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Revision 1
Test/Quality Assurance Plan (T/QAP) for Phase II of the Advanced Septic System Nitrogen Sensor Challenge

Prepared by

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for

U.S. Environmental Protection Agency
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Revision 1, June 4, 2018

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A1 Preface

Revision 1 of the Test/Quality Assurance Plan (T/QAP) for Phase II of the Advanced Septic System Nitrogen Sensor Challenge is an updated version of the March 9, 2018 “final” document. Revisions incorporated in this version have been informed by the preliminary screening test conducted at the Massachusetts Advanced Septic System Test Center (MASSTC) March 26, 2018 to April 4, 2018 and the subsequent data analysis from that study. While the data obtained from the preliminary screening test were sufficient to assess the technology, the project team and the Technical Panel determined that more data would be advantageous in order to fully understand the performance of each sensor technology. This updated version of the T/QAP includes several substantive modifications:

1) The screening test has been lengthened to one month. Assessment against screening goals will remain unchanged and will be based on 7-days of performance. However, the project team recognized the need to provide the developers with a more realistic longer duration test in order to assist in sensor development. The revised one-month screening test retains an embedded 7-day preliminary screen test to determine if a technology performs well enough to pass through to the full six-month field test.

2) An additional tap water spike concentration has been added to the tests. Three spike levels (high, medium and low) will facilitate assessment of sensor performance over the full concentration range (2-60mg/L) in the challenge performance goals.

3) Matrix spikes have been added to the testing scheme. Matrix spikes will aide in assessing matrix effects on sensor performance and ensure that there are sufficient data where analytes are at concentrations in the challenge performance range (2-60 mg/L). During the March 2018 screening test, it was noted that ammonium concentrations in treated septic test fluid fell below the performance range. As a result, those data points could not be used to evaluate sensor accuracy. Low level matrix spikes will bring concentration levels into the performance range and provide more data for evaluation.

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LIST OF ACRONYMS

A2LA	American Association for Laboratory Accreditation
BCDHE	Barnstable County Department of Health and Environment
CB	calibration blank
CCV	continuing calibration verification
$CHCl_3$	chloroform
DC	direct current
DI	de-ionized
DO	dissolved oxygen
DQA	data quality audit
DQI	data quality indicator
DQO	data quality objective
EDD	electronic data deliverable
EPA	U.S. Environmental Protection Agency
H_2SO_4	sulfuric acid
I/A OWTS	innovative and alternative onsite wastewater treatment systems
IB	instrument blank
ICV	initial calibration verification
ID	identification
IEC	International Electrotechnical Commission
IPC	instrument performance check
ISO	International Organization for Standardization
L	liter
LFB	laboratory fortified blank
LFM	laboratory fortified sample matrix
LIMS	Laboratory Information Management System
LRB	laboratory record book
KNO_3	potassium nitrate
MASSDEP	Massachusetts Department of Environmental Protection
MASSTC	Massachusetts Alternative Septic System Testing Center
MCAWW	Methods for Chemical Analysis of Water and Wastes
MCL	maximum contaminant level
MDL	method detection limit
mg	milligrams
mg/L	milligram per liter

MPS	multi-probe sensor
NH_3	ammonia
NH_{4+}	ammonium ion
NH_4Cl	ammonium chloride
NIST	National Institute of Standards Technology
NO_3^-	nitrate
NO_2^-	nitrite
NSF	National Sanitation Foundation
OWTS	onsite wastewater treatment system
PARCCS	precision, accuracy, representativeness, comparability, completeness, and sensitivity
PE	primary treated effluent
PES	performance evaluation sample
PVP	Performance Verification Protocol
QAO	Quality Assurance Officer
QAP	quality assurance plan
QAPP	quality assurance project plan
QA/QC	quality assurance/quality control
QCS	quality control sample
%R	percent recovery
RL	reporting limit
RMO	Records Management Office
RPD	relative percent difference
RSD	relative standard deviation
SM	Standard Methods
SOP	standard operating procedure
TKN	total kjeldahl nitrogen
TN	total nitrogen
TOC	total organic carbon
T/QAP	Test Quality Assurance Plan
TS	treated sewage effluent
TSA	technical systems audit
TW	tap water
USGS	United States Geological Survey

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A PROJECT MANAGEMENT

A4 PROBLEM DEFINITION AND BACKGROUND

Nitrogen loads from conventional residential septic systems can cause critical water quality problems in the northeastern U.S. and elsewhere. In coastal areas, septic systems are a major source of excess nitrogen loading. To protect public health, ecosystems, and water resources, local and state regulators across the U.S. are considering, encouraging, and (in some cases) requiring the widespread installation of advanced septic systems or innovative and alternative onsite wastewater treatment systems (I/A OWTS) designed to remove significant amounts of nitrogen. Regulators, however, need to be sure about the long-term performance of these I/A OWTS technologies. Effective long-term management of advanced nitrogen removal I/A OWTS requires measurement data that provide a real-time indication of proper functioning over the lifetime of the treatment system. An advanced septic system nitrogen sensor package which would measure the nitrogen concentration in I/A OWTS effluent would give regulators, managers, and homeowners improved ability to optimize the performance and maintenance of I/A OWTS technologies. While there are a number of I/A OWTS available, nitrogen sensor packages that can be used in conjunction with these systems are not currently being used commercially.

In January of 2017, U.S. Environmental Protection Agency (EPA) partnered with The Nature Conservancy, the US Geological Survey (USGS) and others to launch Phase I of the "Advanced Septic System Nitrogen Sensor Challenge" to spur the development and design of a low-cost nitrogen sensor package which could measure and monitor the performance of I/A OWTS. Performance goals for the nitrogen sensors (Table A-1) were developed by EPA in consultation with Massachusetts Alternative Septic System Testing Center (MASSTC), the University of Rhode Island, state regulators, The Nature Conservancy, and USGS. Phase I of the Challenge was conducted in early 2017 and solicited sensor designs from technology developers. Eighteen sensor designs were submitted; an expert panel of judges selected three designs as winners and four as honorable mentions. In June 2017, EPA and its partners hosted a Sensor Showcase Day event to bring together interested parties in the water sector, introduced the three Phase I winning sensor designs, and launched Phase II of the Challenge: Septic Sensor Performance Testing. EPA selected Battelle to support Phase II.

Table A-1. Advanced Septic System Nitrogen Sensor Performance Goals

Attribute	Attribute Description	Performance Goals		
		Minimum	Almost Ideal	Ideal
Parameter[1]	What is being measured	NO_3^-, NH_4^+	NO_3^-, NH_4^+, TOC	Total nitrogen (TN)[2]
Installation Price	Price to the homeowner to install	$1,500	$1,250	$1,000

Table A-1. Advanced Septic System Nitrogen Sensor Performance Goals, continued

Attribute	Attribute Description	Performance Goals		
		Minimum	Almost Ideal	Ideal
Data Management	Ability to record and transmit data (i.e., telemetry) for real-time access by practitioners, regulators, and interested stakeholders	Record and automatically transmit data to designated server or cloud	Record and automatically transmit data to designated server or cloud	Record and automatically transmit data to designated server or cloud
Applicability & Accessibility	Applicability of sensor(s) to various innovative/alternative system designs and ease of access to OWTS for installation and maintenance	Located in-situ to provide performance information on the OWTS; must be accessible for maintenance	Located in-situ to provide performance information on the OWTS; must be accessible for maintenance	Located in-situ to provide performance information on the OWTS; must be accessible for maintenance
Frequency of Sensor System Maintenance	How often the sensor(s) need to be maintained	No more than quarterly	No more than semi-annually	No more than annually
Accuracy	Accuracy of sensor measurements to the true measurement	Within 20% of true value[3]	Within 20% of true value[3]	Within 20% of true value[3]
Precision	Repeatability of sensor measurements	≤30% RSD	≤20-30% RSD	≤20% RSD
Range[4]	Range of the detection	2-60 mg N/L	2-60 mg N/L 2-60 mg/L TOC	2-60 mg N/L
Sensor Operating Temperature Range	Temperature range in which the sensor can operate	4° C to 35° C	4° C to 35° C	4° C to 35° C
Deployment	Period of deployment	Continuous	Continuous	Continuous
System Lifetime	Expected life of sensor	5 years	5 to 10 years	10 years

[1] Refer to Section B1.4 for information on the sources of nitrate (NO_3^-), ammonia (NH_4^+), and total organic carbon (TOC).
[2] Total Nitrogen (TN) is defined as the sum of total kjeldahl nitrogen (ammonia, organic and reduced nitrogen) and nitrate-nitrite.
[3] True value is defined as the certified laboratory result for the parameter using approved test methods.
[4] The sensors must be capable of alerting about or otherwise notifying of an over range value.

This Test Quality Assurance Plan (T/QAP) document pertains to Phase II activities, which include screening and field performance testing of sensor prototype packages and verification in accordance with the International Organization for Standardization (ISO) 14034 standard. ISO 14034 provides independent verification of the performance of new innovative environmental technologies that have the potential to improve protection of human health and the environment. The new standard features sections on verification principles, accepted testing practices, and reporting requirements to help create a level

playing field for technological innovators and encourage greater market acceptance of innovative technologies. This standard helps build developer credibility and buyer confidence by providing the marketplace with the assurance that environmental performance claims are valid, credible and supported by high-quality, independent test data.

A5 PROJECT/TASK ORGANIZATION

A5.1 Project Initiator: U.S. Environmental Protection Agency (EPA)

EPA is the project initiator and has the following responsibilities:

- Provide overall Technology Challenge framework and funding,
- Provision of testing and verification objectives,
- Recommendations on membership and direct participation in the Technical Panel,
- Design of the preliminary screening and field performance test procedures, in consultation with MASSTC, which will be incorporated into the T/QAP,
- Review and approval of the T/QAP (this document) and the Verification Plan,
- Review the sensor performance report after the preliminary screening test and work with the Technical Panel and Battelle to determine which sensors will move on to the field performance test,
- Review Technical System Audit (TSA) and Data Quality Assessment (DQA) reports,
- Review Verification Reports and Statements,
- Provision of overall policy guidance and logistical and technical support, as needed,
- Approval of project-related communications to stakeholders and other interested parties.

A5.2 Technical Verification Expert: Battelle

Battelle is the technical verification expert. Following ISO 14034, at the discretion of the independent verification organization (VerifiGlobal), an independent technical verification expert may be selected to review the verification plan, test plan and test report, and to prepare the verification report. EPA selected Battelle to serve in this capacity. Battelle is a member of the VerifiGlobal Alliance performance testing and verification platform and successfully completed the VerifiGlobal Peer Assessment Process in May 2017. The VerifiGlobal Peer Assessment Process Statement of Recognition (#2017001) confirms that VerifiGlobal recognizes the expertise and capabilities of Battelle as a competent body for conducting verification of environmental technology performance claims according to the requirements of ISO 14034, ISO/ International Electrotechnical Commission (IEC) 17020 and ISO/IEC 17025.

This T/QAP will guide the overall performance testing process and related quality assurance requirements ensuring the level of quality required by ISO/IEC 17025 and the Verification Plan. Battelle is responsible for deciding which requirements of ISO/IEC 17025 are relevant and that these requirements are clearly indicated in the T/QAP and the Verification Plan. Battelle is also responsible for controlling the fulfilment of ISO 14034 requirements, including quality management and general test requirements, through a test system assessment, including a test system audit.

In addition, Battelle has the following responsibilities:

- Review of the qualifications of the MASSTC and the Barnstable County Department of Health and Environment (BCDHE) Laboratory,
- Coordination of the Technical Panel and planning and facilitation of Technical Panel meetings,
- Development and facilitation of an informational webinar on the requirements and process for the advanced septic system nitrogen sensor performance screening and field testing,
- Development of the draft and final T/QAP (this document),
- Development of the BCDHE laboratory audit report,
- Oversight of the beginning and conclusion of the screening and the field tests,
- Scheduling and coordinating all the activities of all performance testing participants, including establishing a communication network and providing logistical and technical support as needed,
- Development of the sensor performance reports after the preliminary screening and field performance tests (using data from MASSTC and BCDHE laboratory),
- Review the sensor performance report after the preliminary screening test and work with EPA and the Technical Panel to determine which sensors will move on to the field performance test,
- Conduct of a Technical Systems Audit during the field performance test and deliver a report,
- Conduct of a Data Quality Audit (DQA) after the field performance test and deliver a report,
- Observation of a grab sample(s) collection two days during the field test,
- Verification of the test results, in accordance with ISO 14034, the Verification Plan[1], and the VerifiGlobal Performance Verification Protocol (PVP)[2],
- Preparation of the Verification Report and initial draft of the Verification Statement.

A5.3 Independent Verification Organization: VerifiGlobal

VerifiGlobal is the independent verification organization and has the following responsibilities:

- Review of the qualifications of the MASSTC and the BCDHE laboratory, and the acceptability of test sites, with support from Battelle, as required
- Review of the site-specific test procedure and coordination of its review by qualified technical experts, as needed (e.g., the Technical Verification Expert and/or a Technical Panel)
- Approval of the T/QAP (this document) for verification purposes
- Direction on the implementation of on-site audit of test procedures, as required
- Review and provide input to sensor performance reports as required for verification purposes
- Direction on the verification of performance test results, in accordance with ISO 14034, the

[1] The Verification Plan provides clarity and guidance on the verification, containing an overview of the verification process, instructions for review of the technology, and established checklists that provide guidance to ensure that a comprehensive assessment and verification are undertaken. A separate Verification Plan will be tailored to each sensor technology.

[2] The VerifiGlobal PVP provides a framework and guidance to assist verifiers in verifying technology performance using several checklists that can be used when performing technology verification.

Verification Plan, and the VerifiGlobal PVP.

- Approval and dissemination of the Verification Report and Verification Statement in consultation with the Applicant
- Posting of the Verification Statement on the VerifiGlobal website.

A5.4 Testing Organization: Massachusetts Alternative Septic System Testing Center (MASSTC)

MASSTC is the testing organization. Under ISO 14034, the testing organization is responsible for performing testing of an environmental technology and reporting the test results. The testing organization is responsible, in consultation with EPA, for developing a test procedure (or plan) in accordance with the requirements of ISO 14034 and the Verification Plan, as agreed to by the Verification Organization and the applicant. This test plan has been incorporated into the T/QAP (this document). MASSTC is expected to perform tests according to the T/QAP, ensuring the level of quality required by ISO/IEC 17025 and the Verification Plan.

MASSTC is also expected to fulfil the relevant requirements for quality management with respect to its role in the overall verification process, including quality assurance and quality control (QA/QC) to meet the general test requirements of ISO 14034. The quality management and general test requirements referenced in the ISO 14034 standard are those requirements of ISO/IEC 17025 – 'General requirements for the competence of testing and calibration laboratories', that are considered relevant for the tests to be performed. Accordingly, MASSTC must also ensure that any sampling and analytical testing meet the requirements of ISO/IEC 17025. MASSTC will provide Battelle with a summary data report, comparing the analytical results to each of the sensor readings for specified time-stamped events. In addition, MASSTC will provide the full laboratory reports with quality control information including limits of detection.

In addition, MASSTC has the following responsibilities:

- Design of the preliminary screening and field performance test procedures, in consultation with EPA, which will be incorporated into the T/QAP,
- Implementation of testing according to the T/QAP,
- Controlling access to the area where performance testing is being carried out,
- Maintaining safe conditions at the test site for the health and safety of all personnel involved with performance testing (including compliance with occupational health and safety regulations),
- Assist the developers in setting up the sensors at the beginning of testing, as needed,
- Provide logistical and technical support, as needed,
- Provide Battelle with a summary data report, comparing the analytical results to each of the sensor readings for specified time-stamped events,
- Provide Battelle with the full laboratory reports with quality control information including limits of detection.

A5.5 Analytical Laboratory: Barnstable County Department of Health and Environment (BCDHE) Laboratory

The BCDHE laboratory is the analytical laboratory and has the following responsibilities:

- Calibration of analytical equipment in accordance with an up-to-date quality management plan meeting the requirements of ISO/IEC 17025
- Implementation of sample analysis according to the test procedure as directed by MASSTC and the T/QAP (this document)
- Controlling access to the area where sample analysis is being carried out
- Maintaining safe conditions at the analytical laboratory for the health and safety of all personnel involved with verification testing (including compliance with occupational health and safety regulations)
- Scheduling sample analysis and maintaining records of all analytical data and results for future review and possible audit, as needed
- Reporting on the observed analyte concentrations, as requested.

Note that Battelle reviewed laboratory documents provided by the BCDHE laboratory to establish the Laboratory's compliance with ISO/IEC 17025 when conducting wastewater sample analysis for nitrate (NO_3^-), nitrite (NO_2^-), ammonia as N (NH_3-N), total kjeldahl nitrogen (TKN), and total organic carbon (TOC). Battelle's review was based on the American Association for Laboratory Accreditation (A2LA) C101 General Checklist - ISO/IEC 17025 Laboratory Accreditation Program (dated December 19, 2011); and the A2LA C106 General Checklist: Proficiency Testing for ISO/IEC 17025 Laboratories (dated September 19, 2013).

A5.6 Applicants: Nitrogen Sensor Developers

The various nitrogen sensor developers are the applicants and have the following responsibilities:

- Complete and submit an application for sensor performance screening, testing and verification by the specified due date.
- Review and accept the T/QAP (this document) and Verification Plan
- Provide any available site-specific performance data and information on any previous test site(s), assuming the sensor has been tested/operated
- Provide documentation on the sensor technology, including any operation and maintenance manual(s) and instructions on installation and start-up.
- Operation and maintenance, calibration, and any other adjustment of the sensor technology
- Download and report sensor data/readings at the conclusion of the tests, using a standard spreadsheet provided by Battelle (Appendix E).

A5.7 Advisors: Technical Panel

EPA has determined the need for an independent Technical Panel to provide advice and overall guidance. The Technical Panel has the following responsibilities:

- Provide technical and scientific input to the T/QAP (this document), as guided by Battelle
- Review the draft T/QAP
- Participate in the webinar for informational and question/answer purposes
- Review the Challenge applications submitted by the sensor developers

- Review the sensor performance report after the preliminary screening test and work with EPA and Battelle to determine which sensors will move on to the field performance test
- Review the verification report(s) and statement(s)
- Review final performance and verification reports to help determine best performing sensors.

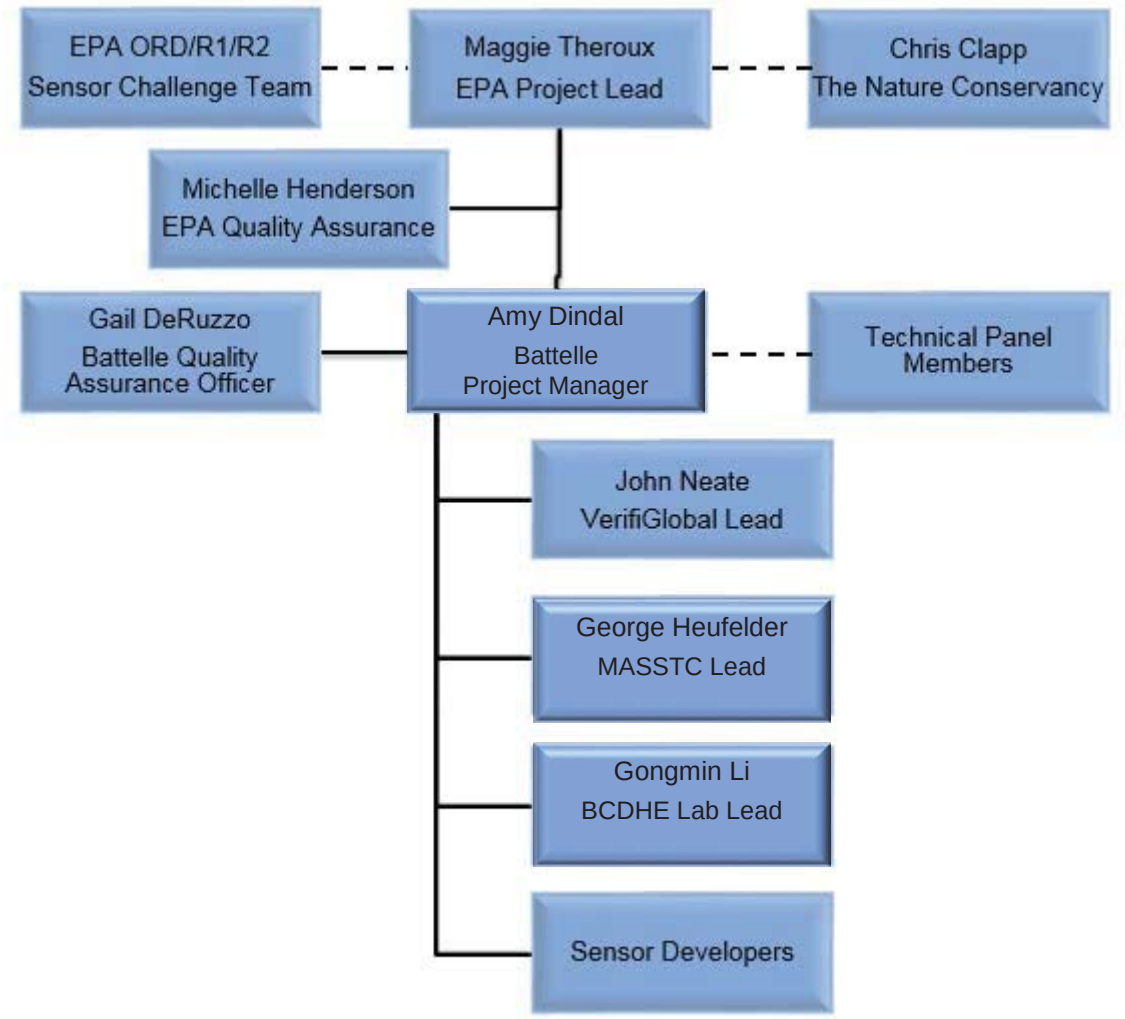

Figure B-1. Nitrogen Sensor Challenge Organizational Chart

A6 TEST PROCEDURE DESCRIPTION

A6.1 Test Description Overview

Prior to the start of testing, Battelle will develop and facilitate a webinar designed to provide interested sensor developers with information on the testing process, including the performance goals, logistical requirements, and test conditions. Phase II testing will be conducted at MASSTC, a National Sanitation Foundation (NSF) certified test facility, and will include a one-month preliminary screening test, followed

by a six-month field performance test. Although the preliminary screening test is a one-month duration, the first 7 days only will be used to evaluate performance for moving to the six-month field performance test by meeting criteria specified in Table A-3. The remaining 2-4 weeks of results will be used by the developers to help them determine their readiness for the six-month field performance test.

Following the webinar, interested parties with sensor technologies will be encouraged to submit an application to participate in the preliminary screening test. This application will be used by Battelle and the Technical Panel to determine whether a sensor is suitable to participate in the preliminary screening test. The application will collect information on the developer's organization, the sensor technology description and functionality, previous testing activities, and commercial readiness information.

There are several logistical requirements for the sensors that will participate in the preliminary and field tests. Table A-2 lists these minimum requirements.

Table A-2. Logistical Requirements of the Sensors

Sensor Attribute	Requirement
Size of Sensor	Overall dimensions no larger than 6" x 6" x 20", where the immersed portion of the device is no more than 6" x 6" x 6[1]
Attachment of Sensor to Test Cell	Attached to the side (side thickness: ~1/4")
Power Supply	UL-listed direct current (DC) requiring no more than 3 amps at 120 volts
Data Output	Capable of collecting and retaining time stamped nitrogen test data for download
Interference	Sensors may not discharge into or in any other way contaminate the test cell contents[2]

[1] External electronics accompanying the sensor can be up to 12" x 12" x 12".
[2] Incidental microscale contamination such as leaching from an antifouling coating or corrosion of a sacrificial anode will be permitted.

Preliminary Screening Test

The no-fault one-month screening test is intended to serve two objectives: 1) assist developers in more realistically assessing their system's performance in real-world conditions and 2) allow the project team to judge the readiness of the system for full field evaluation. A series of tests will be conducted to evaluate dynamic range, precision, accuracy, and stability of the sensors under controlled performance conditions. Tests will be performed in a temperature-conditioned room with a flow-through test tank containing well-mixed spike solutions and septic stream effluent. Instruments will be set up and calibrated by the developer, with assistance provided by MASSTC staff as necessary. Samples of the same effluent will be independently analyzed by the BCDHE laboratory using standard test methods (Appendix C). At the conclusion of the preliminary screening test, Battelle will develop a sensor performance report, based on the first seven days of the one-month screening test, presenting and interpreting the sensor data and the BCDHE laboratory data. The project team will assemble, but not interpret, data for the full month-long

test. This report and the full one-month test data set will be shared with the sensor developers to help them make improvements to their sensors. The developers will have an opportunity to

Developers whose sensors meet basic performance goals during the first seven days of the preliminary screening test will be invited to participate in the field performance test (six-month test). To determine which sensors will be invited to move forward to the field performance test, the Technical Panel (in consultation with EPA and Battelle) will use a more specific subset of performance goals (Table A-3) than those presented in Table A-1:

Table A-3. Subset of Sensor Performance Goals For Moving Forward to the Field Performance Test

Attribute	Performance Goals to Determine Field Performance Test Invitation
Parameter	Measures • NH_4^+ and NO_3^- or • NH_4^+, NO_3^-, and TOC or • TN
Data Management	Internal (local) sensor system data logger successfully collects time stamped data for the screen test
Applicability & Accessibility	Meets test size limits and performs under screen test environmental conditions
Maintenance	No more than one servicing during the preliminary screening test
Accuracy	Within 40% of true value
Precision	≤40% RSD
Range	2-60 mg N/L 2-60 mg/L TOC
Deployment	High frequency (at least hourly) measurement for the duration of the test

Field Performance Test

Field performance testing of the sensors will be conducted during a second extended deployment at MASSTC. A series of tests will be conducted to evaluate dynamic range, precision, accuracy, and stability of the sensors under controlled performance conditions; however, the focus of this test will be on long-term performance and durability of the sensors. Instruments will be set up and calibrated by the sensor developers, with assistance provided by MASSTC staff as necessary. Developers are to supply complete systems capable of operating autonomously for a six-month test. Limited servicing of the instruments will be allowed during the six-month period to address routine maintenance and observed physical damage from natural events and/or repair or replacement as deemed necessary (Section B7.1). Instruments should be set up with self-recording data loggers programmed to record data at regular intervals for at least the specified number of days. Samples of the same effluent will be independently analyzed by the BCDHE laboratory using standard test methods (Appendix C).

Within two weeks of the start of the field performance test, Battelle will conduct a Technical Systems Audit (TSA) (Section C1.1) to ensure that the test is being performed in accordance with the MASSTC's facility Quality Assurance Project Plan (QAPP), this T/QAP, published reference methods, and any Standard Operating Procedures (SOPs) used by MASSTC or the BCDHE laboratory. At the conclusion of the field performance test, Battelle will develop a sensor performance report, presenting and interpreting the sensor data and the BCDHE laboratory data. Battelle will also conduct a DQA (Section C1.2) at the conclusion of the field performance test to determine if the resulting data are of the right type, quality, and quantity to support their intended use.

Verification Reports and Statements

Upon successful completion of the field performance test, a Verification Report will be developed for each sensor, assuming that the sensor company agrees to proceed with verification. The Verification Report will contain a detailed description of the technology; a detailed description of the performance claim including specific parameters, operating conditions and applications; and the results of data assessment and claim verification. A Verification Statement will also be developed based on the final Verification Report for each sensor that completes the field performance test. The Verification Statement is the company's authenticated proof of having successfully completed the verification process. It should contain the company's full corporate/organizational identifier, the verified performance claim, an authorized signature, a certificate number and an effective date. The Verification Statement should also contain a brief description of the verification process and information on the limitations of the verification.

A6.2 Summary of Testing Schedule

Table A-4 shows a general schedule of testing and data analysis and reporting activities. Developers need only complete one successful preliminary screening test. Two different identical screening test periods are currently being offered. The first 7 days of the test will be used to evaluate the sensor's readiness to proceed to the 6-month test based on Table A-3 criteria. The week 2-4 testing for each of the two preliminary screens will not be evaluated but will be tabulated and provided to the developer to aide them in evaluating their readiness for the longer duration field test period.

Table A-4. Estimated Schedule of Testing and Reporting

Task	Activity	Estimated Date
Webinar	Delivery of webinar for sensor developers	July 12, 2018
Verification Plan and T/QAP Development	Delivery of draft T/QAP to EPA	May 31, 2018
	Delivery of final T/QAP to EPA	June 4, 2018
Preliminary Screening Test -1	Installation of sensors for first 1-month test	Day 0 = October 1, 2018
	Conduct Technical Systems Audit	October 9, 2018
	7-day testing results due from laboratory for first test	10 working days from 7th day of test (10/23/18)
	Delivery of Draft Sensor Performance Report for first 7-day test to EPA	2 weeks after data receipt (estimated 11/6/18)

Table A-4. Estimated Schedule of Testing and Reporting, continued

Task	Activity	Estimated Date
	Review comments on Draft Sensor Performance Report for first 7-day test from EPA and Technical Panel	2 weeks after report receipt (estimated 11/20/18)
	Final Sensor Performance Report for first 7-day test to EPA and Sensor Developer	1 week after comment receipt (estimated 11/27/18)
	1-month testing results due from laboratory for first test	10 working days from last day of test (estimated 11/16/18)
	Delivery of Sensor Performance Report for first 1-month test (addendum to 7-day report) to EPA and Sensor Developer	2 weeks after data receipt (estimated 11/30/18)
	Installation of sensors for second 1-month test	Day 0 = January 7, 2019
	7-day testing results due from laboratory for second test	10 working days from 7th day of test (estimated 1/29/19)
	Delivery of Draft Sensor Performance Report for second 7-day test to EPA	2 weeks after data receipt (estimated 2/12/19)
	Review comments on Draft Sensor Performance Report for second 7-day test from EPA and Technical Panel	2 weeks after report receipt (estimated 2/26/19)
	Final Sensor Performance Report for second 7-day test to EPA and Sensor Developer	1 week after comment receipt (estimated 3/5/19)
	1-month testing results due from laboratory for second test	10 working days from last day of test (estimated 2/22/19)
	Delivery of Sensor Performance Report for second 1-month test (addendum to 7-day report) to EPA and Sensor Developer	2 weeks after data receipt (3/8/19)
Field Performance Test	Installation of sensors for 6-month test	May 13-14, 2019
	Conduct Technical Systems Audit	Within two weeks of 6-month test
	Delivery of Technical Systems Audit Report to EPA	2 weeks after audit
	Conduct Periodic QA and Technical Visits by Battelle	TBD
	Conclusion of 6-month test	November 15, 2019
	Delivery of Data Quality Audit Report to EPA	One month after receipt of data

Table A-4. Estimated Schedule of Testing and Reporting, continued

Task	Activity	Estimated Date
	Delivery of Draft Sensor Performance Report for six-month test to EPA	One month after receipt of data
	Review comments on Draft Sensor Performance Report for six-month test from EPA and Technical Panel	2 weeks after report receipt
	Final Sensor Performance Report for six-month test to EPA and Sensor Developer	1 week after comment receipt
Reporting	Delivery of Verification Reports for Sensors to EPA	One month after Final Sensor Performance Reports for six-month test
	Delivery of Verification Statements for Sensors to EPA	One week after Verification Reports Finalized
	Verification Information Posted on VerifiGlobal Website	One week after Verification Statements are completed

Note: The results from the first 7 days of the one-month test will be evaluated to determine if the criteria in Table A-3 have been met and allow the sensor to advance to the six-month field performance test. The remaining 2-4 weeks of the one-month screening test will provide additional data for the developers to assess the longer-term durability of their technology.

A7 QUALITY OBJECTIVES AND CRITERIA

Battelle, MASSTC, the BCDHE laboratory, and the nitrogen sensor developers will follow the technical and QA/QC procedures specified in this T/QAP. The tests described in this T/QAP will evaluate the performance of septic system nitrogen sensors and include a comparison of the sensor data to analytical results from the BCDHE laboratory. Data quality objectives (DQOs) have been established to ensure that the preliminary screening and field performance tests provide suitable data for a robust evaluation of performance. The DQOs for the screening and field performance tests have been established to assess the performance of the nitrogen sensors in relation to their ability to measure NO_3^-, NH_4^+, TOC, and TN. The DQOs are evaluated by the acceptance criteria defined in Section B5.

Assessing the DQOs is also a key component of the verification process. One DQA will be conducted for this project, to confirm the accuracy of the data. The Battelle Quality Assurance Officer (QAO) will also perform a TSA once during the field performance test to confirm that testing and analysis were performed according to the T/QAP.

A8 SPECIAL TRAINING/CERTIFICATIONS

A8.1 Testing Facility Certification

The MASSTC, located in Barnstable, Massachusetts, operates the test facility in accordance with Massachusetts Department of Environmental Protection (MassDEP) pre-treatment facility requirements. The Project Manager of the facility maintains a Massachusetts Wastewater Operator's License of Grade 4-M-Full (License #7591). The two other operators have licenses of Grade 3 or higher. All technical

assistants complete training under direct supervision of the Project Manager on tasks relating to the collection and processing of samples, and collection and recording of field data. Trainees first receive instruction from trainer personnel during normal tasks, then the trainee will perform the tasks with the trainer observing to ensure tasks are performed correctly.

A8.2 Laboratory Certification

The Barnstable County BCHDE Laboratory, located in Barnstable, Massachusetts, maintains potable water and non-potable water certification for all applicable analyses listed in Section B4 required for this project with MassDEP. Their certification number is M-MA009.

A8.3 Personnel Training

All MASSTC technical assistants complete training under direct supervision of the Project Manager on tasks relating to the collection and processing of samples, and collection and recording of field data. Trainees first receive instruction from trainer personnel during normal tasks, then the trainee will perform the tasks with the trainer observing to ensure tasks are performed correctly. A training checklist is used to document training.

BCDHE laboratory staff complete training in the SOPs they are assigned to, including successfully completing an initial demonstration of performance on the SOP. This entails at a minimum: performing an initial calibration of the instrument and successfully passing quality control samples or performance evaluation samples, prior to analyzing samples.

A9 DOCUMENTATION AND RECORDS

The documents for this project will include the laboratory audit report, T/QAP, verification plan, sensor performance reports, technical systems audit report, data audit report, verification report, and verification statement. Project records will include: field log books, laboratory record books (LRBs), supporting laboratory records, sensor data spreadsheets, training records, electronic files (both raw data and spreadsheets), and QA audit files. All data generated during the course of this project must be able to withstand challenges to their validity, accuracy, and legibility. To meet this objective, data will be recorded in standardized formats and in accordance with prescribed procedures. The documentation of all data collection activities must meet the following minimum requirements:

- Data must be documented directly, promptly, and legibly. All reported data must be uniquely traceable to the raw data. All data reduction formulas must be documented, and sample calculations must be carried out and recorded so that the accuracy and validity of any derived or calculated value is not in question.
- Handwritten data must be recorded in dark (blue or black) ink. All original data records include, as appropriate, a description of the data collected, units of measurement, unique sample identification (ID) and station or location ID (if applicable), name (signature or initials) of the person collecting the data, and date of data collection.
- Any changes to the original (raw data) entry must not obscure the original entry and must be made with a single line cross out. The change must be initialed and dated by the person making the change.
- The use of pencil, correction fluid, and erasable pen is prohibited.

At the conclusion of the project, Battelle will transfer the records to permanent storage at Battelle's Records Management Office (RMO). The Battelle QA Officer will maintain all quality records. All Battelle LRBs and reports are stored permanently by Battelle's RMO; all raw data are stored for at least 10 years. Battelle will distribute the final T/QAP and any revisions to the distribution list given in Section A3. Section B10 further details the data management practices and responsibilities.

B DATA GENERATION AND ACQUISITION

B1 EXPERIMENTAL DESIGN

This project will specifically address the verification of nitrogen sensors under advanced septic system treatment conditions by evaluating the accuracy, precision, and range of NO_3^-, NH_4^+, TOC, and TN [operationally defined here as TKN plus nitrite and nitrate] measurements made by each sensor in wastewater mixtures. The project will also assess the ability of the sensors to perform continuous monitoring with minimal intervention. The experimental design incorporates testing to evaluate impacts of waste matrix, temperature, time, septic system and power failure on accuracy, precision, range and completeness. Specifically, the nutrient analyzers will be evaluated for the performance goals summarized in Table A-1 over the duration of the test and discussed in detail in the following section.

In addition to the testing activities specified in this T/QAP, MASSTC staff and, if required, the sensor developer or their designee, will perform regular maintenance and other routine procedures for the sensors. In accordance with the Performance Goals (Table A-1), routine maintenance is limited to three-month intervals for the minimal goal, six months for the almost ideal system, and twelve months for the ideal system. Developers will be allowed to setup their device and provide maintenance at three months. They will also be allowed to reset their device in the event of a test upset, or any act of nature.

B1.1 Nitrogen Sensor Test Cell

The nitrogen sensors will be housed in a sensor test cell, a circular enclosed tub, the exact dimensions of which will be finalized once the characteristics of the sensors being tested are known. However, as depicted in Figure B-2, it is anticipated that the test cell will be constructed of a non-corrosive material

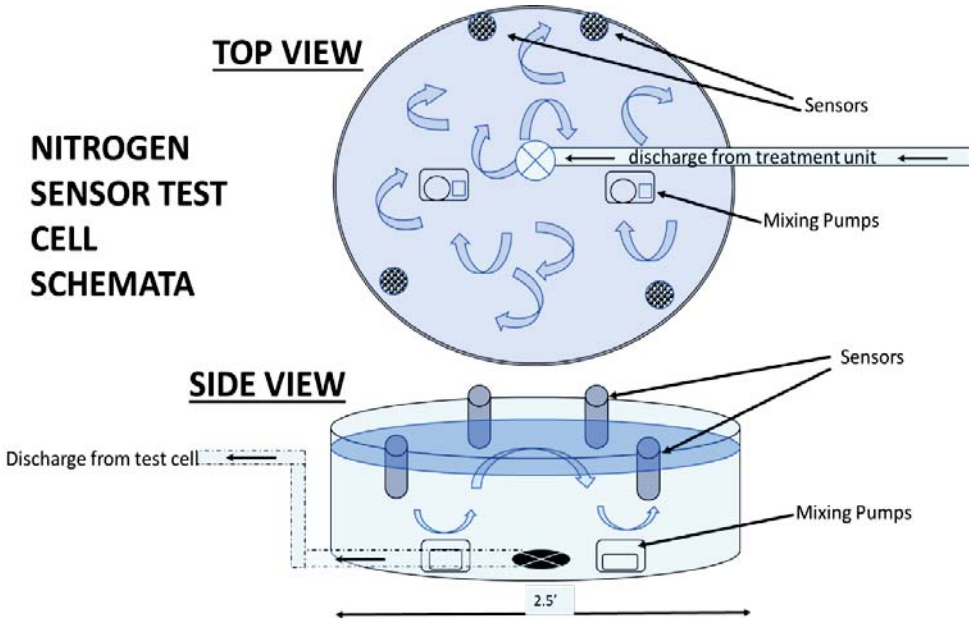

Figure B-2. The Nitrogen Sensor Test Cell Schemata

(i.e., plastic) and measure approximately 2.5 feet in diameter (a similar, larger vessel may be utilized if necessary to accommodate more sensors) and 1 foot in depth[3]. The thickness of the outside wall of the test cell will be approximately ¼-inch. The treatment unit discharge will enter the sensor test cell via flow-through plumbing, which will be situated in the center bottom of the test cell, with the sensors being tested arranged around the outside of the tub. The sensors will be placed no closer than 10 inches to each other. Positive displacement mixing pumps will be used inside the tub to ensure uniform sensor exposure to the challenge solution.

The sensor test cell will be housed inside a trailer on the MASSTC property, to facilitate MASSTC staff moving the trailer to different treatment systems for testing. The trailer will be heated to protect the contents of the sensor test cell from freezing. The temperature range of the fluids that the sensors will be immersed in will be between 4 and 35° C. The ambient temperature to which the electrical control panel would be subjected to would be between -10° C and 40° C.

There will be 120-volt AC power available inside the trailer for those sensors that require external power. Developers who connect to power must do so using a UL-listed direct current (DC) power supply that requires no more than 3 amps at 120 volts. The entire system must provide electrical isolation between the fluid, 120 VAC power, and earth ground to prevent galvanic issues or ground looping with other developers' devices under test. Sensors may not discharge into or in any other way contaminate the test cell contents. Incidental microscale contamination such as leaching from an antifouling coating or corrosion of a sacrificial anode will be permitted.

The sensors will need to be attached to the wall of the test vessel and the developers will need to demonstrate that the unit is secure and will not move during the preliminary screening or field performance tests.

B1.2 Definition of Test Parameters

Sensors will be tested for accuracy, precision, range, and completeness of data return as they are exposed to a range of test fluids over the duration of the test. Data from each sensor will be compared to laboratory data at specific time intervals as described in Tables B-1 and B-2.

Accuracy: Closeness of agreement between the result of a measurement and reference values, as measured using EPA approved methods, defined in Section B4. Accuracy is estimated by comparisons between laboratory (defined as "true") and sensor measured values.

Percent Recovery (%R) is determined by:

$$ {}^{0}/_{0}\,R = \frac{Found\ Concentration}{True\ Concentration} \times 100 $$

Where,

[3] These measurements of the test cell will be finalized once the set of sensors that will be tested is known.

Found Concentration = the found concentration of the test material as determined by the sensor (e.g., low standard, high standard, effluent)

True Concentration = the true concentration of the test material as determined by laboratory analysis (e.g., low standard, medium, high standard, effluent, spiked effluent)

Note: The ±20% goal for the sensor data equates to 80-120% recovery.

A percent recovery will be determined for each sensor reading against each laboratory true value, where the laboratory true values are within the performance goal range of 2-60 mg/L. Where there are replicate results for a test fluid, mean recoveries will be determined by sensor.

Percent Recovery for Laboratory Fortified Matrix Samples is determined differently, taking into consideration the unspiked sample concentration as follows:

$$\%R = \frac{SSR - SR}{SA} \times 100$$

Where,
SSR = Spike sample Result
SR = Unspiked Sample Result
SA = Spike Added

Precision: Closeness of agreement between independent test results obtained under stipulated controlled conditions. Determined by repeated measures (n=3) during study testing with sensors placed in, or exposed to, known stable test fluid conditions. Reported as relative standard deviation (RSD). For laboratory measurements, precision will be determined from laboratory duplicate analyses, where the laboratory results are greater than the reporting limit, and will be reported as relative percent difference (RPD).

Relative standard deviation (RSD) is calculated as:

$$\%RSD = \frac{S}{\bar{x}} \times 100$$

Where,
S = standard deviation (shown below)
$\bar{x}$ = mean of the concentrations
x_i = each individual value used to calculate the mean
N = total number of values

$$S = \sqrt{\frac{\sum_{i=1}^{N}(x_i - \bar{x})^2}{N-1}}$$

As an alternative to %RSD, standard deviation of recovery [s(recovery)] may be used to provide additional precision determination results for single assay results for sensors versus laboratory true values. The same standard deviation equation above will be used with the following changes to the variables:

$\bar{x}$ = mean of the recoveries
x_i = each individual recovery used to calculate the mean

N = total number of recoveries

Relative percent difference (RPD) is calculated as:

$$^{0}/_{0}\, RPD = \frac{|S - D|}{(S + D) / 2} \times 100$$

Where,
S = Sample Result
D = Duplicate Sample Result

Range: Upper and lower level limits of detection and quantification. Determined by an analysis of the variance within repeated sensor readings on a known (prepared, sampled, and analyzed) zero, low, medium and high test solutions of the measurement parameter.

Completeness: Amount of time the sensor can operate in a submerged deployment setting without needed maintenance or recalibration. Successful deployment requires the sensor to perform within the targeted ranges of accuracy defined in Table A-1 throughout the deployment duration. Also, comparisons will be made of the percent data recovered as a proportion of the data that an instrument was designed to collect during its deployment period.

Recovery after loss of Power: Ability of sensor to recover from a complete loss of external power for an 8 to 12-hour period. Successful deployment requires the sensor to return an accurate value for test fluid two hours after power has been restored. Accuracy will be determined as defined above.

B1.3 Test Procedures

The following sections describe the test procedures that will be used to evaluate each of the nitrogen sensor performance parameters listed in Table A-1. The test will include off-line measurement when the test fluid is spiked and on-line effluent measurement. Procedures during the testing phases will be conducted simultaneously for all sensors. Initially the sensor test cell will be filled with tap water, and then spiked sequentially with low, medium and high standards (which will be supplied by EPA), the sensor test cell will be mixed, and time stamped lab samples will be collected at each concentration level. During the off-line part of the test, the flow-thru septic fluid plumbing will be turned off so the test fluid is “off-line” or static.

During the on-line effluent monitoring phase, the flow-through valves will be opened and used to deliver the effluent to the sensors. Section B1.1 describes the characteristics of the sensor test cell, which is the housing vessel for the sensors during testing.

Spiking solutions for the test fluids will be prepared from certified standards or high-purity solids (e.g., potassium nitrate [KNO_3], ammonium chloride [NH_4Cl]) and nicotinic acid (Section B1.4 and B1.6). The sensors will also be tested using OWTS treated sewage effluent, spiked OWTS treated sewage effluent (matrix spike) and primary treated effluent (to simulate OWTS failure). This primary treated effluent is raw untreated sewage which has gone through a primary treatment in a standard septic tank It is not required that the test fluid solutions be prepared quantitatively since all evaluations of analyzer performance specified in this T/QAP will utilize the reference laboratory analysis result for each solution, rather than

the nominal concentration calculated from the sample preparation. However, the test fluid solutions will be prepared as close to the target concentrations outlined in this T/QAP as is feasible.

B1.4 Test Fluid Solutions for Preliminary Screening Test

- Tap water (TW)
- Low Standard (Low Std) – Tap water spiked with:
  - Nitrate solution (KNO_3 preserved with chloroform [$CHCl_3$]): 1-15 mg N/L
  - Ammonia solution (NH_4Cl): 10-15 mg/L
  - Organic nitrogen, nicotinic acid (C_5H_4NCOOH): 10-20 mg N/L
- Medium Standard (Med Std) – Tap water spiked with:
  - Nitrate solution (KNO_3 preserved with $CHCl_3$): 10-40 mg N/L
  - Ammonia solution (NH_4Cl): 10-40 mg/L
  - Organic nitrogen, nicotinic acid (C_5H_4NCOOH): 15-40 mg N/L
- High Standard (High Std) – Tap water spiked with:
  - Nitrate solution (KNO_3 preserved with chloroform [CHCl3]): 30-60 mg N/L
  - Ammonia solution (NH_4Cl): 30-60 mg/L
  - Organic nitrogen, nicotinic acid (C_5H_4NCOOH): 30-60 mg N/L
- Typical advanced OWTS treated sewage effluent (TS)
- Matrix Spike – OWTS treated sewage effluent spiked with:
  - Nitrate solution (KNO_3 preserved with chloroform [CHCl3]): 1-15 mg N/L
  - Ammonia solution (NH_4Cl): 10-15 mg/L
  - Organic nitrogen, nicotinic acid (C_5H_4NCOOH): 10-20 mg N/L
- Primary treated effluent to simulate OWTS failure (PE)

B1.5 Progression of Preliminary Screening Test

Table B-1 shows the schedule of the preliminary screening test, including the types of tests to be performed over the one-month test, what test fluids will be used during each test, the number of sample replicates taken each day, and the total number of analyses for the target parameters. As stated elsewhere, the results from the 7-day test samples will be used to evaluate the performance of the sensor in meeting the criteria in Table A-3 and whether the technology will advance to the six-month field performance test. The remaining 2-4 week testing will be used to provide additional data to the developers to demonstrate longer duration performance.

Table B-1. Preliminary Screening Test Progression

7-Day/1-Month Screen Test

Test Day	Day of Week	1st Test Date (2018)	2nd Test Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
0	Mon-Tues	10/1 and 10/2	1/7 and 1/8	Vendor set-up and calibration	Off-line	None		
1	Wed	10/3	1/9	Accuracy/Precision/ Range	Off-line	TW	1	4
					Off-line	TW + Low Std	3	12
					Off-line	TW + Med Std	3	12
					Off-line	TW + High Std	1	4
2	Thu	10/4	1/10	Accuracy/Precision in matrix	On-line	TS	3	12
					Off-line	TS + Low Std	3	12
3	Fri	10/5	1/11	Alarm	On-line	PE	1	4

Table B-1. Preliminary Screening Test Progression, continued

7-Day/1-Month Screen Test

Test Day	Day of Week	1st Test Date (2018)	2nd Test Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
6	Mon	10/8	1/14	Accuracy following alarm	On-line	TS	1	4
7	Tues	10/9	1/15	Accuracy/Precision/ drift at 7 days	Off-line	TW	1	4
					Off-line	TW + Low Std	3	12
					Off-line	TW + Med Std	3	12
					Off-line	TW + High Std	1	4
7 Day Total							24	96
8	Wed	10/10	1/16	Accuracy/Precision/ Drift in matrix	On-line	TS	3	12
					Off-line	TS + Low Std	3	12
9	Thu	10/11	1/17		On-line	TS		
10	Fri	10/12	1/18		On-line	TS		

Table B-1. Preliminary Screening Test Progression, continued

7-Day/1-Month Screen Test

Test Day	Day of Week	1st Test Date (2018)	2nd Test Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
13	Mon	10/15	1/21		On-line	TS		
14	Tues	10/16	1/22		On-line	TS		
15	Wed	10/17	1/23	Accuracy/Precision/ Drift in matrix	On-line	TS	3	12
					Off-line	TS + Low Std	3	12
16	Thu	10/18	1/24		On-line	TS		
17	Fri	10/19	1/25		On-line	TS		
20	Mon	10/22	1/28		On-line	TS		
21	Tues	10/23	1/29		On-line	TS		
22	Wed	10/24	1/30	Accuracy/Precision/ Drift in matrix	On-line	TS	3	12
					Off-line	TS + Low Std	3	12

Table B-1. Preliminary Screening Test Progression, continued

7-Day/1-Month Screen Test

Test Day	Day of Week	1st Test Date (2018)	2nd Test Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
23	Thu	10/25	1/31	Power Failure (8-hours)	Off-line	TS		
24	Fri	10/26	2/1	Accuracy after power restoration	On-line	TS	1	4
27	Mon	10/29	2/4		On-line	TS		
28	Tues	10/30	2/5		On-line	TS		
29	Wed	10/31	2/6	Accuracy/Precision/ Drift in matrix	On-line	TS	3	12
					On-line	TS + Low Std	3	12
30	Thu	11/1	2/7	Accuracy/Precision/ Linearity/Range	Off-line	TW	1	4
					Off-line	TW + Low Std	3	12
					Off-line	TW + Med Std	3	12

Table B-1. Preliminary Screening Test Progression, continued

7-Day/1-Month Screen Test

Test Day	Day of Week	1st Test Date (2018)	2nd Test Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
					Off-line	TW + High Std	1	4
31	Fri	11/2	2/8	Demobilization				
1 Month Total							57	228

[1] Off-line refers to days when the sensor test cell will not be flow-through.
[2] Drift refers to a change in sensor accuracy over time

During off-line testing, the sensors will be supplied with unspiked tap water, spiked tap water or spiked treated septic effluent (Section B1.4). During on-line tests, the sensors will be supplied with test fluid by continuously feeding the solution into the test vessel. Prior to sampling, on-line effluents are introduced over a 24-hour period to ensure that the test vessel is fully flushed and uniform before a test sample is taken. The test vessel will be flushed three times over a 24-hour cycle. Each test fluid solution, off-line or on-line, will be mixed for a minimum of fifty minutes before a test sample is taken. Preliminary testing demonstrates that the test vessel is fully mixed within one minute.

The proposed test fluid solutions, sequence of testing, and number of replicate tests are shown in Table B-1. The sensor response to the nutrient standards and tests listed in Section B1.4 and Table B-1, respectively, will be used to evaluate accuracy, precision, and range. Appendix F provides a statistical analysis that shows the design of the sampling plan has sufficient replicates (precision data) and spike samples (accuracy data) to demonstrate that a sensor's performance is acceptable.

B1.6 Test Fluid Solutions for Field Performance Test

- Tap water (TW)
- Low Standard (Low Std) - Tap water spiked with:
  - Nitrate solution (KNO_3 preserved with chloroform [$CHCl_3$]): 1-15 mg N/L
  - Ammonia solution (NH_4Cl): 10-15 mg/L
  - Organic nitrogen, nicotinic acid, (C_5H_4NCOOH): 10-20 mg N/L
- Medium Standard (Med Std) – Tap water spiked with:
  - Nitrate solution (KNO_3 preserved with $CHCl_3$): 10-40 mg N/L
  - Ammonia solution (NH_4Cl): 10-40 mg/L

    - Organic nitrogen, nicotinic acid (C_5H_4NCOOH): 15-40 mg N/L
- High Standard (High Std) - Tap water spiked with:
    - Nitrate solution (KNO_3 preserved with chloroform [$CHCl_3$]): 30-60 mg N/L
    - Ammonia solution (NH_4Cl): 30-60 mg/L
    - Organic nitrogen, nicotinic acid (C_5H_4NCOOH): 30-60 mg N/L
- Typical advanced OWTS treated sewage effluent (TS)
- Matrix Spike – OWTS treated sewage effluent spiked with:
    - Nitrate solution (KNO_3 preserved with chloroform [$CHCl_3$]): 1-15 mg N/L
    - Ammonia solution (NH_4Cl): 10-15 mg/L
    - Organic nitrogen, nicotinic acid (C_5H_4NCOOH): 10-20 mg N/L
- Primary treated effluent to simulate OWTS failure (PE)
- Alternate treated sewage effluents will be tested during the course of the study (TS2, TS3, TSx).

B1.7 Progression of Field Performance Test

Table B-2 shows the progression of the field performance test, including the types of test to be performed over the six-month test, what test fluids will be used during each test, the number of sample replicates taken each day, and the total number of analyses for the target parameters.

Table B-2. Field Performance Test Progression

[1] Off-line refers to days when the sensor test cell will not be flow-through.
[2] Drift refers to a change in sensor accuracy over time

6-Month Performance Test Plan

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
0	Mon-Tues 5/13 and 5/14	Vendor set-up and calibration	Off-line	None		
1	Wed 5/15	Accuracy/Precision/Range	Off-line	TW	1	4
			Off-line	TW + Low Std	3	12

Table B-2. Field Performance Test Progression, continued

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
			Off-line	TW + Med Std	3	12
			Off-line	TW + High Std	1	4
2	Thu 5/16	Accuracy/Precision in matrix	On-line	TS	3	12
			Off-line	TS + Low Std	3	12
3	Fri 5/17	Alarm	On-line	PE	1	4
6	Mon 5/20	Accuracy following alarm	On-line	TS	1	4
7	Tues 5/21	Accuracy/Precision/drift at 7 days	Off-line	TW	1	4
			Off-line	TW + Low Std	3	12
			Off-line	TW + Med Std	3	12
			Off-line	TW + High Std	1	4

Table B-2. Field Performance Test Progression, continued

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
8	Wed 5/22	Accuracy/Precision/Drift in matrix	On-line	TS	3	12
			Off-line	TS + Low Std	3	12
9	Thu 5/23		On-line	TS		
10	Fri 5/24		On-line	TS		
13	Mon 5/27		On-line	TS		
14	Tues 5/28		On-line	TS		
15	Wed 5/29	Accuracy/Precision/Drift in matrix	On-line	TS	3	12
			Off-line	TS + Low Std	3	12
16	Thu 5/30		On-line	TS		
17	Fri 5/31		On-line	TS		

Table B-2. Field Performance Test Progression, continued

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
20	Mon 6/3		On-line	TS		
21	Tues 6/4		On-line	TS		
22	Wed 6/5	Accuracy/Precision/Drift in matrix	On-line	TS	3	12
			Off-line	TS + Low Std	3	12
23	Thu 6/6	Power Failure (8-hours)	Off-line	TS		
24	Fri 6/7	Accuracy after power restoration	On-line	TS	1	4
27	Mon 6/10		On-line	TS		
28	Tues 6/11		On-line	TS		

Table B-2. Field Performance Test Progression, continued

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
29	Wed 6/12	Accuracy/Precision/Drift in matrix	On-line	TS	3	12
			On-line	TS + Low Std	3	12
30	Thu 6/13	Accuracy/Precision/Range	Off-line	TW	1	4
			Off-line	TW + Low Std	3	12
			Off-line	TW + Med Std	3	12
			Off-line	TW + High Std	1	4
31	Fri 6/14		On-line	Switch to TS2		
Month 1 Total					57	228

Table B-2. Field Performance Test Progression, continued

Month 2 of 6

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
1-2			On-line	TS2		
3	Mon 6/17	Accuracy/Precision/Drift in matrix	On-line	TS2	3	12
			Off-line	TS2 + Low Std	3	12
4-13	6/18-6/27		On-line	TS2		
14	Fri 6/28	Accuracy/Drift in matrix	On-line	TS2	1	4
15-30	6/29 - 7/14		On-line	TS2		
31	Mon 7/15	Accuracy/Precision/Drift in matrix	On-line	TS2	3	12
			Off-line	TS2 + Low Std	3	12
31	Mon 7/15		On-line	Switch to TS3		
Month 2 Total					13	52

Table B-2. Field Performance Test Progression, continued

Month 3 of 6

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
1	Tues 7/16	Accuracy/Precision/Drift in matrix	On-line	TS3	3	12
			Off-line	TS3 + Low Std	3	12
2-14	7/17 – 7/29		On-line	TS3		
15	Tues 7/30	Accuracy/Drift in matrix	On-line	TS3	1	4
16-28	7/31 – 8/12		On-line	TS3		
29	Tues 8/13	Accuracy/Precision/Drift in matrix	On-line	TS3	3	12
			On-line	TS3 + Low Std	3	12
30	Wed 8/14	Accuracy/Precision/Range	Off-line	TW	1	4
			Off-line	TW + Low Std	3	12
			Off-line	TW + Med Std	3	12
			Off-line	TW + High Std	1	4
31	Thur 8/15		On-line	Switch to TSX		

Table B-2. Field Performance Test Progression, continued

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
Month 3 Total					21	84

Month 4 of 6

1	Fri 8/16	Accuracy/Precision/Drift in matrix	On-line	TSX	3	12
			Off-line	TSX + Low Std	3	12
2-14	8/17 – 8/29		On-line	TSX		
15	Fri 8/30	Accuracy/Drift in matrix	On-line	TSX	1	4
16-28	8/31 – 9/12		On-line	TSX		
29	Fri 9/13	Accuracy/Precision/Drift in matrix	On-line	TSX	3	12
			Off-line	TSX + Low Std	3	12
29	Fri 9/13		On-line	Switch to TSX		
30-31	9/14 – 9/15		On-line	TSX		

Table B-2. Field Performance Test Progression, continued

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
Month 4 Total					13	52

Month 5 of 6

1	Mon 9/16	Accuracy/Precision/Drift in matrix	On-line	TSX	3	12
			Off-line	TSX + Low Std	3	12
2-14	9/17 – 9/29		On-line	TSX		
15	Mon 9/30	Accuracy/Drift in matrix	On-line	TSX	1	4
16-29	10/1 – 10/14		On-line	TSX		
30	Tue 10/15	Accuracy/Precision/Drift in matrix	On-line	TSX	3	12
			Off-line	TSX + Low Std	3	12
31	Wed 10/16		On-line	Switch to TS 1		
Month 5 Total					13	52

Table B-2. Field Performance Test Progression, continued

Month 6 of 6

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
1	Thu 10/17	Accuracy/Precision/Drift in matrix	On-line	TS 1	3	12
			Off-line	TS 1 + Low Std	3	12
2-14	10/18 – 10/30		On-line	TS 1		
15	Thu 10/31	Accuracy/Drift in matrix	On-line	TS 1	1	4
16-28	11/1 – 1/13		On-line	TS 1		
29	Thu 11/14	Accuracy/Precision/Drift in matrix	On-line	TS 1	3	12
			On-line	TS 1 + Low Std	3	12
30	Fri 11/15	Accuracy/Precision/Range	Off-line	TW	1	4
			Off-line	TW + Low Std	3	12
			Off-line	TW + Med Std	3	12
			Off-line	TW + High Std	1	4
30	Fri 11/15	Demobilization				

Table B-2. Field Performance Test Progression, continued

Test Day	Day of Week/ Date (2019)	Test	Phase	Test Fluid	Sample taken at hourly intervals	Total # of analyses (NH4, NO3, TOC, TN)
Month 6 Total					21	84
6-Month Grand Total					138	552

During off-line testing, the sensors will be supplied with unspiked tap water, spiked tap water or spiked treated septic effluent (Section B1.6). During on-line tests, the sensors will be supplied with test fluid by continuously feeding the solution into the test vessel. Prior to sampling, on-line effluents are introduced over a 24-hour period to ensure that the test vessel is fully flushed and uniform before a test sample is taken. The test vessel will be flushed three times over a 24-hour cycle. Each test fluid solution, off-line or online, will be mixed for a minimum of fifty minutes before a test is sample is taken. Preliminary testing demonstrates that the test vessel is fully mixed within one minute.

The proposed test fluid solutions, sequence of testing, and number of replicate tests are shown in Table B-2. The sensor response to the nutrient standards and tests listed in Section B1.6 and Table B-2, respectively, will be used to evaluate accuracy, precision, and range. Appendix F provides a statistical analysis that shows the design of the sampling plan has sufficient replicates (precision data) and spike samples (accuracy data) to demonstrate that a sensor's performance is acceptable.

B2 SAMPLING METHODS

As described above, testing of nitrogen sensors will consist of several off-line and on-line phases (for the preliminary screen test; Table B-1 and the field performance test; Table B-2). MASSTC staff will collect samples throughout the verification test that will be submitted to the BCDHE laboratory for analysis. The samples will be collected following guidelines set in each standard reference method listed in Section B4. The methods describe the appropriate sampling containers, preservation techniques, and maximum holding times. During the off-line testing phase, aliquots of the nutrient and other samples prepared for testing the sensors will be transferred to appropriate sample containers, preserved if necessary, and submitted to the BCDHE laboratory for analysis. During the on-line effluent monitoring phase, grab sample collection will be documented and these grab samples will be compared to the nearest time stamped sensor reading obtained from the developer data logger. Tables B-1 and B-2 summarize the samples to be collected during each of the tests.

Treated effluent samples will be collected using ISCO™ portable or refrigerated liquid samplers and Sigma refrigerated liquid samplers. The samplers use peristaltic pumps and are programmable.

MASSTC staff will manually start the sampler at a designated time on the hour using Verizon clock time and watch the withdrawal and purges. Samples will be withdrawn with a peristaltic pump from the perimeter of the test cell close to the location of the sensors. The withdrawn sample will be immediately transported to the laboratory.

Field measurements of pH, dissolved oxygen (DO), and temperature will be performed on the test fluid immediately before a fluid change, 1 hour after a test fluid change and whenever a sample is taken (if a sample is taken 1 hour after a fluid change, one measurement is adequate). The measurement of these field parameters will be done with a YSI 556 Multi Probe Sensor (MPS) or equivalent by MASSTC personnel. Calibration of the YSI is described in Section B7.1. All calibrations, field observations, and data will be recorded in the sampling log book and reported by MASSTC with the lab and sensor data on the data report spreadsheet supplied by Battelle.

B3 SAMPLE HANDLING AND CUSTODY

B3.1 Testing Facility Sample Handling and Custody

Wastewater samples selected for confirmatory analysis will be collected using methods described in Section B2. Sample aliquots will be transferred into sample containers provided by the BCHDE Laboratory in certified pre-preserved bottles as summarized in Table B-3.

Table B-3. Sample Containers

Test	Bottle Type	Preservative	Holding Time
Ammonia	2 x 250 mL polyethylene bottle	Sulfuric acid (H_2SO_4) to pH<2, Cool to 4˚C	28 days
Total Kjeldahl Nitrogen			
Nitrate and Nitrite	250 mL polyethylene bottle	Cool to 4˚C	48 hours
Total Organic Carbon	40 mL clear or amber glass vial	H_2SO_4 to pH<2, Cool to 4˚C	28 days

Sample containers are labeled with the following information:

- Project name (EPA Nitrogen Sensor Challenge)
- Sensor ID (unique alphanumeric ID, to be determined when sensors are identified)
- Sample ID (see Table B-4)
- Date and Time
- Analysis and Preservatives.

Prior to splitting and sub-sampling the collected grab sample, the volume is determined from graduated markings on the side of the collection vessel to the nearest half-liter. This information is recorded in the appropriate log book. The sample volume is manually agitated vigorously to ensure complete mixing of the sample. The sample is uncapped and poured with continuous, uninterrupted flow from the grab bottle

to the sub-sample bottles. All filled bottles are capped, rinsed externally with fresh tap water, and packed in a cooler with ice to maintain an internal temperature of 4˚C. The chain-of-custody provided by the BCDHE laboratory (Appendix A), is completed by the field personnel and accompanies the cooler with transport to the BCDHE laboratory within a timeframe to allow for holding times to be met for analysis, typically the same day of sample collection. The sampler will relinquish the samples to the laboratory, documented by signature in the appropriate box on the chain-of-custody.

B3.2 Sample ID Convention

Each replicate collected by MASSTC will require a unique alphanumeric identification code. Using the test type, the test fluid, and the replicate numbers, MASSTC will assign a sample ID to each replicate, using Table B-4 as a guide.

Table B-4. Sample ID Naming Convention

Date	Test Type	Test Type Abbr.	Test Fluid	Test Fluid Abbr.	Replicate	Sample ID Number
4-digit date (MMDD)	Accuracy/Precision/Range	APR	Tap Water	TW	1	MMDD-APR-TW-1
4-digit date (MMDD)	Accuracy/Precision/Range	APR	Low Standard	LS	1, 2, or 3	MMDD - APR-LS-1 (or APR-LS-2 or APR-LS-3)
4-digit date (MMDD)	Accuracy/Precision/Range	APR	Medium Standard	MS	1, 2, or 3	MMDD - APR-MS-1 (or APR-MS-2 or APR-MS-3)
4-digit date (MMDD)	Accuracy/Precision/Range	APR	High Standard	HS	1	MMDD - APR-HS-1
4-digit date (MMDD)	Accuracy/Precision/Matrix	ADM	Treated Sewage Effluent + Low Standard	TSx[1] LS	1, 2, or 3	MMDD - ADM-TSx[1]LS-1 (or ADM-TSx[1]LS-2 or ADM-TSx[1]LS-3)
4-digit date (MMDD)	Accuracy/Precision/Matrix	ADM	Treated Sewage Effluent	TSx[1]	1, 2, or 3	MMDD - ADM-TSx[1]-1 (or ADM-TSx[1]-2 or ADM-TSx[1]-3)

Table B-4. Sample ID Naming Convention, continued

Date	Test Type	Test Type Abbr.	Test Fluid	Test Fluid Abbr.	Replicate	Sample ID Number
4-digit date (MMDD)	Alarm	A	Primary Treated Effluent	PE	1	MMDD -A-RS-1
4-digit date (MMDD)	Accuracy following Alarm	AFA	Treated Sewage Effluent	TSx[1]	1	MMDD -AFA-TSx[1]-1
4-digit date (MMDD)	Accuracy/Precision/Drift	APD	Tap Water	TW	1	MMDD-APD-TW-1
4-digit date (MMDD)	Accuracy/Precision/Drift	APD	Low Standard	LS	1, 2, or 3	MMDD-APD-LS-1 (or APD-LS-2 or APD-LS-3)
4-digit date (MMDD)	Accuracy/Precision/Drift	APD	Medium Standard	MS	1, 2, or 3	MMDD-APD-MS-1 (or APD-MS-2 or APD-MS-3)
4-digit date (MMDD)	Accuracy/Precision/Drift	APD	High Standard	HS	1	MMDD—APD-HS-1
4-digit date (MMDD)	Accuracy after power failure	APF	Treated Sewage Effluent	TSx[1]	1	MMDD-APF-TSx[1]-1
4-digit date (MMDD)	Accuracy/Drift	AD	Treated Sewage Effluent	TSx[1]	1	MMDD-AD-TSx[1]-1

[1] The Sample IDs for the Treated Sewage Effluent fluids would specify which effluent it was: '2', '3', or 'X'.

B3.3 Laboratory Sample Handling and Custody

On receipt at the laboratory, samples are examined for breakage and sample integrity (bottles, preservative, sample identification, and condition). If any issues are identified, the Battelle Project Manager will be notified within one business day of receipt. Once the chain-of-custody has been reviewed for clarity and accuracy, the sample shipment is signed as received and the samples are logged into the sample log book and Laboratory Information Management System (LIMS) by the Sample Receiving Person, given a laboratory identification number, and stored refrigerated in a secured area. The internal report form generated following the login process, follows the samples through the laboratory until all analyses are completed. A copy of the completed chain-of-custody is included in the final report. The samples shall remain stored until 30 days after the final report has been issued.

If samples need to be subcontracted to another certified laboratory due to instrument breakdown or laboratory over capacity, the BCDHE laboratory will notify the Battelle Project Manager prior to the samples being shipped to the subcontract laboratory to approve the shipment of the samples. The EPA Project Manager will in turn be alerted of this issue by the Battelle Project Manager if this situation arises.

B4 ANALYTICAL METHODS

Confirmatory analyses of wastewater samples will be completed by the BCDHE laboratory located in Barnstable, Massachusetts. The analyses include:

- Ammonia as Nitrogen by laboratory SOP "Determination of Ammonia Nitrogen in Aqueous Samples by Semi-Automated Colorimetry Gas Diffusion Separation Method". This SOP is based on EPA Methods for Chemical Analysis of Water and Wastes (MCAWW), EPA-600/4-79-020, Revised 1993, Method 350.1. The samples are analyzed with an automated continuous flow analysis instrument (Lachat). The ammonia is separated from the matrix in a diffusion cell across a hydrophobic semi-permeable membrane and absorbed by a flowing acceptor stream. The ammonia reacts with salicylate and hypochlorite in an alkaline phosphate buffer to produce an emerald green color proportional to the ammonia concentration.
- Nitrate and Nitrite as Nitrogen by laboratory SOP "Determination of Inorganic Anions in Aqueous Samples Using Ion Chromatography". The SOP is based on EPA "Determination of Inorganic Anions by Ion Chromatography", Method 300.0, Revision 2.1, August 1993. The anions of interest are separated and measured using a system comprised of an ion chromatographic pump, sample injection valve, and a conductivity detector.
- Total Kjeldahl Nitrogen (TKN) by laboratory SOP "Determination of Total Kjeldahl Nitrogen in Aqueous Samples by Semi-Automated Colorimetry". This SOP is based on EPA MCAWW, EPA-600/4-79-020, Revised 1993, Method 351.2. The samples are analyzed with an automated continuous flow analysis instrument (Lachat). The sample is digested in the presence of sulfuric acid for three hours then analyzed for ammonia. TKN is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate under the conditions of digestion.
- Total Organic Carbon (TOC) by laboratory SOP "Determination of Total Organic Carbon (TOC) in Aqueous Samples Using High-Temperature Combustion Method". This SOP is based on American Public Health Association, American Water Works Association, and Water Environment Federation, "Standard Methods for the Examination of Water and Wastewater", 22nd Edition, 2012, SM 5310B. The sample is injected into a heated reaction chamber of a TOC analyzer where the organic and inorganic carbon is oxidized to carbon dioxide and water. The carbon dioxide is transported in the carrier-gas stream and is measured by a non-dispersive infrared analyzer.

The BCDHE laboratory's SOPs for this project are included in Appendix C.

The analytes, calibration ranges, and detection limits are presented in Table B-5.

Table B-5. Laboratory Target Analytes, Calibration Ranges, and Detection Limits

Analyte	Reported as	Calibration Range[1]	Detection Limit
Ammonia Nitrogen	NH3 as N	0.1 to 20 mg/L	0.082 mg/L
Total Kjeldahl Nitrogen	TKN as N	0.25 to 20 mg/L	0.103 mg/L
Nitrate-Nitrogen	NO_3^- as N	0.1 to 10 mg/L	0.015 mg/L
Nitrite-Nitrogen	NO_2^- as N	0.05 to 5 mg/L	0.035 mg/L
Total Organic Carbon	TOC	1 to 100 mg/L	0.373 mg/L

Note: The laboratory will report uncensored data, qualifying results below the detection limit. Results are typically reported to the lowest calibration standards as ND as the reporting limit (RL).
[1] If the measured concentration of the analyte exceeds the calibration range, the sample will be diluted and reanalyzed.

B5 QUALITY CONTROL

QC sample analyses are used to provide data quality indicators (DQI) to ensure the quality of data obtained during the facility study and laboratory analysis meet the project DQOs. The DQIs are often expressed in terms of precision, accuracy, representativeness, comparability, completeness, and sensitivity (PARCCS). The QC samples to be tested in this study are described below.

B5.1 Laboratory Quality Control

The laboratory Quality Assurance Plan (QAP) and SOPs define the QC samples to be tested for each method. Table B-6 summarizes the QC samples for the methods being performed for this study:

Table B-6. Laboratory Quality Control Sample Summary

QC Parameter	Frequency	Acceptance Criteria	Corrective Action
Laboratory Reagent Blank	1 batch of 20 or fewer samples	< 1/2 method detection limit (MDL) for TOC; <MDL for all other tests	If samples non-detect, no action needed. Otherwise, analyze another blank and re-prepare and reanalyze affected samples.
Laboratory Fortified Blank (LFB)	1 batch of 20 or fewer samples	80-120% R for TOC; 90-110% R for all other tests	Analyze another LFB. If second LFB fails, check an independent reference material. If acceptable, re-prepare and reanalyze affected samples.
Laboratory Fortified Sample Matrix (LFM)	TOC: 5% or 1 batch. All other tests: 10% or 1/batch.	70-130% R for TOC; 80-120% R for NO_3^- /NO_2^-; 90-110% for TKN and NH_3	Check LFB. If LFB acceptable, qualify the data for LFM sample results.
Laboratory Duplicate	TKN and NH_3: 20% or 1/10. TOC and NO_3^- /NO_2^-: 10% or 1/10.	≤20% RPD	Check LFB. If LFB acceptable, qualify the laboratory duplicate results.

Table B-6. Laboratory Quality Control Sample Summary, continued

QC Parameter	Frequency	Acceptance Criteria	Corrective Action
LFB or LFM Precision	TKN and NH_3: 20% or 1/10. TOC and NO_3^- /NO_2^-: 10% or 1/10.	≤20% RPD	Evaluate results to determine source of the difference. Apply qualifiers.
Performance Evaluation Samples (PES)	Once during 6-month study with one batch of study samples	Within acceptance limits of certified reference material	Qualify sample results. Repeat PES analysis.

Note: QC samples are processed and analyzed similarly to test samples in the same analytical batch of 20 or fewer samples. R=Recovery; RPD=relative percent difference

B6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

All sensor developer maintenance visits and work conducted on septic sensors will be recorded in the visitor's log book. Developer's name and date of occurrence will be recorded, as well as MASSTC staff oversight name and date to document maintenance activities. All observations of unusual occurrences, breakdowns, or malfunctions of the sensors will be recorded. All instrumentation used for field measurements by MASSTC staff are visually inspected prior to use to ensure proper operating condition. All observations of breakdowns or malfunctions of equipment are recorded in the appropriate equipment log book. Malfunctions of measurement instruments are often immediately apparent during pre- and post-calibration procedures.

BCDHE laboratory refrigerator temperatures are measured daily and must be within ±2˚C of the required 4˚C. Thermometers are calibrated yearly with a National Institute of Standards and Technology (NIST) certified thermometer. Balances are calibrated daily with NIST traceable weights, which are verified annually. Each analytical system (e.g., LACHAT, TOC analyzer, ion chromatograph) 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, demonstrated by compliance to calibration requirements. Service contracts on the equipment include annual preventive maintenance visits. Maintenance logbooks are utilized to document major and routine maintenance procedures performed on the instruments.

B7 INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

B7.1 Testing Facility Instrument/Equipment Calibration and Frequency

A summary of field equipment calibration and frequency is shown in Table B-7.

Table B-7. Testing Facility Equipment Calibration and Frequency

Equipment	Specification and Frequency	Acceptance Criteria	Corrective Action
Thermometer	Each Round of Sampling	±1˚C (field and reference thermometer)	Use backup thermometer.

Table B-7. Testing Facility Equipment Calibration and Frequency, continued

Equipment	Specification and Frequency	Acceptance Criteria	Corrective Action
	Annual	±1˚C (reference thermometer to NIST)	Obtain a new thermometer.
YSI 556 MPS	Beginning and End of Sampling	DO DI water: 95-105%; DO Na_2SO_3: <0.5 mg/L pH 7.0 solution: ±0.2 pH	Recalibrate and retake field measurements.
ISCO™ and Sigma Auto-Samplers	Monthly	Equal samples, sufficient volume	Service, clean, repair.
Wastewater Volume	Two cycles per day (AM and PM), visually observed and recorded.	±10% of flow	Make adjustments to flow. Document deviations.

Details on the YSI 556 MPS calibration procedure are included in MASSTC's SOP shown in Appendix B.

With regards to the calibration of the sensors themselves, the sensor developers will need to disclose the maintenance interval of their technology and the developers will be allowed to perform calibrations or maintenance activities according to that interval.

B7.2 Laboratory Instrument Calibration and Frequency

Each laboratory SOP describes the requirements for instrument calibration and frequency for the test method. A summary of the requirements is shown in Table B-8.

Table B-8. Laboratory Instrument Calibration Requirements

Requirement	Specification and Frequency	Acceptance Criteria	Corrective Action
Calibration Curve	For NO_3^-/NO_2^-: At least 5 calibration standards. Run every 6 months or when changes occur. If RT drifts more than 10%. Daily, a one- point calibration standard (IPC) verifies the curve remains acceptable.	NO_3^- Range: 0.1 to 10 mg/L; NO_2^- Range: 0.05 to 5 mg/L; R≥0.9950	Recalibrate and/or re-prepare standards.
	For TKN/NH_3: 6-7 calibration standards. Run daily.	TKN Range:0.25 to 20 mg/L; NH_3 Range: 0.1 to 20 mg/L; R≥0.995	
	For TOC: 6 calibration standards are run in triplicate daily.	Range: 1-100 mg/L; %RSD ≤20%	
Quality Control Sample (QCS)	An external/second source standard run following calibration.	±10% of the true value	Recalibrate and/or re-prepare standards.
Instrument Blank (IB) or Calibration Blank (CB)	After calibration curve to verify cleanliness of system.	≤MDL	Clean the system and reanalyze the blank.
Instrument Performance Check (IPC)	For TKN, NH_3, NO_3^-/NO_2^-, Mid-range calibration standard run after daily calibration, after every 10th sample, and at end of run.	±10% of the true value	Reanalyze once. Recalibrate and reanalyze affected samples.
Initial Calibration Verification (ICV) and Continuing Calibration Verification (CCV)	For TOC only: ICV is mid-range calibration standard run after daily calibration. CCV is mid-range calibration standard run after every 10th sample and at end of run.	±10% of the true value	Reanalyze once. Recalibrate and reanalyze affected samples.

B8 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

All supplies are inspected upon delivery and inventoried accordingly. Standards used for instrument calibration are stored according to manufacturer instructions and replaced weekly. Standards are marked with the date the bottle is opened and the expiration date. Certified clean sample bottles are obtained from the BCDHE laboratory and stored on clean, dry, shelves in an upright position.

The BCDHE laboratory orders glassware, supplies, and reagents required to perform the analytical methods from proven developers. Laboratory reagent blanks demonstrate cleanliness of supplies and reagents from these developers. Standards are prepared and tracked in standard logbooks and each standard is given unique identification numbers to track and trace the levels of standards used in

analyses. The BCDHE laboratory produces its distilled water through Milli-Q and Direct-Q water purification systems. The water is monitored daily for specific conductance and resistivity; monthly for residual chlorine and heterotrophic plate count; and annually for metals. A logbook is maintained by the laboratory staff to record and monitor the lab water purification system.

B9 NON-DIRECT MEASUREMENTS

Data and information from a variety of published sources may be used for data processing and non-direct measurements or data comparison. Only information and data from credible published sources will be used and will be referenced accurately in the final report. Sources will include but not be limited to:

- Sensor documentation;
- Wastewater sampling guidelines;
- Health and environmental risk guidelines;
- US EPA analytical methods;
- Other published literature relating to acceptable errors and variances relating to wastewater analysis and reporting;
- Peer reviewed literature relating to sensors; and,
- Standard Operating Procedures of the BCDHE laboratory

B10 DATA MANAGEMENT

B10.1 MASSTC Data Management

All associated log books and log sheets used for the study will be scanned to the project file and included in the final report. Field data measurements will be manually entered in a Microsoft Excel® spreadsheet (Appendix D) by MASSTC. At a minimum, field data will include:

- Sample identification;
- Field parameter (e.g., temperature, DO, pH);
- Result and unit for each parameter;
- Field technician's name/initials; and,
- Date/time measurements taken.

Sensor data will be entered into a Microsoft Excel® spreadsheets (Appendix D and E) by the sensor developer. Sensor data will include:

- Sensor identification;
- Date and time of readings;
- Analytical parameter (e.g., ammonia as N, nitrate as N, total nitrogen, TOC);
- Results and units for each parameter.

Appendix D data will be entered to coincide with the laboratory sample data. Appendix E data will be hourly readings taken throughout the entire test.

Sensor data will be entered into a Microsoft Excel® spreadsheet by each developer. Laboratory data will be entered into the spreadsheet by MASSTC. Following receipt of laboratory data, a review and comparison of sensor data with laboratory data is completed to identify errors (see Section B10.2).

B10.2 BCDHE Laboratory Data Management

The BCDHE laboratory maintains separate logbooks by instrument to record the analyses performed on that instrument. The instrument printouts of each analysis are filed according to the laboratory identification number assigned to the samples. Included in the files are copies of the appropriate chain-of-custody forms, quality control reports, and all calculations of the data. These data will become part of the final data package.

The data report is generated from the LIMS. There are three parts to the lab report: 1) Customer Information, 2) Analytical Information, and 3) Signature and Date. The Customer Information includes the following at a minimum:

- Reporting mailing address;
- Name and address of customer;
- Date collected;
- Type of Sample (e.g., raw or finished);
- Sample Location/ID;
- Original, Resubmitted, or Confirmation; and,
- Sample receipt notes.

The Analytical Information includes the following at a minimum:

- Laboratory Name (BCDHE);
- Laboratory MassDEP Certification Number (M-MA009);
- Sample Matrix;
- Requested Analytes and Respective Results, Maximum Contaminant Levels (MCLs), MDLs, RLs, Analytical Methods, Analytical Dates and Times, Analyst Name or initials, and Lab Sample ID; and,
- Laboratory notes on sample preparation and analysis.

As indicated above, QC sample results and raw data will also become part of the laboratory data report for this project. The sample results are sent to MASSTC on a regular basis. The typical turn-around time for the laboratory ten (10) working days. The data report is first sent to MASSTC to use for data entry into a Microsoft Excel® spreadsheet. Then both the data report in PDF format and the Microsoft Excel® spreadsheet are reviewed for data entry error and reviewed versus sensor data by MASSTC.

Data will be checked for errors three ways: 1) hard copy laboratory data will be compared to field data measurements to detect transcription errors in the spreadsheet; 2) data will be graphed according to analytical parameter and method (i.e., field and laboratory separately) to check for outliers and inconsistent data; and 3) graphical comparison of data will be cross-checked for contradictory results for each parameter between methods. Data entry errors will be corrected as noted. Suspected errors will be verified with the laboratory for investigation or further analysis. Unresolved data discrepancies will be noted in the final report. Once reviewed, all data will be submitted to Battelle for data audit, final evaluation, and reporting. The data will then be uploaded into Battelle's database to generate tables for data review, data calculations, and evaluation for the final report. Data will be submitted to Battelle over the course of the field performance test after analysis of Day 34, Day 92, and Day 188 sampling events.

C ASSESSMENT AND OVERSIGHT

C1 ASSESSMENTS AND RESPONSE ACTIONS

One of the major objectives of the T/QAP is to establish mechanisms necessary to anticipate and resolve potential problems before data quality is compromised. Internal QC measures described in this T/QAP will yield day-to-day information on data quality. The responsibility for interpreting the results of these checks and resolving any potential problems resides with Battelle. Technical staff has the responsibility to identify problems that could affect data quality or usability. Any problems that are identified will be reported to the EPA Project Manager, who will work with the Battelle QAO to resolve any issues. Action will be taken to identify and appropriately address the issue and minimize losses and correct data, where possible. The Battelle QAO will be responsible for ensuring that the audits described in the following subsections are conducted as part of this testing.

Any changes to the approved T/QAP must be reported within 24 hours and documented in a formal deviation submitted to the EPA Project Manager. If approval by these managers is not received within 24 hours of notification, testing will be halted until a suitable resolution has been achieved.

C1.1 Technical Systems Audit

The Battelle QAO, or designee, will perform one TSA during the first one-month screening test and one during the 6-month field performance test, preferably within one week or two weeks of the beginning of the test, respectively. The TSA is being performed in accordance with the MASSTC QAPP, this T/QAP, published reference methods, and any SOPs used by MASSTC or the BCDHE laboratory to ensure that QA/QC procedures are implemented. The Battelle QAO, or designee, will review evaluation methods, compare test procedures to those specified in this T/QAP, and review data acquisition and handling procedures.

The Battelle QAO, or designee, will prepare a TSA report and the findings must be addressed either by modifications of test procedures or by documentation in the evaluation file and evaluation report. The TSA report will be prepared within 10 business days after completion of the audit; the completed audit checklist will be attached to the report. MASSTC will respond to the audit within 10 business days. The Battelle QAO, or designee, will verify that all audit findings and observations have been addressed and that corrective actions are appropriately implemented. A copy of the complete TSA report with corrective actions will be provided to the EPA Project Manager within 10 business days after receipt of the audit response.

C1.2 Data Quality Audit

The Battelle QAO, or designee, will audit at least 10% of the sensor data, 10% of the laboratory data, and 100% of the calibration and QC data for each 1-month screening test and the 6-month field test. A checklist based on the T/QAP will guide the audit (Table C-1).

Table C-1. Data Quality Audit Checklist (Items to be Verified for MASSTC Field Data and BCDHE Laboratory Data)

Component	MASSTC Activities	BCDHE Laboratory Activities
Certifications and Training		
1. Are certifications/licenses current?	✓	✓
2. Do training records for samplers/analyst demonstrate training is completed?	✓	✓
Experimental Design		
3. Was field testing (e.g., pH, DO, temperature) performed according to the T/QAP?	✓	
4. Were field instruments calibrated daily and did they meet criteria?	✓	
5. Were technology dosing events and sampling frequencies completed according to the T/QAP?	✓	
6. Were all T/QAP data collection requirements for the experimental design achieved?	✓	
7. Were the technology sensors operated according to the T/QAP and technical directions provided by the developers?	✓	
8. Were samples collected according to T/QAP procedures?	✓	
9. Are field observations and data recorded in sampling log books?	✓	
Sample Handling and Custody		
10. Is sample custody documented as specified in the T/QAP? a) COC forms document time, date, location, and person collecting the sample. b) COC forms are signed by person relinquishing and receiving samples	✓	✓
Quality Control		
11. Were reference method QC samples run as specified by the method or the T/QAP?		✓
12. Did the reference method QC sample results achieve the acceptance criteria?		✓
13. Were method blank samples <MDL (<1/2 MDL for TOC)?		✓
14. Were LFBs 80-120% R for TOC and 90-110% for other tests?		✓

Table C-1. Data Quality Audit Checklist (Items to be Verified for MASSTC Field Data and BCDHE Laboratory Data), continued

Component	MASSTC Activities	BCDHE Laboratory Activities
15. Were LFMs 70-130%R for TOC; 80%-120% for NO_3/NO_2; 90-110% for TKN and NH_3?		✓
16. Were Laboratory Duplicates and LFB/LFM Precision samples ≤20% RPD?		✓
17. Were PE samples within acceptance limits of certified reference materials?		✓
18. Were test design QC samples run as specified in the T/QAP?	✓	
Analytical Reference Method Requirements (These will be assessed from the laboratory data reports.)		
19. Were samples analyzed according to the reference method or as modified by the T/QAP?		✓
20. Was the reference method instrumentation calibrated according to the reference method or as modified by the T/QAP?		✓
21. Did the calibration and calibration checks meet the acceptance criteria of the reference method or as modified by the T/QAP?		✓
Analytical Reference Laboratory Data Reporting		
22. Do the data packages include all required elements of the T/QAP (Section B10.2)?		✓
23. Does the QA narrative document any laboratory SOP or T/QAP deviations?		✓
24. Are any data associated with failed calibration or QC data flagged in the hard copy and electronic data deliverable (EDD)? 25. Are data flags defined?		✓
26. Are data in the Excel® spreadsheet traceable to the laboratory data report?		✓
27. Is there documentation of internal laboratory review of data per the laboratory QAPP?		✓
28. If errors have been found, has resolution been made with the laboratory?		✓

Table C-1. Data Quality Audit Checklist (Items to be Verified for MASSTC Field Data and BCDHE Laboratory Data), continued

Component	MASSTC Activities	BCDHE Laboratory Activities
29. Are any data associated with failed calibration or QC data flagged in the hard copy and electronic data deliverable (EDD)? 30. Are data flags defined?		✓
31. Are data in the Excel® spreadsheet traceable to the laboratory data report?		✓
32. Is there documentation of internal laboratory review of data per the laboratory QAPP?		✓
33. If errors have been found, has resolution been made with the laboratory?		✓
Technology Calibration and Frequency		
34. Was the technology calibrated according to the T/QAP frequency and criteria defined by the developer?	✓	
35. Did the technology calibration achieve the developer acceptance criteria prior to testing?	✓	
36. Was the calibration stability of the technology verified as specified by the developer?	✓	
Data Management		
37. Is it possible to track data from raw data entries to spreadsheets?	✓	
38. Do the Data Collection Logs include all the elements required in the T/QAP (Section B10.1)?	✓	
39. Is permanent ink used to document manually-recorded data?	✓	
40. Are corrections made by drawing a single line through the entry to be corrected and providing a simple explanation for the correction, along with a date and the initials of the person making the correction?	✓	
41. Has the laboratory adequately documented and addressed non-conformances and problems according to the acceptance criteria and corrective action specified in the T/QAP associated with this data package?		✓

Table C-1. Data Quality Audit Checklist (Items to be Verified for MASSTC Field Data and BCDHE Laboratory Data), continued

Component	MASSTC Activities	BCDHE Laboratory Activities
42. Can project notebook entries be linked to personnel making the entry?	✓	
43. Are data collected by the technology uniquely named and able to be directly linked to the samples as received?	✓	
44. Do the sensor data meet all the elements of the T/QAP (Section B10.1)?	✓	
45. If data are collected electronically, are data saved to a second media (e.g., CD) to prevent data loss?	✓	
46. Are those media labeled to identify the test, data type, and date of collection?	✓	
47. Are project records maintained securely by MASSTC staff during the test?	✓	
48. Are records reviewed at the frequency defined in the T/QAP? 49. Was the reviewer independent of the person who generated the record? 50. Are reviewer initials and date recorded?	✓	
51. Overview of documentation: Are activities recorded in project log books or data sheets detailed enough to enable reconstruction of the verification data?	✓	
52. Review raw data: Is documentation complete? Note any issues or discrepancies vs. the T/QAP.	✓	
53. Has MASSTC done a comparison of sensor data with laboratory data prior to release to Battelle?	✓	
54. Were issues with the comparison documented and resolved?	✓	

The Battelle QAO, or designee, will calculate percent recovery of sensor data versus laboratory true value results for each sensor and sample collected. Precision of replicates will be calculated for sensors and laboratory samples. All data analysis calculations will be checked.

Data audits will verify the transcription of field data collected by MASSTC staff and hard copy laboratory data entered into spreadsheets and report tables. For the BCDHE laboratory, data audits will verify transcription of data from the hard copy summary tables provided with the laboratory data package to the EDD as well as review of calibration and QC sample results vs. the frequency and acceptance criteria

defined in the reference method or as modified by the T/QAP. BCDHE laboratory data will not be re-calculated vs. raw data for this project, rather, the DQA will verify that the laboratory has provided signed documentation that the data and report have been reviewed and approved according to the laboratory's QAP. A final DQA will be conducted for the final report, verifying statements, data tables, and figures vs. the previously-audited data from the MASSTC field and BCDHE laboratory data collection activities.

For each audit, the audit checklist will be provided as an attachment to an e-memo to MASSTC or the BCDHE laboratory within 10 business days after completion of each data audit. Any findings that could impact data integrity will be specifically described in the e-memo. MASSTC or the BCDHE laboratory will respond to the audit within 10 business days. The Battelle QAO or designee will verify that all audit findings have been addressed and that corrective actions are appropriately implemented. A copy of the complete DQA e-memo with corrective actions and checklist will be provided to the Battelle project manager within 10 business days after receipt of the audit response.

C2 DATA EVALUATION

The data evaluation will include precision and evaluation results of each sensor. Because the critical range of concern is 5-20 mg N/L for operation, which is narrower than the performance goals, additional evaluation of precision and accuracy results of the data set may be centered in this range. Average values for the full data set will also be summarized.

A comparison of the sensor data and the laboratory data from the field performance test will be done to assess the overall performance of each sensor. Plotting of senor data versus laboratory measurement data will be done to compare relative changes in concentration over time. Evaluation of trends and reasonableness of direction of change in concentration will be commented on in the final sensor reports.

For the preliminary screening test, only the 7-day results will be evaluated for precision and accuracy and performance criteria to evaluate continuing forward to the 6-month testing phase as defined in Table A-3. The remaining data from the one-month study will be tabulated and graphed to provide additional information to the developers to aid in adjustments to their sensors for the longer duration study.

For the field performance study, evaluation for precision and accuracy and other performance goals in Table A-1 will be reviewed for each sensor for the duration of the study. If a technology does not make the full 6-month test period, evaluation for precision and accuracy and other performance goals in Table A-1 will be reviewed for the 7-day, 1-month, 3-month, and x-month intervals to see when the technology begins to fail.

C3 REPORTS TO MANAGEMENT

Each assessment and audit will be documented in accordance with the STREAMS III Quality Management Plan (Battelle 2018). Assessment reports will include the following:

- Identification of any adverse findings or potential problems
- Space for response to adverse findings or potential problems
- Possible recommendations for resolving problems
- Citation of any noteworthy practices that may be of use to others

- Confirmation that solutions have been implemented and are effective.

The Battelle QAO, during the course of any assessment or audit, will identify to the personnel performing experimental activities any immediate corrective action that should be taken. If serious quality problems exist, the Battelle QAO will notify the Battelle Project Manager, who will issue a stop work order. The results of QA audits will be reported to the Project Manager and, once corrective actions are identified, to EPA. The Battelle QAO will verify that corrective action has been implemented effectively. The final report will include a summary of QC results, QA activities, and the corrective action implemented to minimize impact of QC failures or T/QAP deviations. The T/QAP, verification report(s), and verification statement(s) are reviewed by EPA and select members of the Technical Panel. Upon review and approval, the final verification statement(s) will then be posted on the VerifiGlobal website.

D DATA VALIDATION AND USABILITY

D1 DATA REVIEW

The data review requirements include:

- Verification that all testing is completed as specified in the T/QAP
- Ensuring that each data point is valid, i.e., complies with acceptance criteria specified in the T/QAP
- Records generated during the evaluation will receive a QC/technical review before these records are used to calculate, analyze, or report results
- All data analysis calculations will be checked before the results are incorporated into the draft test report.

D2 VALIDATION AND VERIFICATION METHODS

The Project Manager will compare the data generated to the requirements of the T/QAP to ensure that all testing is completed in accordance with the plan. The required technical review of records generated during the evaluation will be performed by Battelle personnel. MASSTC test personnel will be consulted as needed to clarify any issues about the data records. The review will be documented by the person performing the review by adding his/her initials and date to a hard copy of the record being reviewed. This hard copy will then be returned to the Battelle personnel who will be storing the record. The data generated in this evaluation will be transferred from the data collection forms into an electronic database. DQAs will be performed as specified in Section C1.2.

Verification of the field performance test data for selected sensors will be conducted in accordance with the Verification Plan for a specific sensor following the requirements of ISO 14034 and the VerifiGlobal Performance Verification Protocol. Each technology will have its own verification report and verification statement. Individual verification statements for each technology will be posted on the VerifiGlobal website.

D3 RECONCILIATION WITH USER REQUIREMENTS

This T/QAP and the verification report(s) that may result will be subjected to review by EPA and select members of the Technical Panel. These reviews will assure that this T/QAP and the resulting report(s) meet the needs of potential users and permitters of advanced septic system nitrogen sensors. The final report(s) will be submitted to EPA in Microsoft Word and Adobe pdf format. For sensors that proceed through verification with completion of a final verification report, VerifiGlobal verification statements will be posted on the VerifiGlobal website.

Data obtained during this evaluation will be assessed by comparison with the DQOs contained in Section A6. Data not meeting the DQOs will be considered invalid and will be rejected from use. The results of reconciling the data obtained with the DQOs will be presented in the final report. In addition, any limitations on the data will be presented in the report including the impact or potential impact on the quality of the results. The developers will have an opportunity to review the reports on their technology

prior to finalization of the reports and the verification reports. A draft will be provided to the developers with a 2-week review period before the reports go final.

E REFERENCES

APHA, 2012. American Public Health Association, American Water Works Association, and Water Federation, Standard Methods (SM) for the Examination of Water and Wastewater, 22nd Edition.

BCDHE, 2017. Barnstable County Department of Health and the Environment (BCDHE) Water Quality Laboratory, “Quality Assurance Plan”, Revision 018, October 16, 2017.

BCDHE, 2017. Barnstable County Department of Health and the Environment (BCDHE) Water Quality Laboratory, "Determination of Ammonia Nitrogen in Aqueous Samples by Semi-Automated Colorimetry Gas Diffusion Separation Method", Method 350.1, Revision 004, November 7, 2017.

BCDHE, 2018. Barnstable County Department of Health and the Environment (BCDHE) Water Quality Laboratory, "Determination of Inorganic Anions in Aqueous Samples Using Ion Chromatography", Method 300.0, Revision 015, May 11, 2018.

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BCDHE, 2017. Barnstable County Department of Health and the Environment (BCDHE) Water Quality Laboratory, "Determination of Total Organic Carbon (TOC) in Aqueous Samples Using High-Temperature Combustion Method", Method 5310B, Revision 007, November 7, 2017.

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EPA, 1993. Methods for Chemical Analysis of Water and Wastes (MCAWW), EPA-600/4-79-020.

ISO 14034:2016, Environmental Management – Environmental Technology Verification (ETV)

ISO/IEC 17020:2011, Conformity Assessment – Requirements for the Operation of Various Types of Bodies Performing Inspection

ISO/IES 17025:2017, General Requirements for the Competence of Testing and Calibration Laboratories

MASSTC, 2016. Massachusetts Alternative Septic System Test Center, “Quality Assurance Project Plan (QAPP), June 2016-June 2019.

APPENDIX A

BCDHE Laboratory Chain-of-Custody

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BCDHE Laboratory Chain of Custody will be inserted as PDF in the Final T/QAP. A screen capture is provided below.

CHAIN OF CUSTODY

PAGE___OF____

BARNSTABLE COUNTY DEPARTMENT OF HEALTH & ENVIRONMENT
WATER QUALITY LABORATORY
3195 Main Street/PO Box 427, Barnstable, MA 02630 Phone: 508-375-6605; Fax: 508-362-7103

REPORT GOES TO	BILLING INFORMATION	NOTE
ATTENTION:	ATTENTION:	
COMPANY NAME:	COMPANY NAME:	
ADDRESS:	ADDRESS:	
		TURN-AROUND TIME (Circle) Standard: Ten days
E-MAIL:	E-MAIL:	Rush (day): One Two Three Four Five
PHONE: FAX:	PHONE: FAX:	DELIVERY (Circle) Hard Copy, e-mail, Fax

LAB ID (Lab Use Only)	SAMPLE LOCATION / IDENTIFICATION	COLLECTION DATE	COLLECTION TIME	SAMPLER	Sample Matrix	ANALYSIS									COMMENT

Relinquished By:	Date/Time	Container Type [1]										Temp (°C)	
		Preservative	H_2SO_4										Please print completely and clearly.
Received By:	Date/Time		HNO_3										
			Others[2]										

[1] Container Type: P = Plastics; CG = Clear Glass; AG = Amber Glass; GV = Glass Vial

[2] H = HCl; T = $Na_2S_2O_3$ (THIO); S = Sterile; N = NaOH

APPENDIX B

MASSTC YSI 556 MSP SOP

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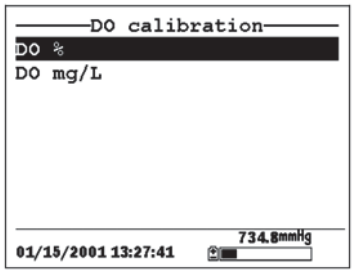
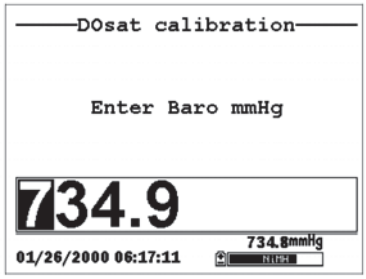
MASSTC Standard Operating Procedure: YSI 550 MPS Calibration Procedure

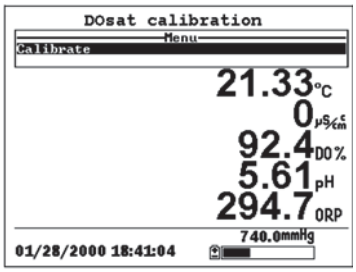
YSI 556 MPS Calibration Procedure
Instrument Start-up

*****Turn on the instrument and let it warm up for 20 minutes. *****

Calibrate DO:

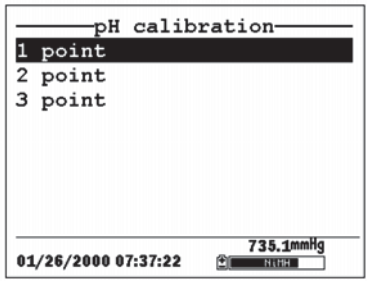
i 

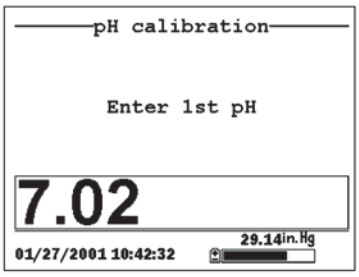
ii. 

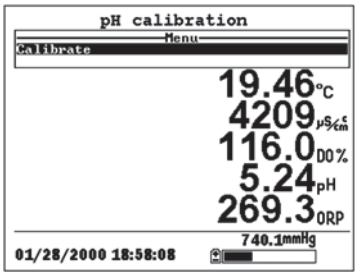
iii. 

a. Choose DO from the calibration menu, then choose DO % (saturation) (i).
b. Press Enter. The DO barometric pressure entry screen is displayed (ii).
c. Very **lightly** screw on the DO calibration cup which should be filled with 1/8 inch of DI water. Make sure sides of the probe are dry. Note: the calibration cup should be engaged **just** enough to hold it in place.
d. Press Enter. The DO% saturation calibration screen is displayed (iii).
e. Wait approximately 10 minutes for the air in the transport/calibration cup to become water saturated and for the temperature to equilibrate. **The DO% should read between 95 and 105% and should show no significant change for approximately 30 seconds.** Press Enter to accept the calibration.
f. Press Enter again and then Esc to get back to the calibration menu.
g. Again, choose DO from the calibration menu, then choose DO mg/L (i).
h. Press Enter. The DO barometric pressure entry screen is displayed (ii).
i. Submerge the probe into the zero DO solution, consisting of a saturated solution of sodium sulfite.
j. Press Enter. The DO mg/L calibration screen is displayed.
k. The DO mg/L should read less than 0.5 mg/L. Press Enter to accept the calibration.

Calibrate pH:

iv. 

v. 

vi. 

a. Put probe in 4.0 solution (change every Monday!) and firmly screw on cup.
b. Choose pH from the calibration menu and then choose **3 point** calibration (iv). Press Enter.
c. Type in appropriate pH for the corresponding temperature (see temperature compensation chart) (v). Press Enter.
d. At the pH calibration screen, wait for reading to stabilize. Press Enter.
e. Press Enter again to get to the 2nd pH calibration screen.
f. Remove the 4.0 solution, spray the probe with DI water, and wipe everything down with a Kim wipe.
g. Put probe in the 4.0 solution (change every Monday!) and screw on cup. Enter the appropriate pH for the corresponding temperature (see temperature compensation chart). Wait for reading to stabilize. Press Enter.
h. Press Enter again to get to the 3rd calibration screen.
i. Remove the 7.0 solution, spray the probe with DI water, and wipe everything down with a Kim wipe.
j. Put the probe in the 10.0 solution (change every Monday) and screw on cup. Enter the appropriate pH for the corresponding temperature (see temperature compensation chart). Wait for the reading to stabilize.
k. Press Enter again and then Esc to get back to the calibration menu.
l. Remove cap, thoroughly spray probe and wipe dry with a Kim wipe.

Calibrate Temperature:
*Temperature is calibrated monthly against a certified NIST thermometer and recorded.

YSI 556 MPS Calibration Procedure
Instrument Open and Close

NOTE: The steps below should be followed twice: 1.) immediately after the instrument has been fully calibrated and 2.) after all readings have been completed.

1. Rinse the probe and probe sensor guard thoroughly with fresh tap water. Make sure the meter is in Run mode.

2. Remove the probe sensor guard if it is in place, rinse the probe with DI water, and carefully dry with a Kim wipe.

3. Place the probe in 7.0 pH solution, screw on the cup, and wait for the reading to stabilize (the reading should correspond to the appropriate temperature on the temperature compensation chart). Once the meter stabilizes, record the value in the meter reading logbook. **Precision criterion: the value should be within ± 0.2 pH units of the temperature compensated value.**

4. With the meter still in Run mode, place the probe in the aeration tank and wait for the reading to stabilize. Record the DO% in the meter reading logbook. **Precision criterion: the value should read between 95 and 105% saturation.**

NOTE: If the opening/closing pH and DO values fall outside of the precision criteria listed above, the meter must be evaluated for potential malfunction and the readings should be re-done.

Acceptable pH vs. Temperature Values for 4.00, 7.00 &10.00 Buffers

Temp in ºC	Buffer 4.01	Buffer 7.00	Buffer 10.01
0	4.00	7.11	10.32
1	4.00	7.11	10.31
2	4.00	7.10	10.29
3	4.00	7.09	10.28
4	4.00	7.09	10.26
5	4.00	7.08	10.25
6	4.00	7.08	10.23
7	4.00	7.07	10.22
8	4.00	7.07	10.21
9	4.00	7.06	10.20
10	4.00	7.06	10.18
11	4.00	7.05	10.17
12	4.00	7.05	10.16
13	4.00	7.04	10.14
14	4.00	7.04	10.13
15	4.00	7.03	10.12
16	4.00	7.03	10.11
17	4.00	7.02	10.10
18	4.00	7.02	10.09
19	4.00	7.02	10.08
20	4.00	7.01	10.06
21	4.01	7.01	10.05
22	4.01	7.01	10.04
23	4.01	7.00	10.03
24	4.01	7.00	10.02
25	4.01	7.00	10.01
26	4.01	6.99	10.00
27	4.01	6.99	9.99
28	4.01	6.99	9.98
29	4.01	6.99	9.98
30	4.02	6.98	9.97
31	4.02	6.98	9.96
32	4.02	6.98	9.95
33	4.02	6.98	9.94
34	4.02	6.98	9.93
35	4.02	6.97	9.93

Reference: This information was taken from the buffer manufacturer insert.
Acceptable accuracy: +/- 0.15 pH units

Acceptable Dissolved Oxygen Concentrates in Relation to Temperature

Temp in ºC	DO (mg/L)	Temperature (ºC)	DOmg/L
0	14.60	23	8.56
1	14.19	24	8.40
2	13.81	25	8.24
3	13.44	26	8.09
4	13.09	27	7.95
5	12.75	28	7.81
6	12.43	29	7.67
7	12.12	30	7.54
8	11.83	31	7.41
9	11.55	32	7.28
10	11.27	33	7.16
11	11.01	34	7.16
12	10.76	35	6.93
13	10.52	36	6.82
14	10.29	37	6.71
15	10.07	38	6.61
16	9.85	39	6.51
17	9.65	40	6.41
18	9.45	41	6.41
19	9.26	42	6.22
20	9.07	43	6.13
21	8.90	44	6.04
22	8.72	45	5.95

Reference: APHA. 1992. Standard methods for the examination of water and wastewater. 18th ed. American Public Health Association, Washington, DC.

Acceptable accuracy: ±10%

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APPENDIX C

BCDHE Laboratory Analytical SOPs

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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:	**Lacey Prior**	Lacey Adams Prior	5/11/18
	Diane Brown	Diane Brown	5/11/18
Laboratory Director:	**Gongmin Lei**	Gongmin Lei	5/11/2018

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 ± 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 ±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 ($C_{\overline{X}}$) and the relative standard deviation (%RSD).	The $C_{\overline{X}}$ 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

Figure 1. Inorganics Primary Standards Logbook

Barnstable County Laboratory

Inorganics Primary Standard Logbook

Date	Analyte	Vendor	Catalog No	Lot No	Concentration	Primary Standard ID	Exp Date	Initial

Figure 2. Inorganics Working Standards Logbook

Barnstable County Laboratory

Inorganics Working Standards Logbook

Date	Analyte	Primary Standard ID	Initial Conc	Amt taken	Final Vol	Final Conc	Solvent	Working Standard ID	Exp Date	Initial

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 004)

November 7, 2017

		Signature	Date
Analyst:	Kelby Karnes	Kelby Karnes	11-07-2017
Laboratory Director:	Gongmin Lei	Gongmin Lei	11/7/2017

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
Level4	1.0	20
Level 5	0.25	80
Level 6	0.1	200
Level 7	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 (80-120%) 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 20% 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 ≤20%. 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.

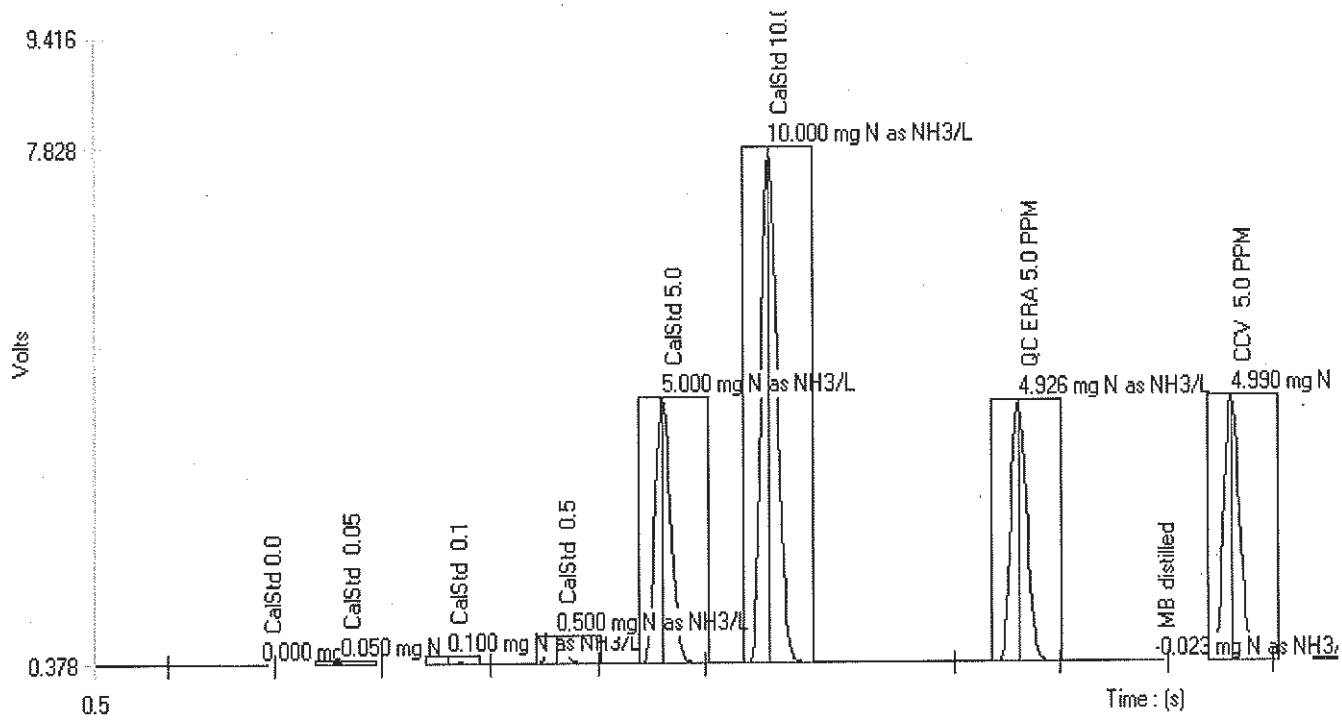

Figure : 1 (Ammonia)

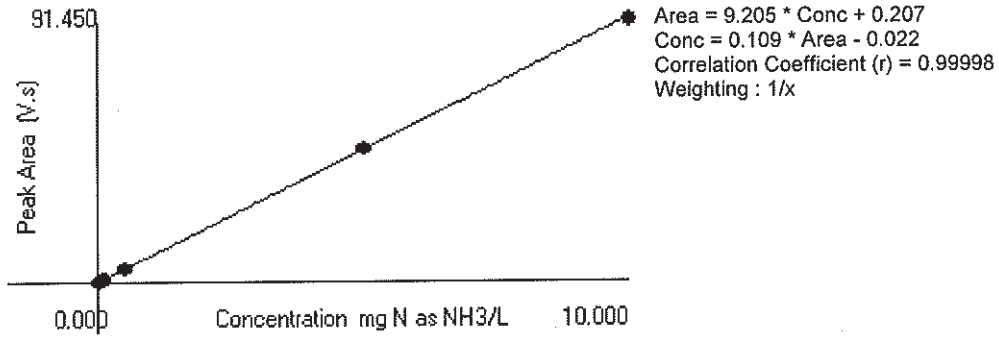

Figure 2.

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:	21.1
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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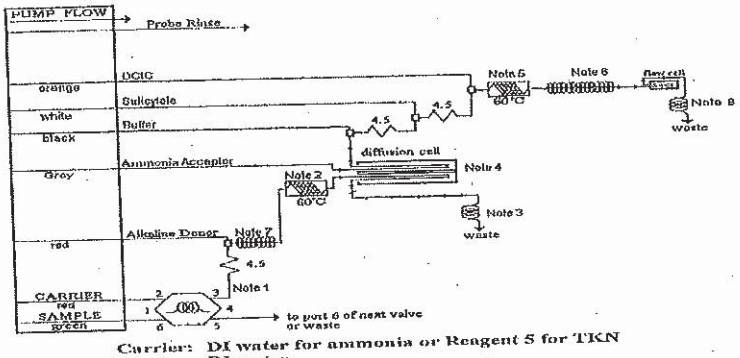
Figure 3.

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

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	$R \geq 0.995$
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 (0.10 mg/L) of Initial Calibration	
7	Level 7 (O mg/L) of Initial Calibration	
8	QCS at 5.0 mg/L	90-110%
9	Blank	
10	CCV at 5.0 mg/L	90-110%
11	MB	
12	LFB at 5.0 mg/L	90-110%
13	Sample 1	
14	Sample 1 – Laboratory Duplicate	$\leq 20\%$
15	Sample 1 - Matrix Spike	80% – 120%
16	Sample 2	
17	Sample 3	
18	Sample 4	
19	Sample 5	
20	Sample 6	
21	Sample 7	
22	Sample 8	
23	Sample 9	
24	Sample 10	
25	Blank	
26	CCV	90-110%
27	MB	
28-35	Sample 11 to Sample 19	
36	Sample 20	
37	Blank	
38	CCV at 5.0 mg/L	90-110%
39	MB	
40	LFB at 5.0 mg/L	90-110%

Barnstable County Department of Health and the
Environment Laboratory

EPA Method 351.2

STANDARD OPERATING PROCEDURE

For

Determination of Total Kjeldahl Nitrogen in Aqueous Samples by Semi-Automated Colorimetry

(Revision 004)

November 7, 2017

		Signature	Date
Analyst:	Kelby Karnes		11/7/17
Laboratory Director:	Gongmin Lei		11/7/2017

STANDARD OPERATING PROCEDURE (SOP)

For

Determination of Total Kjeldahl Nitrogen in Aqueous Samples
by Semi-Automated Colorimetry

1.0 SCOPE AND APPLICATION

1.1 This SOP provides procedure for determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

1.2 The applicable range is 0.25 – 20 mg/L TKN. The range may be extended with sample dilution.

2.0 SUMMARY OF METHOD

2.1 The sample is heated in the presence of sulfuric acid, H_2SO_4 for three hours. The residue is cooled, diluted to 25 mL and analyzed for ammonia. The digested sample may also be used for phosphorus determination.

2.2 Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described.

2.3 Organic Kjeldahl nitrogen is the difference obtained by subtracting the free-ammonia value from the total Kjeldahl nitrogen value.

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 High nitrate concentrations (10x or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

4.2 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

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 Dual Range.

6.2 Glassware – Class A volumetric flasks and pipets as required.

6.3 Block Digestor with Tubes – TKN 50 well AIM600 Block/Controller with rack and 100 mL glass digestion tubes (Environmental Express, Item#: SC900).

6.4 Automated Continuous Flow Analysis Equipment – QuickChem 8500 Series 2 Flow Injection Analysis System (LACHAT Instruments, A Hach Company Brand)

6.4.1 LACHAT XYZ Autosampler.

6.5 BD Kjeldahl Digestion Granules from Environmental Express, Item#. 8032178

6.6 Seal Analytical Teardrop Stoppers, Item No. SC9703

7.0 REAGENTS, CHEMICALS AND STANDARDS

7.1 Potassium Sulfate (K_2SO_4). Fisher, Cat No. P305-500

7.2 Copper (II) Sulfate ($CuSO_4$). Fisher, Cat No. AC422871000

7.3 Sulfuric Acid (H_2SO_4). Fisher, Cat No. A300-212

7.4 Sodium Hypochlorite (NaClO). Cat No. 19-546-929

7.5 Sodium Salicylate $C_6H_4(OH)(COO)Na$. Fisher, Cat No. 50-700-6201

7.6 Sodium Nitroprusside [sodium nitroferricyanide dehydrate, $Na_2Fe(CN)_5NO{\cdot}2H_2O$]. Fisher, Cat No. AC21164-1000

7.7 Sodium Phosphate dibasic heptahydrate ($Na_2HPO_4{\cdot}7H_2O$). Fisher, Cat No. AC20651-5000

7.8 disodium EDTA (ethylenediaminetetracetic acid salt). Fisher, Cat No. BP120500

7.9 Sodium Hydroxide (NaOH). Fisher, Cat No. S613-3

7.10 Ammonium Chloride (NH_4Cl). Fisher, Cat No.[s] AC199975000 and A661-3

7.11 **Reagent Water**: Ammonia free deionized water produced from Millipore Milli-Q Water Purification System.

7.12 Degassing with Helium:

7.2.1 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.

7.2.2 All reagents used in heated chemistry must be degassed.

7.13 Reagent 1: Digestion Solution

In a 1.0-liter volumetric flask, add 134 g potassium sulfate (K_2SO_4) and 7.3 g copper sulfate ($CuSO_4$) in 800 mL water. Then add 134 mL conc. Sulfuric acid (H_2SO_4) and dilute to the mark with reagent water. Stir to mix.

7.14 Reagent 2: Hypochlorite Solution

In a 250 mL volumetric flask, dilute 15 mL 5.25% sodium hypochlorite (NaOCl) to the mark with reagent water. Invert to mix.

7.15 Reagent 3: Salicylate Nitroprusside

In a 1.0-liter volumetric flask, dissolve 150 g sodium salicylate [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$] and 1.0 g sodium nitroprusside [sodium nitroferricyanide dehydrate, $Na_2Fe(CN)_5NO{\cdot}2H_2O$] in about 800 mL reagent water. Dilute to the mark with reagent water and invert to mix. Store in a dark bottle and prepare fresh monthly.

7.16 Reagent 4: Buffer

In a 1.0-liter volumetric flask containing 900 mL reagent water, completely dissolve 35 g sodium phosphate dibasic heptahydrate ($Na_2HPO_4{\cdot}7H_2O$). Next, add 20 g disodium EDTA (ethylenediaminetetracetic acid salt). The EDTA will not dissolve but will form a turbid solution. Finally, add 50 g sodium hydroxide (NaOH), dilute to the mark with reagent water and invert to mix. Degas weekly and prepare fresh monthly.

7.17 Reagent 5: Sodium Hydroxide (0.8M)

In a 1.0-liter volumetric flask, dissolve 32 g sodium hydroxide (NaOH) in about 800 mL reagent water. Dilute to the mark with reagent water and stir to mix.

7.18 Reagent 6: Digestion Diluent (for Carrier and Simulated Standards)

In a 1.0-liter volumetric flask, dissolve 400 mL digestion solution (**Reagent 1**) in about 600 mL reagent water. Dilute to the mark with reagent water and shake to mix.

7.19 Calibration Standards

7.19.1 Stock Standard: 1000 mg/L

In a 1.0 liter volumetric flask, dissolve 3.819 ammonium 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 Calibration Standards:

There are six levels calibration standards and their respective concentrations and preparation procedures are listed as follows:

Level	Volume (mL) Taken from Stock Standard (1000 mg/L, Section 7.19.1)	Final Volume (mL) Diluted with Reagent Water	Concentration (mg/L)
6	10	500	20
5	5	500	10
4	2.5	500	5
3	1.25	500	2.5
2	0.125	500	0.25
1	Reagent Water	Reagent Water	0.0

The calibration standards are digested using the same procedures as actual samples.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples are collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. 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 0.25 mg/L which is the same as L2 in Section 7.9.2. 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 =0.25 mg/L							Unit: mg/L			
	MDL01	MDL02	MDL03	MDL04	MDL05	MDL06	MDL07	MEAN	STDEV	MDL
DATE	10/12/2017	10/12/2017	10/12/2017	10/19/2017	10/19/2017	10/19/2017	10/25/2017			
Conc	0.313	0.291	0.281	0.302	0.246	0.315	0.352	0.30	0.033	0.103

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 10% 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 TKN 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 80-120%.

9.4.1.3 If the recovery falls outside the designated LFM recovery range (80-120%) 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 20% 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 TKN are ≤20%. 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 reagents and standards as described in Section 7.

10.2 Set up TKN manifold as is shown in Section 17.3 of the Lachat Instruments Methods Manual (reference 14.5.)

10.3 Input data system parameters as shown in Section 17 of the Lachat Instruments Methods Manual (reference 14.5.)

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 add reagent lines one by one, ending with the salicylate nitroprusside added last. For removal after analysis, reverse this order with the salicylate 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 10 mg/L. This standard (Ammonium chloride) is ordered from Fisher Scientific, Acros Organics, ACS reagent grade. The procedure for making the QCS is the same as the one for Level 5 of the calibration standards described in (Section 7.9.1, 7.9.2, and 7.9.3). 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 All samples, any quality control samples and the initial calibration standards are digested using the following procedures. At a minimum, two blanks and one standard (LFB) should be prepared in reagent water and carried through the digestion procedure.

11.2 DIGESTION PROCEDURE

11.2.1 To a **25.0 mL** sample add **10 mL digestion solution** (Reagent 1) and mix.

11.2.2 Add 2 to 4 BD Kjeldahl Digestion Granules to each tube.

11.2.3 Place tubes in the preheated block digester for one hour at 200°C. Water from the sample must be boiled off before increasing the temperature.

11.2.4 Place the cold finger, teardrop stopper on the top of the sample tube.

11.2.5 Continue to digest for 2 hours at 380°C. This includes the ramp time (approximately 50 minutes) for the block temperature to come up to 380°C.

11.2.6 Remove the sample tubes from the block and allow about 3 minutes to cool.

11.2.7 Dilute to **25.0 mL** with **reagent water (add 23.5 mL)** to each tube and vortex to mix.

11.2.8 If the samples are not run immediately they should be covered tightly and refrigerated at 4°C.

11.3 SYSTEM START-UP PROCEDURE

11.3.1 Prepare reagent and standards as described in section 7.

11.3.2 Set up manifold as shown in Section 17.3 of the Lachat Instruments Methods Manual.

11.3.3 Input peak timing and integration window parameters as specified in section 17.2 of the Lachat Instruments Methods Manual.

11.3.4 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.

11.3.5 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.

11.3.6 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.

11.3.7 After a stable baseline has been obtained, start the sampler and perform the analysis.

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 sample concentrations are greater than the high standard the digested sample should be diluted with **Reagent 6 (diluent.)** Do not dilute digested samples or standards with reagent water, as this will cause a problem with matix-matching, pH differences.

11.4.3 If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. If this occurs NaOH can be run through the system to attempt to clear clogs, flush system with NaOH for 20 minutes. If clogged tubing cannot be cleared, the tubing should be replaced. To prevent this, prime the system by first placing the buffer transmission line in the buffer solution.

11.4.4 In normal operation nitroprusside gives a yellow background color which combines with the blue indosalicylate to give an emerald green color. This is the normal color of the solution in the waste container.

11.4.5 If the block digestor tubes are not completely dry and have water droplets on them, there exists the possibility of ammonia contamination in the water droplets.

11.4.6 If phosphorus is also determined with the Lachat System, a second helium degassing tube should be used and segregated for the individual chemistries.

11.4.7 If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:

11.4.7.1 Place transmission lines in water and pump to clear reagents first.

11.4.7.2 Place reagent lines in 1M HCl and pump for several minutes

11.4.7.3 Place all lines back into water and pump out HCl.

11.4.8 If digested samples contain turbidity allow to settle prior to analysis, decant sample slowly into test tube.

11.4.9 Alternatively, if turbid conditions persist, filter the digested sample with 0.45uM filter.

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 351.2

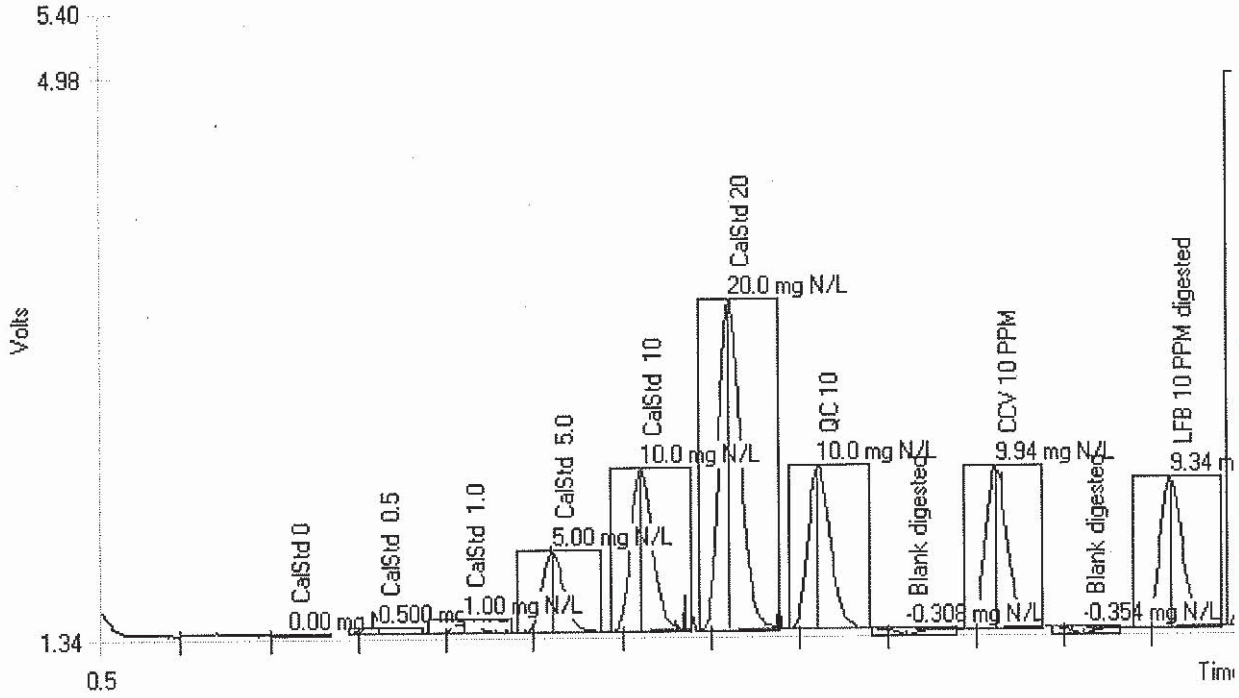
14.2 ASTM, Water(I), Volume 11.01, Method D3590-89, Test Methods for Kjeldahl Nitrogen in Water, p. 447

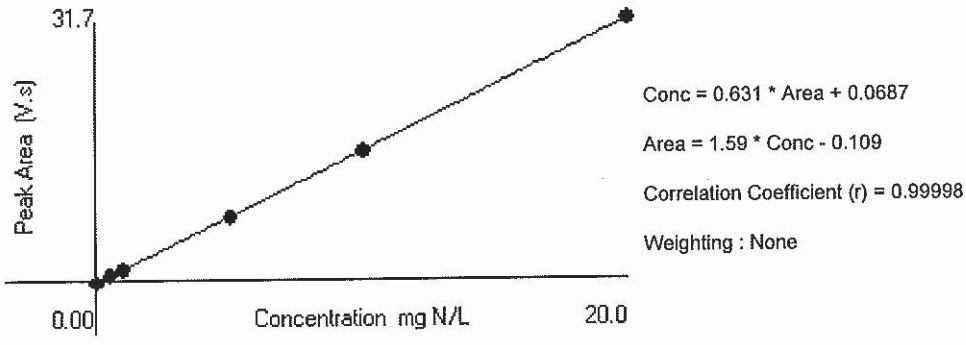
14.3 Code of Federal Regulation 40, Chapter 1, Part 136, Appendix B

14.4 Guidelines and Format for EMSL-Cincinnati Methods. EPA-600/8-83-020, August 1983.

14.5 Lachat Instruments Methods Manual, QuikChem Method 10-107-06-2-L, Revision Date, 14 May 2008

Figure 1.


5.40
4.98
Volts
1.34
0.5
Time
CalStd 0
0.00 mg N/L
CalStd 0.5
0.500 mg N/L
CalStd 1.0
1.00 mg N/L
CalStd 5.0
5.00 mg N/L
CalStd 10
10.0 mg N/L
CalStd 20
20.0 mg N/L
QC 10
10.0 mg N/L
Blank digested
-0.308 mg N/L
CCV 10 PPM
9.94 mg N/L
Blank digested
-0.354 mg N/L
LFB 10 PPM digested
9.34 mg N/L


31.7
Peak Area (V·s)
0.00
Concentration mg N/L
20.0
Conc = 0.631 * Area + 0.0687
Area = 1.59 * Conc - 0.109
Correlation Coefficient (r) = 0.99998
Weighting : None

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 007)

November 7, 2017

		Signature	Date
Analyst:	Ken Ni	Yuan Ni	11/07/2017
Laboratory Director:	Gongmin Lei	Gongmin Lei	11/7/2017

Barnstable County Laboratory

STANDARD OPERATING PROCEDURE (SOP)

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).

1.2

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.

2.2

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.

Level 6: 100 mg/L
Level 5: 50 mg/L
Level 4: 10 mg/L
Level 3: 5 mg/L
Level 2: 1 mg/L
Level 1: 0 mg/L

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.

6.3.5 Quality Control Sample Standard: 1000 mg/L (Ultra Sci; Cat# IQC-106)

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORGAE

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. To determine MDL values, take seven replicate aliquots of this standard and process through the entire analytical method. Perform the calculations as follows and report the concentration values in µg/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].

S = Standard deviation of the replicate analyses.

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 5.0 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 5.0 mg/L to a minimum of 5% of the collected field samples or at least one with every analysis batch, whichever is greater.

8.3.1.1 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 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): 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 particles, the sample will be homogenized using an IKA Ultra-Turrax Homogenizer.

11.4 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.

11.5 Injection volume is 50 µl. Each sample is injected three times, and final concentration is the mean value of three readings.

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_____

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APPENDIX D

Field, Sensor, and Laboratory Data Spreadsheet

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									Sensor Results		Laboratory Results	
Test Day	Test Fluid	Sample ID	Sampling Date	Sampling Time	pH	DO (mg/L)	Temperature ?C	Field Technician	Ammonia (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Nitrate (mg/L)

Instructions:
Battelle will complete the Test Day, Test Fluid, and Sample ID schemes for each test plan.
MASSTC will complete Sampling Date and Time, pH, DO, and Temperature readings and record field technician initials.
The developer will complete the sensor results and BCDHE will complete the laboratory results.
Each developer will have a separate results page.

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APPENDIX E

Sensor Hourly Data Spreadsheet

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Sensor ID	Date of reading	Time of reading	Analytical parameter	Result	Units	Notes and Observations

APPENDIX F

Nitrogen Sensor Challenge Performance Statistics

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Nitrogen Sensor Challenge Sampling Plan Performance, May 2018

Background

This document gauges the performance of the Nitrogen Sensor Challenge's sampling plan. Performance will be expressed as the false positive and false negative rates associated with testing the hypothesis that a sensor's performance is acceptable. In terms of precision and bias, "acceptable performance" means that:

- The true relative bias is at most 20% (mean recovery is between 80% and 120%), where bias is the error (sensor value minus laboratory value) divided by the true value (laboratory value).
- The true standard deviation of recovery (ratio of sensor value to laboratory-derived value) is at most 30%. NOTE: This is similar to, but not exactly the same as the relative standard deviation.

Sensor data (and laboratory data corresponding to samples tested by the sensor) will be used to test the hypothesis that the sensor's performance is acceptable (i.e., the null hypothesis, designated Ho, is true). The hypothesis testing errors - and tolerable error probabilities are as follows:

- False positive = rejecting Ho when it is true (in all respects, including normal error structure) should be limited to 5% for each test (test for bias and test for precision)
- False negatives (failing to reject Ho when it is false) are defined for two alternatives of interest (Ha and Hb):
    - False negative a = failing to reject Ho when Ha is true. Ha: Relative Bias = 1 - mean (Recovery) = +/- 30% (while the standard deviation of recovery is 20% (good) or 30% (tolerable))
    - False negative b = failing to reject Ho when Hb is true. Hb: StdDev(Recovery) = 45%

Our aim is to limit each false negative error rates (rejecting Ho when Ha is true or rejecting Ho when Hb is true) to 10%. In other words, when precision is poor (45% standard deviation of recovery) the probability of rejecting Ho (and rejecting the device) should be at least 0.9 and when recovery is poor (70% or 130%) the probability of rejecting Ho should also be at least 0.9.

Sampling plan

Normally, the sampling plan would be developed *after* specification of objectives. In this case, we have the sampling plan and need to assess the performance of this plan. In the end, if we like the performance, we accept the plan and we can defend it in light of expected performance.

Code

The plan (from Table B.2 of the TQAP) shows that each sensor will perform about 133 (before 5/20/2018, was 55) assays to estimate recovery for each target analyte (NH4, NO3, TOC, TN). Collectively, the 133 (was 55) estimated recoveries are used to derive (and test) mean recovery and the standard deviation of recovery. DI water tests are not used to estimate recovery or the standard deviation of recovery.

Desired probability of the false positive

For each test (of bias and precision, as expressed by mean recovery and standard deviation of recovery), we wish to avoid the false positive (rejecting Ho and declaring the sensor “unacceptable”, when, in fact, the sensor’s true performance is acceptable).

Our concern for this error is great, so we conduct statistical tests at the 5% level. This error rate is directly controlled by selecting the significance levels of the tests. Tests of bias and precision will each be made at the 5% significance level. The two tests are independent, so the overall probability rejecting Ho when a sensor has borderline bias (+/- 20%) and borderline precisions (30% standard deviation of recovery) will be about 10%.

Desired probability of the false negative (1 - power)

A negative is a failure to reject the hypothesis that sensor performance is satisfactory (Ho), when, in truth, sensor performance is poor. We wish to avoid the false negative. Under Ha and Hb, the sensor fails for only one of poor bias and poor precision and our tolerable error rates are:

- Ha: < 10% probability of negative outcome (failing to reject Ho)
- Hb: < 10% probability of negative outcome (failing to reject Ho)

Estimated false negative error rates

R functions *power.t.test()*, *pchisq()* and *qchisq()* are used to derive false negative error rates. Simulation is used as a check of the precision test’s false negative error rates.

Ha: Bias = +/- 30%

Here, bias is unacceptable and precision is borderline acceptable. The sensor fails if mean recovery is found to be significantly greater than 120% or less than 80%.

Student’s t-test is used to test mean recovery. The performance of this test depends on both the magnitude of the unacceptable bias and the standard deviation of recovery. Below, the probability of rejecting Ho (and declaring the sensor performance to be unacceptable) is derived over a range of biases and with two acceptable levels of standard deviation (20% and 30% standard deviation of recovery).

Hide

```
    N <- 145 - 12 # Number of recovery estimates (QC assays). Was 67-12 Alpha
    <- 0.05 # Selected probability of false positive for this test mu.0 <- 1.2
    # Maximum acceptable recovery
    mu.a <- seq(from = 1.2, to = 1.5, by = 0.01) # mean recovery
    delta.a <- mu.a - mu.0 # "delta" in  t-test
    sd.rec.20 <- 0.2 # Good standard deviation of recovery sd.rec.30 <-
    0.3 # Tolerable standard deviation of  recovery
    # For 20% std deviation of recovery
    power.a.20 <- numeric()
    for (i in 1:length(mu.a)) power.a.20[i] <-
        power.t.test(n = N,
                     delta = mu.a[i] - mu.0,
                     sd = sd.rec.20,
                     sig.level = Alpha / 2, # Alpha/2 applies to each side of the
test.


type = "one.sample",
alternative = "one.sided")$power # This is one side of the sy

    mmetric two-sided test.
    # For 30% std deviation of recovery
    power.a.30 <- numeric()
    for (i in 1:length(mu.a)) power.a.30[i] <-
        power.t.test(n = N,
                     delta = mu.a[i] - mu.0,
                     sd = sd.rec.30,
                     sig.level = Alpha / 2, # Alpha/2 applies to each side of the
test.

type = "one.sample",
alternative = "one.sided")$power # This is one side of the sy


    mmetric two-sided test.
    plot(mu.a, power.a.20, type = "l", lwd = 2, xlab = "True Mean Recovery",
         ylab = "Probability of Rejecting Ho", xlim = c(0.5,  1.5))
        points(mu.a, power.a.30, type = "l", lwd = 2, lty = 2, col = "blue")
```

Hide

```
    points(2 - mu.a, power.a.20, type = "l", lwd = 2) # Power curves are symme tric.
    points(2 - mu.a, power.a.30, type = "l", lwd = 2, lty = 2, col = "blue")
```

Hide

```
    points(c(0.7, 1.3), rep(0.9, 2), type = "p",
       col = "red", pch = 3, cex = 2)
    points(rep(0.8, 2), c(0, 1), type =  "l",
           col = "darkgreen", lty = 2)
```

Hide

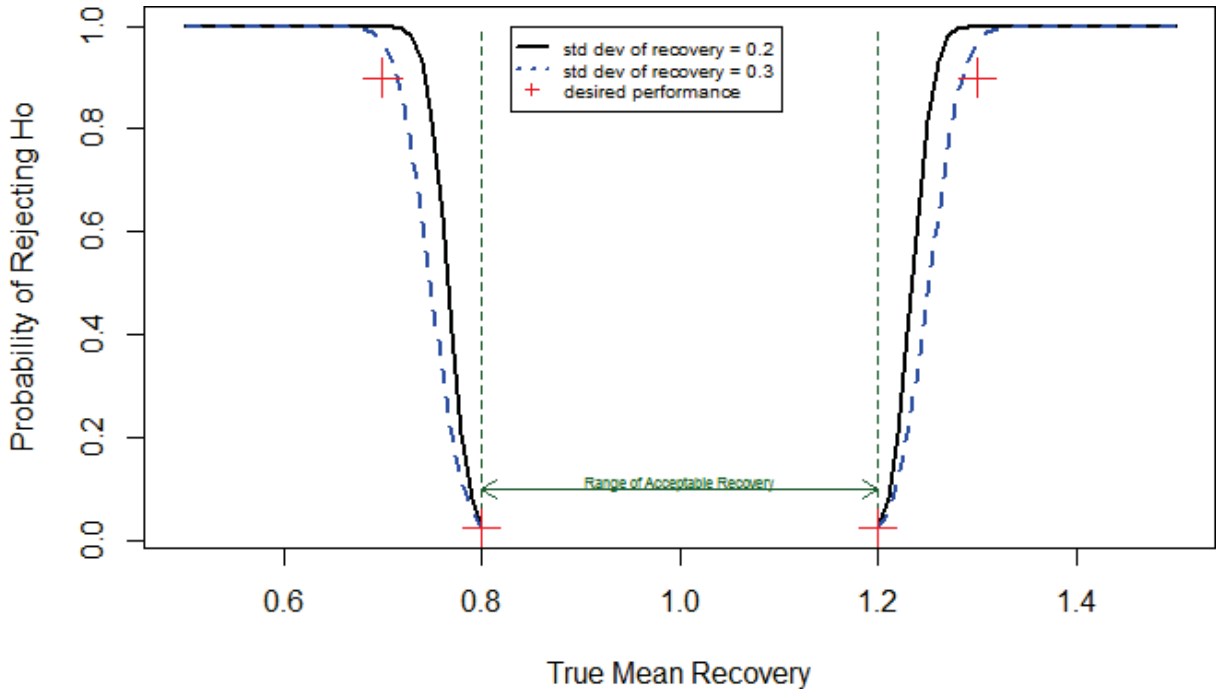
```
    points(rep(1.2, 2), c(0, 1), type = "l",
           col = "darkgreen", lty = 2)
    points(c(0.8, 1.2), rep(Alpha/2, 2), type = "p", pch = 3, cex = 2, col = "r
ed")
```

Hide

```
    # points(rep(1.3, 2), c(0, 1), type = "l", lty = 3, col = "red")
    arrows(x0 = 0.8, x1 = 1.2,
           y0 = 0.1, y1 = 0.1,
           col = "darkgreen", length = 0.1, code = 3)
    text(1, 0.07, pos = 3, col =  "darkgreen",
         labels = "Range of Acceptable Recovery", cex =  0.5)
```

Hide

```
legend(0.83, 1, c("std dev of recovery = 0.2",
                  "std dev of recovery = 0.3",
                  "desired performance"),
       lty = c(1, 3, NA), lwd = c(2, 2, NA),
       col = c("black", "blue", "red"), pch = c(NA, NA, 3), cex = 0.6)
```



Display the first 15 power estimates.

Hide

```
head(cbind(mu.a, power.a.20, power.a.30), 15)
```

```
      mu.a power.a.20 power.a.30
 [1,] 1.20 0.02500000 0.02500000
 [2,] 1.21 0.08264171 0.05724457
 [3,] 1.22 0.20751130 0.11571034
 [4,] 1.23 0.40413203 0.20751130
 [5,] 1.24 0.62920282 0.33233606
 [6,] 1.25 0.81650867 0.47931939
 [7,] 1.26 0.92982982 0.62920282
 [8,] 1.27 0.97966595 0.76156385
 [9,] 1.28 0.99559395 0.86278906
[10,] 1.29 0.99929259 0.92982982
[11,] 1.30 0.99991637 0.96828093
[12,] 1.31 0.99999275 0.98737924
[13,] 1.32 0.99999954 0.99559395
[14,] 1.33 0.99999998 0.99865376
[15,] 1.34 1.00000000 0.99964071
```

At 130% recovery, the probabilities of rejecting Ho are 0.9999 and 0.9683 when the standard deviation of recovery is 20% and 30%, respectively. The corresponding false negative error rates are 0.01% and 3.17.

Hb: StdDev(Recovery) = 45%

Here, we test the standard deviation of recovery using a chi-square test. Unlike the test above, this is a one-tailed test. The test's performance does not depend on mean recovery. A sensor fails only if it's standard deviation of recovery is significantly greater than desired. A sensor with significantly smaller standard deviation of recovery would be considered to have excellent performance and excellence performance would not be a reason for rejecting the sensor.

NOTE: When mean recovery is near 100%, StdDev(Recovery) ~ Relative Standard Deviation (RSD). When mean recovery is low, StdDev(Recovery) < RSD. When mean recovery is high, StdDev (Recovery) > RSD.

The sampling distribution of the variance is chi-squared, with N - 1 degrees of freedom. See NIST Engineering Statistics Handbook, Section 7.2.3 (http://www.itl.nist.gov/div898/handbook/prc/section2/prc23.htm).

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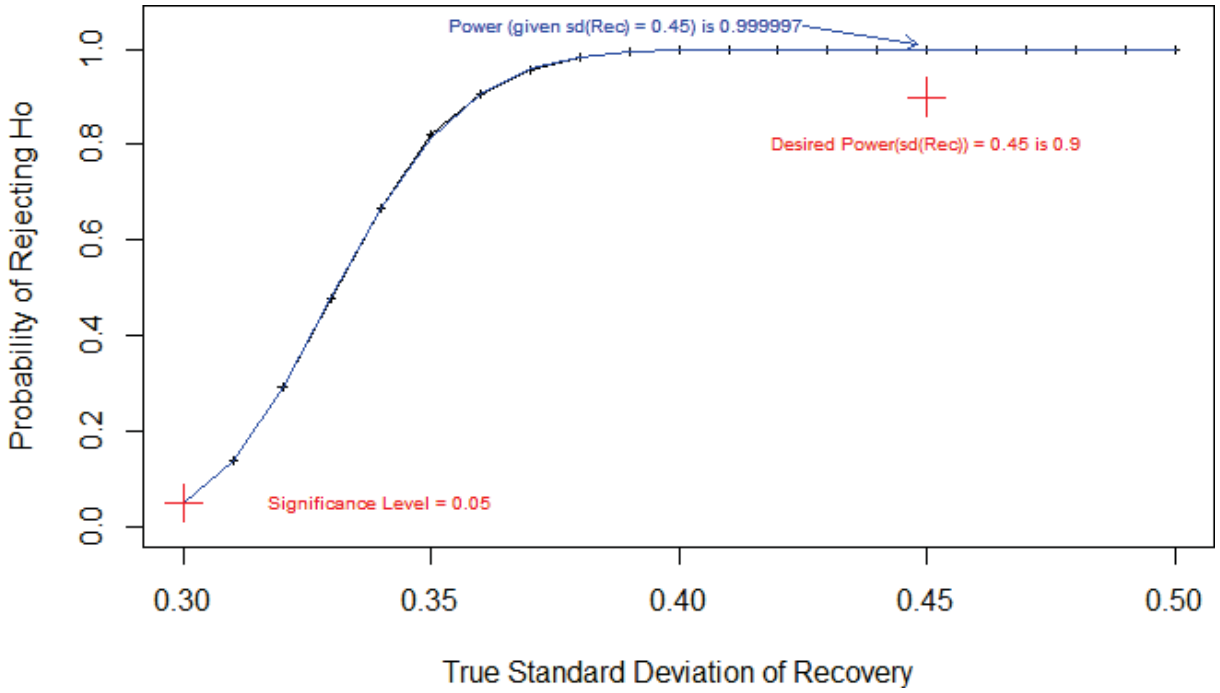

When the true standard deviation of recovery is 45%, the probability of rejecting Ho is 0.999997 and the false negative error rate is negligible.

BATTELLE

It can be done