by 8:30am - House That Is Green: Confirm and pour dose		
10am - House That Is Green ponding		
Lambda ponding; put into electronic spreadsheet		
Epsilon ponding		
GST/Stone ponding		
Friday		
Clean channel and note in journal		
Clean dosing buckets, note in journal		
Lift station pumps checked/cleared, noted in journal		
All active underdrains flushed, noted in journal		
Meter standards emptied & cleaned		
Sample bottles and chains of custody ready for next week; e-mail to lab		
Daily/Other		
Shed cleanouts		
Check SanTOE and NitROE air pressure and note in journal.		
Check water level in CEN5 UV manhole, pump if needed, and journal.		
Daily: Record fridge thermometer temperature.		
Daily: Record any visitors in log.		
Daily: Virus dosing check		
Work trailer clean-up: put stuff away, wipe down counters, sweep and mop floors.		

Weekly Quality Assurance Checklist

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Please review to ensure the following are completed DAILY.

Upon Arrival
Open front gate and/or lift station, if needed
Remove batteries on charger if applicable
Remove phone from charger and carry on your person
Morning Priorities
7:00am Complete morning dosing shed checks/shed counts
Calibrate ProDSS meter in preparation for field samples
Record fridge thermometer temperature
Bring in composite samples from outside and pour
Replace influent sampling carboy (or turn off, ex. Friday)
Take any grab samples (see schedule/bottles)
Set up any composite samplers needed for the next day
Prepare cooler, check samples, sign chains for courier pick up
Take field measurements with ProDSS
Daily Priorities
Check Weekly Quality Assurance list for day-specific tasks
Set up alkalinity/sodium bicabonate feed to DCW
Clean d-box levelers and record on appropriate form
11:00am Second round of shed checks/shed counts, as needed
Check Microsoft Planner for tasks
Daily Shed Check (see checklist by door, this is cleaning and checking one shed per day)
End of Day
Close meter and upload meter readings to Sharepoint
Log any visitors' names in visitor log
Perform afternoon dose count (after 2pm)
Clean carboys that are out (3 volume rinse) and put back on shelf
Remove Nalgene caps from bleach and put into drying bin
Make sure any notes are in the online journal
Close the front gate and lift station, make sure shed doors all closed, channel covers are on
Make sure samplers have enough ice and no issues (no dead batteries or *)
May sure any tools used are back in their proper location
When Leaving
Plug in base phone to charger
Plug in batteries to charger, if needed; put up rope
Leave water dripping if danger of freezing

Massachusetts Alternative Septic System Test Center

Barnstable, Massachusetts

Form

Title: Weekend Quality Assurance Checklist

Effective Date: 2021-01-24	**Number:** MASSTC-FRM-018	**Revision:** 003

Authors

Name: Brian Baumgaertel
Title: MASSTC Director

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Approvals

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Weekend Quality Assurance Checklist

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Revision History

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History	Effective Date
Revision #003: Removed "Friday checklist items addessed" (no longer applicable); added "if no flow" to "Lift station checked". Edits by BB	2021-01-24
Revision #002: Reformatted into common format. Added new checklist items. Revisions by Brian Baumgaertel.	2020-02-07
Revision #001: Revisons by George Heufelder.	2018-08-04
Revision #000: Original Issue	2017-10-05

Weekend Quality Assurance Checklist

Document ID#: MASSTC-FRM-018
Revision#: 003
Released: 2021-01-24
Released By: Brian Baumgaertel

Date: _____ Arrival Time:_____ Departure Time:_____

Task	Initial
General Tasks	
Flow to channel on arrival	
Lift station checked if no flow	
All dosing sheds checked	
Alkalinity added	
Samplers set or checked if applicable	
Prior to Departure	
Lift station secured	
Front gate locked	
Flow to channel OK	
Channel covers in place	
All manhole covers secured	
All shed doors secured	
Water hydrants off; hoses put away if winter	
Tractor & lawnmowers covered/stowed; keys hung in key box	
All faucets dripping if winter	
Lights off	
Doors locked (field and lab)	
Notes / Items to be Addressed in Coming Week	
Final Closeout Initial	
Time	

Appendix 15

EPA STAR Grant #84025901 – MASSTC and MassDEP Quality Assurance Project Plan

Massachusetts Alternative Septic System Test Center

Barnstable, Massachusetts

Form

Title: Chemical Receipt Log

Effective Date: 2020-08-21	**Number:** MASSTC-FRM-014	**Revision:** 001

Authors

Name: Brian Baumgaertel
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Chemical Receipt Log

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Revision #001: Reformatted. Added "MSDS Check" column. Revisions by Brian Baumgaertel.	2020-08-21
Revision #000: Original Issue	2018-02-12

Chemical Receipt Log

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Revision#: 001
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Released By: Brian Baumgaertel

Chemical Name	Lot/Batch Number	Date Rec'd	Rec'd By	Date Opened	Opened By	MSDS Checked	Comments