New Jersey Department of Environmental Protection
Division of Water Monitoring & Standards
PO Box 420, Mail Code 401-04I
Trenton, NJ 08625

QUALITY ASSURANCE PROJECT PLAN
2021 NJDEP/IEC Harbor Monitoring Network

Prepared by: _____ Date: 4-5-21
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NJDEP Bureau of Marine Water Monitoring

Reviewed by: _____ for Kim Cenno Date: 04/07/2021
Kim Cenno, Manager 4 Environmental Protection,
NJDEP Bureau of Environmental Analysis, Restoration and Standards

Approved by: _____ Date: 4/08/21
Evelyn Powers, Executive Director
Interstate Environmental Commission

Approved by: Bruce Friedman Digitally signed by Bruce Friedman Date: 2021.04.27 14:16:42 -04'00' Date: 4/27/21
Bruce Friedman, Director
NJDEP Division of Water Monitoring and Standards

Approved by: _____ Digitally signed by Melissa D. M. Hornsby Date: 2021.04.27 15:31:02 -04'00' Date: 4/27/21
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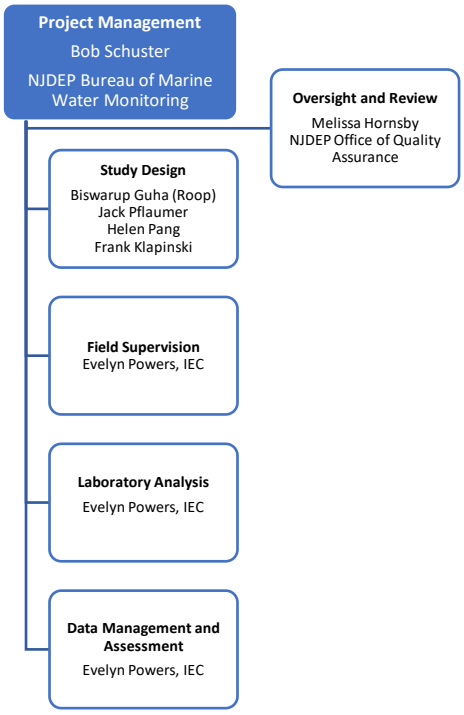
APPENDICIES

Project/Task Organization



Special Training Needs/Certification

The Project Manager will supervise all activities of this project. IEC will hold current certification from the NJDEP Office of Quality Assurance for the Analyze Immediately category during the time of sample collection. The IEC laboratory will hold current certification from the NJDEP Office of Quality Assurance for the parameters specified in this plan under the Water Pollution category during the time of sample analysis.

Principal Data Users

The principal users of this data would be NJDEP for conducting the biennial statewide integrated water quality assessment and develop the Integrated water quality assessment report and list of impaired waters (303(d) list).

Decision Makers

The NJDEP has decision-making authority. The Project Manager must approve any changes to the monitoring locations, analyte, analytical method, sampling and will list these changes as an addendum to this QAPP.

Major Changes

All signatories will review and be made aware of all major changes to this project

1. Project Name: 2021 NJDEP/IEC Harbor Estuary Monitoring Network
2. Project Requested by: Bruce Friedman, Director, NJDEP/DWM&S
3. Date of Request: October 1, 2019
4. Data of Project Initiation: May 1, 2021
5. Project Officer: Bob Schuster, NJDEP/Bureau of Marine Water Monitoring
6. Quality Assurance Officer: Melissa Hornsby, NJDEP/Office of Quality Assurance (OQA)
7. Project Description:

7.1. Problem Definition and Background

The NJDEP Division of Water Monitoring and Standards (DWM&S) is required to assess current water quality conditions in New Jersey's part of the NY/NJ Harbor for the NJDEP Integrated Report to USEPA which is part of NJDEP's Performance Partnership Agreement (PPA) with USEPA. This PPA requirement is mandated by the US Congress under Sections 305(b) and 303(d) of the Federal Clean Water Act (P.L. 92-500). Currently, DWM&S lacks data for parts of the Harbor and is unable to fully assess the waters as required in the PPA.

DWM&S wants to investigate current water quality conditions in waters which were downgraded by the NJDEP from the surface water classification SE1, to SE2 and SE3 in 1985 as part of a Use Attainability Analysis and determine if any classification upgrades are now warranted.

DWM&S wants to obtain preliminary water quality data that will inform the design of future water quality monitoring related to Harbor CSOs and NJDPES permitted discharges that NJDEP will use to:

- Revisit the calibration of the Harbor Eutrophication Model potentially using the Long Island Sound Model funded by USEPA and NY City DEP, and
- Validate the Pathogen Model developed by the Passaic Valley Sewerage Commission

7.2. Objective and Scope Statement

7.2.1. Objective

NJDEP and the Interstate Environmental Commission (IEC) are partnering to set up the Harbor Monitoring Network and implement that network annually going forward. In the first year, IEC will monitor the concentration of bacteria and nutrients in the NJ part of the NY/NJ Harbor Estuary

7.2.2. Scope

The Interstate Environmental Commission (IEC) will:

- Collect and analyze surface water samples for bacteria at a frequency of 5 samples within 30 days during the recreation season months (May– September), and a single, monthly sample during the non-recreation season months (October – April)

- Collect and analyze surface water samples from a transect of the Elizabeth Channel at a frequency of 5 samples within 30 days during the recreation season months (May–September)
- Collect and analyze monthly samples for total (unfiltered) nutrients (year-round).
- Measure field parameters concurrently with the above described samples
- Sampling is not dependent on rainfall or tide stage. However, IEC staff will record the previous 24-hour and 48-hour precipitation totals, as reported at Newark Airport, and will record the tide stage on the field measurement data sheets.

7.3. Data Usage

- NJDEP will use data from this project to assess current water quality conditions in New Jersey's part of the NY/NJ Harbor for the State's Integrated Report to USEPA,
- NJDEP will also use data from this project to investigate current water quality conditions in waters which were downgraded by NJDEP from SE1 to SE2 and SE3 in 1985 as part of a Use Attainability Analysis,
- NJDEP will use data from this project to inform the design of future water quality monitoring that the NJDEP will use to:
  - Revisit the calibration of the Harbor eutrophication model (potentially using the Long Island Sound Model funded by USEPA and NY City, DEP), and,
  - Validate the Pathogen Model developed by the Passaic Valley Sewerage Commission.

7.4. Monitoring Network Design and Rationale

NJDEP staff identified discrete monitoring locations at access points (bridges, piers, etc.) in Integrated Report Assessment Units (AUs, aka 14-digit Hydrologic Unit Codes or HUC14s) lacking monitoring results, at locations previously monitored by NJDEP, the US Geological Survey (USGS), the NJ Harbor Dischargers Group, locations used for modeling studies, and in areas with SE2 and SE3 waters.

IEC staff will make field measurements and collect samples as close as possible to the specific monitoring location coordinates listed in Table 4 and Table 5. IEC staff may have to deviate from monitoring locations due to accessibility issues, safety issues or tide stage. IEC staff will obtain GPS coordinates for each monitoring location and record them on field sheets during each monitoring event, and try to maintain consistency in the monitoring locations throughout this monitoring project.

Specific monitoring locations are listed in Table 4 - Proposed Discrete Monitoring Locations and Table 5 - Proposed Pathogen Transect Monitoring Locations

7.5. Monitoring Parameters and their Frequency of Collection

NJDEP staff selected the parameters (aka, analytes or characteristics) to be monitored, many of which have criterion in the New Jersey Surface Water Quality Standards. NJDEP staff also selected parameters used as inputs to water quality models that NJDEP staff will use to evaluate conditions in the Harbor.

7.5.1. Field Measurements

IEC staff will make field measurements one meter below the surface, and (wherever possible, depending on depth and current), one meter above the bottom (NOTE the depth and current at some monitoring locations may limit the ability of IEC staff to obtain bottom measurements. IEC staff will measure Secchi disk depth (at boat stations only) by lowering the disk all the way to the bottom (if needed).

IEC staff will make field measurements concurrent with the collection of bacteriology and nutrient samples described below. IEC staff will make field measurements at the monitoring locations shown in Table 4 - Proposed Discrete Monitoring Locations and Table 5 - Proposed Pathogen Transect Monitoring Locations

7.5.2. Laboratory Testing

7.5.2.1. Bacteriology

IEC staff will collect grab samples for fecal coliform and enterococcus at sites classified SE, and for E. coli at sites classified FW. IEC staff will collect samples:

- Monthly during the non-recreation season (October - April) at the monitoring locations listed in Table 4 - Proposed Discrete Monitoring Locations,
- at a frequency of 5 times within 30 days during the recreational season months (May – September) at the monitoring locations listed in Table 4 - Proposed Discrete Monitoring Locations,
- From a bacteriology transect in Newark Bay (Elizabeth Channel) at sites NB039, NB102, and NB406 at the frequency of 5 times within 30 days during the recreation season months (May - September). See Table 5 - Proposed Pathogen Transect Monitoring Locations

7.5.3. Nutrients

IEC staff will collect grab samples for nutrients on a monthly basis, year-round at all monitoring locations shown in Table 4 - Proposed Discrete Monitoring Locations. IEC staff will collect sample for total phosphorus and orthophosphate from the Hackensack River monitoring locations.

7.5.4. Chlorophyll a

IEC staff may collect 1 or 2 chlorophyll grab samples each sampling day for a comparison of the laboratory method that involves extracting the chlorophyll to the field measurements of chlorophyll a via a probe attached to a meter.

NOTE: IEC staff will not make field measurements or collect samples based on rainfall or tide stage criteria. Trying to meet a dry weather condition or wet weather requirement for an amount of rain, or trying to make field measurements and collect environmental samples during a particular tide stage would prevent IEC staff from getting the necessary number of field measurements and samples needed for NJDEP staff to assess data for the Integrated Report. IEC staff will record both weather conditions and tide stage on the field notes for each sampling event and monitoring location.

8. Project Fiscal Information

Table 1 - Summary of Project Costs

Item	Cost
Personnel	$75,185
Fringe	$15,396
Travel	$2,100
Equipment	$70,000

Item	Cost
Supplies	$10,720
Professional Services	$21,450
Total Direct	$194,851
Total Indirect	$33,984
TOTAL COSTS	**$228,835**

9. Schedule of Tasks and Products

Table 2 - Schedule of Tasks and Products

Task	Due Date
QA Plan approved	May 1, 2021
MOU between NJDEP and IEC executed	May 1, 2021
Sampling Begins	May 1, 2021
First Quarter Report (Reporting period ending May 31, 2021) & invoice	July 1, 2021
Data entry begins	July 15, 2021
Second Quarter Report (Reporting period ending August 31, 2021) & invoice	October 1, 2021
Sampling Ends	November 30, 2021
Third Quarter Report (reporting period ending November 30, 2021) and invoice:	January 1, 2022
Being Work on Year 2 QAPP and MOU	January 1, 2022
Data Entry Completed	January 31, 2022
Year 2 QAPP and MOU completed	March 31, 2022
Fourth Quarter Report (reporting period ending February 28, 2022) and invoice	April 1, 2022
Implement Year 2 MOU and QAPP	May 1, 2022

10. Sampling Organization and Responsibility

Table 3 - Sampling Organization and Responsibility

Responsibility	Person
Sampling Operations	Evelyn Powers
Sampling QC	Evelyn Powers
Laboratory Analysis	IEC Staff
Laboratory QC	Evelyn Powers
Data Processing Activities	IEC Staff
Data Processing QC	Evelyn Powers
Performance Auditing	NJDEP/OQA
Systems Auditing	NJDEP/OQA

Responsibility	Person
Overall QA	Melissa Hornsby, NJDEP/OQA
Overall Project Coordination	Bob Schuster, NJDEP/BMWMS

11. Data Quality Requirements and Assessments

11.1. Detection and Quantitation Limits

Overall, the goal is to avoid "less than" values (except in the case of a non-detect) and "greater than" value (except in the cases of confluent growth or too numerous to count, aka TNTC) by having IEC staff run the appropriate calibration standards and dilutions. NJDEP recognizes and accepts that Detection and Lower Quantitation Limits will increase should IEC staff need to dilute the samples due to matrix interferences or to achieve higher Upper Quantitation Limits.

11.1.1. Detection Limits

Detection Limits are given in Table 6 - Field Measurement QC Requirements, Table 7 - Bacteria QC Requirements and Methods, and Table 8 - Nutrients and Inorganic QC Requirements and Methods.

11.1.2. Lower Quantitation Limits

Parameters with a criterion that specifies a maximum value in the New Jersey Surface Water Quality Standards must be quantifiable (have a Lower Reporting Limit) less than or equal to the criterion. These parameters include: Ammonia, Enterococcus, E. coli, Fecal coliform, Nitrate, Temperature, pH, Total Phosphorus and Turbidity

11.1.3. Upper Quantitation Limits

Parameters with a criterion that specifies a minimum value in the New Jersey Surface Water Quality Standards must be quantifiable (have an Upper Quantitation Limit) greater than the criterion). These parameters include Dissolved Oxygen and pH.

11.2. Precision and Accuracy

NJDEP staff require the field measurement and laboratory analyses to achieve the precision and accuracy limits in Table 6, Table 7, and Table 8.

11.3. Bias

In order to eliminate bias, IEC staff will clean, inspect and properly calibrate (per the manufacturer's instructions and the NJDEP Laboratory Certification Regulations) all field and laboratory equipment before they are deployed or used by IEC staff. The same properly trained IEC staff will perform the calibration following detailed Standard Operating Procedures (SOPs).

11.4. Representativeness

NJDEP staff selected the sampling sites for this project with the goal of representing the estuary and its tributaries, mostly at current and historic discrete water quality sampling locations from other projects.

11.5. Comparability

IEC will make field measurements and analyzing samples only while holding current certification from the NJDEP Office of Quality Assurances Laboratory Certification Program for the Analyze Immediately and the parameters listed in this QAPP. IEC will be sampling many of the same monitoring locations previously used by the NJ Harbor Dischargers Group, NJDEP and the US Geological Survey.

11.6. Completeness

NJDEP and IEC staff have set up a completeness goal of 100% for this project. NJDEP staff set up the 100% completeness goal to attempt to achieve the maximum from the sampling design.

12. Sampling Procedures

12.1. Field Measurements

IEC staff will make field measurements 1 meter below the surface following the requirements in the manufacturer's operating instructions, the NJDEP Fields Sampling Procedures Manual and the NJDEP Laboratory Certification Regulations. IEC staff will calibrate the meters and they will record all calibration results per the "Regulations Governing the Certification of Laboratories and Environmental Measurements", N.J.A.C. 7:18.

12.2. Lab Measurements

12.2.1. Bacteriology

IEC staff will collect samples 6-12" below the surface into new, sterilized containers following Sections 6.8.2.2.6 (Estuarine Sampling) and 6.8.2.2 7 (Bacteriology) of the NJDEP Field Sampling Procedures Manual, 2005 edition

12.2.2. Nutrients

IEC staff will collect samples 6-12" below the surface in new, single use containers following Sections 6.8.2.2.4 (Point Sampling) and 6.8.2.2.6 (Estuarine Sampling) of the NJDEP Field Sampling Procedures Manual, 2005 edition

12.3. Sample Containers, Preservation and Holding Time

See Table 9 - Sample Collection, Containers, Preservation and Holding Time.

12.4. Sample Custody Procedures

NJDEP staff may use the results from this project for regulatory decisions. Therefore, IEC staff will follow the Chain of Custody procedures described in Section 6.11.3 of the NJDEP Field Sampling Procedures Manual, 2005 edition and the NJDEP Laboratory Certification regulations will be followed for all samples collected and delivered to the laboratory. A sample Chain of Custody Form is shown in Appendix 1

13. Calibration Procedures and Preventive Maintenance

13.1. Field Measurements

There are no quality control standards for Secchi depth. IEC staff will calibrate and check the handheld field meters for dissolved oxygen, pH, chlorophyll a and salinity immediately prior to, and after each weekly use as described above. IEC staff will will make quarterly checks of the temperature measurements of the meter against a NIST certified thermometer. IEC staff will record the results of the calibration checks in the bound logbook for the instrument, initial and date. Similarly, IEC staff will check the calibration of the chlorophyll a probe.

13.2. Laboratory Measurements

13.2.1. Calibration

13.2.1.1. Calibration standards

IEC staff will use one blank and five standards over the expected range of sample target analyte concentrations when the samples are analyzed. Acceptable correlation coefficients are 0.99500 and above.

13.2.1.2. Calibration check standard

IEC staff will use one mid-range standard prepared independently from the initial calibration standards and will analyze the mid-range standard at the beginning of the run, every ten samples, and at the end of every run. IEC staff will reanalyze the samples or flag them as estimated values if the percent recovery is below 90% or above 110%.

13.2.2. Detection and Reporting Limits

13.2.2.1. Method Detection Limit - MDL

IEC staff determine MDLs annually following the method in 40 CFR 136 Part A.

13.2.2.2. Lower Reporting Limit - LRL

IEC staff calculate the laboratory reporting limit from the MDL

13.2.3. Accuracy

13.2.3.1. Matrix spikes

IEC staff adds known amounts of target analytes to random samples, where appropriate for the method. Acceptable percent recovery is 80 – 120%. If acceptable limits are exceeded, IEC staff determine the cause of the exceedance by making a new spike and/or standard curve. IEC staff will then recalibrate the system and reanalyze all samples or flag all samples as Estimated values.

13.2.3.2. Calculation

IEC staff calculate accuracy as percent recovery from the analysis of matrix spike samples as follows:

% Recovery = {[Ms – (Mu/2)]/Ts}*100

Where

Ms = Measured concentration of target analyte in the spiked sample

Mu = Measured concentration of target analyte in the unspiked sample

Ts = "True" concentration of target analyte added to the spiked sample

13.2.4. Precision

13.2.4.1. Laboratory replicates

IEC staff will prepare replicate aliquots of samples for every 20 samples. Acceptable limit for range percent difference are 20%, unless otherwise specified by the method. If acceptable limits are exceeded, IEC staff will determine the cause of the problem. IEC staff will then recalibrate the system and all suspect samples will be reanalyzed or IEC staff will flag the results as estimated values.

13.2.4.2. Calculation

IEC staff estimate precision using the Range Percent Difference (RPD).

RPD = [Absolute Value (R1 – R2)/R3]*100

Where R1 and R2 are replicate values and R3 is the average of the replicates

13.2.5. Contamination Assessment

13.2.5.1. Blanks

IEC staff will analyze one method blank at the beginning of the sample analysis run, after ten samples are analyzed as part of the sample analysis run, and at the end of every run.

13.2.5.2. Instrument/Equipment Calibration and Frequency

As an NJDEP certified laboratory (#NY240), the IEC takes part in independent laboratory proficiency testing annually. If the results of a parameter do not meet the acceptable limits, a new proficiency sample is sent from the company and the parameter is reanalyzed. Certificates are posted in the laboratory. IEC staff record all maintenance in the laboratory's Equipment Maintenance Log. IEC staff will properly discard all expired standards and reagents. IEC staff will calibrate yearly and check quarterly all analytical balances. IEC staff will calibrate and monitor on a quarterly basis all pipettes and replace them when needed. IEC staff will regularly replace the filters for the deionized water set-up. IEC staff will monitor and record daily the temperature of ovens and refrigerators.

13.2.5.3. Inspection/Acceptance of Supplies and Consumables

IEC staff will inventory the lab supplies and consumables monthly. IEC staff will forward the inventory to the Quality Assurance Manager for the laboratory.

IEC staff will inspect the supplies for defects upon receipt and either accept the supplies or return defective supplies. IEC staff will date and properly store accepted chemicals.

14. Documentation, Data Reduction and Reporting

14.1. Documentation

14.1.1. Field Measurements

IEC staff will record all meter calibrations and measurements in a bound field logbook (and initial and date the entry) and on the sample analysis request form.

14.1.2. Laboratory Measurements

IEC staff will record all lab instrument calibrations in a bound laboratory logbook (and initial and date the entry) per the NJDEP Laboratory Certification Regulations.

14.2. Data Reduction and Reporting

14.2.1. Data Reductions

IEC staff will download measurement from the field meters onto a laptop in the field or office. In the absence of a meter with download capabilities, IEC staff will enter the information from a bound field logbook into a spreadsheet and a separate IEC staff person will proofread the data.

IEC staff will review the data monthly to look for any anomalies or areas to target for sampling. If anomalies are present, IEC staff will take a newly calibrated data sonde and/or a calibrated handheld meter to the field to verify the sensors performance. If IEC staff find that the meter is not be functioning properly, they will replace the meter, and IEC will add a qualifier code to the result or remove the data from the archived dataset.

The NJDEP Office of Quality Assurance can coordinate the observation of the process with the Project Officer and the Field Supervisor for auditing purposes.

14.2.2. Reporting

IEC staff will upload the raw data to the USEPA's WQX data system after the data validation is completed (NOTE: data validation is to be completed no later than 60 days after the samples are analyzed). IEC staff will prepare a quarterly report detailing the dates and number of monitoring locations sampled and samples analyzed in the previous quarter and a spreadsheet of the completed results will be sent by IEC staff to the Project Officer by the 15th of the current quarter. The report will include a listing of any issues found and the solution implemented by IEC staff.

14.2.2.1. Field Measurements

IEC staff will compare the field measurements to the pre and post measurement calibrations. If the post calibration values do not meet the acceptance criteria, IEC staff will discard the data and it will not be reported. IEC staff will compare the results from individual monitoring locations to historical values and any anomalies will be

investigated by IEC staff. IEC staff will note anomalous data with a result comment when the data is reported.

14.2.2.2. Laboratory Results

IEC staff will verify the data using the replicate data percent difference. IEC staff will validate the data using the QC data. The QC sample should fall between two standard deviations at the 95th percentile confidence level to be valid.

14.2.2.3. Data Entry

IEC staff will enter the result from this project into EPA's WQX data system following:

USEPA's WQX Best Practices for Sharing Nutrient Data
https://www.epa.gov/waterdata/wqx-nutrients-best-practices-guide

EPA's Full WQX-Web 3.0 Users Guide https://www.epa.gov/waterdata/user-guide-version-30-water-quality-exchange-web

15. Performance and Systems Audits

IEC laboratory and field staff will annually take part in an independent proficiency testing. In addition, IEC staff use quality control samples (with known concentrations), replicate data, and percent recovery to assess the quality of the data. If any of the quality assurance fails, IEC staff take steps to troubleshoot and correct the situation to ensure that the data produced is accurate. IEC staff will replace standards and reagents, will check equipment, and will take other action will be taken to remedy the situation.

The NJDEP Office of Quality Assurance may perform both performance and systems audits of the field and laboratory staff at their discretion during this project.

16. Corrective Action

16.1. Field Measurements

IEC staff will replace field instruments that fail the calibration check with units that do perform within the acceptance limits until IEC staff identifies and corrects the cause of the failure.

16.2. Laboratory Analysis

IEC staff will stop the analysis when an instrument fails its QC criteria. IEC laboratory staff will investigate and correct the source of error. If the error cannot be corrected, IEC staff will notify the Project Manager and the latter will make the decision to either analyze the samples or make arrangements for IEC staff to transport the samples to a different NJDEP Certified Laboratory

17. Reports

IEC staff will report performance evaluations and significant quality assurance problems and solutions to the Project Manager and the Field Supervisor. IEC staff will contact the QA Manager and the Project Manager via email of any problems in the laboratory and the timeline for remediation.

NJDEP and IEC staff will review this QAPP will every year for updates or changes. If updates or changes are necessary, all signatories will be asked to review and approve

Table 4 - Proposed Discrete Monitoring Locations

PRIORITY	SITE	NAME	REASON	LONGITUDE	LATITUDE	STATE	CLASS	TIDAL	LAND_BOAT*	COMMENT
1	BCUA_W4	Hackensack River at NJ Turnpike bridge	Mid Tidal Hack Full Param Site	-74.034277	40.822600	NJ	SE2	YES	BOAT	I-95/NJTP bridge?
2	NJHDG_28	Raritan Bay - confluence with Raritan River and Arthur Kill	Raritan R Mouth Full Param Site	-74.268560	40.490970	NJ	SE1	YES	BOAT	bulkhead 0.25 mi from NJHDG_28?
3	NJHDG_18	Newark Bay	Newark Harb Full Param Site	-74.146830	40.656660	NJ	SE3	YES	BOAT	bulkhead 0.22 mi from NJHDG_18
4	NJHDG_11	Passaic River at Jackson Ave	Lower Tidal Passaic Full Param Site	-74.155660	40.733660	NJ	SE3	YES	LAND	Jackson St. bridge
5	K4	Arthur Kill	Arthur Kill Full Param Site	-74.210373	40.571812	NJ/NY	SE3	YES	BOAT	
6	NJHDG_33	Hudson River	Mid Hudson Full Param Site	-74.025530	40.723510	NJ/NY	SE2	YES	BOAT	
7	21NJDEP1_01378500	Hackensack River at New Milford NJ	Upper Tidal Hack Full Param Site	-74.028946	40.953818	NJ	FW2-NT(C1)	NO	LAND	Oradell Ave. bridge
8	NJHDG_15	Hackensack River	Upper Tidal Hack Full Param Site	-74.084000	40.739500	NJ	SE2	YES	BOAT	Newark Ave/ Rt. 7 bridge?
9	USGS_01407081	Raritan Bay at Keansburg NJ	Raritan Bay S Coast Full Param Site	-74.148060	40.448900	NJ	SE1	YES	LAND	Natco Plant access road
10	NJHDG_30	Raritan Bay	Raritan Bay N Coast Full Param Site	-74.146000	40.520000	NY	SE1	YES	BOAT	
11	NOPH	Upper New York Harbor	Lower Hudson Full Param Site	-74.067134	40.661218	NJ	SE2	YES	BOAT	Port Terminal Blvd.?

PRIORITY	SITE	NAME	REASON	LONGITUDE	LATITUDE	STATE	CLASS	TIDAL	LAND_BOAT*	COMMENT
12	N3B	Upper Hudson River	Upper Hudson Full Param Site	-73.965178	40.821110	NJ/NY	SE2	YES	BOAT	
13	RB	Raritan Bay	Outer Harbor Full Param Site	-74.047400	40.550500	NY		YES	BOAT	
14	NJDEP_CCMPMC0011	Navesink River at Rt 36 near confluence with Sandy Hook Bay	Nav_Shrews Full Param Site	-73.979219	40.396510	NJ	SE1	YES	LAND	Rt. 36 bridge
15	NJHDG_7	Passaic River at Union Ave	Mid Tidal Passaic Full Param Site	-74.124330	40.839830	NJ	FW2-NT	NO	LAND	off River Dr.
16	USGS_01460595	Del & Rar Canal at Landing La at New Brunswick NJ	Upper Tidal Raritan Full Param Site	-74.464160	40.507770	NJ	FW2-NT	NO	LAND	Landing Ln. bridge
17	NJHDG_5	Passaic River at Dundee Dam	Upper Tidal Passaic Full Param Site	-74.120660	40.879500	NJ	FW2-NT	NO	LAND	Ackerman Ave. bridge
18	21NJDEP1_01406580	South River at Rt 535	South River Full Param Site	-74.371544	40.455310	NJ	SE1	YES	LAND	Veterans Memorial bridge
19	EMAP_CS_WQX-NJ05-0020-A	Raritan Bay	Atlantic_Harbor Full Param Site	-73.989900	40.500500	NJ	SE1	YES	BOAT	
20	W10	Sawmill Creek	Sawmill Creek	-74.096158	40.764258	NJ	SE2	YES	BOAT	
21	W5	Hackensack River at Rt 46	Lower Tidal Hackensack	-74.029153	40.850820	NJ	SE1	YES	LAND	Rt. 46 Bridge
22	W6	Hackensack River at University Plaza Drive	Lower Tidal Hackensack	-74.031738	40.898700	NJ	SE1	YES	LAND	University Plaza Drive
23	NJHDG_14	Hackensack River near Hudson Regional Hospital	Lower Tidal Hackensack	-74.078370	40.791900	NJ	SE2	YES	BOAT	

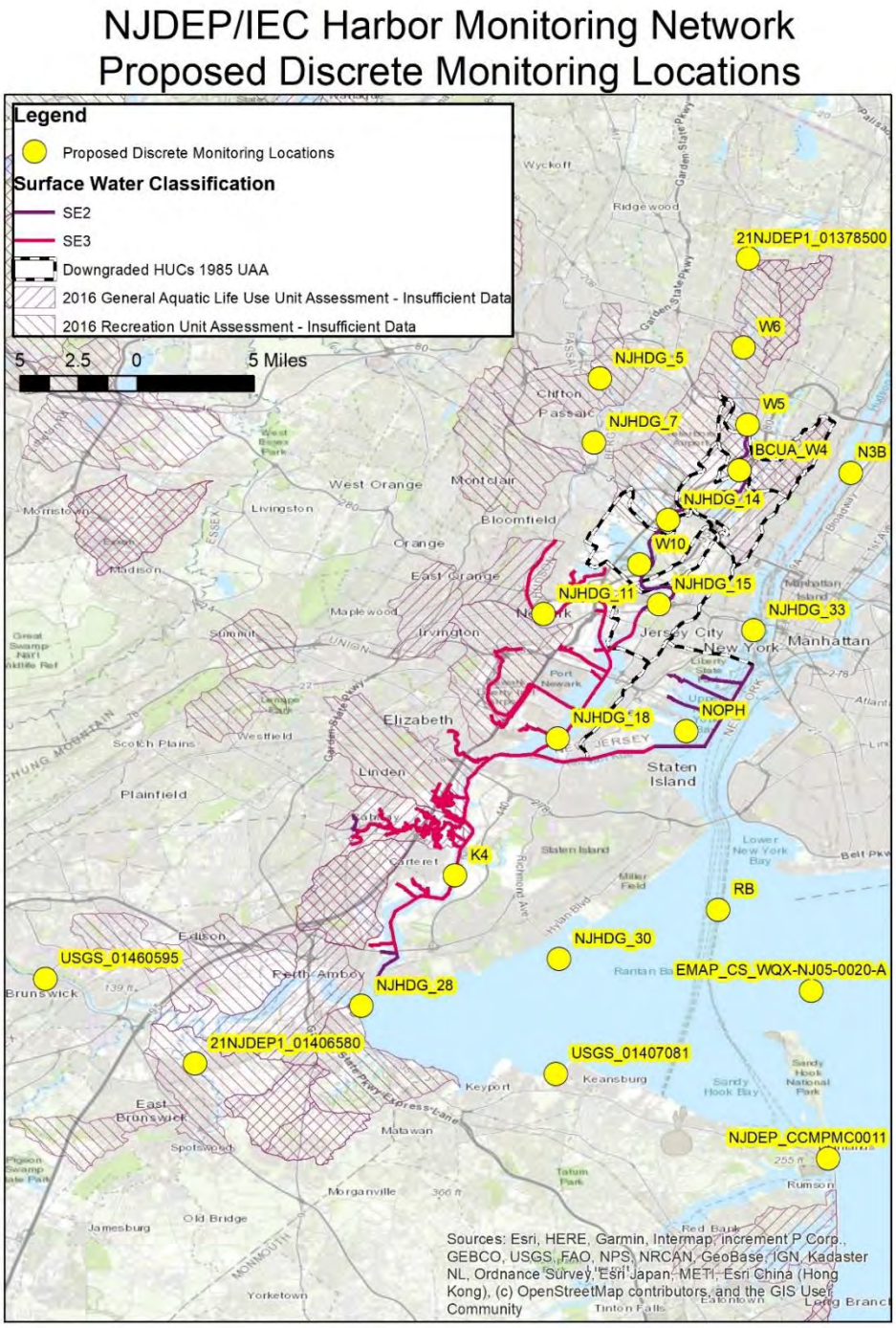


Figure 1 - Proposed Discrete Monitoring Locations

Table 5 - Proposed Pathogen Transect Monitoring Locations

Location	Name	Latitude	Longitude
EPA_REGION_2-NB039_1993_C38	REMAP Campaign: 1993 - AREA: Newark Bay - SITE ID: NB039 - SITE CLUSTER: NB_C38	40.673698	-74.127502
EPA_REGION_2-NB102_1993_C43	REMAP Campaign: 1993 - AREA: Newark Bay - SITE ID: NB102 - SITE CLUSTER: NB_C43	40.688202	-74.158096
EPA_REGION_2-NB406_2013_C39	REMAP Campaign: 2013 - AREA: Newark Bay - SITE ID: NB406 - SITE CLUSTER: NB_C39	40.677101	-74.137100

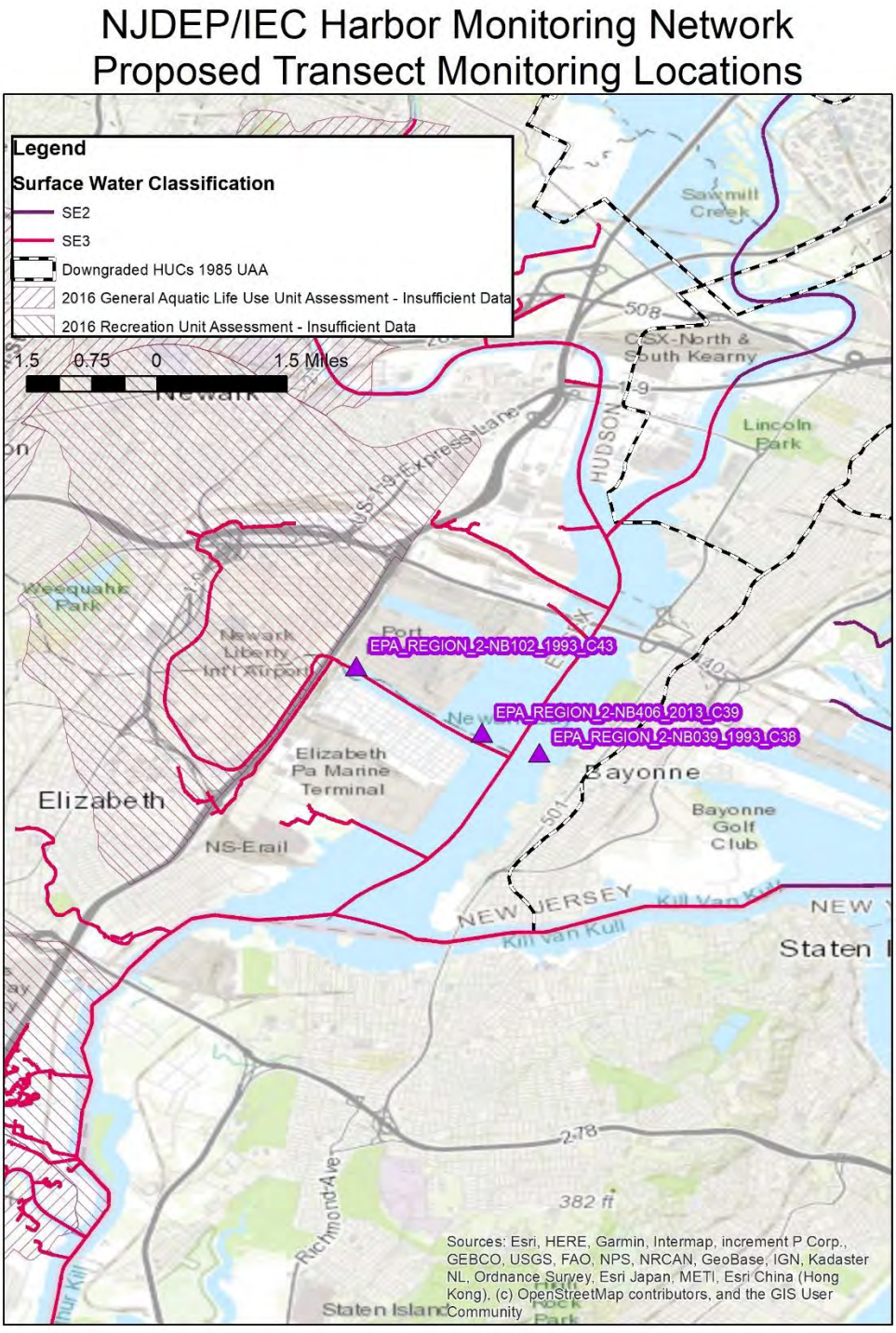


Figure 2 - Proposed Transect Monitoring Locations

Table 6 - Field Measurement QC Requirements

Parameter	Unit	SWQS Criteria	MDL	LRL	UQL	Accuracy	Precision
Chlorophyll a	ug/l	none	0.01	0.01	400	R2>0.999	20%
Dissolved oxygen (DO)	mg/l	> 3.0 mg/l (SE3) > 4.0 mg/l (SE2) > 5.0 mg/l 24-hour average, > 4.0 anytime (FW2-NT, SE1)	0.1	1.0	50	3.8%	10%
Dissolved oxygen saturation	%	none	1.0	10	500	3.8%	5%
pH	None	Between 6.5 – 8.5 SU (FW2-NT, SE)	0.1	4.0	10	1.4%	5%
Salinity	ppt	none	0.1	0.1	70	1.0%	10%
Secchi Disk Depth	m	none	0.1	0.1	5.0	10%	10%
Temperature, water	deg C	≤ 29.4 deg C summer seasonal average (SE) ≤ 31 deg C rolling 7-day average, < 28 daily max (FW2-NT)	-5.0	0.2	50	0.01°C	5%

Table 7 - Bacteria QC Requirements and Methods

Parameter	Unit	SWQS Criteria	MDL	LRL	UQL	Bias	Precision	Method
Enterococcus	CFU/ 100ml	geometric mean* of 35 counts/100 ml(SE1)	1 CFU/100 ml	1 CFU/100 ml	6000** CFU/100 ml	N/A	20%	EPA 1600
Escherichia coli	MPN/ 100ml or CFU/ 100ml	geometric mean* of 126 counts/100 ml (FW)	1.8 MPN/100 mL or 1 CFU/100 ml	1.8 MPN/100 ml or 1 CFU/100 ml	16,000** MPN/100 mL or 8000** CFU/100ml	N/A	50%	SM 9221B.2-06/9221 F-06 or EPA 1103.1
Fecal coliform	CFU/ 100ml	geometric mean* of 770/100 ml (SE2) geometric mean of 1500/100 ml (SE3)	1 CFU/100 ml	1 CFU/100 ml	8000 CFU**	N/A	20%	SM 9222 D-06

* Calculated from at least 5 samples collected from within 30 days

** Higher quantification limits can be achieved if necessary, through further dilution of samples.

Table 8 - Nutrients and Inorganic QC Requirements and Methods

Parameter	Fraction	Unit	SWQS Criteria	MDL	LRL	UQL	Bias	Precision	Method
Chlorophyll a	Filtered and extracted	ug/l	none	0.03	0.09	114	10%	20%	EPA 445.0
Ammonia as N	unfiltered	mg/l	Calculated value using equation – FW2-NT 0.115 ug/l(a); 0.030 ug/l(c) - SE [Unionized ammonia (NH3) as N]	0.008	0.02	4.0*	10%	10%	EPA method 350.1, Revision 2.0
Total Nitrogen as N	Unfiltered	mg/l	none	0.009	0.02	1.5*	10%	20%	Lachat method 31-107-04-4-A#
Nitrite + Nitrate (NO2+NO3 as N)	Unfiltered	mg/l	10 (h) – FW-2 NT	0.013	0.03	1.5*	10%	20%	EPA method 353.2, Revision 2.0
Orthophosphate as P	Unfiltered	mg/l	none	0.00083	0.002	5*	10%	20%	EPA Method 365.1, Revision 2.0
Total phosphorus, mixed forms as P	Unfiltered	mg/l	0.1 – FW2 Non-tidal streams	0.053	0.133	5*	10%	20%	EPA Method 365.1, Revision 2.0
Turbidity	Unfiltered	NTU	15 NTU @ 30-day max/50 NTU anytime max (FW, SE3) 10 NTU @ 30-day max/30 NTU anytime max (SE2, SE3)	0.01	0.62	40*	10%	10%	EPA 180.1, Revision 2.0

*Higher quantification values can be determined if necessary through sample dilution or additional calibration standards.

See Appendix 4, Page 16 Lab SOP for total Nitrogen

Table 9 - Sample Collection, Containers, Preservation and Holding Time

Type	Characteristic	Number of Samples	Sample Collection Procedure	Sample Collection Equipment	Sample Container	Sample Preservation	Holding Time
Field Measurement	Chlorophyll a	811	Mfg. instructions	Field Meter (YSI ProDSS or YSI EXO1)	None	None	None
Field Measurement	Dissolved oxygen (DO)	811	mfg. instruction	Field Meter (YSI ProDSS or YSI EXO1)	None	None	None
Field Measurement	Dissolved oxygen saturation	811	mfg. instructions	Field Meter (YSI ProDSS or YSI EXO1)	None	None	None
Field Measurement	pH	811	mfg. instructions	Field Meter (YSI ProDSS or YSI EXO1)	None	None	None
Field Measurement	Salinity	811	mfg. instructions	Field Meter (YSI ProDSS or YSI EXO1)	None	None	None
Field Measurement	Temperature, water	811	mfg. instructions	Field Meter (YSI ProDSS or YSI EXO1)	None	None	None
Field Measurement	Secchi disk depth	811	mfg. instructions	Secchi disk	None	None	None
Lab Analysis	Turbidity	811	FSPM 6.8.2.2.4	Water bottle	P,G	None	48 hours
Lab Analysis	Enterococcus	811	FSPM 6.8.2.2.7	Water bottle	sterilized	Ice, 4 deg C	8 hours
Lab Analysis	Escherichia coli	811	FSPM 6.8.2.2.7	Water bottle	sterilized	Ice, 4 deg C	8 hours
Lab Analysis	Fecal coliform	811	FSPM 6.8.2.2.7	Water bottle	sterilized	Ice, 4 deg C	8 hours
Lab Analysis	Nitrite + Nitrate as N	276	FSPM 6.8.2.2.4	Water bottle	P,G	Ice, 4 deg C	28 days
Lab Analysis	Total Nitrogen as N	276	FSPM 6.8.2.2.4	Water bottle	P,G	H2SO4 pH<2	28 days
Lab Analysis	Ammonia as N	276	FSPM 6.8.2.2.4	Water bottle	P,G	H2SO4 pH<2	28 days
Lab Analysis	Orthophosphate as P	72	FSPM 6.8.2.2.4	Water bottle	P,G	H2SO4 pH<2	28 days
Lab Analysis	Total phosphorus, mixed forms as P	72	FSPM 6.8.2.2.4	Water bottle	P,G	H2SO4 pH<2	28 days
Lab Analysis	Chlorophyll a	78	FSPM 6.8.2.2.4	FSPM 6.8.2.2.4	Amber or foil wrapped G	Ice, 4 deg C	3 ½ weeks (24 days)

See Table 10 - Number of Measurements and Samples per Month for a breakdown of the total number of samples

Table 10 - Number of Measurements and Samples per Month

Parameter	May	Jun	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	TOTAL
Temperature	130	130	130	130	130	23	23	23	23	23	23	23	811
DO	130	130	130	130	130	23	23	23	23	23	23	23	811
pH	130	130	130	130	130	23	23	23	23	23	23	23	811
Salinity	130	130	130	130	130	23	23	23	23	23	23	23	811
Turbidity	130	130	130	130	130	23	23	23	23	23	23	23	811
Chl a	10	10	10	10	10	4	4	4	4	4	4	4	78
Enterococcus	130	130	130	130	130	23	23	23	23	23	23	23	811
E. coli	130	130	130	130	130	23	23	23	23	23	23	23	811
Fecal coliform	130	130	130	130	130	23	23	23	23	23	23	23	811
NO2+NO3-N	23	23	23	23	23	23	23	23	23	23	23	23	276
NH3-N	23	23	23	23	23	23	23	23	23	23	23	23	276
Total N	23	23	23	23	23	23	23	23	23	23	23	23	276
Ortho P	6	6	6	6	6	6	6	6	6	6	6	6	72
Total P	6	6	6	6	6	6	6	6	6	6	6	6	72

23 regular stations + 3 transect stations x 5 times in 30 days = 130

23 regular stations x 1 time per month = 23

6 Hackensack River stations x 1 time per month = 6

Table 11 - Project Cost

Project Period: 5/1/21 - 04/30/2022

Budget Period: 5/1/21-04/30/2022			**NJDEP/IEC Harbor Monitoring**	**Total**
Personnel	*Hrs*	*Rate*	**$75,185**	**$75,185**
Executive Director /QA	75	$62.82	$4,712	$4,712
Environmental Analyst I (Lab)	350	$25.12	$8,792	$8,792
Environmental Analyst II	700	$31.02	$21,714	$21,714
Environmental Analyst III (Laboratory)	350	$54.19	$18,967	$18,967
Interns (3)	1050	$20.00	$21,000	$21,000
Total Hours	2525			
Fringe			**$15,396**	**$15,396**
22.6% of full-time Personnel			$12,246	$12,246
15% of part-time Personnel			$3,150	$3,150
Travel	*Trips*	*Rate*	**$2,100**	**$2,100**
Sampling: 35 total sampling trips X 2 vehicles X $30/day (tolls and gas)	70	$30.00	$2,100	$2,100
Equipment – Additional Nutrient analyzer			**$70,000**	**$70,0000**
Supplies			**$10,720**	**$10,720**
Bacteriology supplies (media, filters, organisms, reagents)			$2,400	$2,400
pH buffers, conductivity standards, turbidity standards, chlorophyll std			$1,100	$1,100
nutrient analyses supplies and reagents			$4,920	$4,920
probe tip replacements			$2,000	$2,000
ice			$300	$300
Professional Services			**$21,450**	**$21,450**
Research Vessel 33 survey runs- $650.00 per survey	33	$650	$21,450	$21,450
Other			**$0**	**$0**
Total Direct			**$194,851**	**$194,851**
Indirect (45.2% of personnel $80,604)			**$33,984**	**$33,984**
TOTAL COSTS			**$228,835**	**$228,835**
Procurement			**$80,720**	**$80,720**

Personnel: Total hours 2525/1950= 1.29 FTEs. IEC does not provide annual salary and other confidential information.

Fringe Benefits: Costs for health and accident insurance, FICA, unemployment, worker's compensation and retirement are based on historical data and are calculated, annually, as a percentage of personnel costs. Budgeted using current fringe rates which may change.

Travel: Travel costs are based on historical data. IEC travel policies include estimated mileage reimbursement at the federal IRS rate.

APPENDIX 1

CHAIN OF CUSTODY FORM

Interstate Environmental Commission Laboratory

2800 Victory Boulevard, Bldg. 6S Rm. 106

Staten Island, NY 10314 Ph: 718-982-3792

Page _____of _____

SITE/INVESTIGATION #:	Date/Time Sampling Began:
Address:	Date/Time Sampling Ended:
City:	Sampled by:
State: Zip:	Date/Time Transferred to IEC District Lab:
SPDES #	Relinquished By: Received By:

	Sealed				Sample Description		Unsealed	Destroyed	
Sample ID	Time/Date	By	Matrix	Preserva-tive	Grab/Comp	Location	D/T/I	D/T/I	Analyses

Matrix Code: WW= Wastewater SW= Surface (ambient) Water D/T/I= Date/Time/Initials

COMMENTS:_____

_____

_____

APPENDIX 2

YSI METER CALIBRATION SHEET: Pre-Sampling

YSI METER:_____
Sonde S/N:_____
Handhled S/N:_____

pH Calibration Pre-Sampling Date:_____Time:_____Initials:_____

Manufacturer	Buffer Value	Catalog Number	Lot Number	Exp. Date	Pre-Cal	Post Cal (Reading)	mV	Temp. °C
Fisher*	4.0							
Fisher*	10.0							
Fisher*	7.0 (ICV)							

***Change* if different manufacturer/catalog number is used.**

Specific Conductance Calibration Pre Sampling: Date:_____Time:_____Init.:_____

Manufacturer	Specific* Value	Catalog #	Lot Number	Exp. Date	Pre-Cal	Post Cal	Cell Constant

***Use specific conductance calibration value appropriate for your expected range of measurements. Typically 50,000µs/cm is used for salt/brackish water. 10,000µs/cm is used for freshwater.**

Dissolved Oxygen Calibration Pre-Sampling: Date:_____Time:_____Initials:____

Temperature (°C)	Barometric Pressure (mmHg)	Pre Cal %DO	Post Cal %DO	Post Cal mg/L

Winkler mg/L	Meter %	Meter mg/L	ODO gain	ICV (0%)	OPR

OPR true/theoretical value:_____%Recovery:_____

Depth Calibration Pre Sampling: Date:_____Time:_____Initials:_____

Location	Pre Cal	Post-Cal

***Alternate Calibration sheets may be provided/developed for specific programs.**

DISSOLVED OXYGEN
(Optical Luminescent-Based Sensor Method)

1) Test Method

Based on Hach Method 10360

2) Applicable Matrix or Matrices

Optical luminescence-based sensors provide an excellent method for rapid DO analysis in the field. This method is for the measurement of dissolved oxygen (DO) in surface and ground water, and municipal and industrial wastewater.

3) Method Detection Limit

The reporting limits for the specific dissolved oxygen meter currently used in the field for dissolved oxygen surveys (EXO1 sonde) is 0.1mg/L-50 mg/L.

4) Scope and Application

This method is used to measure dissolved oxygen in-situ of ambient waters in conjunction with ambient water quality monitoring surveys. The method is capable of measuring DO in the range of 0.20 to 20 mg/L. See IEC BOD SOP (SOP ID III) for additional procedures related to BOD_5 determination in the laboratory.

5) Summary of Method

The principle of operation of the optical luminescence based dissolved oxygen sensor is on the concept that dissolved oxygen quenches both the intensity and the lifetime of the luminescence associated with a carefully chosen chemical dye. The EXO DO sensor currently utilized in this method operates by shining a blue light of the proper wavelength on this luminescent dye which is immobilized in a matrix and formed into a disk. The blue light causes the immobilized dye to luminesce and the lifetime of this dye luminescence is measured via a photodiode in the probe. To increase the accuracy and stability of the technique, the dye is also irradiated with red light during part of the measurement cycle to act as a reference in the determination of the luminescence lifetime. When there is no oxygen present, the lifetime of the signal is maximal; as oxygen is introduced to the membrane surface of the sensor, the lifetime becomes shorter. Thus, the lifetime of the luminescence is inversely proportional to the amount of oxygen present and the relationship between the oxygen pressure outside the sensor and the lifetime can be

quantified by the Stern-Volmer equation. For most lifetime-based optical DO sensors, this Stern-Volmer relationship ((Tzero/T)-1) versus O_2 pressure is not strictly linear (particularly at higher oxygen pressures) and the data must be processed by polynomial non-linear regression. Fortunately, the non-linearity does not change significantly with time so that, as long as each sensor is characterized with regard to its response to changing oxygen pressure, the curvature in the relationship does not affect the ability of the sensor to accurately measure oxygen for an extended period of time.

6) Definitions

Dissolved Oxygen- The term refers to oxygen which is dissolved in water. Oxygen can become dissolved in water through a variety of methods. Atmospheric oxygen can become dissolved through wave and wind action.

Dissolved Oxygen mg/L- A measurement of oxygen in mg/L.

Dissolved Oxygen %- A measurement of oxygen in percent of saturation.

7) Interferences

Variables that could affect dissolved oxygen measurements include temperature, salinity, and barometric pressure. Temperature and salinity are compensated for during instrument calibration and field use with the use of additional sensors and/or instrument software settings. Barometric pressure relates to the pressure of oxygen in the calibration environment, and barometric pressure changes due to a change in altitude or local weather. Generally, the effect of barometric pressure is overcome by proper sensor calibration to a standard pressure.

8) Safety

There are minimal safety concerns when utilizing this method. When performing Winkler titration, use appropriate personal protective equipment (PPE) including gloves, goggles, and a labcoat to minimize exposure to the reagents utilized in the azide-modification Winkler titration (see BOD SOP).

9) Equipment and Supplies

1. Sample container (only if necessary). Use narrow-mouth glass-stoppered BOD bottles of 300 mL with tapered and pointed ground-glass stoppers.

2. EXO 1 sonde multi-meter equipped with EXO handheld unit and 66 m (216 ft) or a cable of appropriate length for the survey. A fully-equipped back-up meter should be available in case of meter malfunction. This procedure is tailored to the EXO 1 multi-meter equipped with an EXO Optical Do sensor (Item # 599100-01). Other similar meters, such as the YSI ProDSS, may be used. Always review and follow manufacturer-specific meter and sensor instructions for calibration and use.

3. Spare D batteries (4)

10) Reagents and Standards

1. See Dissolved Oxygen Winkler Titration (SOP ID IX) for required reagents for comparing EXO1 readings to Winkler titration.
2. Sodium Sulfite, anhydrous (to zero out DO as ICV and post run CCV)
3. DO standard to use and Initial and Ongoing Precision and Recovery samples. Sample may be prepared in laboratory or commercially procured.

11) Sample Collection, Preservation, Shipment and Storage

Because the EXO1 sonde offer the advantage of analysis in situ they eliminate errors caused by sample handling and storage. For BOD analysis in laboratory using the HACH HQ440d multi meter, transfer water samples in narrow-mouth glass-stoppered BOD bottles of 300 mL capacity with tapered and pointed ground-glass stoppers and flared mouths. Avoid entraining or dissolving atmospheric oxygen.

12) Quality Control

Calibrate meter within 24 hours of use by performing an air %DO calibration and by performing a zero point check with a 0 mg/L DO sample (prepared by adding sodium sulfite to reagent grade water until meter reads the sample as 0mg/L) in the laboratory (see Section 13). Perform an air %DO calibration in the field on day of use. Inspect meter and probe during these checks for any issues that could affect performance such as fouled probes or dull sensor. Inspect meter case for spare parts and batteries. Perform method comparison in the laboratory by comparing to a Winkler titration of dissolved oxygen in aerated reagent-grade water in the lab at least once a month or more often in the case of regular surveys (within 24 hours of start of survey) or if meter malfunction is suspected. Compare the measured values against the readings obtained from the Winkler titration. Winkler readings must be within ±0.3mg/L of the meter value.

Refer to EXO1 Sonde user manual, sections 1.8, 2.1, 2.2, 2.3, 3.1, 3.3, 5.1, 5.3, 6.1, 6.2, 6.3, 6.8, 6.9, 6.16, 6.17 for information of battery replacement, calibration cup installation, sensor

installation, basic sonde calibration, storage, maintenance, rehydration, sensor cap replacement and procedures to maintain the integrity of the sonde and DO sensor.

13) Calibration and Standardization

EXO sensors (except temperature) require periodic calibration to assure high performance. Calibration procedures follow the same basic steps with slight variations. Conduct calibrations in a temperature-controlled laboratory.

Review Basic Calibration in section 5.1 of EXO user manual.

ODO% sat 1 point

Place the sonde with sensor into saturated air:

(a) Saturated air: Ensure that there are no water droplets on the DO sensor or the thermistor. Place into a calibration cup containing about 1/8 inch of water LOOSEN cap so that is vented by loosening the threads. (Do not tighten the cup to the sonde during calibration step.) Wait 15 minutes before proceeding to allow the temperature and oxygen pressure to equilibrate. Keep out of direct sunlight.

Record the current barometric pressure in mm of Hg (Inches of Hg x 25.4 = mmHg) on PRE-Sampling Calibration Sheet.

Press “Calibration” button on handheld.
Scroll down, using arrow up and down buttons on handheld, to select ODO.
Press enter to Select ODO.
Ensure “Calibrate” is highlighted (using arrow up/down buttons on handheld to select/highlight if not) and press enter.
Ensure DO% is highlighted (using arrow up/down buttons on handheld to select/highlight DO% if not).
Press enter.
Observe the readings under live data chart displayed and when they are Stable (or data shows no significant change for approximately 60 seconds), click enter to accept the calibration.

Click Finish Calibration. View the Calibration Summary Screen and QC score. Record ODO gain, and pre-calibration DO% (this is “actual reading” on Calibration summary screen. Click Esc to return to the sensor calibration menu, and record the post-calibration %DO reading.

Record the DO of an ongoing precision and recovery (OPR) prepared in the lab (by preparing air-saturated water) or commercially procured.

Preparation of air-saturated water:

-Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle.
-Allow the water to equilibrate to room temperature (±2ºC).
-With a steady stream of filtered air (10-40 mL per minute), aerate the water for a minimum of 30 minutes.
-At the completion of aeration, let water re-equilibrate to room temperature (±2ºC) for 30 minutes. And note the barometric pressure of the laboratory during preparation (can use EXO1 handheld).
-Transfer the aerated water to a BOD bottle until overflowing and perform Winkler Titration (see BOD SOP ID III) to determine Winkler DO value. Read DO of solution in beaker with meter to determine ODO value.

Analyze a zero% DO solution in the laboratory (see QC section for preparation) as an ICV. Repeat the analysis of a 0% DO solution as a CCV upon returning to laboratory after survey (within 24 hours of survey completion).

In the laboratory within 24 hours of sampling survey, compare the meter reading of an air-saturated sample with a Winkler titration. See BOD SOP (IEC SOP ID III) for directions on Winkler titration. See Method Performance
Rinse the sonde and sensor(s) in tap or purified water and dry.

14) Procedure

Turn the instrument on and wait 5-15 minutes. Remove the calibration cup from the sensor/cable and install the weighted sensor guard to protect the sensor and membrane. Place the sonde in the sample (waterbody) and deploy slowly to the desired depth. Be sure not to hit the bottom hard or sediment may scatter, affecting the reading. If this happens, note the depth, pull the cable with probe attached out of the water, inspect probe and probe housing for debris, and clean if necessary, by pulling the sonde back and forth across the surface of the water, as in a "rowing" motion." Wait at least 3 minutes and re-deploy slowly as you approach the bottom depth Allow the readings to stabilize. Once the values plateau and stabilize, you may record the measurement (as %DO and mg/L) and/or log the data set. The dissolved oxygen reading may drop over time if stirring is ceased. If placing the DO sensor into a stream or fast flowing water it is best to place it perpendicular to the flow and NOT facing into the flow. In deep water, the probe may drift. Weights are stored within the sonde kit and can be anchored onto EXO1 sensor guard to minimize drift.

15) Calculations

Instrument is designed to provide measurements of dissolved oxygen % and dissolved oxygen mg/L and displays these readings on the LCD screen.

16) Method Performance

16.1 See SOP for Winkler Titration. Compare dissolved oxygen results with Winkler Titration method within 24 hours of start of run and record. The readings must correspond to within ±0. 3mg.L of each other. Air-saturated water prepared for Winkler Titration comparison may serve as an OPR sample (see 16.3).0

16.2 Perform a %DO saturation calibration prior to start of sampling run. Analyze a zero standard as a an ICV after calibration and as a CCV after each survey.

16.3 Analyze dissolved oxygen in aerated reagent-grade water OPR sample or commercially procured DO standard prior to each survey (each batch) or if meter malfunction is suspected. Inspect meter and probe during these checks for any issues that could affect performance such as fouled membrane or dull sensor. Inspect meter case for spare parts and batteries. Compare the measured values against the readings obtained from the dissolved oxygen Winkler titration. The readings must correspond to within ±0. 3mg.L of each other.

17) Pollution Prevention

The MSDS for all reagents are stored in a file in the laboratory. All analysts should review the relevant MSDS before using any reagent. Clean up all spills immediately. All cleaning or treatment of probe should be done in the lab, not in the field, to prevent spillage of detergents or reagents into the environment.

18) Data Assessment and Acceptance Criteria for Quality Control Procedures

The quality control measures must include the check for the accuracy of the method within 24 hours of use against the Winkler titration method (SM 4500-O C., IEC SOP ID IX). The acceptance criteria for such QC check must fall within ±0.3mg/L of the value calculated from the titration method.

19) Corrective Actions for Out-of -Control Data

Any out-of-control data must be reported at once to the Executive Director who in turn has to promulgate a corrective action plan in consultation with IEC's QA Officer.

20) Contingencies for Handling Out of Control or Unacceptable Data

The Executive Director or designee, will investigate the problem(s) in depth and take all necessary corrective actions that may include but are not confined to cleaning or replacing membrane, sending instrument for repair to an authorized service center, and/or comparing the measured values against that obtained by the titration method. All analysis of samples will be stopped until the problem is rectified.

21) Waste Management

Order or prepare all reagents in limited volumes that will be used before their expiration dates to minimize producing waste. See Pollution Prevention, section 17, above.

22) References

1. EXO User Manual. August 2014. YSI Incorporated, Yellow Springs, Ohio.
2. HACH Method 10360 Luminescence Measurement of Dissolved Oxygen in Water and Wastewater and for Use in the Determination of BOD_5 and $cBOD_5$

23) Tables

None.

pH

1) Test Method

Based on SM 4500-H B-11

2) Applicable Matrix or Matrices

Non-Potable and Potable Water.

3) Reporting Limit

pH of 0.01 S.U.

4) Scope and Application

The Interstate Environmental Commission regularly analyzes environmental samples for pH in the field at municipal and industrial facilities, as well as in conjunction with ambient water quality surveys aboard the Commission's boat. This method is applicable to surface, and saline waters, domestic and industrial wastes. This method is used by IEC most frequently to monitor the quality of the deionized water, as well as to measure the pH of prepared microbiological media and reagents. In the field, the pH of samples is taken using the Accumet AP 61 portable pH meter, the YSI ProPlus multimeter or the EXO1 sonde. Separate calibration and sample measurement procedures are outlined in the appropriate sections for each specific meter.

5) Summary of Method

The pH of a sample is measured electrometrically, using a combination electrode attached to a pH meter.

6) Definitions

The pH of a solution is defined as:

$pH = -\log a_H$

where a_H is the activity of the hydrogen ion in the solution. For hydrogen ions, the distinction between activity and concentration can usually be ignored.

Substituting the definitions of pH and slope into the Nernst equation, yields a working equation for computing sample pH:

$E = Eo + S_T\ pH$

In practice Eo and S_T may be determined through standardization with buffers of known pH. The

pH value of a sample then may be calculated from this equation and the measured output potential E of the electrode when placed in the sample.

7) Interferences

1. Oil and Grease or particulate matter, by coating the surface of the pH electrode, may interfere by causing sluggish response. These can usually be removed by gentle wiping or detergent washing, followed by deionized water rinsing. An additional treatment with hydrochloric acid (10%) may be necessary to remove any remaining film.

2. The pH meter automatically compensates for the temperature dependence of the electrode's response when measuring pH with an automatic temperature compensation (ATC) probe.

8) Safety

The reagents used in this analysis are not particularly hazardous. However, personal protective equipment including goggles, gloves and a labcoat should be worn at all times. Clean up any drips or spills of buffers or other reagents immediately using paper towels and a general cleanser.

9) Equipment and Supplies

1. Thermo Scientific Orion Star A215 pH/conductivity meter. In field, the Accumet Model AP61 pH meter, YSI Professional Plus multi-meter or EXO1 sonde is used.

2. pH electrode: The laboratory pH meter is equipped with an Orion 8157BNUMD Ross Ultra pH/ATC triode electrode. The field probe used is Fisher/Accumet Catalog #13-620 AP50. Use flat-tipped pH electrode, Accumet Catalog # 13-620-289 with lab pH meter for checking pH of microbiological agar. The EXO1 sonde measures pH with two electrodes combined in the same probe: one for hydrogen ions and one as a reference. The sensor is a glass bulb filled with a solution of stable pH (usually 7) and the inside of the glass surface experiences constant binding of H+ ions. The outside of the bulb is exposed to the sample, where the concentration of

hydrogen ions varies. The resulting differential creates a potential read by the meter versus the stable potential of the reference.

3. Beakers- 50 mL capacity.

4. Stir Plate and magnetic stirrers.

10) Reagents and Standards

1. Potassium Chloride Solution 4M- Saturated with Silver Chloride. Fisher Catalog # SP135.

2. pH Buffer 4.0 (Fisher Catalog # SB101-500)

3. pH Buffer 7.0 (Fisher Catalog # SB107-500)

4. pH Buffer 10.0 (Fisher Catalog # SB115-500)

5. Deionized water in squeeze bottle for rinsing electrode.

11) Sample Collection, Preservation, Shipment and Storage

Samples should be analyzed as soon as possible after sampling. High-purity waters and waters not at equilibrium with the atmosphere are subject to changes when exposed to the atmosphere, therefore the sample containers should be filled completely and kept sealed prior to analysis. Samples may be taken in either glass or plastic containers. They must be analyzed within 15 minutes of collection. Minimum sample volume required for analysis is100 mL.

12) Quality Control

The meter must be calibrated on each day of use. Make sure that the level of electrode filling solution in the electrode is sufficient. The filling solution should be filled at least one inch above the level of the sample in the beaker. Ensure that the electrode filling hole is in the open position and free from crystallization. Field meters are calibrated in the field on the day of use prior to the measurement of any environmental samples. pH meters are calibrated with pH 4.00 and pH 10.0 buffers and the reading of the pH 7.0 buffer is read. The calibration readings of pH 4 and 10 buffer solutions must be within ±0.05 S.U. The pH 7.0 buffer must read 7.0 ±0.10 S.U. Temperatures for all buffers must be recorded with calibration information, along with all information indicated on the log, including date and **time of calibration**. Daily or with each batch of twenty (or fewer samples) one sample must be analyzed in duplicate. The duplicate is

used for QC tracking of RPD. Sample and duplicate measurements must be within ±0.1 pH unit of one another. Do not report the average of sample duplicates, neither for routine samples nor PT samples.

The Field meters are brought into the lab and inspected by the Executive Director or designee monthly. During this monthly check, the pH buffers and deionized rinse water are replaced. The pH meter and kit is checked for cleanliness and the presence of a spare battery, backup pH paper, a digital thermometer (to check pH probe temperature readings if they seem erroneous). The pH meter is calibrated with the pH 4.00 and 10.0 buffer and the pH of the pH 7.00 buffer is read. The pH 7.0 buffer must read 7.0 ±0.10 S.U. The probe is inspected for breakage or crystallization. The ATC probe is calibrated against an NIST thermometer at least annually. A record of these monthly checks is logged into the Field Equipment log manual kept in the lab.

At the end of each analysis day, the meter will be checked with the mid-range buffer (pH 7.0) at the end of the run in addition to the required meter checks.

When in regular use, the pH sensor for the EXO1 should remain installed on the sonde in an environment of water-saturated air. Place approximately 0.5 in (1 cm) of deionized water in the bottom of the calibration cup. Insert the sonde and sensor into the cup and screw it on tightly to prevent evaporation. For long-term storage, remove the sensor from the sonde and insert its sensing end into the bottle that the sensor was shipped in. Install the bottle's o-ring and cap then tighten. This bottle contains a 2 molar solution of pH 4 buffer (tap water may be used as a replacement. DO not store the pH sensor in DI water.) If the pH sensor has been allowed to dry, soak the sensor for several hours (preferably overnight) in a 2 molar solution of KCl solution. If KCl is unavailable, tap water or pH 4 buffer soak may restore function. If the sensor is irreparably damaged, users must replace the sensor module.

13) Calibration and Standardization

Lab Benchtop Thermo Scientific Orion Star A215 pH/conductivity meter-*Clearing Existing Buffer*s- Over time, both an electrode's slope and its zero potential will change. As a general rule of thumb, pH electrodes require a complete re-standardization at least once daily.

1. Make sure that the pH electrode (standard electrode is ORION cat# 8157BNUMD) is attached to the BNC connector on the back of the meter. If using the meter to measure the pH of an agar, the flat-tipped electrode (cat#13-620-289) is used. **This may require replacing the installed electrode**.

2. In the measurement mode, press f1(cal) Press the arrow up button or arrow down button to highlight pH-Channel and press f2(select).

3. Calibrate according to the manufacturer's instruction manual, p. 4, utilizing pH 4 and 10 buffers to calibrate and pH 7 to check the calibration (read only).

- Rinse the electrode(s) in use with deionized water and blot dry with a kimwipe.
- Place in pH buffer 4.00. When the electrode and buffer are ready, press f3(start).
- Wait for the pH value on the meter to stabilize and stop flashing. Press f2(accept) to accept the displayed value, OR:
- Press f3(edit) to access the numeric entry screen and edit the value. **NOTE: Record temperature of buffer and adjust temperature on meter so that the meter indicates the pH value for buffers CORRECTED for buffer temperature.** Use the arrow buttons to highlight a number, a decimal point, or negative sign; press f3(enter) to select the highlighted item and repeat until the value at the measured temperature is shown above the numeric entry screen
- Press f2(done) to exit the numeric entry screen.
- Press f2(accept) to accept the entered value.
- Record calibration value and pH buffer temperature in the pH logbook.

4. Press f2(next) to proceed to the next buffer (pH 10) and repeat the bulleted steps above, recording calibration buffer and temperature with each step. Press f3(cal done) to save and end the calibration. The meter will display the calibration summary including the average slope. Record calibration data in pH calibration logbook.

Measure the pH 7.00 buffer in the measure mode. Record pH 7 buffer reading and buffer temperature.

For both the pH 4.00 and pH 7.00 buffers, measure the mV reading by pressing the **mV** button. Record. Calculate the absolute value of the difference between the pH 4.00 and pH 7.00 readings and record in the pH meter log book.

Field Accumet Model 61 Portable pH Meter-

1. Turn on the meter by pressing the **on/off** button.

2. Press **setup** twice, followed by **enter** to clear existing standards.

3. Open the pH probe, by holding the middle of the probe in one end and twisting the blue band at the top so the hole in the blue band is open. Place the pH probe in the pH 4.00 buffer and press **std.** Let the probe sit for a few seconds and press **std** again. Rinse the probe with deionized water and repeat with pH 10.00 buffer, by pressing **std** twice. Record the reading of the pH 4.00 and 10.00 buffers after calibration, along with pH buffer temperature. Rinse the probe with deionized water, place in the pH 7.00 buffer, allow to stabilize and record the reading of the pH 7.00 buffer and temperature of pH 7 buffer. The pH 7.00 buffer must read 7.00 ±0.10 SU. Perform a check with the pH 7.00 buffer at end of use for the day. The pH 7.00 buffer must read 7.00 ±0.10 SU for acceptability

YSI Professional Plus Multi-meter-

Turn on meter. Press "**Cal**." Highlight probe ID (ISE1 pH) by scrolling down with the up/down arrows until "ISE1pH" is highlighted. Press enter. The message line will show the instrument is "ready for point 1." Place the sensor in pH 4 buffer solution. The instrument should automatically recognize the buffer value and display it at the top of the calibration screen. If the calibration value is incorrect, the auto buffer recognition setting in the Sensor Setup menu may be incorrect. If necessary, highlight the **Calibration Value** and press enter to input the correct buffer value.

Once the pH and temperature readings stabilize, highlight **Accept Calibration** and press enter to accept the first calibration point. Record the pH buffer value and temperature of each pH buffer used to calibrate the meter. The message line will then display "Ready for point 2." To continue with the 2nd point, place the sensor in pH 10 buffer solution. The instrument should automatically recognize the 2nd buffer value and display it at the top of the calibration screen. If necessary, highlight the **Calibration Value** and press enter to input the correct buffer value. Once the pH and temperature readings stabilize, highlight **Accept Calibration** and press enter to confirm the second calibration point. Record the pH buffer value and pH buffer temperature of each pH buffer used in the calibration. Press **Cal** to complete the calibration. Read pH 7 buffer reading and buffer temperature and record.

YSI EXO1 SONDE pH Calibration:

1. Begin with a clean, dry probe installed on the EXO sonde. Install the clean calibration guard over the probe(s). Go to the Calibrate menu in KOR software. Select the sensor

you are going to calibrate from the list. Next select the parameter (pH) you are going to calibrate from the list. In the next menu, select a 2-point calibration. Pour of pH 4 buffer up to the first line in a pre-rinsed calibration cup. Carefully immerse the probe end of the sonde into the solution, making sure the sensor's glass bulb is in solution by at least 1 cm. Allow at least 1 minute for temperature equilibration before proceeding.

2. In the Calibrate menu, select pH (may need to select twice). Select the number of points desired for calibration (2 points). Enter the values (4 and 10) of the pH buffers that will be used for the calibration. Observe the temperature reading above the standard value. The actual pH value of all buffers varies with temperature. Record the pH buffer temperature. Enter the correct value from the bottle label for your calibration temperature for maximum accuracy.

3. Click start Calibration. Observe readings under Current and Pending data points and when they are Stable (or data shows no significant change for approximately 40 seconds), click Apply to accept this calibration point. Confirm that the Pending data value is close to the Setpoint value. Click Proceed and wait for the software to prompt you to move the sensor to the next buffer solution.

4. Rinse the sensor and calibration cup with deionized water. Pour the next buffer (10) up to the first line and carefully immerse the probe end of the sonde into the solution. Allow at least 1 minute for temperature equilibration before proceeding. Record pH 10 buffer temperature. Repeat the calibration procedure and click Apply when the data are stable.

5. Click Complete. View the Calibration Summary screen and read and record the QC score. Click Exit to return to the sensor calibration menu, and then the back arrows to return to main Calibrate menu if additional sensors are to be calibrated. Rinse the sensor in tap or purified water.

6. Read a pH 7.0 buffer as a sample. pH 7.0 buffer must read 7.0±0.1 SU. Record pH 7 buffer value and temperature.

14) Procedure

Specific procedures for each meter are outlined below. The manufacturer's manual for each meter should be referred to for more detailed measurement procedures. All data for pH measurements, whether performed in the field or in the laboratory (i.e. PT and DOC samples)

must be recorded on the appropriate data sheet to ensure incorporation and recording of all required supporting information. One pH measurement is performed in duplicate in each analytical batch. See QC section.

Thermo Scientific Orion Star A215 pH/conductivity meter.

Measuring pH procedures-See manufacturer's instruction manual, page 5.

Make sure the pH-Channel is selected.

1. Rinse the pH electrode, conductivity cell, and any other electrodes in use with deionized water, blot dry with a lint-free tissue and place into the sample.

2. Start the measurement and wait for it to stabilize.

a. If the meter is in AUTO-READ mode (default setting), press MEASURE (ESC) button to start the measurement. When the AR icon stops flashing, record the applicable measurement parameters and temperature of the sample. Press MEASURE (ESC) again to start a new measurement. AUTO-READ is the mode used for most applications.

b. If the meter is in continuous mode, the meter will immediately start taking a measurement and update the display whenever the measurement changes. Wait for the display to show **ready** and record the applicable measurement parameters and temperature of the sample.

c. If the meter is in timed mode, the meter will log measurements at the preselected time interval, regardless of the measurement stability. The meter will update the display whenever the measurement changes, so the applicable measurement parameters and temperature of the sample can be recorded when the display shows **ready.**

3. Remove the electrodes from the sample, rinse with deionized water, blot dry and place into the next sample. Repeat steps 2 through 3 for all samples.

Additional information on the use of the Orion Star A215 meter can be found in the manufacturer's instruction manual

Field Portable Accumet model 61 pH Meter/YSI Professional Plus Multi-meter and EXO1 Sonde

1. After calibrating with the pH 4.00 and pH 10.00 buffers, and recording the reading of the pH 7.00 buffer, place the field pH probe either directly in the water being measured, where practical and safe to do so. Provide moderate stirring, and when the reading is stable, record. If it is not

possible to place the pH probe in the water, or the water being measured is a rapid flow such as from a spout or turbulent effluent stream, collect the sample in a clean, dry plastic or glass jar attached to a sampling stick. Rinse the jar 3 times with the water being measured, then collect a sample for pH analysis. Record all data on the appropriate field data sheet.

15) Calculations

None.

16) Method Performance

Each analyst must perform an initial demonstration of capability (IDC) prior to performing analysis on environmental samples. The IDC consists of analyzing a quality control sample, procured by the Quality Executive Director (QC Officer), or designee, and analyzed four times by the analyst. Thereafter, each analyst must perform a CDC, consisting of a sample analyzed in duplicate. The results of the IDC and CDC must be within the manufacturer's determined acceptance limits. In addition to in-house quality control measures, the laboratory performs two proficiency test samples procured by providers approved by NJDEP OQA. PT samples must be rotated through all field meters in use. Typically, the Interstate Environmental Commission has between two and four field meters in use, so each meter should be used to analyze a PT at least once every two years. Record in the analysis log book the model and serial number of the meter used.

17) Pollution Prevention

Clean up any spills immediately with paper towels and a general cleanser.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

Quality Control samples must yield results within the manufacturer's acceptance limits. The pH of the 7.00 buffer must read 7.00 ±0.1 S.U. As part of the calibration procedure the mV reading of the pH 4.00 buffer and pH 7.00 buffer are measured. The absolute value of the difference of these two measurements should be between >160 mV. This check of the electrode slope verifies acceptable electrode response. Duplicate readings, when measured, should be within 20% of each other.

19) Corrective Actions for Out-of-Control Data

Out-of-control data must be reported at once to the Senior Manager, who in turn will promulgate a corrective action plan.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

The Executive Director will investigate the problem(s) at depth and take all necessary corrective actions that may include, but not be limited to: obtaining fresh QC standards from one or more suppliers and determining the accuracy of measurements, measuring standard deviations, consulting a service engineer and arranging on-site maintenance or repairing service. All analyses of samples will be stopped until the problem is rectified.

21) Waste Management

Dispose of measured samples and buffers down the drain, flushing with cold water. Samples that have been measured to be acidic (pH < 5.00) should be neutralized with 1 N NaOH prior to disposal.

22) References

Standard Methods for the Examination of Water and Wastewater, section 4500 H-B. APHA, AWWA, WEF, 2011.

Fisher Scientific. Accumet AP Series Handheld pH/mV/Ion Meter Instruction Manual. Rev. 1. 12/03

Fisher Scientific. Accumet pH/Temperature, Combination, Refillable Electrodes (Cat # 13-620-AP50) manual. October 2004.

Thermo Scientific Orion Star A215 Benchtop pH/Conductivity Meter Instruction Sheet, 2011.

YSI Professional Plus Multi-meter user manual. Revision D, March 2009. YSI Incorporated.

EXO1 Sonde user manual, August 2014. YSI Incorporated.

The NELAC Institute, 2016. 2*016 TNI Standards, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis.*

23) Tables, Diagrams, Flowcharts and Validation Data

None.

APPENDIX 3
Field Standard Operating Procedures

SALINITY
(Electrical Conductivity Method)

1) Test Method
Based on Standard Methods, 20th Edition, 2520 A and B and EPA Method 120.1

2) Applicable Matrix or Matrices
Salinity is an important property of industrial and natural waters. This field parameter can be useful for assessing the source or origin of effluents and of the mixing between fresh and marine waters in coastal regions.

3) Method Detection Limit
The salinity range for the specific meters currently used (YSI ProDSS and EXO1) is 0-70 ppt (manufacturer expresses range as conductivity, 200 ms/cm)

4) Scope and Application
This method is used to measure salinity in-situ of ambient waters in conjunction with ambient water quality monitoring surveys such as the Long Island Sound Survey.

5) Summary of Method
Salinity by definition is the ratio of the mass of dissolved salts in a given mass of solution. Thus, salinity values are commonly expressed as "grams of salt / kilograms of water" and recorded as ‰ or ppt. The experimental determination of the salt content by drying and weighing may result in a loss of sample, leaving the only reliable way to determine the true or absolute salinity is to make a complete chemical analysis. This method is time-consuming and cannot yield the precision necessary for accurate work, thus salinity is determined by using indirect methods involving the measurement of a related physical property such as conductivity, density, sound speed, or refractive index with known empirical relationships to salinity. Due to its high sensitivity and ease of measurement, the electrical conductivity method is used in the field to calculate salinity.

6) Definitions
Conductivity – The term refers to the measure of the ability of an aqueous solution to carry an electric current. The ability depends on the presence of ions; on their total concentration, mobility and valence; and on the temperature of measurement.

7) Interferences
1. The conductivity sensor will provide quick readings as long as the entire sensor is submerged and no air bubbles are trapped in the sensor area.

2. Drifting readings or an inability to calibrate the sensor may indicate that deposits have formed on the electrode. Occasional cleaning of the sensor and the openings which allow sample access to the conductivity electrodes may be required to maintain accuracy and increase responsiveness.

3. Avoid any environment that contains substances that may attack probe materials such as concentrated acids, caustics, and strong solvents.

8) Safety

This method requires the use of a sodium chloride conductivity calibration standard. To protect the analyst from contact with the standard, appropriate personal protective equipment, including goggles and gloves should be worn.

The following first-aid procedures should be used as a guideline in the event of contact with NaCl. For more information, refer to the reagent-specific MSDS, which is on file in the laboratory.

Eyes:
In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

Skin:
Wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops.

Ingestion:
Do NOT induce vomiting unless directed to do so by medical personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

Inhalation:
If inhaled, remove to fresh air. Get medical attention if symptoms appear.

9) Equipment and Supplies

1. YSI ProDSS or YSI EXO1 multi-meter with 10 foot and/or 100 foot cable (depending on anticipated depth of waterbody to be monitored)

2. Spare D batteries (4)

3. Spare Conductivity/Temperature sensor and replacement tool kit. ProDSS part # 626902; EXO1 part # 599870)

4. Traceable Conductivity Standards (see reagents)

5. Calibration cup (YSI)

6. Ring stand (to assist with calibration in lab)

7. Deionized water

10) Reagents and Standards

1. Traceable Conductivity Standard 5 µs/cm at 25 °C: Manufactured by Ricca Chemical and purchased from Fisher Scientific (Catalog # 2236.02-16).

2. Traceable Conductivity Standard 100 µs/cm at 25 °C: Manufactured by Ricca Chemical and purchased from Fisher Scientific (Catalog # 5877-16).

3. Traceable Conductivity Standard 1413 µs/cm atc25°C. Purchased through Fisher Scientific (catalog # 09-328-11)

4. Traceable Conductivity Standard 10,000 µs/cm at 25°C. Purchased from Fisher Scientific (Catalog # 09-328-4)

5. Traceable Conductivity Standard 50,000 µs/cm at 25°C. Purchased from Fisher Scientific (Catalog # 2248-1)

Standards used for salinity calibration should be in the expected range of sample values. For Long Island Sound (brackish water) sampling, the 50,000µs/cm standards are typically used. For freshwater 10,000 µs/cm is typically used. Alternate sources of calibration standards may be used if they have been evaluated and determined to meet the data quality needs of the laboratory. Manufacturer's certificates of analysis are kept on file in the laboratory.

11) Sample Collection, Preservation, Shipment and Storage

Because electrodes offer the advantage of analysis in situ they eliminate errors caused by sample handling and storage. If sampling is required, collect samples according to standard field methods.

12) Quality Control

1. Occasional cleaning of the sensor may be necessary to maintain accuracy and increase the responsiveness. Regularly check electrode for formed deposits, cracks or other issues that could affect performance. Use a mild detergent (laboratory grade soap or bathroom foaming tile cleaner) with the manufacturer's supplied cleaning brush if necessary. Rinse thoroughly with clean water, then check the response and accuracy of the conductivity cell with a calibration standard.

2. No special storage is required. Sensors can be stored dry or wet as long as solutions in contact with conductivity electrodes are not corrosive (for example, chlorine bleach). Sensors should be cleaned prior-to and after long-term storage. Long-term storage temperature: -5 to 70 °C.

3. Commercially purchased reagents must be used by the manufacturer's expiration date and certificates of analysis kept on file in the laboratory.

4. In the event that the electrode does not calibrate or read correctly, bring it to the attention of the Executive Director and see the YSI ProDSS or EXO1 user manual for instructions on electrode replacement.

5. The YSI ProDSS meters are checked weekly in the laboratory when in routine use (typically during the Long Island Sound Surveys from June-September). Lab checks include general inspection of equipment condition, presence of spare batteries and parts, spare probes as well as a calibration with traceable conductivity standards. Meters are checked monthly in the laboratory when not in routine use (September-May) to ensure proper working order, battery condition, and to address any maintenance concerns. Ensure handheld is fully charged (>90%) before packing up for field use. Sonde voltage can be checked by scrolling to "status" on the handheld. Ideally, voltage should be >2.0 volts. Proactive replacement of the D batteries in the sonde is recommended if voltage is <2.0 volts to avoid battery replacement in the middle of field work.

13) Calibration and Standardization

On each day of use, calibrate the conductivity (salinity) meter (YSI ProDSS or YSI EXO1 multimeter) before taking sample measurements. All calibrations should be completed at a temperature which is as close as possible to the sample temperature. The following instructions for calibration are adapted from the YSI ProDSS Instruction Manual. Always follow manufacturer and model-specific calibration, maintenance and storage instructions.

1. Place the sensor into a fresh, traceable conductivity calibration solution in the range of the expected sample values (10,000 µs/cm for Long Island Sound) using the specialized calibration container connected. The solution must be filled to the top line on the calibration cup. Ensure the entire conductivity sensor is submerged in the solution. A ring stand may be used for stability.
2. Press **Cal.** Highlight **Conductivity** and press enter. Highlight the desired calibration method: **Specific Conductance**, **Conductivity,** or **Salinity.** YSI recommends calibrating in specific conductance for greatest ease. Calibrating by any of these methods will calibrate the meter for the other two units of measurement as well.
3. Highlight "Calibration value" and press enter to input the value of the calibration standard. Then, once the temperature and conductivity readings stabilize, highlight "Accept Calibration" and press enter. Or, press "Esc" to cancel the calibration.

4. After completing the calibration, the message line at the bottom of the screen will display "Calibrating Channel…" and then "Calibration accepted." If there is an error with the calibration, the screen will alert you that the calibration may yield inaccurate readings and you must re-calibrate.

At the end of calibration, check electrode against an additional known conductivity standard in the range of expected measurement, making sure to rinse the sensor thoroughly with deionized water before taking a reading. The electrode should read ± 5% of the known standard value.

14) Procedure
Turn the instrument on and wait 5-15 minutes. Remove the plastic cup from the sensor/cable and install the sensor guard to protect the sensor and membrane. Install weights if necessary (they screw onto the metal probe guard) to facilitate sinking the probe to the desired depth. Completely submerge the probe in the sample to be measured and give the probe a quick shake to release any air bubbles. Allow the temperature readings to stabilize. Once the values plateau and stabilize, record the measurement and/or log the data set.

15) Calculations
The instrument is designed to provide measurements of salinity in ‰ or ppt and displays these readings on the LCD screen.

16) Method Performance
Analyze conductivity standard in the lab at least once a month or more often during routine surveys or if meter malfunction is suspected. The electrode should read ± 5% of the known standard value.

17) Pollution Prevention
All cleaning or treatment of probe should be done in the lab, not in the field, to prevent spillage of detergents or reagents into the environment. If possible, use low-phosphorus detergents to prevent pollution of local water bodies caused by nutrient loading.

18) Data Assessment and Acceptance Criteria for Quality Control Procedures
Certificates of Analysis for conductivity calibration standards will remain on file in the laboratory in QC Log 27: Chemistry Certificates of Analysis.

19) Corrective Actions for Out-of -Control Data
Any deviation from the protocol or a questionable result will be brought to the attention to IEC's Laboratory Director/QC Officer who will promulgate a corrective action plan.

20) Contingencies for Handling Out of Control or Unacceptable Data
The QC officer will investigate the problem(s) in depth and take all necessary corrective actions. This may include but is not confined to cleaning or replacing the electrode or sending instrument

for repair to an authorized service center. All analysis of samples will be stopped until the problem is rectified.

21) Waste Management
Order and prepare all standards in limited volumes that will be used before their expiration dates to minimize producing waste.

22) References

1. Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 9020-A and B. pp (2-48) to (2-49). APHA, AWWA, WEF, 1998.
2. Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 2510-A. p (2-44). APHA, AWWA, WEF, 1998.
3. EPA Method 120.1
4. YSI Professional Plus User Manual. 2009. YSI Incorporated, Yellow Springs, Ohio.

23) Tables
Not Applicable.

TEMPERATURE

1) Test Method

Based on SM (21) 2550B

2) Applicable Matrix or Matrices

Non-Potable and Potable Water.

3) Reporting Limit

The reporting limit for temperature is 0.1°C. However, the lowest detection limit depends on the graduation of the thermometer used. The only thermometers that reach this reporting limit are those that are graduated in 0.1°C increments. The reporting limit for any given thermometer will be whatever the lowest graduation increment is on that thermometer. The maximum reporting limit also depends on the range of the thermometers used. Currently, the thermometers used in IEC TDS ovens is the thermometer with the highest range, from 0º to 200°C. For field work, temperature is measured by a thermistor-type thermometer attached to the pH meter. The temperature range of the field pH meters (Fisher Model AP61) is 0 to 100°C. The range for the EXO1 is -5 to 50°C. The reporting limit for both of these meters is 0.1°C.The range for the ProDSS -5°C to 70°C. The reporting limit for the ProDSS temperature sensor is 0.2°C

4) Scope and Application

The Interstate Environmental Commission regularly analyzes environmental samples taken at municipal and industrial facilities, as well as samples collected in conjunction with ambient water quality surveys aboard the Commission's boat for temperature using this method. This method is applicable to surface, and saline waters, domestic and industrial wastes. The IEC uses this method to measure the temperature of ambient water and wastewater in the field. In the lab, the method is used to monitor the temperature of refrigerators, waterbaths, incubators, coolers as well as to monitor the temperature of samples and standards during analytical processes (digestions, distillations, etc.) as required in specific analytical SOPs .

5) Summary of Method

Temperature is read using a Celsius thermometer or thermistor-type thermometer that displays a temperature on an LED screen. Thermometers must be checked at least annually against an NIST traceable thermometer

6) Definitions

Temperature is defined as the degree of hotness or coldness of a substance measured on a definite scale.

7) Interferences

Most interferences can be avoided by using good quality thermometers, annually checked against a NIST certified thermometer that is used with its certificate and correction chart. Thermometers must be regularly checked for imperfections such as chipped glass or gaps in the mercury column that could interfere with accurate readings. Thermometers calibrated for total immersion must be completely immersed to the depth of the etched circle around the stem just below the scale level.

8) Safety

Handle thermometers with extreme care to avoid breakage. Broken thermometers containing mercury must be cleaned up using a mercury spill kit. Notify the Associate Laboratory Director who will supervise the cleanup of the mercury spill. All technicians present in the laboratory must be notified of the location of the spill.

9) Equipment and Supplies

Temperature can be read using a thermometer or thermistor-type thermometer that displays a temperature on an LED screen. In the field, temperature measurement is made using one of two meters. The Fisher Scientific Accumet® AP 61 Handheld pH/mV/Ion meter is used in routine municipal and industrial treatment plant sampling events. The electrode used with these meters is the Accumet® pH/Temperature, Combination Refillable Electrode, Fisher Catalog number 13-620-AP50. The YSI ProPlus Multimeter Oxygen, Conductivity, Salinity, and Temperature System, the EXO1 Sonde, or the ProDSS is used to measure temperature for ambient sampling events. Specifications are contained in the manufacturer's instruction manuals for the AP61, AP50 electrode, EXO1 and the ProDSS sensors.

10) Reagents and Standards

There are no reagents for temperature. All laboratory and field thermometers are checked annually against the NIST-traceable thermometer. The NIST-traceable thermometer is sent out for re-certification annually.

11) Sample Collection, Preservation, Shipment and Storage

In the field temperature measurements should ideally be taken directly, by submerging the pH probe or YSI probe into the water, if it is practical and safe to do so. For ambient water quality surveys, temperature reading should be taken 1 foot below the surface, unless the Quality Assurance Project Plan specifies particular depths. Samples can be collected in plastic or glass bottles, if the water cannot be reached directly by the probe. The method of collection depends on the location of the sampling site. The sample must be collected in a clean dry glass or plastic container. The sample may be taken by directly dipping the sampling container by hand into the water (if the field technician can do so safely) or using a bottle attached to a sampling stick. The sampling vessel should be rinsed out three times with portions of the water to be sampled. Temperature must be analyzed immediately (15 minutes or less from the time of sample collection). The sample aliquot should then be discarded in the field. Under no circumstances should a sample aliquot, having had a thermometer or temperature measuring probe inserted into the bottle for the purpose of measuring temperature, be used for any other analysis.

12) Quality Control

All thermometers- field and lab, must be checked against a precision thermometer or thermistors certified by the National Institute of Standards and Technology (NIST) annually. To do this, place the NIST thermometer alongside the device to be checked (the "working" thermometer), in the location where that device is normally used (e.g. refrigerator, 44.5°C water bath). Leave both thermometers side by side until they reach constant temperatures. Read the measurement on the NIST thermometer, apply any correction factor that has been determined from its annual recertification at that temperature, then read the measurement on the thermometer to be calibrated. The difference between the corrected NIST thermometer reading and the reading of the working thermometer is the correction factor for that working thermometer. As an example, if the NIST thermometer reads 44.7°C at a device set at 44.5°C, and the NIST thermometer is determined at its annual re-certification to have a correction factor of -0.1°C at 44.5°C, the analyst would subtract 0.1°C from the reading of the NIST thermometer to arrive at a reading of 44.6°C. The analyst would then use this as the NIST reading, and compare it to the reading of

the working thermometer, to arrive at a correction factor for the working thermometer. Using the example of the 44.6°C corrected NIST reading above, if a working thermometer next to it stabilizes at a reading of 44.4°C, the analyst would add 0.2°C to reach the NIST-traceable reading of 44.6°C. The thermometer would therefore have a correction factor of +0.2°C. Each thermometer must be tagged with its unique identification number, correction factor, temperature at which the annual NIST check was made, date of the check, and the initials of the analyst performing the check.

In the case of field meters, a monthly check is done in the lab, by the associate laboratory director, comparing the pH probe temperature reading to the NIST traceable thermometer.

The Certificates from the annual certification of the NIST thermometer are retained on file in the lab in the black 3-ring binder labeled "Service records, Service contracts and Calibration Certificates."

13) Calibration and Standardization

See Quality Control, Section 12, above, for requirements for calibrating thermometers.

14) Procedure

In the laboratory, the temperature of instruments (waterbaths, incubators, ovens, etc.) is monitored with thermometers that are permanently situated within the instrument. In the case of the waterbaths, the thermometers are inserted into a special thermometer opening in the hood of the waterbaths. The thermometer is immersed directly into the waterbath up to the immersion level etched on the thermometer. For refrigerators and dry incubators, the thermometer is immersed in deionized water in an ehrlenmeyer flask up to the immersion level etched on the thermometer. Care must be taken, especially in the case of dry incubators, to make sure that the level of water in the flask does not fall below the immersion point due to evaporation. In the case of the ovens, the thermometers are placed in beakers containing sand.

As part of daily routine laboratory quality control practices, the temperatures of the waterbaths, dry incubators, and refrigerators are checked twice a day, at least four hours apart. The temperatures are read directly off of the thermometer, the applicable correction factor applied, and the corrected temperature reading recorded in the temperature logbook.

Temperatures of environmental samples are measured in the field immediately upon collection. The sample is collected as described above in Section 11, Sampling, Preservation,

Shipment, and Storage. If using the Accumet AP 61 pH/mV/Ion meter, immerse the probe directly into the sample and provide moderate stirring. Turn the meter on by pressing the on/off button. Wait until the temperature readout, displayed on the screen as EC, stabilizes. This should take less than 10 seconds. The temperature is always displayed on the AP61 screen while the meter is on, and changes as the temperature of the sample changes. Therefore it is critical to read and RECORD the temperature as soon as a stable reading is displayed.

If using the YSI ProDSS or EXO Sonde, immerse the probe in the solution (or water body at desired depth), provide moderate stirring, and turn on the meter. Like the AP61, the temperature of the sample will always be displayed on the screen as long as the meter remains on, and will change as the temperature of the sample changes. Read and record the first stable reading that the meter displays.

15) Calculations

None Required.

16) Method Performance

There are no proficiency samples available or required for temperature. Method performance is monitored by recording and reviewing data collected from daily checks of laboratory thermometers and annual checks of field and lab thermometers against the NIST thermometer.

17) Pollution Prevention

Under no circumstances are mercury filled thermometers to be disposed of in the regular waste. Broken thermometers must be cleaned up using a mercury spill kit under the direct supervision of the Executive Director or Senior Environmental Analyst. The Executive Director or designee will then arrange for a pickup of this waste by a licensed chemical waste company. The IEC currently uses Triumverate Environmental, Inc. for the removal of chemical wastes.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

The correction factor for all laboratory and field thermometers must not be more than ±0.7°C. In the event that this criterion is not met, the thermometer should be rechecked, and if the criteria is still not met, a new thermometer shall be procured, calibrated against an NIST thermometer and put into service. The NIST traceable thermometer correction factors must not exceed ±0.3°C at

any temperature. In the event this criteria is not met, another NIST thermometer shall be procured.

19) Corrective Actions for Out-of-Control Data

Thermometers that are malfunctioning or appear to be malfunctioning must be reported at once to the IEC's Executive Director/QC Officer or Senior Environmental Analyst, who in turn has to promulgate a corrective action plan.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

The QC Officer will investigate the problem(s) at depth and take all necessary corrective actions that may include, but not be limited to: comparing the thermometer against the NIST traceable thermometer, determining the accuracy of measurements, or replacing the thermometer, probe, or meter. All analyses of samples will be stopped until the problem is rectified. If the temperature device in question is monitoring a laboratory apparatus (incubator, waterbath, etc.), another thermometer, checked against the NIST thermometer at the temperature of use and assigned a correction factor, must be put into use in the apparatus.

21) Waste Management

See Pollution Prevention, Section 17, for information on managing mercury waste (broken thermometers). Environmental samples collected and solely used for temperature analysis in the field can be discarded in the field.

22) References

Standard Methods for the Examination of Water and Wastewater, Section 2550B 21th Edition. APHA, AWWA, WEF, 2005.

EXO1 user manual, August 2014. YSI Incorporated.

Accumet AP61 user manual.

The NELAC Institute, 2016. 2016 TNI Standard, Volume 1: *Management and Technical Requirements for Laboratories Performing Environmental Analysis.*

23) Tables, Diagrams, Flowcharts and Validation Data

None.

APPENDIX 3
Field Standard Operating Procedures

SECCHI DISK DEPTH

1) Test Method

Secchi Disk Depth, SOP ID FIELD IV, Revision 1, January 2015.

2) Applicable Matrix or Matrices

This method is applicable to open water, lakes, ponds, reservoirs and other slow-moving waterways. Monitoring water clarity in river waters is more difficult with a secchi disk as the current can interfere with a stable reading.

3) Method Detection Limit

0.5 feet

4) Scope and Application

The IEC uses this method as an in situ measurement to assess water clarity in the far western Long Island Sound as well as other ambient water quality projects, as needed.

5) Summary of Method

Secchi disk depth is measured by lowering a round weighted circular disk 20 cm in diameter with four alternating blank and white quadrants painted on the surface. The disk is lowered into the water from a boat until the point at which it disappears. It is then raised until it reappears. The depths, in feet, at which the Secchi disk disappears and reappears is averaged and the average recorded as the Secchi disk depth (also called Secchi depth).

6) Definitions

Secchi disk- a round weighted circular disk 20cm in diameter (approximately 8 inches) with four alternating black and white quadrants, which is suspended on a rope graduated at 0.5 foot intervals.

7) Interferences

This procedure may disrupt fish and microscopic organisms, such as phytoplanktona and zooplankton, which can interfere with other sample collections (such as chlorophyll a). Secchi disk depth should be taken after these samples have been collected, but before any activity that may disturb bottom sediments to avoid increasing turbidity at the location. Ideally, secchi disk measurements should be taken between 10am and 2 pm to minimize glare. When this is not

possible, the time the measurement is taken and, in the case of a series of surveys (such as the Long Island Sound surveys), take each station's secchi disk depth measurement at approximately the same time. Ideally the same analyst should take the Secchi disk depth at the same station throughout the season. As this is not always possible, at one station per survey, two analysts should measure Secchi disk depth to ensure consistent readings. The analyst should remove sunglasses but continue to wear any clear prescription glasses. The measurement should be taken on the shaded side of the boat to reduce glare. Where there is no shade, the analyst should keep their back to the sun to block glare and reflection.

8) Safety

All boat surveys consist of a minimum of two analysts for safety. Life vests and stable, flat, rubber soled shoes should be worn at all times on the boat.

9) Equipment and Supplies

Secchi disk secured to a weight and appropriately graduated rope (0.5ft increments) (The Science Source or other suitable supplier)

Spare Secchi disk in case of loss or damage

Long Island Sound Field Data Sheet or other project-specific data sheet.

Clipboard

Charter Boat with depth finder, GPS

Life jackets

Pen

Clock

Gloves

10) Reagents and Standards

No reagents or standards are required for Secchi disk depth measurement.

11) Sample Collection, Preservation, Shipment and Storage

Secchi disk depth is an *in situ* measurement. No samples are collected, preserved, shipped or stored for this method

12) Quality Control

While the procedure of this test is very simple, certain basic quality control requirements are still applicable. Procedures listed in section 7) Interferences as well as section 14 should be reviewed and followed to ensure accurate, consistent, and quality results. At two stations per run, duplicate Secchi disk measurements are taken. At one station per run, a second analyst performs the Secchi disk depth measurement to ensure consistent readings.

13) Calibration and Standardization

The rope used to suspend the Secchi disk is graduated in 0.5 foot increments.

14) Procedure

The field analyst should remove sunglasses (prescription or non-prescription) but continue to wear any clear prescription glasses. Determine which side of the boat is exposed to the sun. The measurement should be taken on the shaded side of the boat to reduce glare. Where there is no shade, the analyst should keep their back to the sun to block glare and reflection. When taking a measurement the Secchi disk should remain in the water directly below your hand holding the rope attached to the Secchi disk. If the current or wind causes the disk to drift, extra weight should be added to the disk to avoid skewed measurements when read at an angle. Unwind the rope attached to the Secchi dis. Place your foot gently on the end of the rope to prevent loss of Secchi disk overboard. Lower the Secchi disk over the side of the boat into the water until the pattern on the Secchi disk just disappears from view. Read aloud the depth at the water surface from the graduated rope to the nearest 0.5 foot to the second analyst. The second analyst records this number on the datasheet. Lower the disk approximately 0.5ft more and pull the rope, gently out of the water until the pattern on the Secchi disk just reappears. Read aloud the depth at the water surface from the graduated rope to the second analyst. The second analyst records this number, and averages and record the result.

15) Calculations

The depth at which the Secchi disk disappears and the depth at which the Secchi disk reappears is averaged.

16) Method Performance

See Quality Control, section 12

17) Pollution Prevention

This analysis poses little potential for pollution.

18) Data Assessment and Acceptance Criteria for QC Measures

Duplicate Secchi disk depth measurements should vary by no more that 5%. In the case of very shallow Secchi disk depths, this criteria may not be able to be measured, as the rope is graduated in 0.5 ft increments. This acceptance criteria applies both to duplicate measurements taken by the same analyst as well as duplicate measurements taken by the second analyst.

19) Corrective Actions for Out-of-Control Data

If duplicate readings are not within acceptance criteria, a third measurement should be taken. If this measurement is also out of the acceptable range, the boat position on the GPS should be checked to make sure the boat has not drifted. If second analyst measurements are not within acceptance criteria, the analysts should jointly perform a Secchi disk depth measurement to ensure consistent procedures.

20) Contingencies for Handling Out-of-Control Data or Unacceptable Data

Measurements that do not meet acceptance criteria should be identified with an appropriate qualifying QC note on the final report. Consistent issues with acceptance criteria indicate a problem with the procedure. The QC officer should review the procedure with all analysts, and accompany the analysts on the next survery to ensure appropriate procedures are being followed and there is not a problem with the equipment (Secchi disk or boat)

21) Waste Management

This procedure produces no hazardous waste.

22) References

USEPA 2012. *Volunteer Lake Monitoring: A Methods Manual*. Office of Water Regulations and Standards, Washington, D.C. EPA 440-4-91-002
http://www.epa.gov/volunteer/lake/lakevolman.html

USEPA 2012. *Using a Secchi Disk or Transparency Tube*.
http://water.epa.gov/type/rsl/monitoring/155.cfm

Rhode Island Department of Enviornmental Management, 2011. *Standard Operating Procedure for Secchi Disk Measurements (SOP WR-W-7)*Office of Water Resources, Providence, RI.

23) Tables, Diagrams, Flowcharts, and Validation Data

APPENDIX 3
Field Standard Operating Procedures

None.

Ammonia

1. TEST METHOD

Based on EPA Method 350.1, Revision 2.0

2. APPLICABLE MATRIX OR MATRICES

The method is applicable for non-potable water (ambient surface water and wastewater).

3. METHOD DETECTION LIMIT

The IEC method detection limit is 0.008 mg/L as NH_3. The reporting limit is 0.02 mg/L (MDLs updated March 2021).

4. SCOPE AND APPLICATION

4.1 This method covers the determination of ammonia in surface waters, and domestic and industrial wastes. IEC will be using for the analysis of samples collected in western LIS (brackish samples) as well as periodic samples collected from wastewater treatment plants, as applicable.

5. SUMMARY OF METHOD

5.1 This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, and sodium hypochlorite to form indophenol blue to form monochloramine, which, in the presence of phenol, catalytic amounts of nitroprusside (nitroferricyanide), and excess hypochlorite gives indophenol blue. The formation of monochloramine requires a pH between 8 and 11.5. At higher pH, ammonia may begin to oxidize to nitrate. At pH greater than 9.6, some precipitation of calcium and magnesium as hydroxides and carbonates occurs in seawater. EDTA added to the buffer prevents this from occurring. The indophenol blue measured at 630 nm is proportional to the original ammonia concentration.
The method is calibrated using standards prepared in deionized (laboratory reagent grade) water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed.

If distillation is required, the sample is buffered at pH of 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds, and is distilled into a solution of boric acid.

6. DEFINITIONS

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

6.1 CALIBRATION BLANK (CB) – A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

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

6.3 INSTRUMENT PERFORMANCE CHECK SOLUTION (ICP) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.

6.4 LABORATORY SPIKED BLANK (LSB) -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB 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

6.5 LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like 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 correct for background concentrations.

6.6 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

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

6.8 MATERIAL SAFETY DATA SHEETS (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.

6.9 METHOD DETECTION LIMIT (MDL) – The lowest level at which an analyte can be detection with 99 percent confidence that the analyte concentration is great than zero.

6.10 LIMIT OF QUANTITATION (LOQ) – The Laboratory performs a Limit of Quantitation (LOQ) study on an annual basis for analytes associated with chemistry methods. The validity of LOQ is confirmed by successful analysis of a Laboratory Fortified Blank (LFB) at approximately 2X the reporting limit. The acceptance criteria for each analyte is + 30% of the true value. After this study is completed, it is reviewed and approved by the Laboratory Management. A summary of all LOQ study performance is maintained.

6.11 QUALITY CONTROL SAMPLE (QCS) – A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. THE QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently form the normal preparation process.

6.12 STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method anlaytes prepared in the laboratory using assay reference materials or purchased from a reputable commercial source.

7. INTERFERENCES

7.1 Calcium and magnesium ions may precipitate if present in sufficient concentration. EDTA is added to the sample in-line to prevent this problem.

7.2 Color, turbidity, and certain organic species may interfere. Turbidity may removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without color formation. See System Note 11 for specific instructions.

7.3 Sulfide may interfere at levels greater than 2 mg H_2S/L. Samples containing concentrations greater than this should be diluted.

7.4 Salinity does not normally interfere in this method. This may be verified by running the samples through the manifold with all reagents pumping, except hypochlorite, which is replaced by deionized water. The resulting concentrations are then compared to those obtained for samples determined with color formation.

7.5 The salt effect (salinity influence on absorbance) is less than 2%.

7.6 Residual Chlorine, if present, must be removed by pretreatment of the sample with sodium thiosulfate or other suitable method before distillation.

7.7 Cyanate, which may be encountered in certain industrial effluents, will hydrolyze to some extent even at the Ph of 9.5 at which distillation is carried out.

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

See System Note 11.2 in Lachat Method for additional information.

8. SAFETY

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

8.2 Each laboratory is responsible for maintaining a current awareness file of the Occupational Health and Safety Act (OSHA) regulation regarding the safe hand ling of the chemicals specified in this method. A reference fi le of Material Safety Data sheets(MSDS) is made available to all personnel involved in the chemical analysis.

8.3 The following chemicals have the potential to be highly toxic or hazardous, for detailed explanation consult the MSDS.

5.3.1. Sodium hydroxide

5.3.2. Phenol

9. EQUIPMENT AND SUPPLIES

9.1 Balance – analytical, capable of accurately weighing to the nearest 0.0001 g.

9.2 Glassware – Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

9.3 Flow injection analysis equipment (Lachat 8500 series 2) designed to deliver and react sample and reagents in the required order and ratios.

9.3.1. Lachat Autoanalyzer

9.3.2. Multichannel proportioning pump

9.3.3. Reaction unit or manifold

9.3.4. Colorimetric detector

9.3.5. Data system

9.4 Special Apparatus

6.4.1. Seawater Accessories Kit Lachat Part No. 50969 – RAS or 50970 – ASX510 (for brackish samples)

6.4.2. PVC PUMP TUBES MUST BE USED FOR THIS METHOD.

10. REAGENTS AND STANDARDS

10.1 PREPARATION OF REAGENTS

Use reagent-grade (ammonia-free) water for all solutions.

Degassing with helium:

To prevent bubble formation, DO NOT DEGAS USING AN INVASIVE PROCEDURE SUCH AS A WAND TO AVOID CONTAMINATION. Degas by vacuum or sonication. DO NOT DEGAS PHENATE, HYPOCHLORITE, OR STANDARDS.

Reagent 1. Buffer Chelating Agent

By Volume: In a 1 L volumetric flask, dissolved 50.0 g disodium ethylenediamine tetraacetate (Na_2EDTA) and 11.0 g sodium hydroxide (NaOH) in about 900 mL DI water. Stir to mix and dilute to the mark with DI water. Degas as above. Prepare fresh monthly sample.

By Weight: To a tared 1 L container, add 966 g DI water. Add 50.0 g disodium ethylenediamine tetraacetate (Na_2EDTA) and 11.0 g sodium hydroxide (NaOH). Stir to mix. Degas as above. Prepare fresh monthly.

Preferably use Lachat Ammonia Buffer, EDTA/NaOH Solution CAT#52017.

Reagent 2. Phenate Reagent

CAUTION: Wear gloves. Phenol causes skin burns and is rapidly absorbed into the body through the skin. Do not degas this reagent.

By Volume: In a 1 L volumetric flask, dissolved 83 g crystalline phenol (C_6H_5OH) in approximately 500 mL DI water. While stirring, slowly add 32 g sodium hydroxide (NaOH). Cool, dilute to the mark with DI water when cool, and invert to mix. The color of this reagent

darkens with age, increasing the baseline absorbance. Prepare fresh reagent after 72 hours. Prepare fresh every 3-5 days and discard when turns dark brown.

By Weight: To a tared 1 L container, add 888 g DI water. Add 83 g crystalline phenol (C_6H_5OH). While stirring, slowly add 32 g sodium hydroxide (NaOH). Cool and stir to mix. The color of this reagent darkens with age, increasing the baseline absorbance. Prepare fresh every 3-5 days and discard when turns dark brown.

Preferably use Lachat Sodium Phenolate Reagent CAT#52005.

Reagent 3. Sodium Hypochlorite

By Volume: Dilute 250 mL sodium hypochlorite (SS290-1 hypochlorite solution, 4-6% NaOCl from Fisher) to 500 mL with DI water. Prepare fresh daily.

By Weight: Dilute 250 g sodium hypochlorite (SS290-1 hypochlorite solution, 4-6% NaOCl form Fisher) to 500 g with DI water. Prepare fresh daily.

Preferably use Lachat Hypochlorite Reagent CAT#52007.

Reagent 4. Sodium Nitroprusside

By Volume: Dissolve 0.5 g sodium nitroprusside in 1 liter DI water. Prepare fresh every 1-2 weeks.

By Weight: Dissolve 0.5 g sodium nitroprusside in 1 liter DI water. Prepare fresh every 1-2 weeks.

Or use Lachat Sodium Nitroprusside Reagent CAT#52006 (Lachat Ammonia reagent set CAT # 52904 contains the above listed reagent, as an alternative option).

10.2 PREPARATION OF STANDARDS

Standard 1. Stock Ammonia Standard, 100 mg /L

By Volume: In a 1 L volumetric flask dissolve 0.3818 g ammonium chloride (NH_4Cl) FISHER CAT #3384-12 (Macron) in about 800 mL DI water. Dilute to the mark with DI water and invert to mix.

Standard 2. Working Stock Standard 10.0 mg /L

Dilute 10.0 mL of Stock Ammonia Standard to100 mL with DI water.

Standards 3. Working Standards.

Dilute the following mL of working stock standard to 100 mL. The upper and lower value of the standard curve should not be modified. If project requires a different range the upper and lower standards may be modified but reporting limit must also be adjusted accordingly. Samples above the high standard (+10%) must be diluted. The mid-range standards listed below are recommended but may be modified by analyst.

mL Working Stock	mg/L Ammonia
40.0	4.00
20.0	2.00
10.0	1.00
5.00	0.50
1.00	0.10

See EPA Method 350.1, Revision 2 for list of additional reagents if distillation is required.

11. SAMPLE COLLECTION, PRESERVATION AND STORAGE

11.1 There is no single preservation method that may be recommended of all types of samples. The analyst must examine each situation critically and treat this information as a guide.

11.2 If samples must be chemically preserved, samples should be cooled, and adjusted to pH < 2 with H_2SO_4. And store at 4 °C in glass or polyethylene. Analyze within 28 days. Samples collected from western Long Island Sound sampling and stored in polyethylene bottles may be stored frozen up to 1 year as per QAPP.

11.3 Researchers have found serious errors when investigating the effects of filtration. The analyst should examine sample preparation and preservation techniques before routine testing.

12. QUALITY CONTROL

12.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

12.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

12.2.2. Linear Calibration Range (LCR) --- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed and expected. The initial demonstration of linearity must use sufficient standards to insure that the

resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by +/- 10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

12.2.3. Quality Control Sample (QCS) or Laboratory Control Sample (LCS)– When beginning the use of this method, quarterly or more frequently as required to meet data-quality needs, verify the calibration standards are acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/- 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with ongoing analyses. This solution is also utilized as the Laboratory Fortified Blank (LFB) with each batch of samples.

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

$$MDL = t \times S$$

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, t = 2.528 for twenty-one replicates]. S= standard deviation of the replicate analyses.

MDLs are determined annually, when a new operator begins work, or whenever there is a significant change in the background or instrument response. The reporting limit is 2-5X the calculated MDL and must be verified at least annual, or more frequently as required for data-quality needs.

See Method Performance Section 16 for additional quality control analyses required with each run.

13. CALIBRATION AND STANDARDIZATION

13.1 Prepare a series of standards, covering the desired range, and a blank by dilution suitable volumes of standard solution (suggested range in Section 10.2).

13.2 Calibrate the instrument as described in Section 14.

13.3 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

13.4 After the calibration has established, it must be verified by the analysis of a suitable

quality control sample (QCS) as a Laboratory Fortified Blank (LFB) and calibration blank. If measurements exceed +/- 10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check. QCS's are typically analyzed with each batch of samples.

14. PROCEDURE

14.1 CALIBRATION PROCEDURE

14.1.1 Prepare reagent and standards as described in section 10.

14.1.2 Set up manifold as shown in Lachat Ammonia Manual.

14.1.3 Input data system parameters as shown in Lachat Ammonia Manual.

14.1.4 Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieve.

14.1.5 Place samples and/or standards. Input the information required by the data system, such as concentration, replicates, ets. (See section 14.2).

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

14.2 SYSTEM NOTES

14.2.1 For information on system maintenance and troubleshooting refer to the Troubleshooting Guide in the System Operation Manual. This guide is also available on request from Lachat.

14.2.2 Allow 15 minutes for heating module to warm up to 60 ° C.

14.2.3 Allow 15 minutes with reagents pumping for the system to equilibrate.

14.2.4 To check for gross carrier contamination:

A. Monitor the baseline on the system unit screen.

B. Equilibrate the pumping system with DI water in all reagent lines.

C. Equilibrate the pumping system with reagents in the appropriate lines.

D. The increase in absorbance should be 0.02 V or less.

14.2.5 A backpressure coil (100 cm x 0.5 mm (0.022") i.d. Teflon tubing) is used to prevent air bubble formation.

14.2.6 All reagent containers should be covered with Parafilm after insertion of the transmission lines to prevent contamination from airborne ammonia.

14.2.7 Reagent recipes from other automated wet chemistry analyzers should not be substituted.

14.2.8 If sample tube or standard container materials other than polystyrene are used, standards

and samples in these containers should be analyzed to investigate absorption or contamination.

14.2.9 The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

14.2.10 if the detection limit is greater than that specified in the method the following outline should be followed.

A. Verify standards preparation procedures.

B. Verify that a 630 nm filter is being used.

C. Verify that the sample loop is completely filled by running dye.

D. Verify that the reagents are being added in the correct order.

E. Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.

F. Prepare fresh reagents. Take care to be sure that the EDTA is completely dissolved.

14.2.11 If the samples are colored or are suspected to show a background absorbance, this interference should be subtracted. This can be done by using the following procedure:

A. Calibrate the system in the normal manner.

B. Disable the check standards or DQM features and analyze the samples.

C. Place reagent and carrier lines in DI water and allow the baseline to stabilize.

D. Inject samples again without recalibrating.

E. Subtract the “background” concentration from the original concentration to give the correct concentration.

Corrected Concentration = Original Concentration – Background Concentration

14.2.12 It is critical that the peak be detected on the “flat top” of the standard peaks. If the window is not on the “flat top”, the peak start time should be adjusted.

14.2.13 To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater, as is and spiked at 1 or 2 low levels, can be used to set timing parameters.

14.2.14 For low level analysis it is recommended that samples be analyzed in replicate from each sample cup. This is done by entering Replicates = 2 when entering sample information.

14.2.15 Use consumer bleaches with caution. Proprietary additives may contribute to staining of tubing and data quality.

14.2.16 Add reagents in the order that they appear on the manifold to reduce staining.

Data from Lachat instrument is back up periodically.

15. CALCULATIONS

15.1 Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

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

15.3 Report results in mg/L or µg/L.

16. METHOD PERFORMANCE

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

16.2. Laboratory Fortified Blank (LFB) – The laboratory must analyze at least one LFB (also referred to as a Quality Control Sample, QCS, or Laboraotory Control Sample, LCS, with each batch of samples. Calculate accuracy as percent recovery (Section 18). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judge out of control, and the source of the problem should be identified and resolved before continuing analyses.

16.3. The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%.

16.4. Instruments 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 tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/- 10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/- 10%. If the calibration cannot be verified within the specific limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

A demonstration of capability (DOC) should be performed each time there is a significant

change in the chemistry of the method, a major modification to an existing instrument, or a new instrument is installed. An initial DOC is performed by each analyst designated to analyze samples using this method. An annual check must subsequently be performed and documented for each analyst using this method. Ongoing demonstration of capability consists of four replicate analyses on a QC sample or one satisfactory analysis of a proficiency sample.

17. POLLUTION PREVENTION

17.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the recycling as the next best option. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
For information about pollution prevention consult the IEC Laboratory's Health and Safety Manual.

18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 Laboratory Spike Sample Matrix (LSM) – The laboratory must spike, in duplicate, minimum of 10% of routine samples. In each case the LSM aliquots must be duplicate of the aliquot used for sample analyses. The spiking level shall be at 1 to 5 times higher than the background concentration of the sample.

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

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

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

18.2 If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control (sect. 18.3), the recovery problem encountered with the LSM is judge to be either matrix or solution related, not system related.

18.3 Computer the relative percent difference (RPD) between the two LSM results and compare the value to the designated RPD recovery range of 10%. The RPD may be calculated using the following equation:

$$RPD = \frac{(D_1 - D_2)}{(D_1 + D_2)/2} \times 100$$

Where D1= concentration of analyte in the sample, D2= concentration of analyte in the second (duplicate) sample.

18.4 if the RPD falls outside the designated recovery range and the laboratory performance for that analyte is shown to be in control (sect 18.3), the recovery problem encountered with the LSM is judge to be either matrix or solution related, not system related.

18.5 Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions.

19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions.

20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

See Quality Control, Section 12 for a description of contingencies for handling out-of-control or unacceptable data (corrective actions).

21. WASTE MANAGEMENT

The USEPA requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. IEC urges staff to protect the air, water and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any water discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult IEC's Health and Safety Manual and the College of Staten Island's Chemical Hygiene Plan.

22. REFERENCES

3.1 Method for Determination of Inorganic Substances in Environmental Samples, Rev. 2.0, August 1993, Method 350.1

23.2 Lachat Instruments QuickChem Method 10-107-06-1-B, Revision date September 18, 2008.

23.3 Standard Method for Examination of Water and Wastewater, Method 4500-NH3 Flow Injection Analysis, 21th Edition, 1997.

23. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA:

NONE.

See Lachat Ammonia Manual**.**

APPENDIX 4
Lab Standard Operating Procedures

Nitrate , Nitrite, Nitrate+Nitrite and Total Nitrogen

1) Test Method

This SOP is based on EPA method 353.2, Revision 2.0 for Nitrate and Nitrite and Lachat method 31-107-04-4-B for Total Nitrogen.

2) Applicable Matrix or Matrices

Non-potable waters.

3) Method Detection Limit

The MDL and reporting limit for Nitrate (NO3 as N) is 0.018 mg/L (MDL) and 0.045 mg/L (RL)
The MDL and reporting limit for Nitrite (NO2 as N) is 0.009 mg/L (MDL) and 0.02 mg/L (RL)
The MDL and reporting limit for Nitrate-Nitrite (as N) is 0.013 mg/L (MDL) and 0.03 mg/L (RL)
The MDL and reporting limit for Total Nitrogen is 0.009 mg/L (MDL) and 0.02 mg/L (RL)
(MDLs update March 2021)

4) Scope and Application

This method is applicable to surface and saline waters, and domestic and industrial waste waters. This analysis is performed by Flow Injection analysis (FIA).

5) Summary of Method

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone can also be determined by removing the cadmium column. Total Nitrogen can be determined by adding a persulfate digestion prior to analysis through the cadmium column.

6) Definitions

In waters and wastewaters the forms of nitrogen of greatest interest are, in order of decreasing oxidation state: nitrate, nitrite, ammonia and organic nitrogen. All these forms of nitrogen are

biochemically interconvertible and are components of the nitrogen cycle. Nitrate generally occurs in trace quantities in surface water. It is an essential nutrient for many photosynthetic autotrophs and in some cases has been identified as the growth-limiting nutrient. Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation state of ammonia to nitrate and in the reduction of nitrate.

7) Interferences

7.1 Residual chlorine can produce a negative interference by limiting reduction efficiency. Before analysis, samples should be checked and if required, dechlorinated with sodium thiosulfate. Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.

7.2 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

7.3 Sample turbidity may interfere. Remove turbidity first by filtration with 0.45 um pore diameter membrane filter prior to analysis

8) Safety

8.1 The toxicity and carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be minimized by good laboratory practices, e.g. wear proper protective equipment, safety glasses, gloves, lab coat and working inside hoods whenever possible.

Refer to the Interstate Environmental Commission Laboratory Health and Safety Manual and CSI Chemical Hygiene Plan for specific guidelines.

For detailed explanations consult the Material Safety Data Sheets (MSDS), available in the Laboratory.

The following chemicals have the potential to be highly toxic or hazardous, for detailed explanations consult the MSDS:

Cadmium
Hydrochloric acid
Phosphoric acid
Sulfuric acid

9) Equipment and Supplies

9.1 Lachat 8500 QuikChem® Autoanalyzer , manifold reaction unit, colorimetric detector, 520 nm wavelength filter and Omnion software data system.

9.2 Cadmium Reduction Column

9.3 Autoclave

9.4 Miscellaneous laboratory apparatus such as volumetric pipettes, flasks, etc.

10) Reagents and Standards

10.1 Digestion Reagent (Total Nitrogen only):
In a 1L volumetric flask dissolve 10 g potassium persulfate, 6.0 g boric acid, 3.0 g sodium hydroxide in approximately 800 mL DI water. Dilute to the mark and mix. Prepare fresh monthly and store in plastic.

10.1.1 Sodium persulfate Digestion Reagent recommended by Lachat for brackish and sea waters. (Total Nitrogen only):
In 1 volumetric flask, dissolve 50 g sodium persulfate in about 800 mL DI water. Stir until dissolved. Dilute to the mark, and invert to mix. Prepare fresh monthly. Store in plastic.

Analysis Reagents:

10.2 Sodium Hydroxide, 15N
Slowly add 150 g NaOH to 250 mL DI water with constant stirring. This solution will get very hot. Cool and store in plastic bottle.

10.3 Ammonium Chloride Buffer, pH 8.5
Dissolve 85.0 g ammonium chloride and 1.0 g disodium ethylenediamine tetraacetic acid dihydrate in approximately 800 mL DI water in a 1 L flask. Adjust the pH to 8.5 with 15 N sodium hydroxide. Dilute to the line and mix. Or preferably use Lachat Ammonia Chloride Buffer, pH 8.5 Reagent CAT#52003.

10.4 Sulfanilamide color reagent
Add approximately 600 mL DI water to a 1 L flask. Then add 100 mL of 85% phosphoric acid, 40.0 g sulfanilamide and1.0g N-(1-naphthyl) ethylenediamine dihydrochloride. Stir until dissolved, approximately 30 minutes. Dilute to the line and mix. Store in a dark bottle. This solution is stable for 1 month. Or preferably use Lachat Sulfanilamide Reagent 1 CAT#52022 and Sulfanilamide Reagent 2 CAT#52023. Mix together.

10.5 Dechlorinating reagent
Dissolve 0.35g sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_20$) in 100 ml DI water.

Standard Preparation: Prepare fresh weekly or purchase equivalent from approved vendors

(obtain and retain certificates of analysis on file in the laboratory)

10.6 Stock Nitrate Standard, 100 mg/L
Dissolve 0.722 g potassium nitrate FISHER CAT# P383-500 in approximately 600 mL DI water in a 1 L flask. Add 2 mL chloroform, dilute to the line and mix. This solution is stable for 6 months.

10.7 Stock Nitrite Standard, 100 mg/L

Dissolve 0.493 g sodium nitrite FISHER CAT# S347-500 in approximately 800 mL DI water in a 1 L flask. Add 2 mL chloroform, dilute to the line and mix. Alternately, use a commercially prepared stock solution.

10.8 Working Nitrate Standard, 10 mg/L
Dilute 10.0 mL stock nitrate standard up to 100 mL with DI water.

10.9 Cadmium Efficiency Check Standard, Nitrate, 1.0 mg/L. Dilute 10 ml of 10 mg/L NO3 standard up to 100 mL with DI water.

10.10 Working Nitrite Standard, 10 mg/L
Dilute 10.0 mL stock nitrite standard up to 100 mL with DI water.

10.11 Cadmium Efficiency Check Standard, Nitrite, 1.0 mg/L. Dilute 10 ml of 10 mg/L NO2 standard up to 100 mL with DI water.

10.12 Working Standards:

Dilute the following amount of working stock standard to 100 mL. The upper and lower value of the standard curve should not be modified. If a project requires a different range the upper and lower standards may be modified but the reporting limit must also be adjusted accordingly. Samples above the high standard (+10%) must be diluted.

The mid-range standards listed below are recommended but may be modified by the analyst. If preparing standards for total nitrogen only, there is no need to use both the nitrate and nitrite working standard. Either one can be used but the total amount of standard used must remain the same as in the table below.

mL Working Nitrate Stock	mL Working Nitrite Stock	mg/L NO_3+NO_2
1.00	1.00	0.10
2.00	2.00	0.20
5.00	5.00	0.50
10.0	10.0	1.00
15.0	15.0	1.50

BS/BSD Solution:
Obtain solutions from ERA or other reliable sources. Prepare according to instructions supplied by the manufacturer.

All purchased and prepared standards and reagents are recorded in the chemical inventory logbook. All containers must be labeled with the Name, ID#, concentration, preparation date or date received, and expiration date (where applicable).

11) Sample Collection, Preservation, Shipment and Storage

For the Unified Water Study, samples are field-filtered according to UWS-approved QAPP and sampling SOP. Filters are then packaged, frozen and forwarded to IEC laboratory as per established UWS procedure and approved QAPP. See UWS QAPP and sampling SOP for further details.

11.1 Samples are to be collected in plastic or glass containers and must be refrigerated to 4^0C. The holding time for Nitrate and Nitrite as separate analytes is 48 hours.

11.2 Samples for Nitrate + Nitrite and Total Nitrogen analysis are to be preserved to pH <2 with sulfuric acid. The holding time for Nitrate + Nitrite and Total Nitrogen is 28 days. Samples collected from Western LIS monitoring surveys may be frozen for up to one year (after filtration) as per QAPP.

12) Quality Control

12.1 Calibration Curve

Acceptance Criteria - A minimum of 5 standards and a blank must be used to generate the calibration curve. The correlation coefficient must be >0.995. The % residual for each standard should be 0 ± 10% with the exception of the lowest standard which is at the reporting limit. The % residual for this standard may be ± 30%. A calibration curve should be generated at least every six months or following any major change or repair of the instrument. The calibration curve

must be verified on each working day by the analysis of an IPC standard and blank.

Corrective Action - If the correlation coefficient of the calibration curve, consisting of at least five standards and a blank, is <0.995, the calibration is disallowed. The analysis must be terminated, and repeated after correcting the problem. % residuals for the standards are monitored so that any possible instrument or dilutor troubleshooting may be performed. If the IPC check does not pass, the instrument should be recalibrated.

12.2 Instrument Performance Check (IPC) Standard or Initial Calibration Verification (ICV) and Continuing Calibration Verification (CCV) Standard.

Acceptance Criteria - Analyze the IPC solution for all determinations immediately following each calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution immediately following calibration must verify that the instrument is within $\pm$10% of the true value. Subsequent analyses of the continuing IPC solution must be within $\pm$10% of the true value.

Corrective Action - If the calibration cannot be verified within the specified limits, analysis must be discontinued, the cause determined and/or in the case of drift the instrument re-calibrated. All samples following the last acceptable IPC solution must be reanalyzed.

12.3 Laboratory Reagent Blank (LRB), Prep Blank (PB), Initial Calibration Blank (ICB) and Continuing Calibration Blank (CCB)

Acceptance Criteria - Analyze a blank along with each batch of 20 or fewer samples. All LRB/PB/ICB/CCB results must be < the Reporting Limit.

Corrective Action - If the results of the LRB/PB/ICB/CCB are > the Reporting Limit then all associated samples with a concentration of $\leq$10x the amount found

in the LRB/PB/ICB/CCB should be reprepared and reanalyzed (sample results ≥10x the amount found in the LRB/PB/ICB/CCB are not considered to be affected by the blank contamination or drift).

If the samples cannot be reprepared, then all affected sample results must be either 1) qualified accordingly, or 2) the reporting limit is raised to the amount found in the blank. Check with the team leader/section chief to determine which option should be used.

12.4 Laboratory Fortified Blank (LFB), Blank Spike/Blank Spike Duplicate (BS/BSD) or Quality Control Samples (QCS). (Also referred to as Laboratory Control Samples-LCS)

Acceptance Criteria - Analyze two LFB/BS/BSD/QCS samples with each batch of 20 or fewer samples. Calculate accuracy as percent recovery using the following equation:

$$\% \text{ Recovery} = \frac{\text{LFB/BS/BSD/QCS}}{\text{s}} \times 100$$

where:

LFB/BS/BSD/QCS = control sample results determined by laboratory

s = concentration equivalent of analyte added to fortify the LFB/BS/BSD/QCS solution.

The % recovery of the LFB/BS/BSD/QCS for samples analyzed under other programs should be within 85-115%. The relative percent difference (RPD) of the duplicates should not exceed 20% for aqueous standards.

Corrective Action - If the % recovery or RPD results are outside the required control limits, the affected samples should be reprepared and reanalyzed. If the samples cannot be reprepared, then all affected sample results must be qualified accordingly.

12.5 Laboratory Fortified Matrix (LFM) or Matrix Spike (MS) Recovery Acceptance Criteria. For samples analyzed under other programs, e.g. Ambient Water, prepare one LFM/MS per matrix for an analytical batch of 20 samples or less regardless of the number of different projects that comprise the analytical batch. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. Calculate the percent recovery, corrected for background concentration measured in the unfortified sample aliquot as per the equation below. The recovery should be 80- 120%. Calculate percent recovery using the following equation:

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

where: R = percent recovery,
Cs = fortified sample concentration,
C = sample background concentration, and
s = conc. equivalent of spike added to sample.

Corrective Action - If the % recovery of the LFM/MS is outside the required control limits, and the laboratory performance is shown to be in control, the recovery problem encountered is judged to be matrix related, not system related. The native sample result of the sample used to produce the LFM/MS must be qualified accordingly.

Note: The % recovery of the LFM/MS is not evaluated if the result of the unfortified sample concentration is >1X the level used to fortify the sample.

13) Calibration and Standardization

See Quality Control Section 12.1 for the procedure for preparing the calibration curve.

14) Procedure

14.1 Sample Preparation

Sample preparation is documented in the Sample Preparation Log Book

Screen all samples received under the NPDES program for chlorine prior to analysis. If chlorine is present, dechlorinate with sodium thiosulfate.

For samples requiring Total Nitrogen, pipet 10mL of sample into a culture tube. Add 5 mL of digestion solution, cap and vortex. Digest the samples in the autoclave for 30 minutes at 15 psi.

Note: If the final value of a sample or BS/BSD is higher than 2.0 mg/L, make an initial dilution prior to digestion since above this level there will be insufficient persulfate for complete oxidation.

14.2 Instrument Set-up

Turn on the Lachat instrument and install the proper manifold for the analysis to be performed. If nitrate and/or nitrite are to be reported separately, install both the nitrate + nitrite manifold and the nitrite only manifold. If only NO_3+NO_2 or Total Nitrogen is to be reported, use only the NO_3+NO_2 manifold. Consult the Lachat methods manual for installation details.

Make sure the cadmium column on the NO_3+NO_2 manifold is off line, then pump Milli-Q water through the system and check for leaks and smooth flow. Perform any routine maintenance if necessary. Once instrument is stable, remove reagent lines from the water and place in the proper reagent receptacle. Turn on the cadmium reduction column once all reagents are flowing. **Do not run water thru the cadmium column!**

Instrument Maintenance and Repair records are entered into the Lachat Instrument Maintenance Log.

14.3 Sample Analysis

Click on the Omnion icon. From file, open the method file for the analysis being performed. Update the run worksheet with the correct sample IDs. Make any changes to the method that may be necessary, i.e. changes in standard concentrations, etc.

All Lachat methods have been configured to alert the user if the QC criteria Method Performance section 16 has been met. If the criteria is not met, follow the corrective action in the appropriate section.

Enter all standards and samples into the run worksheet protocol. Enter any necessary

dilutions into the worksheet such as those done during the distillation step or those due to high level standards or samples.

When ready to start analysis, click on the start button at the top of the Windows screen.

Once the run is finished, click on tools, custom report and open format. Choose standard report and click OK. Preview the report and make any required changes before printing. Print the report and exit from the reporting area.

Data from the Lachat instrument is backed up periodically.

Close the flow to the cadmium column, then remove all reagent lines and place into a DI water receptacle. Flush system with water for at least 15 minutes. Remove lines from water and allow air to pump through system. Release tension on pump tubing by lowering arms on pump.

15) Calculations

15.1 Integration

Since the peak expectation window may shift within a method if any of the instrument conditions change, an analyst may need to reintegrate data after the run is finished. Some of the instrument conditions that affect timing are ones that will either speed up or slow down the flow of sample between the autosampler and detector such as changing pump tubing or removing a clog from a reagent line. If the peak expectation window is adjusted for any sample in a run, the adjustment is made to each sample and standard in the entire run. When setting the peak expectation window, the entire area of the subject peak and only the area of the subject peak should be integrated. If any analytical run is altered after analysis such as modifying the peak expectation window, save the data file using the original date/time stamp and add the word reprocessed to the end so that the original and modified data file are saved. Refer to the Lachat User Manual for specific instructions on operating this software.

Sample results are calculated by the Omnion 3.0 software supplied with the Lachat autoanalyzer. The calculations are performed by determining the area of each sample or standard peak that falls within a set peak expectation window. The peak expectation window is determined separately for each method based upon the time needed for a sample to travel from the autosampler to detector and also the width of each peak.

The efficiency of the cadmium column must be calculated each time Nitrate, Nitrite and Nitrogen are analyzed. The efficiency should be between 90-110%. If the efficiency is outside of this range, stop the analysis and perform required system or column maintenance before continuing.

$$\% \text{ cadmium efficiency: } \frac{1.0 \text{ mg/L } NO_3 \text{ result}}{1.0 \text{ mg/L } NO_2 \text{ result}} \times 100$$

16) Method Performance

A demonstration of capability (DOC) should be performed each time there is a significant change in the chemistry of the method, a major modification to an existing instrument, or a new instrument is installed. An initial DOC is performed by each analyst designated to analyze samples using this method. An annual check, which consists of replicate analyses of four QC samples or satisfactory analysis of one proficiency sample, must subsequently be performed and documented for each analyst using this method.

16.1 Demonstration of Capability

A demonstration of capability study must be performed and documented for each analyst using this method. The study should consist of the analysis of four standards which are from a source independent of the standard curve. The results of the standards must be within the acceptance criteria for BS/BSD samples in section 14. If samples are analyzed under more than one program, the acceptance criteria used should be the tighter limits. The % RSD should be within 20%. The results of the accuracy and precision study (true value, % recovery, standard deviation and % RSD) are maintained by the Quality Assurance Officer for each analyst and are located in the IEC Laboratory Active Employee Training Manual.

Continuing Demonstration of Capability

An annual continuing demonstration of capability study must be performed and documented. It may consist of either successfully analyzing a PT sample or analyzing 2 sets of BS/BSD standards to within control limits as stated in section 12The results of the continuing accuracy and precision study (true value, % recovery, standard deviation and % RSD or final report from the PT provider) are maintained by the Quality Assurance Officer for each analyst and are located in the IEC Laboratory Active Employee Training Manual.

16.2 Method Detection Limit (MDL)

An MDL Study was conducted for this method. The study is based on the requirements listed in 40 CFR Part 136 Appendix B. The MDL Study comprised the analysis of seven reagent grade water samples fortified at a level between 2-3x the detection limit. The results of the MDL determination (true value, average concentration, standard deviation and calculated MDL) are maintained by the Quality Assurance Officer for each method and are located in the Lachat analysis Log Book.

16.3 Limit of Quantitation (LOQ)

The Laboratory performs a Limit of Quantitation (LOQ) study on an annual basis for analytes associated with chemistry methods. The validity of LOQ is confirmed by successful analysis of a Laboratory Fortified Blank (LFB) at approximately 2X the reporting limit. The acceptance criteria for each analyte is ± 30% of the true value. After this study is completed, it is reviewed and approved by the Laboratory Management. A summary of all LOQ study performance is maintained.

17) Pollution Prevention

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the recycling as the next best option.

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

For information about pollution prevention consult the IEC Laboaratory's Health and Safety Manual

18) Data Assessment and Acceptance Criteria for Quality Control Measures

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions.

19) Corrective Actions for Out-of-Control Data

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

See Quality Control, Section 12 for a description of contingencies for handling out-of-control or unacceptable data (corrective actions)

21) Waste Management

The IEC requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. IEC urges staff to protect the air, water and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any water discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

22) References

EPA Methods for the Determination of Inorganic Substances in Environmental Samples, Rev. 2.0 August 1993, Method 353.2

Lachat QuikChem Automated Ion Analyzer Methods Manual, Method 10-107-04- 1-A,B,C. July 2008.

Standard Methods for the Examination of Water and Wastewater, Method 4500- NO_3^- I Cadmium Reduction Flow Injection Method, 21st Edition, 2000

23) Tables, Diagrams, Flowcharts and Validation Data

None.

See Lachat Manual QuickChem Method 31-107-04-4-A,B,C (above).

TOTALPHOSPHOROUS/ORTHOPHOSPHATE

1. Test method

1.1 This SOP is based on EPA Method 365.1, Revision 2.0. Determination of phosphorous by semi-automated colorimetry.

2. Applicable Matrix or Matrices

This method is applicable to surface and saline waters, and domestic and industrial waste waters.

3. Method Detection Limits

MDL Orthophosphate is 0.00083 mg/L Reporting Limit 0.002 mg/ L MDL Total Phosphorus is 0.053 mg/L Reporting Limit 0.133 mg/L

4. Scope And Application

4.1 This analysis is performed by Flow Injection analysis (FIA).

4.2 The orthophosphate method determines total orthophosphate or if the sample is filtered through a 0.45 micron pore size filter, the result is termed dissolved orthophosphate. The difference between the result of a sample determined directly and filtered is termed insoluble (particulate) orthophosphate

4.3 The Total Phosphorus method determines total phosphorus, or if the sample is filtered through a 0.45micron pore size filter, the result is termed total dissolved phosphorus. The method are based on reactions that are specific for the orthophosphate (PO_4^{3-}) ion.

5. Summary of Method

The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample. Polyphosphates may be converted to the orthophosphate form by sulfuric acid digestion and organic phosphorous may be converted to orthophosphate by persulfate digestion. The determination of Total phosphorus (or total dissolved phosphorus) is performed by

performing this method on digested samples, and utilization of a Total Phosphorus manifold.

6. Definitions

Phosphorus is a nutrient that occurs in natural waters and in wastewaters almost solely as phosphates. Small amounts of orthophosphate or certain condensed phosphates are added to some water supplies during treatment. Larger quantities are added through use of detergents and fertilizers. Phosphorus is essential to the growth of organisms and can be the limiting nutrient for primary productivity of an organism.

7. Interferences

7.1 Orthophosphate: Only orthophosphate forms a blue color in this test. Polyphosphates and organic phosphorus compounds are not recovered. The sulfuric acid in the molybdate reagent does not have enough contact time with polyphosphates to hydrolyze them. The PO_4^{3-} ion reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form an antimony-phosphomolybdate complex. This complex is reduced with ascorbic acid to forma blue complex which absorbs light at 880nm. The absorbance is proportional to the concentration of PO_4^{3}- in the digested sample. Sample turbidity must be removed by filtration immediately after sampling for orthophosphate samples

7.2 Total Phosphorus: Samples for dissolved total phosphorous should be filtered only after digestion. Silica forms a pale blue complex, which also absorbs at 880nm, this interference is generally insignificant as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg P/L positive error in orthophosphate. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glass.

7.3 High concentrations of iron may interfere by consuming some of the reducing agent, resulting in low recoveries. This interference is eliminated by the addition of sodium bisulfite. Sample color that absorbs in the photometric range used for analysis will also interfere

7.4 Arsenate is a positive interference. If it is known to be present it can also be eliminated by the addition of sodium bisulfite.

7.5 This method is very sensitive to contamination. Detergent can contain extremely high concentrations of phosphorous. All glassware must be scrupulously cleaned

and acid washed before use.

8. Safety

8.1 The toxicity and carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be minimized by good laboratory practices, e.g. wear proper protective equipment, safety glasses, gloves, lab coat and working inside hoods whenever possible.

8.2 Refer to the IEC Laboratory Health and Safety Manual and College of Staten Island Chemical Hygiene Plan for specific guidelines.

8.3 For detailed explanations consult the Material Safety Data Sheets (MSDS), available in the Laboratory.

8.4 The following chemicals have the potential to be highly toxic or hazardous, for detailed explanations consult the MSDS.

8.4.1 Sulfuric Acid
8.4.2 Dodecyl Sulfate

9. Equipment and Supplies

9.1 Balance

9.2 Glassware-Class A volumetric flasks, pipettes or plastic containers as required. Samples may be stored in plastic or glass. All glassware used in the determination of phosphate should be washed with hot 1:1 HCl and rinsed with distilled water. Preferably, this glassware should be used only for the determination of phosphate and after use it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with 1:1 HCl is only required occasionally.

9.3 Lachat Autoanalyzer, manifold reaction unit (heating unit), colorimetric detector, 880 nm wavelength filter and Omnion software data system.

9.4 Autoclave

9.5 Miscellaneous laboratory apparatus such as volumetric pipettes, flasks, etc.

10. Reagents And Solutions

10.1 Reagents

10.1.1 Stock Ammonium Molybdate Solution

Dissolve 40.0 g ammonium molybdate tetrahydrate in approximately 800 mL of water in a 1 liter flask. Dilute to the line and mix. Store in plastic and refrigerate.

10.1.2 Stock Antimony Potassium Tartrate Solution
Dissolve 3.0g antimony potassium tartrate (potassium antimonyl tartrate hemihydrate) or 3.22 g antimony potassium tartrate (potassium antimonyl tartrate trihydrate) in approximately 800 mL of water in a 1 liter flask.
Dilute to the line and mix. Store in a dark bottle and refrigerate.

10.1.3 Molybdate Color Reagent
Add approximately 500 mL water to a 1 liter flask. Slowly add 21.0 mL of concentrated sulfuric acid while mixing. When the solution can be comfortably handled, add 72.0 mL of Stock Antimony Potassium Tartrate Solution and 213 mL of Ammonium Molybdate Solution. Dilute to 1 liter and mix. Use HACH LACHAT Color reagent CAT# 52002 as alternative.

10.1.4 Ascorbic Acid Reducing Solution, 0.33 M
Dissolve 60.0 g granular ascorbic acid in approximately 700 mL of water in a 1-liter flask. Dilute to the mark and mix. Add 1.0 g sodium dodecyl sulfate and mix. Prepare fresh weekly and discard if the solution becomes yellow. Use HACH LACHAT Ascorbic Acid reagent CAT# 52020 as alternative.

10.1.5 Carrier, Sulfuric Acid, 0.13M
Add approximately 500 mL water to a 1-liter flask. Add 7.2 mL of concentrated sulfuric acid. Dilute to the mark and mix.

10.1.6 Carrier, Dodecyl Sulfate (Recommended by Lachat for Brackish and Seawater analysis).
To 1 Liter volumetric flask containing about 900 mL of DI water, add 1.0g of dodecyl sulfate. Dilute to the mark. Stir to mix. Do not degas this reagent.

10.1.7 Ammonium Persulfate Digestion Solution
Dissolve 8.0 g of ammonium persulfate in 50 mL DI water. While mixing, slowly add 6.2 mL of concentrated sulfuric acid and dilute to 100 ml. Make fresh weekly.

10.1.8 Sodium Persulfate Digestion Solution (Recommended by Lachat for Brackish and Seawater Analysis).

In 1 Liter volumetric flask, dissolve 50 g sodium persulfate in about 800 mL DI water. Stir until dissolved. Dilute to the mark, and invert to mix. Prepare fresh monthly. Store in plastic.

If necessary, prevent bubble formation by degassing all prepared solutions except the standards with helium. Use He at 140kPa (20 lb/in^2). Preferably, purchase equivalent solutions which should not require degassing.

10.2 Standard Preparation

10.2.1 Stock Phosphorous Standard, 100 mg/L
In a 1-liter volumetric flask, dissolve 0.4396g anhydrous potassium phosphate monobasic (KH_2PO_4) FISHER CAT# P382-500, that has been dried for one hour at 105ºC.

10.2.2 Working stock standard, 10 mg/L
Dilute 10.0 mL of Stock Phosphorous Standard to 100 mL with DI water.

10.2.3 Working Standards

Dilute the following mL of working stock standard to 100 mL. The upper and lower value of the standard curve should not be modified. If a project requires a different range the upper and lower standards may be modified but the reporting limit must also be adjusted accordingly. Samples above the high standard (+10%) must be diluted. The mid-range standards listed below are recommended but may be modified by the analyst.

mL Working Stock	mg/L Phosphorous
50.0	5.00
40.0	4.00
20.0	2.00
10.0	1.00
5.0	0.500
1.0	0.100

Note: Select and prepare the required standards according to the analysis and linear range being used.

10.2.4 BS/BSD Solution

Obtain solutions from ERA, EMSL or other reliable sources. Prepare according to instructions provided by the supplier.

10.3 All purchased and prepared standards and reagents are recorded in the chemical inventory logbook (purchased) or the standards preparation logbook (prepared) which assigns a unique ID# to each. All containers must be labeled with the Name, ID#, concentration, preparation date and expiration date (where applicable).

11. Sample Collection, Preservation, Storage and Holding Time

For the Unified Water Study, samples are field-filtered according to UWS-approved QAPP and sampling SOP. Filters are then packaged, frozen and forwarded to IEC laboratory as per established UWS procedure and approved QAPP. See UWS QAPP and sampling SOP for details.

11.1 Sample containers may be of plastic or glass. All bottles must be thoroughly cleaned and acid rinsed. The volume collected should be sufficient to insure a representative sample and allow for replicate analysis if required. Samples for orthophosphate should be filtered immediately upon collection, with a maximum holding time of 48 hours.

11.2 Samples must be refrigerated to 4^0C.

11.3 Samples for total phosphorous are preserved by the addition of sulfuric acid to a pH of<2 and analyzed within 28 days of collection. This is accomplished by adding no more than 2 mL concentrated H_2SO_4 per liter and verifying that the pH is less than 2. If the pH is still greater than 2, more sulfuric acid is added until the pH is <2.

11.4 Samples for orthophosphate are not preserved and must be analyzed within 48 hours of collection.

11.5 Digested samples may be store up to one month in screw cap tubes.

11.6 Samples collected from Western LIS monitoring survey may be frozen for up to one year (after filtration) as per QAPP.

12. Quality Control

a. Calibration Curve

Acceptance Criteria - A minimum of 5 standards and a blank must be used to generate the calibration curve. The correlation coefficient must be >0.995. The % residual for each standard should be 0 ± 10% with the exception of the lowest standard which is at the reporting limit. The % residual for this standard may be ± 30%. A calibration curve should be generated at least every run or following any major change or repair of the instrument. The calibration curve must be verified on each working day by the analysis of an IPC standard and blank. Analyze at least five standards and a blank using a first order calibration curve. If any verification data exceeds the initial values by ± 10%, reanalyze the standards. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion. The LCR must be verified every six months or whenever a significant change in instrument response is observed.

Corrective Action - If the correlation coefficient of the calibration curve, consisting of at least five standards and a blank, is <0.995, the calibration is disallowed. The analysis must be terminated, and repeated after correcting the problem. % residuals for the standards are monitored so that any possible instrument or dilutor troubleshooting may be performed. If the IPC check does not pass, the instrument should be recalibrated.

b. Instrument Performance Check (IPC) Standard or Initial Calibration Verification (ICV) and Continuing Calibration Verification (CCV) Standard.

Acceptance Criteria - Analyze the IPC solution for all determinations immediately following each calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution immediately following calibration must verify that the instrument is within ±10% of the true value. Subsequent analyses of the continuing IPC solution must be within ±10% of the true value.

Corrective Action - If the calibration cannot be verified within the specified limits, analysis must be discontinued, the cause determined and/or in the case of drift the instrument re-calibrated. All samples following the last acceptable IPC solution must be reanalyzed.

c. Laboratory Reagent Blank (LRB), Prep Blank (PB), Initial Calibration Blank (ICB) and Continuing Calibration Blank (CCB)

Acceptance Criteria - Analyze a blank along with each batch of 20 or fewer samples. All LRB/PB/ICB/CCB results must be < the Reporting Limit.

Corrective Action - If the results of the LRB/PB/ICB/CCB are > the Reporting Limit then all associated samples with a concentration of <10x the amount found in the LRB/PB/ICB/CCB should be reprepared and reanalyzed (sample results ≥10x the amount found in the LRB/PB/ICB/CCB are not considered to be affected by the blank contamination or drift).

If the samples cannot be reprepared, then all affected sample results must be either 1) qualified accordingly, or 2) the reporting limit is raised to the amount found in the blank. Check with the team leader/section chief to determine which option should be used.

d. Laboratory Fortified Blank (LFB), Blank Spike/Blank Spike Duplicate (BS/BSD) or Quality Control Samples (QCS)

Acceptance Criteria - Analyze two LFB/BS/BSD/QCS samples with each batch of 20 or fewer samples. Calculate accuracy as percent recovery using the following equation:

$$\% \text{ Recovery} = \frac{\text{LFB/BS/BSD/QCS}}{\text{s}} \times 100$$

where:

LFB/BS/BSD/QCS = control sample results determined by laboratory

s = concentration equivalent of analyte added to fortify the LFB/BS/BSD/QCS solution.

The % recovery of the LFB/BS/BSD/QCS for samples analyzed under NPDES program should be within 10% of true value. The relative percent difference (RPD) of the duplicates should not exceed 20% for aqueous standards.

Corrective Action - If the % recovery or RPD results are outside the required control limits, the affected samples should be reprepared and reanalyzed. If the samples cannot be reprepared, then all affected sample results must be qualified accordingly.

e. Laboratory Fortified Matrix (LFM) or Matrix Spike (MS) Recovery Acceptance

Criteria:
For samples analyzed under NPDES program, prepare one LFM/MS per matrix for an analytical batch of 10 samples or less regardless of the number of different projects that comprise the analytical batch. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. Calculate the percent recovery, corrected for background concentration measured in the unfortified sample aliquot as per the

equation below. The % recovery for NPDES or DW samples should be 90-110%. The recovery for all other programs should be 80- 120%.

Corrective Action - If the % recovery of the LFM/MS is outside the required

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

where: R = percent recovery,
Cs = fortified sample concentration,
C = sample background concentration, and
s = conc. equivalent of spike added to sample.

control limits, and the laboratory performance is shown to be in control, the recovery problem encountered is judged to be matrix related, not system related. The native sample result of the sample used to produce the LFM/MS must be qualified accordingly.

Note: The % recovery of the LFM/MS is not evaluated if the result of the unfortified sample concentration is >1X the level used to fortify the sample.

13. Calibration

See Section 12 Quality Control for Calibration Procedures.

14. Procedure

14.1 Sample Preparation

14.1.1 Sample preparation is documented in the Sample Digestion Log Book.

Digestion procedure for total phosphorous

14.1.2 Check sample pH before digestion and record on digestion bench sheet. Samples should be preserved to a pH of <2.

14.1.3 Measure 10 mLs of each standard and sample into a digestion tube and add 1.0 mL of persulfate digestion solution. Vortex.

14.1.4 Heat for 30 minutes in an autoclave at 121oC (15-20 psi).

14.1.5 Cool and remix on vortex mixer. If the samples are turbid after digestion, filter prior to analysis

14.2 Instrument Operating Conditions

14.2.1 Turn on the Lachat instrument and install the proper manifold for the analysis to be performed. Use the shorter heater coil and set the temperature to 37o C. Consult the Lachat methods manual for installation details if necessary.

14.2.2 Pump Milli-Q water through the system and check for leaks and smooth flow. Perform any routine maintenance if necessary. Once instrument is stable, remove reagent lines from the water and place in the proper reagent receptacle.

14.2.3 Instrument Maintenance and Repair records are entered into the Instrument Maintenance Log.

14.3 Sample Analysis

14.3.1 Click on the Omnion icon. From file, open the method file for the analysis being performed. Update the run worksheet with the correct sample IDs. Make any changes to the method that may be necessary, i.e. changes in standard concentrations, etc.

14.3.2 Place standards and samples as per the run worksheet protocol. Enter any necessary dilutions into the worksheet such as those done during the distillation step or those due to high level standards or samples. When diluting samples, use the appropriate volume of digestion solution so that the matrix matches that of the standard curve.

14.3.3 When ready to start analysis, click on the start button at the top of the Windows screen.

14.3.4 All Lachat methods have been configured to alert the user if the QC criteria in sections 14.1-14.3.5 has been met. If the criteria are not met, follow the corrective action in the appropriate section.

14.3.5 Once the run is finished, click on tools, custom report and open format. Choose standard report and click OK. Preview the report and make any required changes before printing. Print the report and exit from the reporting area.

14.3.6 Data from the Lachat instrument is backed up periodically.

14.3.7 Remove all reagent lines and place into a DI water receptacle. Flush system with water for at least 15 minutes. Remove lines from water and allow air to pump through system. Release tension on pump tubing by lowering arms on pump.

15. Calculations

15.1 Integration

Since the peak expectation window may shift within a method if any of the instrument conditions change, an analyst may need to reintegrate data after the run is finished. Some of the instrument conditions that affect timing are ones that will either speed up or slow down the flow of sample between the sampler and detector such as changing pump tubing or removing a clog from a reagent line. If the peak expectation window is adjusted for any sample in a run, the adjustment is made to each sample and standard in the entire run. When setting the peak expectation window, the entire area of the subject peak and only the area of the subject peak should be integrated. If any analytical run is altered after analysis such as modifying the peak expectation window, save the datafile using the original date/time stamp and add the word reprocessed to the end so that the original and modified datafile are saved. Refer to the Lachat User Manual for specific instructions on operating this software.

15.2 Calculations

Sample results are calculated by the Omnion 3.0 software supplied with the Lachat autoanalyzer. The calculations are performed by determining the area of each sample or standard peak that falls within a set peak expectation window. The peak expectation window is determined separately for each method based upon the time needed for a sample to travel from the sampler to detector and also the width of each peak.

16. Method Performance

A demonstration of capability (DOC) should be performed each time there is a significant change in the chemistry of the method, a major modification to an existing instrument, or a new instrument is installed. A DOC is performed by each analyst designated to analyze samples using this method. An annual check, consisting of satisfactory performance of four replicate QC samples or one proficiency test sample, must subsequently be performed and documented for each analyst using this method.

a. Accuracy and Precision

i. Demonstration of Capability

A demonstration of capability study must be performed and documented for each analyst using this method. The study should consist of the analysis of four standards which are from a source independent of the standard curve. The results of the standards must be within the acceptance criteria for BS/BSD samples in section 14. If samples are analyzed under more than one program, the acceptance criteria used

should be the tighter limits. The % RSD should be within 20%. The results of the accuracy and precision study (true value, % recovery, standard deviation and % RSD) are maintained by the Quality Assurance Officer for each analyst and are located in the IEC Laboratory Active Employee Training Manual.

ii. Continuing Demonstration of Capability

An annual continuing demonstration of capability study must be performed and documented. It may consist of either successfully analyzing a PT sample or analyzing 2 sets of BS/BSD standards to within control limits as stated in section 13.1.1. The results of the continuing accuracy and precision study (true value, % recovery, standard deviation and % RSD or final report from the PT provider) are maintained by the Quality Assurance Officer for each analyst and are located in the IEC Laboratory Active Employee Training Manual.

b. Method Detection Limit (MDL)

An MDL Study was conducted for this method. The study is based on the requirements listed in 40 CFR Part 136 Appendix B. Specific procedures for conducting an MDL study can be found in SOP # G-8. The MDL Study comprised the analysis of seven reagent grade water samples fortified at a level between 2-3x the detection limit. The results of the MDL determination (true value, average concentration, standard deviation and calculated MDL) are maintained by the Quality Assurance Officer for each method and are located in the Laboratory.

c. Limit of Quantitation (LOQ)

The Laboratory performs a Limit of Quantitation (LOQ) study on an annual basis for analytes associated with chemistry methods. The validity of LOQ is confirmed by successful analysis of a Laboratory Fortified Blank (LFB) at approximately 2X the reporting limit. The acceptance criteria for each analyte is ± 30% of the true value. After this study is completed, it is reviewed and approved by the Laboratory Management. A summary of all LOQ study performance is maintained in the Laboratory.

17. Pollution Prevention

17.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The IEC has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation.

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

18. Data Assessment and Acceptance Criteria for Quality Control Measures

18.1 Laboratory Spike Sample Matrix (LSM) – The laboratory must spike, in duplicate, minimum of 10% of routine samples. In each case the LSM aliquots must be duplicate of the aliquot used for sample analyses. The spiking level shall be at 1 to 5 times higher than the background concentration of the sample.

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

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

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

18.2 If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control (sect. 18.3), the recovery problem encountered with the LSM is judge to be either matrix or solution related, not system related.

18.3 Computer the relative percent difference (RPD) between the two LSM results and compare the value to the designated RPD recovery range of 10%. The RPD may be calculated using the following equation:

$$RPD = \frac{(D_1 - D_2)}{(D_1 + D_2)/2} \times 100$$

Where D1= concentration of analyte in the sample, D2= concentration of analyte in the second (duplicate) sample.

18.4 If the RPD falls outside the designated recovery range and the laboratory performance for that analyte is shown to be in control (sect 18.3), the recovery problem encountered with the LSM is judge to be either matrix or solution related, not system related.

18.5 Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the

ability to perform the method acceptably.

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions.

19. Corrective Actions for Out-of-Control Data

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions

20. Contingencies for Handling Out-Of-Control Data

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions

21. Waste Management

The IEC requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any water discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

22. References

Lachat QuikChem Automated Ion Analyzer Methods Manual, Method 10-115-01-1-E (2001) Determination of Total Phosphorus by Flow Injection Analysis (Acid Persulfate Digestion Method)

Lachat QuikChem Automated Ion Analyzer Methods Manual, Method 10-115-01-1-A (2007) Determination of Ortho Phosphate in Waters by Flow Injection Analysis Colorimetry

Standard Methods for the Examination of Water and Wastewater, Method 4500-P G. Flow Injection Analysis for Orthophosphate, 21st Edition, 1999.

Standard Methods for the Examination of Water and Wastewater, Method 4500-PH. Flow Injection Analysis for Total Phosphorous (Proposed), 20th Edition, 1998

23. Tables, Diagrams, Flowcharts, And Validation Data

See Lachat Methods 10-115-01-1-E (2001) and Lachat Method 10-115-01-1-A (2007) in Lachat methods manual

Enterococci In Water By Membrane Filtration Using Membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI)

1) Test Method

Based on EPA Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI).

2) Applicable Matrix or Matrices

This test can be used for the enumeration of enterococci in non-potable, fresh, estuarine, marine, and shellfish growing waters.

3) Method Detection Limit

Because a wide range of sample volumes and dilutions can be analyzed by the membrane filtration technique, a wide range of enterococci levels in water can be detected and enumerated. Detection limits will vary depending on the volumes and dilutions used in filtration.

4) Scope and Application

This method is not routinely used by the Interstate Environmental Commission laboratory, however may be used for special ambient water quality monitoring projects. Proficiency samples are analyzed semi-annually and continuing demonstrations of capability are analyzed annually to maintain certification. This method describes a membrane filter (MF) procedure for the detection and enumeration of the enterococci bacteria in non-potable (rev.1/03/11) water. The enterococci test measures the bacteriological quality of recreational waters. Epidemiological studies have led to the development of criteria which can be used to promulgate recreational water standards based on the established relationship between health effects and water quality. The significance of finding enterococci in recreational water samples is the direct relationship between the density of enterococci in the water and swimming-associated gastroenteritis studies of marine and fresh water bathing beaches. The test for enterococci can be applied to potable, fresh, estuarine, marine, and shellfish growing waters. The enterococci test is recommended as a measure of ambient fresh and marine recreational water quality.

5) Summary of Method

This method provides a direct count of bacteria in water based on the development of colonies on the surface of the membrane filter. A water sample is filtered through the membrane which retains the bacteria. Following filtration, the membrane containing the bacterial cells is placed on a selective medium, mEI agar, and incubated for 24 hr. at 41.0±0.5C. All colonies (regardless of

color) of 0.5 mm diameter with a blue halo are recorded as enterococci colonies. Magnification and a small fluorescent lamp are used for counting to give maximum visibility of colonies.

6) Definitions

In this method, enterococci are those bacteria which produce colonies of 0.5mm diameter with a blue halo after incubation on mEI agar. The blue halo should not be included in the colony diameter measurement. Enterococci include *Enterococcus faecalis*, *E. faecium*, *E. avium, E. gallinarium* and their variants. The genus *Enterococcus* includes the enterococci formerly assigned to the Group D fecal streptococci.

7) Interferences

Water samples containing colloidal or suspended particulate materials can clog the membrane filter and prevent filtration, or cause spreading of bacterial colonies which could interfere with identification of target colonies.

8) Safety

Wear appropriate personal protective equipment, including lab coat, goggles, and gloves. Avoid breathing dust of dehydrated media. Wear a respirator and/or prepare broth in the hood. Autoclave all spent tubes at 121C±2C for 30 minutes. Dispose all spent tubes and spent plates in to red biohazardous medical waste container, located underneath BOD bench. See Pollution Prevention and Waste Management sections for more information on the safe disposal of microbiological waste. Take extreme precaution when handling micoorganisms used for positive and negative control tests. The manufacturers MSDS's are retained and should be reviewed prior to inoculating these organisms. Mouth-pipetting is prohibited.

9) Equipment and Supplies

1. Glass lens with magnification of 2-5x or stereoscopic microscope.
2. Lamp, with a cool, white fluorescent tube.
3. Hot plate/stir plate combination plate (Isoterm by Fisher Scientific).
4. Pipets, sterile, T.D. bacteriological plastic, of appropriate volume.
5. Graduated cylinders, 100-1000 ml, covered with aluminum foil or kraft paper and sterile.
6. Membrane filtration units (filter base and funnel), plastic, wrapped with aluminum foil or kraft paper and sterilized.
7. Line vacuum, electric vacuum pump, or aspirator for use as a vacuum source.
8. A stainless steel filter manifold to hold a number of filter bases is optional.
9. Flask (or carboy) for safety trap placed between the filter flask and the vacuum source.
10. Forceps, straight or curved, with smooth tips to handle filters without damage.

11. Ethyl Alcohol, absolute 200 proof A.C.S. reagent (ACROS Cat # 61509-0040) in a small, wide-mouth container, for sterilizing forceps.
12. Electric incinerator unit for sterilizing loops and needles.
13. Thermometer, checked against a National Institute of Standards and Technology (NIST) certified thermometer, or one that meets the requirements of NIST Monograph SP 250-23.
14. Petri dishes, sterile, plastic, 9 x 50 mm, with tight-fitting lids.
15. Dilution tubes marked at 9 ml may be used for dilutions.
16. Flasks, borosilicate glass, screw-cap, 250-2000 ml volume.
17. Membrane filters, sterile, white, grid marked, 47 mm diameter, with 0.45Φm pore size.
18. Inoculation loops, at least 3 mm diameter, Sterile, disposable plastic loops are used.
19. Incubator maintained at 41.0 ±0.5C.
20. Waterbath maintained at 50.0C for tempering agar.
21. Test tubes, 16 x 150 mm, borosilicate glass or plastic.
22. Caps, aluminum or autoclavable plastic, for 16 mm diameter test tubes.
23. Test tubes, screw-cap, borosilicate glass, 16 x 125 mm or other appropriate size.
24. Whirl-Pak® bags.
25. Autoclave (Tuttnauer 2540E-B/L Heidolph Brinkmann).
26. CE Colony Counter Pen Cat. # 17649 Lot #0811498.
27. Balance (Mettler Toledo PB303).
28. pH meter, with flat-tip probe.
29. Refrigerator, maintained at 1.0-4.0 C for storing broth.
30. Sterilizer indicator tape (Fisher Catalog # 11-875-50).

10) Reagents and Standards

1. *Enterococcus faecalis* ATCC #19433 BioBalls (BTF PTY Precise Microbiology, Sydney, Australia).

2. *Escherichia coli* ATCC #11775 BioBalls (BTF PTY Precise Microbiology, Sydney, Australia)

3. *S. mitis (*Fisher Catalog # 23-001164)

4. pH 4.00, 7.00 and 10.00 buffers for calibrating the pH meter

5. Sodium Chloride

6. 1N NaOH for adjusting pH of buffer

7. Plate Count Agar (for QC check on dilution water)

8. Reagent- grade distilled or deionized water.

9. Phosphate buffered saline (PBS)
Composition:

Monosodium phosphate	0.58g
Disodium phoshate	2.5g
Sodium chloride	8.5g
Reagent-grade water	1.0L

Dissolve the reagents in 1 L of reagent-grade water and dispense in appropriate amounts for dilutions in screw cap bottles or culture tubes, and/or into containers for use as rinse water. Autoclave after preparation at 121± 2C and 15 PSI for 15 minutes. Final pH should be 7.4±0.2. Alternatively, phosphate buffered saline can be purchased from Fisher Scientific, Catalog # BP 2438-20. Purchased phosphate buffered saline must be autoclaved and sterility-checked before use.

10. mEI Agar -BD/DIFCO ®mEI agar. BD Reference # 214884. Purchased through Fisher Scientific, catalog # B14885. Prepare according to manufacturer's directions (on bottle), making sure to add the appropriate reagents after sterilization (see below).

11. Nalidixic Acid: Mix 0.24 g nalidixic acid (Fisher Cat. # BP 908-25) in 5 ml reagent-grade sterile distilled water, add a few drops of 0.1N NaOH to dissolve; add to the mEI medium.

12. Triphenyltetrazolium chloride (TTC): Add 0.1 g of TTC (Fisher Cat. # T-413) to 10 ml of reagent-grade distilled water, and warm to dissolve. Filter-sterilize the solution, and add 2 ml per liter of medium.

Preparation of mEI Agar Plates: Pour the mEI agar into 9x50 mm petri dishes to a 4-5 mm depth (approximately 4-6 ml), and allow to solidify. Final pH of medium should be 7.1± 0.2. Store in a refrigerator.

13. Brain Heart Infusion Broth (BHIB). BD Bacto™ Brain Heart Infusion (BD Reference # 237500)

Preparation: Combine ingredients except reagent-grade distilled water. Dissolve 37 g of combined ingredients in 1 L of reagent grade water. Dispense in 8-10 ml volumes in screw-cap tubes and autoclave at 121±2.0C (15 lb pressure) for 15 min. If the medium is not used the same day as prepared and sterilized, heat in boiling water bath for several min to remove absorbed oxygen, and cool quickly without agitation, just prior to inoculation. The final pH should be 7.4 ±0.2.

14. Brain Heart Infusion Broth (BHIB) with 6.5% NaC1
Composition:
BHIB with 6.5% NaC1 is the same as BHIB above, but with additional NaCl.
Preparation: Add 60.0 g NaC1 per liter of medium. Since most commercially available

dehydrated media contain sodium chloride, this amount is subtracted from the 65 g per liter required to make a final concentration of 6.5% NaCl.

15. Brain Heart Infusion Agar (BHIA).

Can be purchased commercially prepared through Fisher Scientific (Difco Brain Heart Infusion

Agar, Fisher Catalog # DF0418-15-9)

Composition:
BHIA contains the same components as BHIB above with the addition of 15.0 g of agar per L of BHIB.
Preparation: Suspend 52 g dehydrated BHIA in 1 L of reagent-grade distilled water. Heat to boiling until the ingredients are dissolved. Dispense 10 ml of medium in screwcap test tubes, and sterilize for 15 min at 121±2°C (15 lb pressure). After sterilization, slant until solid. Final pH should be 7.4 ± 0.2.

16. Bile Esculin Agar (BEA)

Can be purchased commercially prepared through Fisher Scientific.

Composition:
Bacto Beef Extract 3.0 g
Bacto Peptone 5.0 g
Bacto Oxgall 40.0 g
Bacto Esculin 1.0 g
Ferric Citrate 0.5 g
Bacto Agar 15.0 g
Reagent-Grade Distilled Water 1.0 L

Preparation: Combine dry ingredients. Add 64.0 g of dry ingredients to 1 L reagent-grade distilled water, and heat to boiling to dissolve completely. Dispense 10-mL volumes in tubes for slants or larger volumes into flasks for subsequent plating. Autoclave at 121±2°C (15 lb pressure) for 15 min. Overheating may cause darkening of the medium. Cool in a 50°C waterbath, and dispense into sterile petri dishes. Final pH should be 6.6 ± 0.2. Store in a refrigerator.

17. Azide Dextrose Broth (ADB)-The dehydrated azide dextrose broth is commercially available and may be procured from a certified supplier (Becton Dickinson, formerly Difco Laboratories, Catalog no. 238710). Dissolve 34.7g of the above dehydrated broth in 1L of de-ionized water. Sterilize at 121±2^{o}C for 15 minutes.

11) Sample Collection, Preservation, and Storage

Samples for microbiological analysis should be collected directly into 125 ml sterilized bottles. Bottles are labeled with sterilization date and sealed heat-sensitive tape that indicates the bottles have reached sterilization temperature. Remove the cap, immediately prior to sampling, taking care so that there is no contamination to the inside of the cap or the bottle. If sampling from a spigot, which is the case at some municipal wastewater treatment plants and industrial facilities, place the open bottle directly below but not touching the spigot, fill the bottle to the neck, allowing sufficient airspace for mixing, close the tap and cap the bottle. If sample must be taken from a waste stream or effluent channel, dip the open bottle, either using a gloved hand or clamped to a sampling stick, into the waterbody, with the mouth of the sample bottle facing upstream or opposite the direction of any noticeable current or flow. Bottles may need to be clamped to "sludge nabber" sampling sticks to facilitate sampling if water cannot safely be accessed by reaching or wading. Composite samples should not be collected, since such samples do not display the range of values found in individual samples. The sampling depth for surface water samples should be 6-12 inches below the water surface. Sample containers should be positioned so that the mouth of the container is pointed away from the sampler or sample point. After removal of the container from the water, a small portion of the sample should be discarded to allow for proper mixing before analysis. The 125 ml sterilized bottle must be clean and sterile (autoclaved for 15 minutes at 121C±2C). Remove the cap, taking care so that there is no contamination to the inside of the cap or the bottle. Pour the sample into the bottle, leaving enough air space in the bottle so that it can be properly shaken before inoculation. Some samples that are known to contain chlorine must be dechlorinated. For sampling chlorinated wastewater effluents, add sufficient sodium thiosulfate to a clean, sterile sample bottle to give a concentration of about 100 mg/L in the sample. For most chlorinated wastewater samples, 2 ml of a 0.025 N solution of sodium thiosulfate should be sufficient. This is purchased and standardized quarterly in the lab. The sample is then capped, labeled with date, time, place and sampler initials and placed inside a cooler on ice. Ice or refrigerate samples at a temperature of <6EC during transit. The cooler must contain a temperature blank, consisting of a sealed clear bottle, filled with deionized water, with a thermometer attached to the cap, extending into the sample. Samples must be transported to the lab, filtered and incubated within 8 hours of sample collection. Upon receipt, the condition of the sample, including any abnormalities or departures from standard condition, must be recorded in sample login book and on chain of custody. All microbiological samples shall be considered acceptable if they arrive with a temperature blank ranging from just above freezing to 6EC. Samples that are hand delivered to the laboratory immediately after collection may not meet this criterion. In these cases, the samples shall be considered acceptable if there is evidence that the chilling process has begun, such as arrival on ice. Upon arrival in the lab, the residual chlorine is measured in all samples requiring field dechlorination prior to inoculation to check the efficacy of the sodium thiosulfate. Residual Chlorine must be <0.1 mg/L.

12) Quality Control

IPR- Initial Precision and Recovery

An initial demonstration of laboratory capability must be performed through performance of the initial precision and recovery (IPR) analyses prior to the analysis of any environmental samples.

Initial precision and recovery (IPR)- The IPR analyses are used to demonstrate acceptable method performance (recovery and precision) and should be performed by the laboratory before the method is used for monitoring field samples. IPR samples should be accompanied by an acceptable method blank and appropriate media sterility checks.

Prepare four, 100- ml samples of PBS and spike each sample with E. faecalis BioBalls ATCC #19433 according to the following directions: Aseptically add 1 BioBall™ to 100 mL of PBS and mix by vigorously shaking the sample bottle a minimum of 25 times. Filter and analyze the spiked sample according to the method Procedure, section 14 and calculate the number of enterococci per 100 mL according to section 15.

Calculate the percent recovery (R) for each IPR sample using the appropriate equation for BioBalls recovery in section 15, calculations.

Using the percent recoveries of the four analyses, calculate the mean percent recovery and the relative standard deviation (RSD) of the recoveries. The RSD is the standard deviation divided by the mean, multiplied by 100.

Compare the mean recovery and RSD with the corresponding IPR criteria in Table 1, below. If the mean and RSD for recovery of enterococci meet acceptance criteria , system performance is acceptable and analysis of field samples may begin. If the mean recovery or the RSD fall outside of the required range for recovery, system performance is unacceptable. In this event, identify the problem by evaluating each step of the analytical process, media, reagents and controls, correct the problem and repeat the IPR analyses.

Table 1. Initial and Ongoing Precision and Recovery (IPR and OPR) Acceptance Criteria

Performance Test	BioBall™ Acceptance Criteria
Initial precision and recovery (IPR) -Mean Percent Recovery -Precision (as maximum relative standard deviation)	 85%-106% 14%
Ongoing precision and recovery (OPR) as percent recovery	78%-113%

OPR- Ongoing Precision and Recovery

To demonstrate ongoing control of the analytical system, the laboratory must routinely process and analyze spiked PBS samples. The laboratory must analyze one OPR sample after every 20 field and matrix spike samples or one per week that samples are analyzed, whichever occurs more frequently. OPR samples must be accompanied by an acceptable method blank and appropriate media sterility checks. The OPR analysis is performed as follows:

Spike one (1) 100 ml PBS sample with *E. faecalis* ATCC # 19433 BioBall™ according to the spiking procedure described above for the IPR. Filter and process each OPR sample according to the method Procedure, Section 14 and calculate the number of enterococci per 100 ml according to Section 15, Calculations.

Calculate the percent recovery (R) for each IPR sample using the appropriate equation for BioBalls recovery in section 15, calculations.

Compare the OPR result (percent recovery) with the corresponding OPR recovery criteria in Table 1, above. If the OPR result meets the acceptance criteria for recovery, method performance is acceptable and analysis of field samples may continue. If the OPR result falls outside of the acceptance criteria, system performance is unacceptable. In this event, identify the problem by evaluating each step of the analytical process, media, reagents, and controls, correct the problem and repeat the OPR analysis.

As part of the laboratory QA program, results for OPR and IPR samples should be charted and updated records maintained in order to monitor ongoing method performance. The laboratory should also develop a statement of accuracy for Method 160 by calculating the average percent (R) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from R - $2s_r$ to R+$2s_r$.

Matrix Spikes (MS)

MS analyses are performed to determine the effect of a particular matrix on enterococci recoveries. The laboratory analyzes one MS sample when disinfected wastewater samples are first received from a source from which the laboratory has not previously analyzed samples. Subsequently, 5% of field samples (1 per 20) from a given disinfected wastewater source must include and MS sample. MS samples must be accompanied by the analysis of an unspiked field sample sequentially collected from the same sampling site, an acceptable method blank, and appropriate media sterility checks. When possible, MS analyses should also be accompanied by an OPR sample. The MS analysis is performed as follows:

Prepare two, 100 ml field samples that were sequentially sampled from the same site. One sample will remain unspiked and will be analyzed to determine the background or ambient

concentration of enterococci for calculating MS recoveries. The other sample will serve as the MS sample and will be spiked with *E. faecalis* ATCC # 19433 BioBall™

Select sample volumes based on previous analytical results or anticipated levels of enterococci in the field sample in order to achieve the recommended target range of enterococci (20-60 CFU, including spike) per filter. It is recommended that three dilutions be analyzed to ensure that a countable plate is obtained for the MS and associated unspiked sample. If possible, 100 ml of sample should be analyzed.

Spike the MS sample volumes with a BioBall™ by aseptically adding the BioBall™ to the sample and mix by vigorously shaking the sample bottle a minimum of 25 times. Immediately filter and analyze the sample according to the method Procedure, Section 14.

Note: When analyzing smaller sample volumes (e.g., <20 ml), 20-30 ml of PBS should be added to the funnel or an aliquot of sample should be dispensed into a 20-30 ml dilution blank prior to filtration. This will allow even distribution of the sample on the membrane.

For the MS sample, calculate the number of enterococci (CFU/100 ml) according to Section 15, Calculations and adjust the colony counts based on any background enterococci observed in the unspiked matrix sample.

Calculate the percent recovery (R) for the MS sample (adjusted based on ambient enterococci in the unspiked sample) using the equation in Section 15, calculations for samples spiked with BioBalls™.

Compare the MS result (percent recovery) with the appropriate method performance criteria in table 2, below. If the MS recovery meets the acceptance criteria, system performance is acceptable and analysis of field samples from this disinfected wastewater source may continue. If the MS recovery is unacceptable and the OPR sample result associated with this batch of samples is acceptable, a matrix interference may be causing the poor results. If the MS recovery is unacceptable, all associated field data must be flagged.

Note: Acceptance criteria for MS recovery (Table 2) are based on data from spiked disinfected wastewater matrices and are not appropriate for use with other matrices (e.g., ambient water).

Table 2. Matrix Spike Precision and Recovery Acceptance Criteria

Performance Test	BioBall™ Acceptance Criteria
Percent Recovery for MS	63%-110%

The Laboratory will record and maintain a control chart comparing MS recoveries for all matrices (if applicable-if more than one matrix is analyzed) to batch-specific and cumulative

OPR sample results analyzed by this method. These comparisons help to recognize matrix effects on method recovery and may also help to recognize inconsistent or sporadic matrix effects from a particular source.

Positive and Negative Controls

Positive and negative controls must be analyzed with each batch of mEI agar prepared prior to use. An OPR sample may take the place of a positive control.

Negative controls are conducted by filtering a dilute suspension of viable E.coli (From BioBall™ or Microbiologics® swab or pellet) and filtering and processing according to the method procedure, Section 14. Viability of the negative control should be demonstrated by using a non-selective media (e.g. tryptic soy agar). If the negative control fails to exhibit the appropriate response (e.g. growth of enterococci colonies on mEI agar or non-growth in non-selective media), check and/or replace the associated media or reagents, and/or the negative control, and reanalyze the appropriate negative control.

Positive controls are conducted by filtering a dilute suspension of viable *E. faecalis* (From BioBall™ or Microbiologics® swab or pellet) and filtering and processing according to the method procedure, section 14. If the positive control fails to exhibit the appropriate response (e.g. no growth of enterococci colonies on mEI agar), check and/or replace the associated media or reagents, and/or the positive control, and re-analyze the appropriate positive control.

Controls for verification media- All verification media must also be tested with appropriate ositive and negative controls whenever a new batch of media and/or reagents are used, and with each batch of samples verified. E. faecalis is an appropriate positive control for all of the verification medias used in this procedure. E. coli is an appropriate negative control for all of the verification medias used in this procedure. See MF (TC/FC) check list.

13) Calibration and Standardization

Check temperatures in incubators twice daily (with at least 4 hours between checks) to ensure operation within stated limits and record in temperature logbook. Check thermometers at least annually against the NIST certified thermometer. Check mercury columns for breaks daily. Calibrate the pH meter on the day of broth preparation using pH buffer 4.00 and 10.00 using the flat-tipped electrode. Measure the pH 7.00 buffer. Record calibration and reading in pH meter calibration logbook. . The NIST thermometer is sent to Kessler Thermometer Corp. in Amityville, NY once a year for certification. The autoclave maximum-registering thermometer is sent to Kessler Thermometer Corp. in Amityville, NY once a year for certification. The performance of the PB303 balance used to weigh dehydrated media is checked on each day of use with 0.05 gram, 20.0 gram and 50.0 gram NVLAP certified weights. The balance is

calibrated once a year by a service representative from Mettler-Toledo, Columbus, OH. The balance weights are calibrated once a year by Troemner in Thorofare, NJ.

14) Procedure

Prepare the mEI agar as directed in Section 10, Reagents and Standards. Mark the petri dishes and report forms with sample identification and sample volumes. Set up the filtration apparatus: Connect the manifold to one hole in the cap of a carboy via tubing. The second hole in the cap of the carboy (with a shorter tube extending into the carboy) is connected to a vacuum source. Make sure the filtration funnel assembly has been autoclaved. Attach the bottom of the filtration assembly to the manifold. There are three spaces in the manifold. This enables up to three samples (or three dilutions or duplicates of a sample) to be filtered simultaneously. Place a sterile membrane filter on the filter base, grid-side up and attach the funnel to the base so that the membrane filter is now held between the funnel and the base.

Method Blank -Filter a 50-mL volume of sterile PBS and place the filter on a mEI agar plate. Invert and incubate at 41±0.5E for 24±2 hours. Absence of growth indicates freedom of contamination from enterococci. Filter and analyze a method blank with each batch of samples (with a batch being no more than 20 samples).

Filtration Blank- Filter a 50-ml volume of sterile PBS before beginning sample filtrations. Place the filter on a TSA (Tryptic Soy Agar) plate, invert and incubate for 24±2 hours at 35.0± 0.5C. Absence of growth indicates sterility of the PBS buffer and filtration assembly.

Media Sterility Check- Test the media sterility by incubating one unit (plate or tube) from each batch of medium (TSA, mEI agar or verification media) and observing for growth. Absence of growth indicates media sterility. Repeat this check every day samples are analyzed.

Filter Sterility Check- Place at least one membrane filter per lot of filters on a TSA plate, and incubate for 24±2 hours at 35.0±0.5C. Absence of growth indicates sterility of the filter. Repeat this check every day that samples are analyzed.

Samples- Shake the sample bottle vigorously about 25 times to distribute the bacteria uniformly, and measure the desired volume of sample or dilution into the funnel. Select sample volumes based on previous knowledge of the pollution level, to produce 20-60 enterococci colonies on membranes. Analyze a minimum of three dilutions to ensure that a countable plate is obtained. Typically 1 mL, 10 mL and 100 mL sample volumes are filtered. Smaller sample size or sample dilutions can be used to minimize the interference of turbidity or high bacterial densities. Multiple volumes of the same sample or sample dilutions may be filtered.

Note: When analyzing smaller sample volumes (e.g.< 20 ml), 20-30 ml of PBS or phosphate-buffered dilution water should be added to the funnel or an aliquot of sample should be dispersed

into a dilution blank prior to filtration. This will allow more even distribution of the sample on the membrane to aid in counting of colonies.

Pour the sample into the filtration funnel, measuring using a graduated cylinder or wide-mouth volumetric pipet, turn on vacuum source, open filter valve and filter sample. Rinse the sides of the funnel at least twice with 20-30 ml of sterile buffered rinse water. Turn of the vacuum and remove the filter funnel from the filter base.

Using sterile forceps (flame in bacticinerator®), aseptically remove the membrane filter from the filter base, and roll it onto the mEI Agar to avoid the formation of bubbles between the membrane and the agar surface. Reseat the membrane if bubbles occur. Run the forceps around the edge of the filter outside the area of filtration, close to the edge of the dish, to be sure that the filter is properly seated on the agar. Close the dish, invert, and incubate at 41.0±0.5C for 24±2 hours.

Note: *If the medium is prepared in loose lid petri dishes, they should be incubated in a tight-fitting container (plastic food container with snap top lid) containing a moistened paper towel to prevent dehydration of the membrane filter.*

After incubation, count and record colonies on those membrane filters containing, if practical, 20-60 colonies with a blue halo, at least 0.5 mm in diameter. Use magnification for counting and a small fluorescent lamp to give maximum visibility of colonies.

Analyst colony counting variability- Laboratories with two or more analysts should compare each analyst's colony counts from one positive field sample per month. Colony countns should be within 10% between analysts. Each analyst should perform duplicate colony counts of a single membrane filter each month. Duplicate colony counts should be within 5% for a single analyst. If no positive field samples are available, an OPR sample may be substituted for these determinations.

Verification Procedure- The laboratory must verify 10 typical colonies (positive) and 10 atypical colonies (negative) per month or 1 typical colony and 1 atypical colony from 10% of all positive samples, whichever is greater. Using a sterile inoculating loop or needle, transfer growth from the centers of the colony into a BHIB tube and onto a BHIA slant. Incubate broth for 24±2 hours and agar slants for 48±3 hours at 35.0C±0.5EC. After a 24 hour incubation, transfer a loopful of growth from each BHIB tube to BEA, BHIB, and BHIB with 6.5% NaCl.

Incubate BEA and BHIB with 6.5% NaCl at 35.0±0.5C for 48±3 hours.

Incubate BHIB at 45.0C ± 0.5C for 48±3 hours.

Observe all verification media for growth. After 48 hour incubation, perform a Gram stain using growth from each BHIA slant. Gram-positive cocci that grow and hydrolyze esculin on BEA (i.e., produce a black or brown precipitate), and grow in BHIB with 6.5% NaCl at 35EC±0.5EC and BHIB at 45EC±0.5EC are verified as enterococci.

Alternatively, commercially available multi-test identification systems (e.g., Vitek®) may be used to verify colonies. Such multi-test identification systems should include esculin hydrolysis and growth in 6.5% NaCl.

15) Calculations

Use the following general rules to calculate the enterococci count per 100 ml of sample: Select the membrane filter with an acceptable number of colonies (regardless of colony color) with a blue halo (20-60) and at leat 0.5 mm in diameter. Calculate the number of enterococci per 100 mL according to the following general formula:

$$\text{Enterococci/100 mL} = \frac{\text{Number of enterococci colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

BioBall™ Spike Recovery Calculation- Calculate percent recovery (R) of spiked enterococci (CFU/100 mL) according to the following equation.

$$R = 100 \times \frac{(Ns-Nu)}{T}$$

Where,

R= Percent recovery
Ns= Enterococci (CFU/100 mL) in the spiked sample
Nu= Enterococci (CFU/100 mL) in the unspiked sample
T= True spiked enterococci (CFU/ 100 mL) in spiked sample based on the lot mean value provided by manufacturer.

16) Method Performance

All measurements of Method Performance outlined in Section 12, Quality Control must be used. See EPA Method 1600, Section 15.0, Method Performance, for information on Method Validation data.

17) Pollution Prevention

The solutions and reagents used in this method pose little threat to the environment when

recycled and managed properly. Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed. All used broth and agar is autoclaved for 30 minutes at 121C±2.0C after use. All waste generated from microbiological testing, including disposable pipettes, transfer loops, agar plates, gloves are disposed of in the red biohazard bins. Benches are cleaned with a disinfectant before and after use for microbiological testing. See Safety, Section 8, and Waste Management, Section 21 for more information on procedures related to pollution prevention.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

Information regarding data assessment and acceptance criteria for quality control measures are outlined in Section 12, Quality Control.

19) Corrective Actions for Out-of-Control Data

Samples received above 6.0 C or outside of the 8 hours (from the collection time to the incubation time) holding time indicate problems with transport. The sampler/transporter must report the problem to the Laboratory Director, who will determine, in consultation with the Quality Assurance Officer, whether the analysis should proceed. If problems with transport are indicated by the above quality control data, the Laboratory Director and the Quality Assurance Officer must review sample transport procedures with the sampler. These may include, but not be limited to, checking the accuracy of the temperature blank thermometer, changing the size of the cooler and/or amount of ice used in transport. If consistent problems adhering to the six hour holding time arise, the Laboratory Director and Quality Assurance Officer should review with the designated Project Manager the feasibility of the sample site in regards to transport requirements. If the batch QC fails for any batch of broth, the broth must be discarded and remade. More corrective actions for out-of-control data are discussed below in Section 20.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

The Laboratory Director and QA Officer will jointly investigate the problem(s) at depth and take all necessary corrective actions that may include, but not be limited to: obtaining fresh QC standards from one or more suppliers and determining the accuracy of measurements, measuring standard deviations, consulting a service engineer and arranging on-site maintenance or repairing service. All analyses of samples will be stopped until the problem is rectified. Samples received at temperatures above 6.0 C or outside of the 8hour (from the collection time to the incubation time) holding time cannot be used for regulatory purposes. The results of the positive and negative quality control checks are vital in determining the validity of data. A negative control culture which generates a positive result indicates contamination of the analysis equipment, supplies and/or reagents. A blank with growth also indicates a contamination problem. A positive control culture which generates a negative result indicates that the broth is not able to support the indicated organism growth. Any batch of broth that fails the above QC checks must be discarded.

21) Waste Management

It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. Samples, reference materials, and equipment known or suspected to have viable enterococci, or any other bacteria, attached or contained must be sterilized prior to disposal (autoclaved at 121 C and 15 PSI for 30 minutes). This waste is then stored in red biohazard bins until pickup by an approved regulated waste company. Currently, the laboratory uses Stericycle on an as-needed basis for removal of waste.

22) References

Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI). April 2005. EPA-821-R-04-023. U.S. Environmental Protection Agency, Office of Water (4303T), 1200 Pennsylvania Avenue, NW, Washington D.C.

Standard Methods for the Examination of Water and Wastewater, 20th edition, Sections 9010,9020, 9030, 9040, 9050, 9060,. APHA, AWWA, WEF, 1992.

IEC Quality Control Manual, Revision 8, January 2015.

NELAC Quality Systems Checklist (NELAC Manual Chapter 5 Checklist), Revision a, Based on 2003 NELAC Standards.

23) Tables, Diagrams, Flowcharts and Validation Data

See Next Page.

Table 1. Key Quality Control Practices

Item	Action	Minimum Frequency
Reagent water	Monitor quality	See Table 2
Bench surface	Monitor for contamination	Monthly
Air in workplace	Monitor bacterial density	Monthly
Thermometers	Check accuracy	annually
Balances	Check accuracy	Each use
Balances	Service and recalibrate	Annually

pH meter	Standardize	Each day of use
Autoclave	Check performance (using autoclave tape, maximum registering thermometer)	Each use
Refrigerator	Check temperature	Daily (2x)- 4 hours apart
Membrane Filtration Equipment	Checks for leaks and surface scratches	Each use
Biohazard hood	Monitor air	Monthly
	Inspect for airflow	Quarterly
Incubator	Check temperature	Twice daily 4 hours apart
Glassware	Inspect for cleanliness, chips and etching	Each use
	Check pH	Each batch
	Conduct inhibitory residue test	Annually
Dilution water bottles	Check pH	Each batch
Media	Check pH and appearance	Each use
Autoclave	Check performance (using steri-amps)	Monthly
Plate Counts	Perform Duplicate Analyses	Each batch
	Repeat Counts (+Second analyst)	Each batch

Table 2. Quality of Reagent Water Used in Microbiology Testing

Test	**Monitoring Frequency**	**Maximum Acceptable Limit**
Chemical Test:		
Conductivity	Monthly	>0.5 megohms resistance or <2 μmhos/cm at 25°C
pH	With each day of use	5.5-7.5
Total organic carbon	Annually	<1.0 mg/L
Heavy metals, single (Cd, Cr, Cu, Ni, Pb, and Zn)	Annually	<0.05 mg/L
Heavy metals, total	Annually	<0.10 mg/L
Ammonia/organic nitrogen	Anually	<0.10 mg/L
Total chlorine residual	Monthly or with each use	<0.01 mg/L
Bacteriological test:		
Heterotrophic plate count	Monthly	<1000CFU/mL
Use test	Annually	Student's *t* <=2.78

Table 3. Time and Temperature for Autoclave Sterilization

Material	Time at 121ºC
Membrane filters and pads (if not purchased sterile)	10 min
Carbohydrate-containing media (lauryl tryptose, BGB broth, etc.)	12-15 min
Contaminated materials and discarded cultures	30 min
Membrane filter assemblies (wrapped)	
Sample collection bottles (empty)	15 min
Buffered dilution water	15 min
Rinse water, volume >100 mL	Adjust for volume

Table 4. Holding Times for Prepared Media

Medium	Holding Time
Membrane filter (MF) broth in screw-cap flasks at 4EC	96 hours
MF agar in plates with tight-fitting covers at 4EC	2 weeks
Agar or broth in loose-cap tubes at 4ºC	2 weeks
Agar or broth in tightly closed screw-cap tubes or other sealed containers	3 months
Poured agar plates with loose-fitting covers in sealed plastic bags at 4ºC	2 weeks
Large volume of agar in tightly closed screw-cap flask or bottle at 4ºC	3 months

Membrane Filter Technique for Coliform Organisms (Fecal and Total Coliforms)

1) Test Method

Based on Standard Methods 9222B-2006 (Total Coliform), and 9222D-2006 (Fecal Coliform)

2) Applicable Matrix or Matrices

Non-potable water.

3) Method Detection Limit

Method Detection Limits depend on the selection of sample size. Size of the sample will be governed by expected bacterial density. An ideal sample volume will yield 20 to 80 coliform colonies and not more than 200 colonies of all types on a membrane filter surface. Analyze non-potable waters by filtering 3 different sample volumes, depending on expected bacterial density. When less than 10 mL of sample is to be filtered, add at least 10 mL sterile dilution water to the funnel before filtration. This increase in water volume aids in uniform dispersion of the bacterial suspension over the entire effective filtering surface.

$$\text{MDL} = 1 \times \frac{100\text{ml}}{\text{volume filtered (ml)}}$$

4) Scope and Application

The Interstate Environmental Commission laboratory uses this method for ambient water quality monitoring associated with special projects. Proficiency samples are analyzed semi-annually and continuing demonstrations of capability are analyzed annually to maintain certification. The MF technique is applicable to a wide range of non-potable waters, although there are significant interferences that must be taken into consideration. These are discussed below, in Interferences, section 7.

5) Summary of Method

Water samples are filtered onto membrane filters. The filters are then transferred onto coliform-specific agars (mFC Agar for fecal coliforms, m-Endo Agar LES for total coliforms) and incubated for 24 hours. The number of coliform colonies per plate is counted and the number of colonies per 100 ml of sample is calculated, based on the volume filtered

6) Definitions

As related to the MF technique, the coliform group is defined as those facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that develop red colonies with a metallic sheen (golden) within 24 h at 35.0C on an Endo-type agar (for Total Coliforms). Fecal Coliforms form colonies on M-FC medium (after filtration and incubation for 24±2 hours in a 44.5C waterbath) that are various shades of blue.

7) Interferences

The MF procedure has limitations when testing waters with high turbidity or large numbers of non-coliform (background) bacteria. When MF methods have not been used previously in the determination of fecal and total coliforms, parallel tests over a three-month period should be performed with the method the laboratory is currently using to demonstrate applicability and comparability. Turbidity caused by the presence of algae, particulates or other matter may not permit testing of a sample volume sufficient to yield significant results. Low coliform estimates may be caused by the presence of high numbers of noncoliforms or of toxic substances. The MF technique is applicable to the analysis of saline waters, but not of wastewaters that have received only primary treatment followed by chlorination because of turbidity in high volume samples or wastewaters containing toxic metals or toxic organic compounds such as phenols

8) Safety

Wear appropriate personal protective equipment, including lab coat, goggles, and gloves. Avoid breathing dust of dehydrated media. Wear a respirator and/or prepare broth in the hood. Autoclave all spent tubes at 121C±2C for 30 minutes. Dispose all spent tubes and spent plates in to red Stericycle regulated medical waste container. See Pollution Prevention and Waste Management sections for more information on the safe disposal of microbiological waste. Take extreme precaution when handling micoorganisms used for positive and negative control tests. The manufacturer's MSDS's are retained and should be reviewed prior to inoculating these organisms.

9) Equipment and Supplies

1. 125 ml plastic bottles for sampling (autoclaved with tape sealing lid of bottle).
2. 5 ml and 10 ml sterile, serological disposable pipets, graduated in 0.1 ml increments, containing cotton plug (Fisher Catalog #'s 13-676-10C and 13-676-10F, respectively).
3. 16 ml polystyrene round-bottom test tubes, with caps, for sample dilution (Fisher Catalog # 959-38B).
4. Disposable, pre-sterilized plastic petri dishes, 60 x 15 mm (Fisherbrand Catalog # 08-757-13A).

5. Filter holding assembly consisting of 3-place stainless steel manifold, connected to a carboy, which is also connected to a vacuum source.
6. Millipore plastic 250 ml funnel assemblies (wrapped in aluminum foil and autoclaved before use).
7. Millipore 0.45µm pore size, white, gridded, 47 mm diameter membrane filters (Millipore Cat. # HAWG04700)
8. Forceps, smooth flat, without corrugations on the inner sides of the tips. Sterilize before use by dipping in 95% ethanol and flaming.
9. 35.0C dry incubator ±0.5C.
10. 44.5C waterbath, ±0.2C.
11. pH meter, with flat-tipped probe.
12. Electric incinerator unit for sterilizing loops and needles.
13. Refrigerator, maintained at 1-4C for storing broth.
14. Autoclave (Tuttnauer 2540E-B/L Heidolph Brinkmann).
15. Sterilizer indicator tape (Fisher Catalog # 11-875-50).
16. Balance (Mettler Toledo PB 303).
17. Glass or Stainless Steel beaker/vessel for preparation of agar.
18. Hot plate/stir plate combination plate (Isotemp by Fisher Scientific).
19. Microscope equipped with low-power (10 to 15x magnification) with light source. American Optical Co. Part # 56C-103 and Scienceware mini lightbox.
20. CE Colony Counter Pen Cat. # 17649 Lot # 081498.

10) Reagents and Standards

1. Dehydrated Difco M-Endo Agar LES (BD Ref. #273620). For Total Coliform MF analysis.
2. Dehydrated Difco m FC Agar (BD Ref # 267720). For Fecal Coliform MF analysis. Equivalent, prepared agar plates or ampules may be purchased through HACH or other supplier. Purchased prepared media must be tested with quality control samples prior to use with environmental samples or proficiency samples.
3. Non -denatured ethanol. 200 proof. (Acros Cat # 61509-0040). For addition to M-Endo Agar LES.
4. Difco Rosolic acid (1 g) (BD Ref. # 232281). For addition to M-FC Agar. Make a 1% solution of Rosolic Acid by adding 1g rosolic acid to 80 mL deionized water and 20 mL 1 N NaOH.
4. *Enterococcus faecalis* (Fisher Catalog # 23-264424)
5. *Escherichia coli* (Fisher Catalog # 23-264397)
6. pH 4.00, 7.00 and 10.00 buffers for calibrating the pH meter
7. Magnesium Chloride
8. Potassium Dihydrogen Phosphate
9. 1N NaOH.
10. Plate Count Agar (for QC check on dilution water)
11. Buffered dilution water:

a). Phosphate buffer solution: Dissolve 34.0g potassium dihydrogen phosphate (KH_2PO_4) in 500mL distilled water, discard if turbid. Adjust to pH 7.2±0.5 with 1N sodium hydroxide (NaOH), and dilute to1 L with distilled water.
b). Magnesium chloride buffer: Dissolve 81.1g $MgCl_2 \cong 6H_2O$ to 1 liter distilled water
Preparation of buffered dilution water: Add 1.25 ml stock phosphate buffer solution and 5.0 ml magnesium chloride solution to 1 L distilled water. Autoclave at 121C±2C for 15 minutes. See quality control section for sterility check on buffered water.

11) Sample Collection, Preservation, Shipment and Storage

Samples for microbiological analysis should be collected directly into 125 ml sterilized bottles. Bottles are labeled with sterilization date and sealed heat-sensitive tape that indicates the bottles have reached sterilization temperature. Remove the cap, immediately prior to sampling, taking care so that there is no contamination to the inside of the cap or the bottle. If sampling from a spigot, which is the case at some municipal wastewater treatment plants and industrial facilities, place the open bottle directly below but not touching the spigot, fill the bottle to the neck, allowing sufficient airspace for mixing, close the tap and cap the bottle. If sample must be taken from a waste stream or effluent channel, dip the open bottle, either using a gloved hand or clamped to a sampling stick, into the waterbody, with the mouth of the sample bottle facing upstream or opposite the direction of any noticeable current or flow. Bottles may need to be clamped to "sludge nabber" sampling sticks to facilitate sampling if water cannot safely be accessed by reaching or wading. Composite samples should not be collected, since such samples do not display the range of values found in individual samples. The sampling depth for surface water samples should be 6-12 inches below the water surface. Sample containers should be positioned so that the mouth of the container is pointed away from the sampler or sample point. After removal of the container from the water, a small portion of the sample should be discarded to allow for proper mixing before analysis. The 125 ml sterilized bottle must be clean and sterile (autoclaved for 15 minutes at 121C±2C). Remove the cap, taking care so that there is no contamination to the inside of the cap or the bottle. Pour the sample into the bottle, leaving enough air space in the bottle so that it can be properly shaken before inoculation. Some samples that are known to contain chlorine must be dechlorinated. For sampling chlorinated wastewater effluents, add sufficient sodium thiosulfate to a clean, sterile sample bottle to give a concentration of about 100mg/L in the sample. For most chlorinated wastewater samples, 2 ml of a 0.025 N solution of sodium thiosulfate should be sufficient. This is purchased and standardized quarterly in the lab. The sample is then capped, labeled with date, time, place and sampler initials and placed inside a cooler on ice. Ice or refrigerate samples at a temperature of <6.0C during transit. The cooler must contain a temperature blank, consisting of a sealed clear bottle, filled with deionized water, with a thermometer attached to the cap, extending into the sample. Samples must be transported to the lab, filtered and incubated within 8 hours of sample collection. Upon receipt, the condition of the sample, including any abnormalities or departures from standard condition, must be recorded in sample login book and on chain of custody. All

microbiological samples shall be considered acceptable if they arrive with a temperature blank ranging from just above freezing to 6.0C. Samples that are hand delivered to the laboratory immediately after collection may not meet this criterion. In these cases, the samples shall be considered acceptable if there is evidence that the chilling process has begun, such as arrival on ice. Upon arrival in the lab, the residual chlorine is measured in all samples requiring field dechlorination prior to inoculation to check the efficacy of the sodium thiosulfate. Residual Chlorine must be <0.1 mg/L.

12) Quality Control

- Samples are transported to the lab on ice and incubated within 8 hours of collection.
- The temperature of the temperature blank is checked upon arrival at the lab and the temperature is recorded in the sample log-in book.
- Quality control samples are available commercially and are currently procured from MicroBiologics. Microorganisms are inoculated into the appropriate broth according to the manufacturer's direction. Microorganisms may be in the form of lyophilized disks or swabs depending on availability. Run a positive control, a negative control, and a blank on each batch of broth prior to using broth. To test mFC agar and M-Endo LES Agar, *Escherichia coli* is used as a positive control; *klebsiella aerogenes* is used as a negative control for mFC agar; *staphylococcus aureus* is used as a negative for mEndo and *Enterococcus faecalis* is used as a negative control. The plates are incubated according to the procedures described for the fecal coliform and total coliform MF tests. The agar is tested both prior to use.
- A blank, consisting of a plate containing only agar, is run alongside each set of positive and negative controls to check for sterility. Commercial availability of the organisms used in the above quality control checks may vary. Appropriate microorganisms for each media are listed in Standard Methods, 22th Edition, and table 9020:VI. "Control Cultures for Microbiological Tests".
- Check the sterility of each batch of buffered dilution water. Put 1 ml dilution water into an agar plate and pour 10 ml of a non-selective agar, such as Tryptic Soy Agar, over it. See Section 16) Method Performance for information on sample duplicates, internal QC samples and external proficiency test samples to be used as quality control measures.
- One filter from each new lot of membrane filters muct be checked for sterility by incubation at 35.0C for 24 hours on Trypic Soy Agar.
- For each filtration series, prepare one beginning and one ending sterility check for each laboratory sterilized filtration unit by filtering 50-100 ml of buffered dilution water through the funnel and incubating the membrane according to the test method (M-FC agar for fecal coliforms and M-Endo Agar LES for Total Coliforms). Repeat the above sterility test every 10 samples for a series larger that 10 samples.
- Duplicate colony counts must be performed on each batch.
- Counts must be repeated by a second analyst on each performed batch.

- Check effectiveness of the autoclave sterilization procedure monthly with a run of biological indicators (Raven Prospore® *Geobacillus stearothermophilus* self-contained ampules).
- Insert a maximum registering thermometer (contained in a beaker of water) in the middle of items to be autoclaved. Ensure that the thermometer has been "shaken down" to below 90°C before placing in autoclave. Read the maximum temperature upon removing the thermometer from autoclave and record in media preparation book.
- Use temperature sensitive tape with the contents of each autoclave run to indicate that the autoclave contents have been processed.
- Record date, contents (materials processed), maximum temperature reached, pressure, time in sterilization mode, total run time, expiration date, initial and final pH, and analysts initials in media prep logbook.
- Maintain records of autoclave maintenance in the equipment logbook.
- Check the autoclave timing device monthly against a stopwatch, record actual time elapsed.

See Section 23, Tables 1-5 for more information on routine quality control performed on laboratory equipment and reagents. See *The Interstate Environmental Commission's Quality Control Manual* for more information on the IEC's quality control program.

13) Calibration and Standardization

Calibrate the pH meter on the day of broth preparation using pH buffer 4.00 and 10.00 using flat-tipped electrode. Measure the pH 7.00 buffer. The pH 7.00 buffer must read 7.00±0.05. Record calibration and reading in pH meter calibration logbook. Each lot of disposable pipets must be checked for accuracy by weighing several volumes of deionized water. All thermometers used in the refrigerators, incubators, and waterbaths are checked once a year at test temperature against a standards thermometer. The standards thermometer is sent to Kessler Thermometer Corp. in Amityville, NY once a year for certification. The autoclave maximum-registering thermometer is sent to Kessler Thermometer Corp. in Amityville, NY once a year for certification. The performance of the Mettler Toledo PB303 balance used to weigh dehydrated media is checked on each day of use with 0.05 gram, 20.0 gram and 50.0 gram NVLAP weights. The balance is calibrated once a year by a service representative from Mettler-Toledo, Columbus, OH. The balance weights are calibrated every 3 years by Troemner in Thorofare, NJ. Calibrate CE Colony Counter Pen monthly with: 10 counts; 20 counts; 50 counts. Record calibration and reading in Laboratory Accuracy logbook (L23).

14) Procedure

Standard Total Coliform Membrane Filter Procedure

A. Prepare M-Endo Agar LES.

Weigh 51g of the powder in 1 L of purified water containing 20 ml of 200 proof (NOT denatured) ethanol in glass or stainless steel sterilized via autoclave beaker. Mix thoroughly. Heat with frequent agitation and heat to near boiling to completely dissolve the powder, then promptly remove from heat and cool to between 45.0C and 50.0C. DO NOT AUTOCLAVE. pH of agar must be 7.2± 0.2. Dispense in 5-7 mL quantities, using sterile, serological disposable pipet.into lower section of 9 x 60-mm plastic petri dishes. Do not expose poured plates to direct sunlight; refrigerate in the dark, preferably in sealed plastic bags or other containers to reduce moisture loss. Discard used medium after 2 weeks or sooner if there is evidence of moisture loss, medium contamination or medium deterioration.

B. Selection of Sample Size

Size of sample will be governed by expected bacterial density. At least one sample volume must yield 20 to 80 total coliform colonies on a membrane-filter surface, 20-60 fecal coliform colonies on a membrane filter, but no more than 200 colonies in either case. Analyze non-potable water by filtering three different volumes, depending on expected bacterial density. Typically 1 mL, 10 mL, and 100 mL are filtered. See SM (22th Edition) Table 9222:I in Appendix for Suggested Sample Volumes. When less than 10 ml of sample (diluted or undiluted) is to be filtered, add approximately 10 ml sterile dilution water to the funnel before filtration or pipet the sample volume into a sterile dilution bottle, then filter the entire dilution. This increase in water volume aids in uniform dispersion of the bacterial suspension over the entire effective filtering surface.

C. Sterile Filtration Units

Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination. A filtration series is considered to be interrupted when an interval of 30 minutes or longer elapses between sample filtrations. After such interruption, treat any further sample filtration as a new filtration series and sterilize all membrane filter holders in use.

D. Filtration of Sample

Using sterile forceps, place a sterile membrane filter (grid side up) over porous plate of receptacle. Carefully place matched funnel unit over receptacle and lock it in place. Filter sample under partial vacuum. With filter still in place, rinse the interior surface of the funnel by filtering three 20-30 ml portions of sterile dilution water. Rinsing between samples prevents carryover contamination. Upon completion of final rinse and the filtration process disengage vacuum, unlock and remove funnel, immediately remove membrane filter with sterile forceps, and place it on a M-Endo Agar LES plate (labeled with sample ID and volume filtered) with a rolling motion to avoid entrapment of air. Cover petri dish, invert, and incubate for 22 to 24 hours at 35.0 ± 0.5C. Insert a sterile rinse water sample (100 ml) at the beginning, the end, and every 10 samples of the filtration series to check for possible cross-contamination or contaminated rinse water. Incubate the rinse water control membrane culture under the same conditions as the sample. Run 100 ml of a buffer rinse through the filter unit after the filter is removed to prevent carryover between samples.

E. Counting

To determine colony counts on membrane filters, use a low-power (10 to 15X magnification) binocular wide-field dissecting microscope or other optical device, with a cool white fluorescent light source directed to provide optimal viewing of sheen. The typical coliform colony has a pink to dark-red color with a metallic surface sheen. Count both typical and atypical coliform colonies. The sheen area may vary in size from a small pinhead to complete coverage of the colony surface. Atypical colonies can be dark red, mucoid, or nucleated without sheen. Generally, pink, blue, white or colorless colonies lacking sheen are considered noncoliforms. The total count of colonies (coliform and noncoliform) on an Endo-type medium has no consistent relationship to the total number of bacteria present in the original sample. A high count of non-coliform colonies may interfere with the maximum development of coliforms. Refrigerating cultures (after 22 hours of incubation at 35.0 ± 0.5C) with high densities of non-coliform colonies for 0.5 to 1 hour before counting may deter spread of confluence while aiding sheen discernment. Samples of disinfected water or wastewater effluent may include stressed organisms that grow relatively slowly and produce maximum sheen in 22 to 24 hours. Organisms from undisinfected sources may produce sheen at 16 to 18 hours, and the sheen subsequently may fade after 24 to 30 hours.

F. Coliform Verification

Verify at least 10 sheen colonies (and representative atypical colonies of different morphologic types) from a positive water sample every month that the test is run on environmental samples. Adjust counts on the basis of verification results.

Verification Procedure-

Transfer growth from each colony and place in Lauryl Tryptose broth; incubate the Lauryl Tryptose broth at 35.0 ± 0.5C for 48 hours. Gas formed in Lauryl Tryptose broth and confirmed in Brilliant Green Lactose broth within 48 h verifies the colony as a coliform. Simultaneous inoculation of both media for gas production is acceptable. Inclusion of EC broth inoculation for incubation at 44.5± 0.2C for 24 hours will provide information on the presence of fecal coliforms. See the IEC SOP for Fecal and Total Coliform, (IEC SOP Manual Section XI) for more information on the broth preparation for this verification procedure.

Fecal Coliform Membrane Filter Procedure

A. Prepare M-FC Agar (BD Ref # 267720)

Suspend 52 grams of the powder in 1 L of purified water containing 10 ml 1 % rosolic acid in 0.2N NaOH. Mix thoroughly, heat to near boiling, promptly remove from heat and cool to below 50.0C in waterbath. DO NOT STERILIZE BY AUTOCLAVING. Add Final pH should be 7.4 ± 0.2. Dispense 5 to 7 ml quantities into 60 x 15 mm sterile, polystyrene petri dishes. Refrigerate finished medium, preferably in sealed plastic bags or other containers to reduce moisture loss, and discard unused agar after 2 weeks. Test each agar lot for satisfactory

performance and sterility with positive and negative controls as described in Quality Control, Section 12.

B. Selection of Sample size

Size of sample will be governed by expected bacterial density. An ideal sample volume will yield 20 to 60 fecal coliform colonies on a membrane-filter surface. When the bacterial density of the sample is unknown, filter several volumes or dilutions to achieve a countable density. See SM (22th Edition) Table 9222:IV in Appendix for Suggested Sample Volumes. When less than 10 ml of sample (diluted or undiluted) is to be filtered, add approximately 10 ml sterile dilution water to the funnel before filtration or pipet the sample volume into a sterile dilution bottle, then filter the entire dilution. This increase in water volume aids in uniform dispersion of the bacterial suspension over the entire effective filtering surface.

C. Sterile Filtration Units

Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination. A filtration series is considered to be interrupted when an interval of 30 minutes or longer elapses between sample filtrations. After such interruption, treat any further sample filtration as a new filtration series and sterilize all membrane filter holders in use.

D. Filtration of Sample

Using sterile forceps, place a sterile membrane filter (grid side up) over porous plate of receptacle. Carefully place matched funnel unit over receptacle and lock it in place. Filter sample under partial vacuum. With filter still in place, rinse the interior surface of the funnel by filtering three 20-30 ml portions of sterile dilution water. Rinsing between samples prevents carryover contamination. Upon completion of final rinse and the filtration process disengage vacuum, unlock and remove funnel, immediately remove membrane filter with sterile forceps, and place it on a M-FC Agar plate (labeled with sample ID and volume filtered) with a rolling motion to avoid entrapment of air. Cover petri dish, place in waterproof plastic bags, invert, and submerge petri dishes for 24±2 hours in a 44.5 ± 0.2C waterbath. Anchor dishes below water surface to maintain critical temperature requirements. Place all prepared cultures in the waterbath within 30 minutes after filtration. Insert a sterile rinse water sample (100 ml) at the beginning, the end, and every 10 samples of the filtration series to check for possible cross-contamination or contaminated rinse water. Incubate the rinse water control membrane culture under the same conditions as the sample. Run 100 ml of a buffer rinse through the filter unit after the filter is removed to prevent carryover between samples.

E. Counting

Colonies produced by fecal coliform bacteria on M-FC medium are various shades of blue. Non-fecal coliform colonies are gray to cream-colored. Normally, few nonfecal coliform colonies will be observed on M-FC Agar because of selective action of the elevated temperature. Count colonies with a low-power (10 to 15X magnification) binocular wide-screen optical device. See

the verification procedure above, under the procedure for Standard Total Coliform Membrane Filtration, for details on the fecal coliform verification procedure. Verify every month the procedure is run by picking at least 10 blue colonies from one positive sample. Verify in Lauryl Tryptose broth and EC broth as described in IEC SOP Manual Section XI.

15) Calculations

Compute the count, using membrane filters with 20 to 80 coliform colonies and not more than 200 colonies of all types per membrane, by the following equation:

$$\text{(Total) coliforms/100 mL} = \frac{\text{coliform colonies counted X 100}}{\text{mL sample filtered}}$$

$$\text{(Fecal) coliforms/100 mL} = \frac{\text{coliform colonies counted X 100}}{\text{mL sample filtered}}$$

If no coliform colonies are observed, report the coliform colonies counted as "<1 coliform/100 mL."

For verified coliform counts, adjust the initial count based upon the positive verification percentage and report as "verified coliform count/100 mL."

$$\text{Percentage verified coliforms} = \frac{\text{number of verified colonies}}{\text{total number of coliform colonies subjected to verification}} \text{ X } 100$$

16) Method Performance

Method performance is checked by numerous methods. 5% of samples are analyzed in duplicate to measure precision of the method. Positive controls, negative controls, and blanks are run before each batch of media is used and with each batch of samples. Analysts must perform an initial demonstration of capability through the satisfactory analysis of four (4) replicate samples and demonstrate ongoing, or continuing capability each year through the satisfactory analysis of a proficiency sample or four (4) replicate samples procured by the Laboratory Director. The Interstate Environmental Commission's laboratory participates in semi-annual proficiency tests administered by the NYSDOH.

17) Pollution Prevention

All used broth and agar is autoclaved for 30 minutes at 121ºC±2C after use. All waste generated from microbiological testing, including disposable pipettes, transfer loops, agar plates, gloves are disposed of in the red biohazard bins. Benches are cleaned with a disinfectant before and after use for microbiological testing. See Safety, Section 8, and Waste Management, Section 21 for more information on procedures related to pollution prevention.

18) Data Assessment and Acceptance Criteria for Quality Control Measures
Duplicate counts must vary by no more than 10% between analysts. Duplicate counts by the same analyst must vary by no more than 5%. All positive control QC checks must yield a positive result. All negative control QC checks and all blanks must yield a negative result. See Section 23, Table 2, for acceptance criteria for water to be used in microbiological testing.

19) Corrective Actions for Out-of-Control Data
Samples received above 6.0C or outside of the 8 hour holding time (from the moment of collection to the sample incubation) indicate problems with transport. The sampler/transporter must report the problem to the Laboratory Director, who will determine whether the analysis should proceed. If problems with transport are indicated by the above quality control data, the Laboratory Director must review sample transport procedures with the sampler. These may include, but not be limited to, checking the accuracy of the temperature blank thermometer, changing the size of the cooler and/or amount of ice used in transport. If consistent problems adhering to the eight hour holding time arise, the Laboratory Director will review with the designated Project Manager the feasibility of the sample site in regards to transport requirements. If the batch QC fails for any batch of broth, the broth must be discarded and remade.

20) Contingencies for Handling Out-of-Control or Unacceptable Data
The Laboratory Director will investigate the problem(s) at depth and take all necessary corrective actions that may include, but not be limited to: obtaining fresh QC standards from one or more suppliers and determining the accuracy of measurements, measuring standard deviations, consulting a service engineer and arranging on-site maintenance or repairing service. All analyses of samples will be stopped until the problem is rectified. Samples received at temperatures above 6.0C or outside of the 8 hour holding time (from the moment of collection to the sample incubation) cannot be used for regulatory purposes. The results of the positive and negative quality control checks are vital in determining the validity of data. A negative control culture which generates a positive result indicates contamination of the analysis equipment, supplies and/or reagents. A blank with growth also indicates a contamination problem. A positive control culture which generates a negative result indicates that the broth is not able to support the indicated organism growth. Any batch of broth that fails the above QC checks must be discarded.

21) Waste Management
See IEC SOP manual, revision 1 January 2002, page 12 of 15, for information regarding pickup of biowaste generated by microbiological testing.

22) References
Standard Methods for the Examination of Water and Wastewater, 21th edition, Sections9020, 9030, 9040, 9050, 9060, 9222 A,B and D. APHA, AWWA, WEF, 1992.

Standard Methods for the Examination of Water and Wastewater, 21th edition, Section 9020B, Table 9020:V. 2005
Standard Methods for the Examination of Water and Wastewater, 21th edition, Section 9222B, Table 9222:I. 2005
Standard Methods for the Examination of Water and Wastewater, 21th edition, Section 9222D, Table 9222:III. 2005
Standard Methods for the Examination of Water and Wastewater, 22th edition, Section 9020,9222 . Table 9220: VI; 9222:IV. 2012
Difco Manual, 11th Edition, Difco Laboratories, 1998.
IEC Quality Control Manual, Revision 5, October 2012.
NELAC Quality Systems Checklist (NELAC Manual Chapter 5 Checklist), Revision a, Based on 2003 NELAC Standards.

23) Tables, Diagrams, Flowcharts and Validation Data

Table 1. Key Quality Control Practices

Item	Action	Minimum Frequency
Reagent water	Monitor quality	See Table 2
Bench surface	Monitor for contamination	Monthly
Air in workplace	Monitor bacterial density	Monthly
Thermometers	Check accuracy	Annually
Balances	Check accuracy	Each day of use
Balances	Service and recalibrate	Annually
pH meter	Standardize	Each day of use
Autoclave	Check performance (using autoclave tape, maximum registering thermometer)	Each use
Refrigerator	Check temperature	Daily (2x)- 4 hours apart
Membrane Filtration Equipment	Checks for leaks and surface scratches	Each use
Biohazard hood	Monitor air	Monthly
	Inspect for airflow	Quarterly
Incubator	Check temperature	Twice daily 4 hours apart
Glassware	Inspect for cleanliness, chips and etching	Each use
	Check pH	Each batch
	Conduct inhibitory residue test	Annually
	BTB Test	Each batch
Dilution water bottles	Check pH	Each batch
Media	Check pH and appearance	Each use

Autoclave	Check performance (using steri-amps)	Monthly
Plate Counts	Perform Duplicate Analyses	Monthly
	Repeat Counts (+Second analyst)	Monthly

Table 2. Quality of Reagent Water Used in Microbiology Testing

Test	Monitoring Frequency	Maximum Acceptable Limit
Chemical Test:		
Conductivity	Monthly	>0.5 megohms resistance or <2 µmhos/cm at 25°C
pH	Each batch	5.5-7.5
Total organic carbon	Monthly	<1.0 mg/L
Heavy metals, single (Cd, Cr, Cu, Ni, Pb, and Zn)	Annually	<0.05 mg/L
Heavy metals, total	Annually	<0.10 mg/L
Ammonia/organic nitrogen	Annually	<0.10 mg/L
Total chlorine residual	Monthly or with each use	<0.01 mg/L
Bacteriological test:		
Heterotrophic plate count	Monthly	<1000CFU/mL
Use test	Annually	Student's *t* <=2.78

Table 3. Time and Temperature for Autoclave Sterilization

Material	Time at 121°C
Membrane filters and pads	10 min
Carbohydrate-containing media (lauryl tryptose, BGB broth, etc.)	12-15 min
Contaminated materials and discarded cultures	30 min
Membrane filter assemblies (wrapped)	
Sample collection bottles (empty)	15 min
Buffered dilution water	15 min
Rinse water, volume >100 mL	Adjust for volume

Table 4. Holding Times for Prepared Media

Medium	Holding Time
Membrane filter (MF) broth in screw-cap flasks at 4EC	96 hours
MF agar in plates with tight-fitting covers at 4EC	2 weeks
Agar or broth in loose-cap tubes at 4°C	2 weeks
Agar or broth in tightly closed screw-cap tubes or other sealed containers	3 months

Poured agar plates with loose-fitting covers in sealed plastic bags at 4°C	2 weeks
Large volume of agar in tightly closed screw-cap flask or bottle at 4°C	3 months

Table 5. Control Culture for Microbiological Tests

	Control Culture	
Group	**Positive**	**Negative**
Total coliforms	*Eschericha coli*	*Staphylococcus aureus*
	Enterobacter aerogenes	*Pseudomonas* sp.
Fecal coliforms	*E. coli*	*E. aerogenes*
		E. faecalis
Escherichia coli	*E. coli*	*E. aerogenes*
Fecal streptococci	*Enterococcus faecalis*	*Staphylococcus aureus*
		E. coli
Enterococci	*E. Faecalis*	*S. mitis/salivarius*

Escherichia Coli MPN PROCEDURES

1) Test Method

Based on Standard Methods 9221B.2-2006/9221F-2006. Standard Methods (20) 9221F specifically describes the confirmed test procedure for *e.coli.* Standard Methods (20) sections 9221 A-E relate to other multiple-tube fermentation techniques for members of the coliform group, such as fecal coliforms and total coliforms. The presumptive phase of the method for total coliform (Section 9221B) is the same as the presumptive phase for *e.coli.*

2) Applicable Matrix or Matrices

Tests for *E. coli* (using EC-MUG medium) are applicable for the analysis of drinking water, surface water, ground water and wastewater.

3) Method Detection Limit

The method detection limit depends on the dilutions of the test sample used in inoculating media and the number of tubes inoculated per dilution. A maximum of 4 dilutions may be planted to cover the expected MPN range in the sample. Dilutions are prepared through serial 1:10 dilutions of the sample. To calculate MPN results of the sample, **three** of the four planted dilutions are selected. See Section 15, Calculations, and Standard Methods, 20th edition, Section 9221 C. 2. for instructions of choosing dilutions to be used in the calculation.

If 10 ml, 1 ml and 0.1 ml dilutions (in 5 tubes each) are selected for the calculation, the lower detection limit is 1.8 MPN/100ml and the upper detection limit is 1,600 MPN/100ml.

If 1 ml, 0.1ml and 0.01ml dilutions (in 5 tubes each) are selected for the calculation, the lower detection limit is 18 MPN/100ml and the upper detection limit is 16,000 MPN/100ml. If 0.1ml, 0.01ml and 0.001ml dilutions (in 5 tubes each) are selected for the calculation, the lower detection limit is 180 MPN/100ml and the upper detection limit is 160,000 MPN/100ml.

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4) Scope and Application

The Interstate Environmental Commission regularly analyzes environmental samples for both fecal streptococcus and enterococcus in conjunction with ambient water quality surveys aboard the Commission's boat as well as for special projects. *Escherichia coli* is a member of the fecal coliform group of bacteria. This organism in water indicates fecal contamination. Enzymatic assays have been developed that allow for the identification of this organism. In this method *E. coli* is defined as coliform bacteria that possess the enzyme β-glucoronidase and are capable of cleaving the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) with the corresponding release of the fluorogen when grown in EC-MUG medium at 44.5°C within 24± 2 hours or less. The procedure is used as a confirmatory test after prior enrichment in a presumptive medium for total coliform bacteria. This test is performed as a multiple-tube procedure.

5) Summary of method

Test tubes containing fermentation tubes are loaded with 10 ml of Lauryl Tryptose broth and sterilized by autoclave. One ml of a test water (or test water diluted with sterile dilution water) is added, and the tubes are incubated. At the end of the presumptive incubation period (48±3 hours or less), the tubes are scored for production of gas. Positive lauryl tryptose broth tubes are used to inoculate tubes containing EC-MUG medium. Inoculated EC-MUG tubes are incubated in a water bath maintained at 44.5 ± 0.2°C for 24±2 hours. The presence of a bright blue fluorescence is considered a positive response for *e. coli* The most probable number (MPN) *e.coli* in the water sample is estimated from the number of positive EC-MUG tubes.

6) Definitions

Escherichia coli is a member of the fecal coliform group of bacteria. *E. coli* is a member of the indigenous fecal flora of warm-blooded animals. The occurrence of *E. coli* is considered a

specific indicator of fecal contamination and the possible presence of enteric pathogens. Enzymatic assays have been developed that allow for the identification of this organism. In this method, *E. coli* are defined as coliform bacteria that posess the enzyme β-glucoronidase and are capable of cleaving the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) with the corresponding release of the fluorogen when grown in EC-MUG medium at 44.5°C within 24±2 hours or less. The procedure is used as a confirmatory test after prior enrichment in a presumptive medium for total coliform bacteria (LTB). Tests for *E.coli* (using EC-MUG medium) are applicable for the analysis of drinking water, surface and groundwater, and wastewater. *E. coli* is a member of the indigenous fecal flora of warm-blooded animals. The occurrence of *E. coli* is considered a specific indicator of fecal contamination and the possible presence of enteric pathogens. See Scope and Application, Section 4, for definitions of organisms. See *Difco Manual* pp 170-171 for definitions of media components.

7) Interferences

Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents. The arbitrary choice of 48±3 hours to assess gas production misses slow-growing members of the coliform family. The media used are not completely selective for coli. Other bacteria may also grow. MPN tables are based on the assumption on a Poisson distribution (random distribution). If the sample bottle is not vigorously shaken before sampling, the most probable number will be an underestimate. A positive control consisting of a known *E. coli* (MUG-positive) culture, a negative control consisting of a thermo-tolerant Klebsiella pneumoniae (MUG-negative) culture, and an uninoculated medium control must be used not only for QC requirements, but to interpret the results and to avoid confusion of weak auto-fluorescence of the medium or test tubes as a positive response.

8) Safety

Wear appropriate personal protective equipment, including lab coat, goggles, and gloves. Avoid breathing dust of dehydrated media. Wear a respirator and/or prepare broth in the hood. Store all tubes, positive and negative, in fume hood after analysis until autoclaving. Autoclave all spent tubes at 121EC±2EC for 30 minutes. Dispose all spent tubes in to red medical biohazardous waste bin underneath the BOD bench. See Pollution Prevention and Waste Management sections for more information on the safe disposal of microbiological waste. Take extreme precaution when handling micoorganisms used for positive and negative control tests. Inoculate in the fume hood, dip inoculating stick in bleach, autoclave and dispose of in red biohazard bins. The manufacturers MSDS's for all reagents are retained and must be reviewed prior to inoculating these organisms.

9) Equipment and Supplies

1. 125 ml plastic bottles for sampling (autoclaved with tape sealing lid of bottle).
2. 5 ml and 10 ml sterile, serological disposable pipets, graduated in 0.1 ml increments, containing cotton plug (Fisher Catalog #'s 13-676-10C and 13-676-10F, respectively).
3. 16 ml polystyrene round-bottom test tubes, with caps, for sample dilution (Fisher Catalog # 959-38B).
4. Mechanical pipetter set to dispense 10 ml broth.
5. 10x75 mm fermentation tubes (Fisher Catalog # 14-961-25).
6. 16x150 mm disposable borosillicate glass test tubes (used for inoculum volumes less that 10 ml). (Fisher Catalog# 14-961-31).
7. 18x150mm disposable borosillicate glass test tubes (used for 10 ml inoculum size). (Fisher Catalog # 14-961-32).
8. 16x150mm screw-cap tubes with caps (for broth to be stored up to 3 months-less than 10 ml inoculum). (Fisher Catalog # 14–95-7-76-C and 03-340-77D)

9. 20x150mm screw-cap tubes with caps (for broth to be stored up to 3 months-10 ml inoculum). (Fisher Catalog # 14-95-7-76E and 03-340-77E).

10.Transfer loops (10µl, sterile, plastic, disposable) (Fisher Catalog # 13-07-03).

11. 35.0°C dry incubator ±0.5°C.

12. 44.5°C waterbath, ±0.2°C.

13. Test tube racks, rust-resistant, capable of holding specified test tubes sizes.

14. pH meter

15. Refrigerator, maintained at 1-4EC for storing broth.

16. Autoclave (Tuttnauer 2540E-B/LHeidolph Brinkmann).

17. Sterilizer indicator tape (Fisher Catalog # 11-875-50)

18. Metal loose-caps for broth to be stored less than 2 weeks.

19. Balance (Mettler Tolledo)

21. Glass or Metal beaker/vessel for preparation of broth.

22. Hot plate/stir plate combination plate. (Isotemp by Fisher Scientific).

23. 6W long-wave UV lamp (365 nm).

10) Reagents and Standards

1. Lauryl Tryptose Broth (Manufactured by BD/Difco, purchased from Fisher, Catalog # DF-0241-17-0)

2. EC-MUG Medium (Manufactured by BD/Difco, DF 0022-17-5)

3. *Escherichia coli* (Fisher Catalog # 23-264397)

4. *Klebsiella pneumoniae* (Fisher Catalog # 23-00172)

5. pH 4.00, 10.00, 7.00 buffers for calibrating the pH meter.

6. Magnesium Chloride for preparation of buffered dilution water.

7. Potassium Dihydrogen Phosphate for preparation of buffered dilution water.

8. 1N NaOH for adjusting pH of buffer

9. Tryptic Soy Broth (for QC check on dilution water and sample bottles)

Preparation of Lauryl Tryptose Broth: The dehydrated lauryl tryptose broth is commercially available and is procured through Fisher Scientific (see Reagents and Standards, above). Dissolve 35.6g of the dehydrated media powder in 1 liter of deionized water. **If 10ml portions are to be planted (to reach lower, <1.8 MPN/100 ml detection level), double strength broth**

must be prepared and used for this dilution only. The pH is measured before and after sterilization. The broth is pipetted, using the automatic pipetter, into the appropriate sized test tubes (see Equipment and Supplies) each containing a fermentation tube. The test tube size chosen depends on the inoculum size and whether the broth will be used within 2 weeks. Inoculum sizes of 10 ml require the larger sized test tubes (see Equipment and Supplies). Broth used within 2 weeks can be stored in loose-capped tubes. Broth that will not be used within 2 weeks must be stored in screw-cap tubes. The tubes should be capped as soon as possible after dispensing and sterilized within 2 hours. The final pH of lauryl tryptose broth must be 6.8±0.2.
Preparation of EC-MUG Broth: 37.1g. EC-MUG Medium is added per 1 liter of deionized water. Warm slightly with stirring to dissolve completely. The broth is pipetted into 16x150mm test tubes each containing an inverted 10x75mm test tube. The pH of the broth is measured before and after sterilization. The tubes should be capped as soon as possible after dispensing and sterilized within 2 hours. Sterilize at 121±2°C for 15 minutes. The final pH of the broth must be 6.9±0.2 at 25°C.

Preparation of Buffers:
a) Phosphate buffer solution: Dissolve 34.0g potassium dihydrogen phosphate (KH_2PO_4) in 500mL distilled water, adjust to pH 7.2±0.5 with 1N sodium hydroxide (NaOH), and dilute to1 L with distilled water.
b) Magnesium chloride buffer: Dissolve 81.1g $MgCl_2 \cong 6H_2O$ in 1 liter deionized water.
Preparation of buffered dilution water: Add 1.25mL stock phosphate buffer solution and 5.0 ml

magnesium chloride solution to 1 L deionized water. Autoclave at 121C±2.0C for 15 minutes. Store refrigerated until used. See Quality Control, section 12, for sterility check on buffered water.

11) Sample Collection, Preservation, Shipment and Storage

Samples for microbiological testing are collected directly into sterilized 125 ml bottles. Bottles may need to be clamped to "sludge nabber" sampling sticks to facilitate sampling if water cannot safely be accessed by reaching or wading. The 125 ml fecal coliform bottle must be clean and sterile (autoclaved for 15 minutes at 121°C±2°C). Bottles are labeled with sterilization date and contain heat sensitive tape which indicates the item has been autoclaved. Remove the cap, immediately prior to sampling, taking care so that there is no contamination to the inside of the cap or the bottle. If sampling from a spigot, which is the case at some municipal wastewater treatment plants and industrial facilities, place the open bottle directly below but not touching the spigot, fill the fecal bottle to the neck, allowing sufficient airspace for mixing, close the tap and cap the bottle. If sample must be taken from a waste stream or effluent channel, dip the open bottle, either using a gloved hand or clamped to a sampling stick, into the waterbody, with the mouth of the sample bottle facing upstream or opposite the direction of any noticeable current or flow. Leave enough air space in the bottle so that it can be properly shaken before inoculation. Cap the bottle immediately and label with investigation # and sample #, location, time of sampling and sampler's initials and placed in a cooler containing ice and a cooler thermometer. Some samples that are known to contain chlorine must be dechlorinated. In this case, add sodium thiosulfate to give a concentration of about 100mg/L. This is purchased from Fisher Scientific (Catalog # SS370-1). The cooler must contain a temperature blank, consisting of a sealed clear bottle, filled with deionized water, with a thermometer attached to the cap, extending into the sample. Samples must be transported to the lab, processed and incubated within 8 hours of sample collection. Upon receipt, the condition of the sample, and temperature, including any abnormalities or departures from standard condition, must be recorded in sample login book and

on chain of custody. All microbiological samples shall be considered acceptable if they arrive with a temperature blank ranging from just above freezing to 6°C. Samples that are hand delivered to the laboratory immediately after collection may not meet this criterion. In these cases, the samples shall be considered acceptable if there is evidence that the chilling process has begun, such as arrival on ice. Upon arrival in the lab, the residual chlorine is measured in all samples requiring field dechlorination prior to inoculation to check the efficacy of the sodium thiosulfate.

12) Quality Control

See QC from TC/FC by MF SOP X Section 12 (bulleted list).

Samples are transported to the lab on ice and inoculated within 8 hours of collection. The temperature of the temperature blank is checked upon arrival at the lab and the temperature is recorded in the sample log-in book. Quality control samples are available commercially and are currently procured from MicroBiologics. Microorganisms are inoculated into the appropriate broth according to the manufacturer's direction. Microorganisms may be in the form of lyophilized disks or swabs depending on availability. Run a positive control, a negative control, and a blank on each batch of broth prior to using broth and along with each batch of samples. To test Lauryl Tryptose Broth, *Escherichia coli* is used as a positive control and *Enterococcus faecalis* is used as a negative control. To test EC-MUG medium, *Escherichia coli* is used as a positive control and *Klebsiella pneumoniae* is used as a negative control. The tubes are incubated at 35°C ± 0.5°C and inspected at 24 and 48 hours. The broth is tested prior to use. A blank, consisting of a tube containing only broth, is run alongside each set of positive and negative controls. Commercial availability of the organisms used in the above quality control checks may vary. Appropriate microorganisms for each media are listed in Standard Methods, 20th Edition, table 9020:V. "Control Cultures for Microbiological Tests".

Check the sterility of each batch of buffered dilution water by inoculating 1 mL of dilution water into Tryptic Soy Broth. Incubate positive control cultures at 35EC ±0.5EC and inspect at 24

hours and 48 hours for turbidity. See Section 16) Method Performance for information on sample duplicates, internal QC samples and external proficiency test samples to be used as quality control measures.
See Section 23, Tables 1-5 for more information on routine quality control performed on laboratory equipment and reagents. See *The Interstate Environmental Commission's Quality Control Manual* for more information on the IEC's quality control program.

13) Calibration and Standardization

Calibrate the pH meter on the day of broth preparation using pH buffer 4.00 and 10.00. Measure the pH 7.00 buffer. Record calibration and reading in pH meter calibration logbook. All thermometers used in the refrigerators, incubators, and waterbaths are checked once a year at test temperature against a standards thermometer. The standards thermometer is sent to Kessler Thermometer Corp. in Amityville, NY once a year for certification. The autoclave maximum-registering thermometer is sent to Kessler Thermometer Corp. in Amityville, NY once a year for certification. The performance of the PB303 balance used to weigh dehydrated media is checked on each day of use with 0.05 gram, 20.0 gram and 50.0 gram NVLAP certified weights. The balance is calibrated once a year by a service representative from Mettler-Toledo, Columbus, OH. The balance weights are calibrated once a year by Troemner in Thorofare, NJ.

14) Procedure

1. Presumptive Procedure:

If the broths have been refrigerated prior to use, incubate them in the 35°C incubator overnight prior to use. If it is not possible to incubate tubes overnight (e.g. samples brought in on Monday), incubate the broths for a minimum of 1 hour prior to inoculating. Inspect tubes for air bubbles prior to beginning inoculation. Prepare and inoculate a maximum of 4 decimal-point (e.g. 10, 1, 1.0, 0.1, 0.01, 0.001, 0.0001 ml of sample) dilutions of the test water sample. Dilutions should be inoculated that cover the range of the expected MPN values. Shake the

sample bottle vigorously, at least 25 times before withdrawing aliquots for inoculations. If 1.0ml is the first (lowest) dilution, add 1 ml of sample to each of five or three test tubes containing 10 ml Lauryl Tryptose broth and 1 ml to dilution tube containing 9 ml dilution water to prepare 0.1 ml sample. Similarly, shake the dilution tube containing the 0.1 ml sample and add 1 ml of 0.1 ml sample to each of five or three tubes containing 10 ml Lauryl Tryptose broth and 1 ml to a dilution tube containing 9 ml dilution water to prepare the 0.01 ml dilution sample. Add 1 ml of this dilution to each of five or three tubes containing Lauryl Tryptose broth to prepare the final sample series. Continue in the same way for the 0.001 and 0.0001 dilutions. Incubate the inoculated fermentation tubes at 35±0.5°C in the dry incubator. At the end of 24±2 hours, shake each tube gently and examine it. If no gas has been trapped in the inverted tube, repeat this step at the end of 48±3 hours from inoculation. Record the presence or absence of gas formation at each examination of the tubes. Formation within 48±3 hours of gas in any amount in inner fermentation tubes constitutes a positive Presumptive Test. The appearance of bubbles must not be confused with actual gas production. If gas is formed due to fermentation, the broth will become cloudy. Active fermentation is shown by the appearance of small bubbles in the medium near the inner vial when the tube is shaken.

2. Confirmed Procedure

Transfers are made using a sterile, disposable 10 µl plastic loop from all positive lauryl tryptose broth tubes after 24±2 hours (and newly positive tubes after 48±3 hours) to EC-MUG medium. When making such transfers, first gently shake the presumptive tube or mix by rotating. Inoculation loops are used once and discarded in red biohazard bins. Inoculated EC-MUG tubes are incubated in a water bath at 44.5±0.2°C for 24±2 hours. Place the tubes into the bath within 30 minutes of inoculation. The water depth in the incubator should be sufficient to immerse the tube to the upper level of the medium. Examine all tubes exhibiting growth for fluorescence using a long-wavelength (6W, 365nm) UV lamp. The presence of bright blue fluorescence is considered a positive response for *e.coli*. A positive control consisting of a known *E. coli* (EC-

MUG) positive culture, a negative control consisting of a thermo tolerant Klebsiella pneumoniae (MUG-negative) culture, and an uninoculated medium control must be run prior to the broth being used. Record the presence or absence of gas formation at each examination of the tubes. Calculate MPN from the number of EC-positive tubes.

15) Calculations

Estimate the *E. coli* densities from the number of tubes in each dilution that produce a positive result in the EC-MUG Medium, respectively. The most probable number (MPN) of coliforms are computed using the attached MPN chart (SM(20) Table 9221.IV). If 10 ml, 1 ml and 0.1 ml inoculum sizes are used, the MPN is read and reported as the number beside the corresponding combination of positives. If 1 ml, 0.1 ml and 0.01 ml inoculum sizes are used, read and report an MPN 10 times the number beside the corresponding combination of positives. The following formula summarizes this calculation for any series of decimal-point dilution:

$$\text{MPN value (from MPN table)} \times \frac{10}{\text{largest volume tested}} = \text{MPN/100 ml}$$

16) Method Performance

Method performance is checked using numerous methods. 5% of all samples are analyzed in duplicate to measure precision of the method. Positive controls, negative controls, and blanks are run before each batch of media is used. Analysts perform a demonstration of capability each year on a proficiency sample or other sample procured by the Laboratory Director. The Interstate Environmental Commission's laboratory participates in both internal and external proficiency tests.

17) Pollution Prevention

All used broth and agar is autoclaved for 30 minutes at 121°C±2°C after use. All waste generated from microbiological testing, including disposable pipettes, transfer loops, agar plates, gloves are disposed of in the red biohazard bins. Benches are cleaned with a disinfectant before and after use for microbiological testing. See Safety, Section 8, and Waste Management, Section 21 for more information on procedures related to pollution prevention.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

Ideally all samples analyzed in duplicate should vary by no more than 15%. All positive control QC checks must yield a positive result. All negative control QC checks and all blanks must yield a negative result. See Section 23, Table 2, for acceptance criteria for water to be used in microbiological testing.

19) Corrective Actions for Out of Control Data

Samples received above 6°C or outside of the 8 hour holding time indicate problems with transport. The sampler/transporter must report the problem to the Laboratory Director who will determine whether the analysis should proceed. If problems with transport are indicated by the above quality control data, the Laboratory Director must review sample transport procedures with the sampler. These may include, but not be limited to, checking the accuracy of the temperature blank thermometer, changing the size of the cooler and/or amount of ice used in transport. If consistent problems adhering to the eight hour holding time arise, the Technical Director should review with the designated Project Manager the feasibility of the sample site in regards to transport requirements. If the batch QC fails for any batch of broth, the broth must be discarded and remade.

20) Contingencies for Handling Out-of-Control Data

Samples received at temperatures above 6°C or outside of the 8 hour holding time cannot be used for regulatory purposes. The results of the positive and negative quality control checks are vital

in determining the validity of data. A negative control culture which generates a positive result indicates contamination of the analysis equipment, supplies and/or reagents. A blank with growth also indicates a contamination problem. A positive control culture which generates a negative result indicates that the broth is not able to support the indicated organism growth. Any batch of broth that fails the above QC checks must be discarded. Even if the results are not to be used for regulatory purposes, the results must be qualified.

21) Waste Management

See IEC SOP manual, revision 1 January 2002, page 12 of 15, for information regarding pickup of biowaste generated by microbiological testing.

22) References

Standard Methods for the Examination of Water and Wastewater, 20th edition, Sections9020, 9030, 9040, 9050, 9060, 9221A-C. APHA, AWWA, WEF, 1998.

Standard Methods for the Examination of Water and Wastewater, 20th edition, Section 9020B, Table 9020:V, p9-10.

Difco Manual, 11th Edition, Difco Laboratories, 1998.

IEC Quality Control Manual, Revision 1, January 2002.

23) Tables, Diagrams, Flowcharts, Validation Data

Table 1. Key Quality Control Practices

Item	Action	Frequency
Reagent water	Monitor quality	See Table 2
Bench surface	Monitor for contamination	Monthly
Air in workplace	Monitor bacterial density	Monthly

Thermometers	Check accuracy	annually
Balances	Check accuracy	Each use
Balances	Service and recalibrate	Annually
pH meter	Standardize	Each day of use
Autoclave	Check performance (using autoclave tape, maximum registering thermometer)	Each use
Refrigerator	Check temperature	Daily (2x)
Incubator	Check temperature	Twice daily
Glassware	Inspect for cleanliness, chips and etching	Each use
	Check pH	Each batch
	Conduct inhibitory residue test	Annually
Dilution water bottles	Check pH	Each batch
Media	Check pH and appearance	Each use
Autoclave	Check performance (using steri-amps)	Monthly

Table 2. Quality of Reagent Water Used in Microbiology Testing

Test	**Monitoring Frequency**	**Maximum Acceptable Limit**
Chemical Test:		
Conductivity	Monthly	>0.5 megohms resistance or <2 μmhos/cm at 25°C
pH	Each day of use	5.5-7.5
Total organic carbon	Annually	<1.0 mg/L
Heavy metals, single (Cd, Cr, Cu, Ni, Pb, and Zn)	Annually	<0.05 mg/L
Heavy metals, total	Annually	<0.10 mg/L
Ammonia/organic nitrogen	Annually	<0.10 mg/L
Total chlorine residual	Monthly	<0.01 mg/L
Bacteriological test:		
Heterotrophic plate count	Monthly	<1000CFU/mL
Use test	Annually	Student's t <=2.78

Table 3. Time and Temperature for Autoclave Sterilization

Material	Time at 12ƒC
Carbohydrate-containing media (Lauryl Tryptose, BGB broth, etc.)	12-15 min
Contaminated materials and discarded cultures	30 min
Sample collection bottles (empty)	15 min
Buffered dilution water, 99 ml in screw-cap bottle	15 min
Rinse water, volume >100 ml	Adjust for volume

Table 4. Holding Times for Prepared Media

Medium	Holding Time
Agar or broth in loose-cap tubes at 4°C	2 weeks
Agar or broth in tightly closed screw-cap tubes or other sealed containers	3 months
Poured agar plates with loose-fitting covers in sealed plastic bags at 4°C	2 weeks
Large volume of agar in tightly closed screw-cap flask or bottle at 4°C	3 months

Table 5. Control Culture for Microbiological Tests

Currently, Microbiologics® Kwik-Stik™ Microorganisms are used for positive and negative QC tests. See Microbiologics® Product Insert, attached, for directions for use.

	Control Culture	
Group	**Positive**	**Negative**
Total coliforms	*Eschericha coli*	*Staphylococcus aureus*
	Enterobacter aerogenes	*Pseudomonas* sp.
Fecal coliforms	*E. coli*	*E. aerogenes*
		Streptococcus faecalis
Escherichia coli	*E. coli*	*E. aerogenes*
Fecal streptococci	*Enterococcus faecalis*	*Staphylococcus aureus*
		E. coli
Enterococci	*S. faecalis*	*S. mitis/salivarius*

Attached at the end of this SOP are the following photocopied tables and charts

Standard Methods, 18th edition, Table 9221.IV (p.9-52)

Standard Methods, 14th Edition, Table 908:II.

Standard Methods, 20th edition, Table 9020B

Escherichia coli (E. coli) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC)

1) Test Method

Based on EPA Method 1103.1: *Escherichia coli (E. coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC)

2) Applicable Matrix or Matrices

This test can be used for the enumeration of *Escherichia coli* in ambient waters. Based on the high Method 1103.1 false positive and false negative levels when compared to Method 1603 in interlaboratory studies, Method 1103.1 is not approved for the analysis of disinfected wastewater.

3) Method Detection Limit

Because a wide range of sample volumes and dilutions can be analyzed by the membrane filtration technique, a wide range of *Escherichia coli* levels in water can be detected and enumerated. Detection limits will vary depending on the volumes and dilutions used in filtration.

4) Scope and Application

Proficiency samples are analyzed semi-annually and continuing demonstrations of capability are analyzed annually to maintain certification. This method describes a membrane filter (MF) procedure for the detection and enumeration of *Escherichia coli* bacteria in ambient waters. This method is being added to the scope of IEC parameters in order to assist the NJDEP Bureau of Marine Water Monitoring.

5) Summary of Method

This method provides a direct count of *E. coli* in water based on the development of colonies on the surface of the membrane filter. A water sample is filtered through the membrane which retains the bacteria. Following filtration, the membrane is placed on a selective and differential medium, mTEC agar, incubated at 35°C±0.5°C for 2±0.5 hours to resuscitate injured or stressed bacteria, and then incubated in a 44.5°C±0.2°C waterbath for 22±2 hours. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. After 15 minutes, yellow, yellow-green, or yellow-brown colonies are counted with the aid of a fluorescent lamp and a magnifying lens.

6) Definitions

E. coli is a common inhabitant of the intestinal tract of warm-blooded animals, and its presence in water samples is an indication of fecal pollution and the possible presence of enteric pathogens. In this method, E. coli are those bacteria which produce colonies that remain yellow, yellow-green, or yellow-brown on a filter pad saturated with urea substrate broth after primary culturing on mTEC medium after the appropriate incubation time and temperatures.
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7) Interferences

Water samples containing colloidal or suspended particulate materials can clog the membrane filter and prevent filtration, or cause spreading of bacterial colonies which could interfere with identification of target colonies.

8) Safety

Wear appropriate personal protective equipment, including lab coat, goggles, and gloves while preparing, using, and disposing of cultures, reagents, and materials and while operating sterilization equipment. Avoid breathing dust of dehydrated media. Wear a respirator and/or prepare broth in the hood. Store all plates, positive and negative, in fume hood after analysis until autoclaving. Autoclave all spent plates at 121EC±2°C for 30 minutes. Autoclave plates in sealed, red plastic autoclave bags. See Pollution Prevention and Waste Management sections for more information on the safe disposal of microbiological waste. Take extreme precaution when handling micoorganisms used for positive and negative control tests. The manufacturers MSDS's are retained and should be reviewed prior to inoculating these organisms. Mouth-pipetting is prohibited.

9) Equipment and Supplies

1. Glass lens with magnification of 2-5x or stereoscopic microscope.
2. Lamp, with a cool, white fluorescent tube.
3. Hand tally or electronic counting device.
4. Pipets, sterile, T.D. bacteriological plastic, of appropriate volume.
5. Graduated cylinders, 100-1000 ml, covered with aluminum foil or kraft paper and sterile. Alternatively, dilutions may be made directly into sterilized filtration funnels (which are periodically calibrated using graduated cylinders).
6. Membrane filtration units (filter base and graduated funnel), plastic, wrapped with aluminum foil and sterilized.
7. Line vacuum, electric vacuum pump, or aspirator for use as a vacuum source.
8. A stainless steel filter manifold to hold a number of filter bases.
9. Ultraviolet unit for sanitization of the filter funnel between filtrations.

10. Flask (or carboy) for safety trap placed between the filter flask and the vacuum source.
11. Forceps, straight or curved, with smooth tips to handle filters without damage.
12. Ethanol, methanol or isopropanol in a small, wide-mouth container, for sterilizing forceps.
13. Electric incinerator unit for sterilizing loops and needles (Bacticinerator™).
14. Thermometer, checked against a National Institute of Standards and Technology (NIST) certified thermometer, or one that meets the requirements of NIST Monograph SP 250-23.
15. Petri dishes, sterile, plastic, 9 x 50 mm, with tight-fitting lids.
16. Dilution tubes marked at 9 ml, for dilutions. Graduated cylinders, 100 ml for verifying funnel graduations if funnels are used for dilutions.
17. Flasks, borosilicate glass, screw-cap, 250-2000 ml volume.
18. Membrane filters, made of cellulose ester, sterile, white, grid marked, 47 mm diameter, with 0.45µm pore size, 47 mm diameter.
19. Inoculation loops, at least 3 mm diameter, Sterile, disposable plastic loops are used.
20. Incubator maintained at at 35°C±0.5°C.
21. Waterbath maintained at 44.5°C±0.2°C
22. Waterbath maintained at 50EC for tempering agar.
23. Screw-capped test tubes, 20 x 150 mm, borosilicate glass or plastic (for LTB and verification media)
24. Test tubes, 10 x 75mm, borosilicate glass (inverted tubes for LTB and EC)
25. Caps, aluminum or autoclavable plastic, for 20 mm diameter test tubes.
26. Test tubes, borosilicate glass, 16 x 125 mm or other appropriate size.
27. Whirl-Pak® bags.
28. Filter paper
29. Autoclave or steam sterilizer capable of achieving 121°C [15 lb pressure per square inch (PSI)] for 15 minutes.
30. Colony (plate) spreader for IPR/OPR

10) Reagents and Standards

1. Purity of Reagents: Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, reagents shall conform to the specifications of the Committee of Analytical Reagents of the American Chemical Society. The agar used in preparation of the culture media must be of microbiological grade.

2. Whenever possible, use commercial culture media as a means of quality control. When preparing media and reagents, manufacturer's instructions typically are based on the preparation of 1 L volumes. The preparation of smaller volumes may be more practical based on sample set size and to reduce waste. When preparing volumes other than those specified, be sure to double-check calculations to ensure the appropriate weight and/or volume of reagents are being used to produce the correct concentration.

3. Purity of Water: Reagent-grade water conforming to specifications in Standard Methods for the Examination of Water and Wastewater, Section 9020. Laboratory Deionized water is used and is monitored using the appropriate QC checks outlined in Table 2.

4. Phosphate buffered saline (PBS)
Composition:

Monosodium phosphate	0.58g
Disodium phopshate	2.5g
Sodium chloride	8.5g
Reagent-grade water	1.0L

Dissolve the reagents in 1 L of reagent-grade water and dispense in appropriate amounts for dilutions in screw cap bottles or culture tubes, and/or into containers for use as rinse water. Autoclave after preparation at 121E± 2EC and 15 PSI for 15 minutes. Final pH should be 7.4±0.2.

Note: The initial and ongoing precision and recovery (IPR and OPR) performance criteria established for Method 1103.1 were determined using spiked PBS samples (Section 9.3). Laboratories must use PBS when performing IPR and OPR sample analyses. However, phosphate-buffered dilution water may be substituted for PBS as a sample diluents and final rinse buffer.

5. Phosphate buffered dilution water:

Composition of stock phosphate buffer solution:	34.0g
Monopotassium phosphate (KH_2PO_4)	500ml

Preparation: Dissolve KH_2PO_4 in 500 ml reagent-grade water. Adjust the pH of the solution to 7.2 with 1N NaOH, and bring the volume to 1 L with reagent-grade water. Sterilize by filtration or autoclave at 121°C (15 PSI) for 15 minutes.

Preparation of stock magnesium chloride solution: Add 38g anhydrous $MgCl_2$ or 81.1g $MgCl_2$•$6H_20$ to 1 L reagent-grade water. Sterilize by filtration or autoclave at 121°C (15 PSI) for 15 minutes.

After sterilization, store the stock solutions in the refrigerator until used. Handle aseptically. If evidence of mold or other contamination appears, the affected stock solution should be discarded and a fresh solution should be prepared.

Working phosphate buffered dilution water: Mix 1.25 ml of the stock phosphate buffer and 5 mL of the $MgCl_2$ stock per liter of reagent-grade water. Dispense in appropriate amounts for

dilutions in screw-cap bottles or culture tubes, and/or into containers for use as rinse water. Autoclave at 121°C (15PSI) for 15 minutes. Final pH should be 7.0±0.2.

6. mTEC agar (HACH Catalog # 22811276). Add 45.3g of dehydrated media to 1 L of reagent-grade water, mix thoroughly, and heat with agitation to dissolve. Autoclave at 121°C (15PSI) for 15 minutes, and cool in a 50°C waterbath. Pour 4-6 ml of medium into each 9 x 50 mm petri dish to a 4-5 mm depth, and allow to solidify. Final pH should be 7.3±0.2. Store in a refrigerator (2 weeks for tight-fitting petri dishes). Commercially prepared poured agar plates may also be procured. Retain certificates of analysis for all media and reagents.

7. Urea substrate medium:
Composition:

Urea	2.0g
Phenol Red	0.01g
Reagent-grade water	100 ml

Add reagents to 100 ml reagent-grade water and mix thoroughly to dissolve. Adjust to pH 5.0±0.2 with 1 N HCl. The substrate solution should be a straw-yellow color at this pH. The substrate solution should be stored at 6-8°C for no more than 1 week.

8. Tryptic Soy Agar (TSA): For preparing spiking stock culture for IPR/OPR
BD/Difco™ Reference# 236940 (Fisher Catalog# DF0369-15-8). Suspend 4g of the powder in 100 mL of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes and cool in a 50°C waterbath. Pour the medium into each 15 x 100 mm petri dish to a 4-5 mm depth, and allow to solidify. Final pH should be 7.3±0.2.

9. Lauryl Tryptose Broth (LTB): For spiking IPR/OPR
BD/Difco™ Ref erence#224150 (Fisher Catalog #DF0241-17-0). Suspend 35.6g of the powder in 1 L of purified water. Mix thoroughly. Warm slightly to completely dissolve the powder. Dispense into 20x150 mm test tubes containing inverted fermentation vials. Cap and autoclave at 121°C for 15 minutes. Cool the broth as quickly as possible. Final pH should be 6.8±0.2.

10. Nutrient agar (NA): For verification procedure
BD/Difco™ Reference # 212000 (Fisher Catalog#DF0001-15-2). Follow manufacturer's instructions on bottle. Suspend specified weight of dehydrated agar in 1 L of reagent-grade water, mix thoroughly, and heat to boiling to dissolve completely. Dispense in screw-cap tubes, and autoclave at 121°C for 15 minutes. Remove the tubes and slant. Final pH should be 6.8±0.2.

11. Tryptic/trypticase soy broth (TSB): for verification procedure
BD/Difco™ Ref erence# 211768 (Fisher Catalog# B11768). Dissolve 30g of the powder in 1 L of purified water. Mix thoroughly. Warm gently until solution is completely dissolved.

Dispense in screw-cap tubes, and autoclave at 121°C for 15 minutes. Final pH should be 7.3±0.2.

12. Simmons citrate agar slants: for verification procedure
BD Reference #212455 (Fisher Scientific Catalog # B12455). Follow manufacturer's instructions on bottle. Suspend specified weight to1 L of reagent-grade water, mix thoroughly, and heat to boiling to dissolve completely. Dispense into screw-cap tubes, and autoclave at 121°C (15 PSI) for 15 minutes. After sterilization, slant until solid. Final pH should be 6.9±0.2.

13. Tryptone water: for verification procedure

Composition:

Tryptone	10.0g
Sodium chloride	5.0g
Reagent-grade water	1.0L

Add reagents to 1L of reagent grade water and mix thoroughly to dissolve. Dispense in 5 –mL volumes into tubes, and autoclave at 121°C (15 PSI) for 15 minutes. Final pH should be 7.3±0.2.

14. EC broth: for verification procedure
BD/Difco Reference # 231430, (Fisher Scientific Catalog # DF0314-17-2). Dissolve 37g of powder in 1 L of purified water. Mix thoroughly. Warm slightly to completely dissolve the powder. Dispense into tubes containing inverted fermentation vials. Autoclave at 121°C for 15 minutes. Note: Do not use if the inverted tubes are not completely filled with medium after sterilization.

15. Oxidase reagent

Composition:
N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride, 1% aqueous solution (1 g per 100 mL sterile reagent-grade water). Note: Prepared oxidase test slides are commercially available.

16. Kovaks indole reagent
Fisher Scientific Catalog # MB0209A

17. Control cultures:
Positive control: *Escherichia coli* ATCC#11775. (Kwik-stiks™ manufactured by Microbiologics are used to prepare stock slants. New slants are prepared monthly from Kwik-stiks™).

Negative control: *Enterococcus faecalis* ATCC # 19433. (Kwik-stiks™ manufactured by Microbiologics are used to prepare stock slants. New slants are prepared monthly from Kwik-stiks™).

18. pH 4.00, 7.00 and 10.00 buffers for calibrating the pH meter

19. 1N NaOH and 1N HCl for adjusting pH of stock phosphate buffer solution and urea substrate, respectively

20. Plate Count Agar (for QC check on dilution water and air)

21. Reagent- grade deionized water.

11) Sample Collection, Preservation, and Storage

Samples for microbiological analysis should be collected as grab samples directly into 125 mL sterilized bottles. The 125 ml sterilized bottle must be clean and sterile (autoclaved for 15 minutes at 121°C±2ºC), and checked for sterility using TSB broth. Bottles are labeled with sterilization date and sealed heat-sensitive tape that indicates the bottles have reached sterilization temperature. Remove the cap, immediately prior to sampling, taking care so that there is no contamination to the inside of the cap or the bottle. If sample must be taken from a waste stream or effluent channel, dip the open bottle, either using a gloved hand or clamped to a sampling stick, into the waterbody, with the mouth of the sample bottle facing upstream or opposite the direction of any noticeable current or flow. Bottles may need to be clamped to "sludge nabber" sampling sticks to facilitate sampling if water cannot safely be accessed by reaching or wading. The sampling depth for surface water samples should be 6-12 inches below the water surface. After removal of the container from the water, a small portion of the sample should be discarded to allow headspace for proper mixing before analysis. The sample is then capped, labeled with date, time, sample location, analysis and sampler initials and placed inside a cooler on ice. A properly completed IEC Laboratory chain of custody must accompany all samples from time of sampling to arrival at laboratory. Ice or refrigerate samples at a temperature of <10ºC during transit. Do not freeze the samples. Use insulated containers to assure proper maintenance of storage temperature. Take care that sample bottles are not totally immersed in water during transit or storage (i.e. drain excess water through cooler valve prior to transport to lab). The cooler must contain a temperature blank, consisting of a sealed clear bottle, filled with deionized water, with a thermometer attached to the cap, extending into the sample. The temperature of the temperature blank is read and recorded in the sample log-in book upon arrival at laboratory. Samples must be transported to the lab, processed and incubated within 8 hours of sample collection. Upon receipt, the condition of the sample, including any abnormalities or departures from standard condition, must be recorded in sample login book and on chain of custody. Sample login information includes All microbiological samples shall be considered acceptable if they arrive with a temperature blank ranging from just above freezing to 10ºC. Samples that are hand delivered to the laboratory immediately after collection may not

meet this criterion. In these cases, the samples shall be considered acceptable if there is evidence that the chilling process has begun, such as arrival on ice.

Additional detail regarding sample collection, including sampling techniques and selection of sites and frequency is discussed in EPA Method 1103.1, section 3.

12) Quality Control

The IEC Laboratory operates a formal Quality Assurance program. The Quality Control manual outlines this program. All documentation regarding instrument and equipment maintenance, performance, and QC checks is outlined in the Quality Control Manual and individual analytical SOPs. Tables 1-4, at the end of this SOP, outline major microbiological QC requirements.

IPR- Initial Precision and Recovery

An initial demonstration of laboratory capability must be performed through performance of the initial precision and recovery (IPR) analyses for each analyst performing the analysis prior to the analysis of any environmental samples.

Initial precision and recovery (IPR) - The IPR analyses are used to demonstrate acceptable method performance (recovery and precision) and should be performed by the laboratory before the method is used for monitoring field samples. IPR samples should be accompanied by an acceptable method blank and appropriate media sterility checks.

Prepare a stock culture by inoculating a TSA slant with *E. coli* ATCC#11775 and incubating at 35°C±3°C for 20±4 hours. This stock culture may be stored in the dark at room temperature for up to 30 days.

Undiluted Spiking Suspension. Prepare a 1% solution of Lauryl Tryptose broth (LTB) by combining 99 ml of sterile PBS and 1 ml of sterile single-strength LTB in a sterile screw-cap bottle or re-sealable dilution water container. Inoculate the 1% LTB using a small amount of growth from the stock culture. Disperse the inoculum by vigorously shaking the broth culture and incubate at 35°C±0.3°C for 20±4 hours. This culture is referred to as the undiluted spiking suspension and should contain approximately 1.0 x 10^7 to 1.0 x10^8 *E. coli* colony-forming units (CFU) per ml of culture.

Mix the undiluted spiking suspension thoroughly by shaking the bottle a minimum of 25 times and prepare a series of dilutions (4 total) in the following manner:

Dilution “A”- Aseptically transfer 1.0 ml of the undiluted spiking suspension to 99 ml of sterile PBS and mix thoroughly by shaking the bottle a minimum of 25 times. This is spiking suspension dilution “A” and 1 ml contains 10^{-2} ml of the original undiluted spiking suspension.

Dilution "B"- Aseptically transfer 1.0 ml of dilution "A" to 99 ml of sterile PBS and mix thoroughly by shaking the bottle a minimum of 25 times. This is spiking suspension dilution "B" and 1 ml contains 10^{-4} ml of the original undiluted spiking suspension

Dilution "C"- Aseptically transfer 11.0 ml of dilution "B" to 99 ml of sterile PBS and mix thoroughly by shaking the bottle a minimum of 25 times. This is spiking solution suspension dilution "C" and 1 ml contains 10^{-5} ml of the original undiluted spiking suspension.

Dilution "D"-Aseptically transfer 11.0 ml of dilution "C" to 99 ml of sterile PBS and mix thoroughly by shaking the bottle a minimum of 25 times. This is spiking suspension dilution "D" and 1 ml contains 10^{-6} ml of the original undiluted spiking suspension.

Add 0.3 ml of the spiking suspension dilution "D" to 100 ml of PBS and mix thoroughly by shaking the bottle a minimum of 25 times. The volume of undiluted spiking suspension added to each 100 ml sample is 3.0 x 10-^{7} m per 100 ml of sample which is referred to as $V_{spikedper\ 100mL}$ below. Filter the spiked sample and analyze the filter according to the Procedure Section 14.

Calculate the percent recovery R for each IPR sample using the equation in Section 15, Calculations.

Using the percent recoveries of the four analyses, calculate the mean percent recovery and the relative standard deviation (RSD) of the recoveries. The RSD is the standard deviation divided by the mean, multiplied by 100.

Compare the mean recovery and RSD with the corresponding IPR criteria in the Table, below. If the mean and RSD for recovery of *E. coli* meet acceptance criteria, system performance is acceptable and acceptance and analysis of field samples can begin. If the mean or the RSD fall outside of the required range for recovery, system performance is unacceptable. In this event, identify the problem by evaluating each step of the analytical process, media, reagents, and controls, correct the problem and repeat IPR analyses.

Initial and Ongoing Precision and Recovery (IPR andOPR) Acceptance Criteria

Peformance test	Lab-prepared spike acceptance criteria
Initial precision and recovery (IPR) • Mean percent recovery • Precision (as maximum relative standard deviation)	 76-124% 41%
Ongoing precision and recovery (OPR) as percent recovery	54-146%

Enumeration of Spiking Suspension

Prepare Trypticase Soy Agar (TSA) spread plates, in triplicate, for spiking suspension dilutions "B" "C" and "D".
Note: agar plates must be dry prior to use. To ensure that the agar surface is dry, plates should be made several days in advance and stored inverted at room temperature or dried using a laminar-flow hood.

Mix dilution "B" by shaking the bottle a minimum of 25 times. Pipet 0.1 ml of dilution "B" onto surface of each of each TSA plate in triplicate using colony spreader to ensure uniform distribution.

Mix dilution "C" by shaking the bottle a minimum of 25 times. Pipet 0.1 ml of dilution "C" onto the surface of each TSA plate in triplicate using colony spreader to ensure uniform distribution.

Mix dilution "D" by shaking the bottle a minimum of 25 times. Pipet 0.1 ml of dilution "D" onto the surface of each TSA plate in triplicate using colony spreader to ensure uniform distribution.

Note: Ensure that the inoculum is evenly distributed over the entire surface of the plate.

Allow the inoculum to absorb into the medium of each plate completely. Invert plates and incubate at 35°C±0.5°C for 20±4 hours.

Count and record number of colonies per plate. Refer to section 14 Procedure for calculation of *E. Coli* concentration in the undiluted spiking suspension. The number of *E. Coli* (CFU/mL) in the undiluted spiking suspension will be calculated using all TSA plates yielding counts within the countable range of 30 to 200 CFU per plate. .

OPR- Ongoing Precision and Recovery

To demonstrate ongoing control of the analytical system, the laboratory must routinely process and analyze spiked PBS samples. The laboratory must analyze one OPR sample after every 20 field and matrix spike samples or one per week that samples are analyzed, whichever occurs more frequently. OPR samples must be accompanied by an acceptable method blank and appropriate media sterility checks. The OPR analysis is performed as follows:

Spike one (1) 100 mL PBS sample with *E. coli* ATCC # 11775 according to the spiking procedure described above for the IPR. Filter and process each OPR sample according to the

method Procedure, Section 14 and calculate the number of *E. coli* per 100 ml according to Section 15, Calculations.

Calculate the percent recovery (R) for each IPR sample using the equation in section 15, calculations.

Compare the OPR result (percent recovery) with the corresponding OPR recovery criteria in Table 1, above. If the OPR result meets the acceptance criteria for recovery, method performance is acceptable and analysis of field samples may continue. If the OPR result falls outside of the acceptance criteria, system performance is unacceptable. In this event, identify the problem by evaluating each step of the analytical process, media, reagents, and controls, correct the problem and repeat the OPR analysis.

As part of the laboratory QA program, results for OPR and IPR samples should be charted and updated records maintained in order to monitor ongoing method performance. The laboratory should also develop a statement of accuracy for Method 1103.1 by calculating the average percent (R) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from R - $2s_r$ to R+$2s_r$.

Positive and Negative Controls

Negative controls- The laboratory must analyze negative controls to ensure that the mTEC agar and urea substrate are performing properly. Negative controls should be analyzed whenever a new batch of media or reagents is used. On an ongoing basis, the laboratory should perform a negative control every day that samples are analyzed.

> Negative controls are conducted by filtering a dilute suspension of viable *E. faecalis* and analyzing as in section 14, Procedure.
>
> If the negative control fails to exhibit the appropriate response, check and/or replace the associated media or reagents, and/or the negative control and reanalyze the appropriate negative control

Positive controls- The laboratory must analyze positive controls to ensure that the mTEC agar and urea substrate are performing properly. Positive controls should be analyzed whenever a new batch of media or reagents is used. On an ongoing basis, the laboratory should perform a positive control every day that samples are analyzed. An OPR sample may take the place of a positive control.

> Positive controls are conducted by filtering a dilute suspension of viable *E. coli* and analyzing as described in Section 14, Procedure.

If the positive control fails to exhibit the appropriate response, check and/or replace the associated media or reagents, and/or the positive control and reanalyze the appropriate positive control.

Controls for verification media-All verified media should be tested with appropriate positive and negative controls whenever a new batch of media and/or reagents are used. On an ongoing basis, the laboratory should perform positive and negative controls on the verification media with each batch of samples submitted to verification. Examples of appropriate controls for verification media are provided in the following table:

Medium	Positive Control	Negative Control
Cytochrome oxidase reagent	*P. aeruginosa*	*E. coli*
Kovacs indole reagent	*E. coli*	*E. aeruginosa*
Simmons citrate agar	*E. aeruginosa*	*S. flexneri*
EC broth (44.5°C±0.2°C)	*E. coli*	*E. aeruginosa*

Colony verification-The laboratory should verify 10 typical colonies (positive) and 10 atypical colonies (negative) per month or 1 typical colony and 1 atypical colony from 10% of all positive samples, whichever is greater.

Filter sterility check-Place at least one membrane filter on a TSA plate, and incubate for 24±2 hours at 35°C±0.3°C. Absence of growth indicates sterility of the filter. On an ongoing basis, the laboratory should perform a sterility check every day that samples are analyzed.

Method Blank-Filter a 50 mL volume of sterile PBS or phosphate-buffered dilution water, place the filter on an mTEC agar plate and process according to the procure in section 14. Absence of growth indicates freedom of contamination from the target organism. On an ongoing basis, the laboratory should perform a method blank every day that samples are analyzed.

Media Sterility Check- The laboratory should test media sterility by incubating one unit (tube or plate) from each batch of medium (TSA, mTEC, urea substrate, and verification media) as appropriate and observing for growth. Absence of growth indicates media sterility. On an ongoing basis, the laboratory should perform a media sterility check every day that samples are analyzed.

Analyst colony counting variability-Laboratories with two or more analysts should compare each analyst's colony counts from one positive field sample per month. Colony counts should be within 10% between analysts. Laboratories with a single analyst should have the analyst perform duplicate colony counts of a single membrane filter each month. Duplicate colony counts should be within 5% for a single analyst. If no positive field samples are available, a OPR sample may be substituted for these determinations.

13) Calibration and Standardization

Check temperatures in incubators twice daily (with at least 4 hours between checks) to ensure operation within stated limits and record in temperature logbook. Check thermometers at least annually against the NIST certified thermometer. Check mercury columns for breaks daily. Calibrate the pH meter on the day of broth preparation using pH buffer 4 and 10 using the flat-tipped electrode. Measure the pH 7 buffer. Record calibration and reading in pH meter calibration logbook. The NIST thermometer is sent to Kessler Thermometer Corp. in Amityville, NY once a year for certification. The autoclave maximum-registering thermometer is sent to Kessler Thermometer Corp. in Amityville, NY once a year for certification. The performance of the PB303 balance used to weigh dehydrated media is checked on each day of use with 0.05 gram, 20.0 gram and 50.0 gram NVLAP certified weights. The balance is calibrated once a year by a service representative from Mettler-Toledo, Columbus, OH. The balance weights are calibrated every year by Troemner in Thorofare, NJ.

14) Procedure

14.1 Prepare the mTEC agar and urea substrate as directed in Section 10, Reagents and Standards. Mark the petri dishes and report forms with sample identification and sample volumes. Set up the filtration apparatus: Connect the manifold to one hole in the cap of a carboy via tubing. The second hole in the cap of the carboy (with a shorter tube extending into the carboy) is connected to a vacuum source. Make sure the filtration funnel assembly has been autoclaved. Attach the bottom of the filtration assembly to the manifold. There are three spaces in the manifold. This enables up to three samples (or three dilutions or duplicates of a sample) to be filtered simultaneously.

14.2 Set up and complete all the QC checks described in section 13 Quality Control (Filter sterility check, Method Blanks, Filtration Blank, media sterility check).

14.3 Place a sterile membrane filter on the filter base, grid-side up and attach the funnel to the base so that the membrane filter is now held between the funnel and the base.

14.4 Shake the sample bottle vigorously at least 25 times to distribute the bacteria uniformly, and measure the desired volume of sample or dilution into the funnel.

14.5 Select the sample volumes based on previous knowledge of *E. coli* concentration, to produce 20-80 E. coli colonies on the membranes. It is recommended that a minimum of three dilutions be analyzed to ensure that a countable plate (20-80 *E. coli* colonies) is obtained. For shellfish water analysis, utilize 30 mLs for at least one dilution to achieve the necessary detection limit of 3 CFU/100 mL.

14.6 Smaller sample volumes or sample dilutions can be used to minimize the interference of turbidity or for high bacterial densities. Multiple volumes of the same sample or sample dilutions should be filtered.

Note: When analyzing smaller sample volumes (e.g., <20 ml) 20-30 ml of PBS or phosphate-buffered dilution water should be added to the funnel or an aliquot of sample should be dispensed into a dilution blank prior to filtration. This will allow even distribution of the sample on the membrane.

14.7 Filter the sample, and rinse the sides of the funnel at least twice with 20-30 ml of sterile buffer. Turn off the vacuum, and remove the funnel from the filter base.

14.8 Use sterile forceps to aseptically remove the membrane filter from the filter base, and roll it onto the mTEC agar to avoid the formation of bubbles between the membrane and the agar surface. Reseat the membrane if bubbles occur. Run the forceps around the edge of the filter outside the area of filtration, close to the edge of the dish, to be sure that the filter is properly seated on the agar. Close the dish, invert, and incubate at 35°C±0.5°C for 2 ± 0.5 hours.

14.9 After a 2± 0.5 hour incubation at 35°C±0.5°C, transfer the plates to a Whirl-Pak® bag, seal the bag, and submerge in a 44.5°C±0.2°C waterbath for 22±2 hours.

Do not overfill the Whirl-Pak® bag because this will prevent proper sealing allowing liquid to enter the bag and possibly contaminate the plates.

14.10 After 22±2 hours, remove the plate from the waterbath. Saturate an absorbent pad in a petri dish with urea substrate medium. Aseptically transfer the membrane from mTEC agar to the absorbent pad saturated with urea substrate medium, and allow to sit at room temperature for 15-20 minutes.

14.11 After incubationon the urea substrate at room temperature, count and record colonies on those membrane filters containing 20-80 yellow, yellow-green, or yellow-brown colonies on the mTEC agar.

Verification Procedure-

14.12 Yellow, yellow-green, or yellow-brown colonies from the urea test are considered "typical" *E. coli.* Verification of typical and atypical colonies may be required in evidence gathering and is also recommended as a means of quality control. The verification procedure follows.

14.13 Using a sterile inoculating loop or needle, transfer growth from the centers of at least 10 well-isolated typical and 10 well isolated atypical colonies to nutrient agar plates or slants and to

Tryptic/Trypticase Soy broth. Incubate the agar and broth cultures for 24±2 hours at 35°C±0.5°C.

14.14 After incubation, transfer growth from the nutrient agar slant and perform cytochrome oxidase test. If the area where the bacteria were applied turns deep purple within 15 seconds, the test is positive.

Note: Use only platinum, plastic or wooden applicators to perform the oxidase test. Do not use iron or other reactive wire because it may cause false positive reactions.

14.15 Transfer growth from the Tryptic/Trypticase soy broth tube to Simmons citrate agar, tryptone water and an EC broth fermentation tube

Incubate the Simmons citrate agar for 4 days at 35°C±2.0°C in an aerobic atmosphere. A positive reaction is indicated by growth with an intense blue color on the slant. *E. coli* is citrate negative, and thus for this organism on this medium there should be either no growth or trace growth with no change in agar color (i.e. medium remains dark green).

Incubate the EC broth at 44.5°C±0.2°C in a waterbath for 24±2 hours. The water level must be above the level of the EC broth in the tube. A positive test is indicated by turbidity and production of gas as seen in the inner durham tube.

Incubate the tryptone water for 18-24 hours at 35°C±2.0°C with loosened caps. After the incubation period, add 0.5 ml of Kovacs indole reagent and shake the tube gently. Allow the tubes to stand for 5-10 minutes at room temperature. A positive test for indole is indicated by a deep red color which develops in the alcohol layer on top of the broth.

14.16 *E. coli* are oxidase-negative, citrate-negative, EC growth and gas-positive, and indole-positive.

14.17 Alternatively, commercially available multi-test identification systems may be used to verify colonies. Inoculate the colonies into an identification system for *Enterobacteriaceae* that includes lactose fermentation, σ-nitrophenyl-β-D-galactopyranoside (ONPG) and cytochrome oxidase test reactions.

15) Calculations

15.1 Use the following general rules to calculate the *E. coli* count per 100 ml of sample:

Select the membrane filter with an acceptable number of colonies (20-80 yellow, yellow-green, or yellow-brown colonies on the urea substrate, and calculate the number of *E. coli* per 100 mL according to the following general formula:

$$\textit{E. coli}/100\ \text{mL} = \frac{\text{Number of E. coli colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

15.2 Recovery calculations for samples spiked with laboratory-prepared spiking suspension

15.2.1 Calculate the concentration of E. coli (CFU/mL in the undiluted spiking suspension according to the following equation. Example calculations are provided in EPA Method 1103.1, Table 3.

$$\textit{E. coli}_{\text{undiluted spike}} = (CFU_1 + CFU_2 + \ldots + CFU_n)/(V_1 + V_2 + \ldots V_n)$$

Where,

$\textit{E. coli}_{\text{undiluted spike}}$ = *E. coli* (CFU/mL) in undiluted spiking suspension

CFU= Number of colony forming units from TSA plates yielding counts within the countable range of 30 to 300 CFU per plate

V= Volume of undiluted sample on each TSA plate yielding counts within the countable range of 30 to 300 CFU per plate

n= Number of plates with counts within the countable range (30 to 300 CFU/plate)

15.2.2 Calculate true concentration (CFU/100 mL of spiked *E. coli* ($T_{\text{spiked } \textit{E. coli}}$) according to the following equation. Example calculations are provided in EPA Method 1103.1, Table 4.

$T_{\text{spiked } \textit{E. coli}}$ = (*E. Coli* undiluted spike) x (V spiked per 100 mL sample)

Where,

$T_{\text{spiked } \textit{E. coli}}$= Number of spiked *E. coli* (CFU/100 mL)

$\textit{E. coli}_{\text{undiluted spike}}$ = *E. coli* (CFU/mL) in undiluted spiking suspension

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

15.2.3 Calculate percent recovery (R) of spiked *E. coli* (CFU/100 mL) according to the following equation.

$$R = 100 \times \frac{(Ns-Nu)}{T}$$

Where,

R= Percent recovery
Ns= *E. coli* (CFU/100 mL) in the spiked sample
Nu= *E. coli* (CFU/100 mL) in the unspiked sample
T= True spiked *E. coli* (CFU/ 100 mL) in spiked sample.

16) Method Performance

All measurements of Method Performance outlined in Section 12, Quality Control must be used. See EPA Method 1103.1, Section 15.0, Method Performance, for information on Method Validation data.

17) Pollution Prevention

The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly. Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed. All used broth and agar is autoclaved for 30 minutes at 121EC±2EC after use. All waste generated from microbiological testing, including disposable pipettes, transfer loops, agar plates, gloves are disposed of in the red biohazard bins, with pickup arranged with Stericycle Inc, periodically. Benches are cleaned with a disinfectant before and after use for microbiological testing. See Safety, Section 8, and Waste Management, Section 21 for more information on procedures related to pollution prevention.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

Information regarding data assessment and acceptance criteria for quality control measures are outlined in Section 12, Quality Control.

19) Corrective Actions for Out-of-Control Data

Samples received above 10EC or outside of the 6 hour holding time indicate problems with transport. The sampler/transporter must report the problem to the Quality Control Officer (The Laboratory Director), who will determine, in consultation with the Quality Assurance Officer, whether the analysis should proceed. If problems with transport are indicated by the above quality control data, the Quality Control Officer and the Quality Assurance Officer must review

sample transport procedures with the sampler. These may include, but not be limited to, checking the accuracy of the temperature blank thermometer, changing the size of the cooler and/or amount of ice used in transport. If consistent problems adhering to the six hour holding time arise, the Quality Control Officer and Quality Assurance Officer should review with the designated Project Manager the feasibility of the sample site in regards to transport requirements. If the batch QC fails for any batch of broth, the broth must be discarded and remade. More corrective actions for out-of-control data are discussed below in Section 20.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

The QC and QA Officers will jointly investigate the problem(s) at depth and take all necessary corrective actions that may include, but not be limited to: obtaining fresh QC standards from one or more suppliers and determining the accuracy of measurements, measuring standard deviations, consulting a service engineer and arranging on-site maintenance or repairing service. All analyses of samples will be stopped until the problem is rectified. Samples received at temperatures above 10EC or outside of the 6 hour holding time cannot be used for regulatory purposes. The results of the positive and negative quality control checks are vital in determining the validity of data. A negative control culture which generates a positive result indicates contamination of the analysis equipment, supplies and/or reagents. A blank with growth also indicates a contamination problem. A positive control culture which generates a negative result indicates that the broth is not able to support the indicated organism growth. Any batch of broth that fails the above QC checks must be discarded.

21) Waste Management

It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. Samples, reference materials, and equipment known or suspected to have viable bacteria, attached or contained must be sterilized prior to disposal (autoclaved at 121C and 15 PSI for 30 minutes). This waste is then stored in red biohazard bins until pickup by an approved regulated waste company. Currently, the laboratory uses Stericycle, Inc. on an as-needed basis for removal of waste.

22) References

Method 1103.1 *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC). March 2010. U.S. Environmental Protection Agency, Office of Water (4303T), 1200 Pennsylvania Avenue, NW, Washington D.C. EPA 821-R-10-002

Standard Methods for the Examination of Water and Wastewater, 20th edition, Sections 9010,9020, 9030, 9040, 9050, 9060,. APHA, AWWA, WEF, 1992.

IEC Quality Control Manual, Revision 5, October 2012.

NELAC Quality Systems Checklist (NELAC Manual Chapter 5 Checklist), Revision a, Based on 2003 NELAC Standards And EL-VI-2009-ISO2009 TNI Accreditation Standards.

23) Tables, Diagrams, Flowcharts and Validation Data

See Next Page.

Table 1. Key Quality Control Practices

Item	Action	Minimum Frequency
Reagent water	Monitor quality	See Table 2
Bench surface	Monitor for contamination	Monthly
Air in workplace	Monitor bacterial density	Monthly
Thermometers	Check accuracy	annually
Balances	Check accuracy	Each use
Balances	Service and recalibrate	Annually
pH meter	Standardize	Each day of use
Autoclave	Check performance (using autoclave tape, maximum registering thermometer)	Each use
Refrigerator	Check temperature	Daily (2x)- 4 hours apart
Membrane Filtration Equipment	Checks for leaks and surface scratches	Each use
Biohazard hood	Monitor air	Monthly
	Inspect for airflow	Quarterly
Incubator	Check temperature	Twice daily 4 hours apart
Glassware	Inspect for cleanliness, chips and etching	Each use
	Check pH	Each batch
	Conduct inhibitory residue test	Annually
Reusable/Disposable pipets	Check sterility	Each lot
Dilution water bottles	Check pH	Each batch
Sample bottles, filtration funnels	Sterilize, check sterility	Each batch
Filtration funnels	check accuracy with class A graduated pipets	Periodically
Media	Check pH and appearance, sterility, Positive/negative control checks	Each use
Membrane filters	recovery compared to previously acceptable	Each lot

	Lots, lot # and date of receipt recorded	
Autoclave	Check performance (using steri-amps)	Each run
Plate Counts	Perform Duplicate Analyses	Weekly
	Repeat Counts (+Second analyst)	Monthly

Table 2. Quality of Reagent Water Used in Microbiology Testing

Test	Monitoring Frequency	Maximum Acceptable Limit
Chemical Test:		
Conductivity	Monthly	>0.5 megohms resistance or <2 µmhos/cm at 25ºC
pH	With each day of use	5.5-7.5
Total organic carbon	Annually	<1.0 mg/L
Heavy metals, single (Cd, Cr, Cu, Ni, Pb, and Zn)	Annually	<0.05 mg/L
Heavy metals, total	Annually	<0.10 mg/L
Ammonia/organic nitrogen	Annually	<0.10 mg/L
Total chlorine residual	Monthly	<0.01 mg/L
Bacteriological test:		
Heterotrophic plate count	Monthly	<1000CFU/mL
Suitability Test	Annually	Suitable

Table 3. Time and Temperature for Autoclave Sterilization

Material	Time at 121ºC
Membrane filters and pads (if not purchased sterile)	10 min
Carbohydrate-containing media (Lauryl Tryptose, BGB broth, etc.)	12-15 min
Contaminated materials and discarded cultures	30 min
Membrane filter assemblies (wrapped)	
Sample collection bottles (empty)	15 min
Buffered dilution water	15 min
Rinse water, volume >100 mL	Adjust for volume

Table 4. Holding Times for Prepared Media

Medium	Holding Time
Membrane filter (MF) broth in screw-cap flasks at 4EC	96 hours

MF agar in plates with tight-fitting covers at 4EC	2 weeks
Agar or broth in loose-cap tubes at 4C	2 weeks
Agar or broth in tightly closed screw-cap tubes or other sealed containers	3 months
Poured agar plates with loose-fitting covers in sealed plastic bags at 4C	2 weeks
Large volume of agar in tightly closed screw-cap flask or bottle at 4C	3 months

TURBIDITY

(Nephelometric Method)

1) Test Method

Based on EPA Method 180.1 Rev. 2.0.

2) Applicable Matrix or Matrices

Non-Potable Water.

3) Method Detection Limit

The measuring range of the turbidimeter is from 0 to 4000 nephelometric units (NTU). However, 40 NTU is the highest recommended detection limit. Turbidity exceeding this range may be measured by diluting the sample with deionized water. The lower detection limit that IEC reports is 0.01 NTU. Reporting Limit is 1 NTU

4) Scope and Application

The Interstate Environmental Commission regularly analyzes environmental samples from municipal and industrial facilities for turbidity. This method is used by IEC to determine turbidity in non-potable waters, including surface and saline waters, domestic and industrial wastewaters.

5) Summary of Method

The method compares the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension. Higher intensities of scattered light correspond to higher turbidities. Readings in NTUs are made in a nephelometer. StablCal™ Stabilized Formazine Suspension Standards, which are available commercially (HACH Company) are used to standardize the instrument.

6) Definitions

Turbidity- Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample. Turbidity in water is

caused by suspended matter, such as clay, silt, finely divided organic and inorganic matter, soluble colored organic compounds and plankton and other microscopic organisms.

Nephelometer- A nephelometer (also referred to as a turbidimeter) is an instrument that contains an optical system to measure the degree to which a sample scatters light. The optical system consists of an 870 ±30 nm light emitting diode (LED) assembly, lenses and apertures to focus the light, a 90E detector to monitor scattered light, forward-scatter light detector, transmitted light detector, and a LED monitor detector.

7) Interferences

1. Floating debris and coarse sediments which settle out rapidly will give low readings.

2. Finely divided air bubbles will affect results in a positive manner.

3. Dissolved substances which absorb light will negatively interfere with measurements.

8) Safety

The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Formazin standards may cause eye and skin irritation or allergic skin reaction. Wear personal protective equipment (lab coat, goggles and gloves) for protection.

The MSDS, supplied by the manufacturer, for the Stabilized Formazin Turbidity Standards procured through HACH, as well as any other secondary standards, are kept on file in a binder readily available to all laboratory staff (yellow binder mounted on wall near door to microbiology room). All staff members should review the MSDS of a chemical before working with it.

9) Equipment and Supplies

1. Hach 2100N Turbidimeter

2. Sample tubes to fit Hach 2100N Turbidimeter

3. Kimwipes for wiping outside of sample vials.

10) Reagents and Standards

HACH StablCal Calibration Set for 2100N Catalog # 2662105.

Water, ACS Reagent grade, ASTM Type I, ASTM Type II (RICCA Chemicals Catalog No. 9150-5, purchased thru Fisher) or other suitable ASTM I, ASTM II type water.

Second Source standard for ICV (Currently HF Scientific Catalog 52180) or similar.

11) Sample Collection, Preservation, Shipment and Storage

Samples can be collected in plastic (polyethylene or equivalent) or glass bottles. All bottles must be thoroughly cleaned and rinsed with turbidity free water. Volume of sample collected should be sufficient to insure a representative sample, allow for replicate analysis, and minimize waste disposal. A minimum of 100 ml must be collected, with sufficient air space to allow the sample to be shaken in the bottle effectively (–1 cm headspace at top of bottle).

No chemical preservation is required. Transport the sample on ice to the laboratory. Store the sample in a laboratory refrigerator between at 4.0±2C. Samples maintained at 4.0±2C may be held for up to 48 hours from collection.

12) Quality Control

1. All samples must be analyzed in duplicate.

2. Each analyst must perform an initial demonstration of capability (IDC) before analyzing any environmental sample. This consists of a quality control sample, purchased through a certified vendor, analyzed four times. Each analyst must also perform a continuing demonstration of capability (CDC) annually, consisting of analyzing a quality control sample once. An external proficiency test is acceptable as a CDC for the analyst that performs that proficiency.

3. Laboratory must analyze at least one laboratory reagent blank (LRB) with each batch of samples. Data produced are used to assess contamination from laboratory environment.

4. The calibration is checked prior to each use, after every 10 samples, and at the end of the sample run with a CCV

13) Calibration and Standardization

1. Turn the turbidimeter on and allow it to warm up for 30 minutes. NOTE: Instrument warm up not required as per manufacturer's instruction manual. Optical and electronic stabilization, according to the manual, is instantaneous. Regardless, it is IEC's SOP to warm-up the instrument for a minimum of 30 minutes.

2. If, at any point in the calibration, the turbidimeter displays an error message (indicated by the letter E followed by a number, eg E1), or the analyst suspects that the instrument is malfunctioning, stop calibration and call either the laboratory director or associate laboratory director over to investigate the problem before proceeding.

3. Press the **CAL** key. The S0 annunciator lights. The NTU value of the dilution water used in the previous calibration is displayed.

4. Wipe the exterior of the vile with kimwipes and place the blank standard in the turbidimeter. Press the **ENTER** key. The instrument counts down from 60 to 0 seconds and beeps.

5. The instrument automatically increments to the next standard, displays the expected value (e.g., 20 NTU) and the S1 annunciator lights. Remove the sample cell from the cell holder.

6. Shake the 20 NTU standard well and wipe off the finger prints from the exterior of the vile. Place the 20 NTU standard vial in the turbidimeter as above. Press the **ENTER** key. The instrument display counts down from 60 to 0 seconds, and makes a measurement. The instrument automatically increments to the next standard, the display shows 200.0 NTU, and the S2 annunciator lights. Remove the sample cell from the holder.

7. Shake the 200 NTU standard well and wipe off the finger prints from the exterior of the vial. Place the 200 NTU standard from the calibration set into the turbidimeter as above. Press the **ENTER** key. The instrument counts down from 60 to 0 seconds, and then makes a measurement. The instrument automatically increments to the next standard, the display shows 1000 NTU, and the S3 annunciator lights. Remove the sample cell from the holder.

8. Shake the 1000 NTU standard well and wipe off the exterior of the vial with kimwipes. Place the 1000 NTU standard into the turbidimeter as above. Press the **ENTER** key. The instrument counts down from 60 to 0 seconds, and then makes a measurement. The instrument automatically increments to the next standard, the display shows 4000 NTU, and the S4 annunciator lights. Remove the sample cell from the holder.

9. Shake the 4000 NTU standard well and wipe off the exterior of the vial. Place the 4000 NTU standard into the turbidimeter as above. Press the **ENTER** key. The instrument counts down from 60 to 0 seconds, and then makes a measurement. The display automatically increments back to the blank (<0.1 NTU) standard value. The S0 annunciator lights, and the previously measured value of the dilution water is displayed.

10. Press the **CAL** key. The instrument makes calculations based on the new calibration data, stores the new calibration and returns the instrument to the measurement mode.

11. The calibration is performed prior to each days use.

12. Check the calibration by reading the 20 NTU Second Source Stabilized Formazin Standard as a sample (see procedure, below). The standard should read between 19-21 NTU (±5%). Check the laboratory reagent blank (LRB). Record the results in analyst's logbook.

14) Procedure

1. Collect a representative sample in a clean container. Fill the sample cell to the line (approximately 30 ml). Take care to handle the sample cell from its top. Wipe the sample cell with kimwipe™ to remove water spots and fingerprints.

2. Make sure the filter is in place. This is a cartridge that slides in the space just behind the sample compartment and is labeled "HACH FILTER." Refer to the turbidimeter's manual for more information.

3. Select the range by pressing the **RANGE** key (manufacturer recommended setting). Pressing the Range key changes the decimal point to which the meter reads the sample. Values between 0 and 1 should be reported to the nearest 0.05 NTU, values between 1 and 10 should be reported to the nearest 0.1 NTU, values between 10 and 40 should be reported to the nearest 1 NTU, values between 40 and 100 should be reported to the nearest 5 NTU and values between 100 and 400 should be reported to the nearest 10 NTU.

4. Select the SIGNAL AVERAGE ON setting by pressing the **SIGNAL AVG** key. When the SIGNAL AVERAGE is on, the instrument's microprocessor compiles a number of readings and averages the result. The averaged value is calculated and displayed approximately once every second.

5. Turn the RATIO ON by pressing the **RATIO** key. The instrument requires the RATIO to be on when turbidity values are >40 NTU.

6. Place cell in sample compartment and close lid to compartment. The instrument will display the result momentarily. Read and record the results.

15) Calculations

None Required.

16) Method Performance

Calibrate the meter on each day of use prior to analyzing samples. Check the calibration by analyzing a mid-range calibration standard. Analysts must perform an initial demonstration of capability through the satisfactory analysis of four (4) replicate samples and demonstrate ongoing, or continuing capability each year through the satisfactory analysis of a proficiency sample or four (4) replicate samples procured by the Laboratory Director. The laboratory participates in semi-annual proficiency test studies supplied by NYSDOH. Any unsatisfactory performance in these studies must be investigated to determine their root cause and a corrective action plan must be promulgated.

17) Pollution Prevention

Make sure all standard vials are tightly capped at all times. Store standard vials between uses in the plastic, foam-insulated box supplied by HACH to prevent breakage. Use the standard kit until its expiration date, unless degradation is suspected. Dispose of standards as described in Waste Management, Section 21 of this SOP.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

The acceptance criteria for all QC checks must fall within the manufacturer's acceptance limits or, if those limits are not provided, within ±10% of their certified values. Duplicate samples must not differ by more than 10%.

19) Corrective Actions for Out-of-Control Data

Out-of-control data must be reported at once to the IEC's Laboratory Director, who in turn has to promulgate a corrective action plan in consultation with IEC's Quality Assurance (QA) Officer.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

The Laboratory Director will investigate the problem(s) at depth and take all necessary corrective actions that may include, but not be limited to: obtaining fresh QC standards from one or more suppliers and determining the accuracy of measurements, measuring standard deviations, consulting a service engineer and arranging on-site maintenance or repairing service. All analyses of samples will be stopped until the problem is rectified.

21) Waste Management

Dilute the standard solution with excess water, making a weaker than 5% solution. Open the cold water tap completely and slowly pour the material to the drain. Flush the system with plenty of water. Empty containers: Rinse three times with an appropriate solvent (water). Dispose of empty containers as normal trash.

22) References

MSDS No.: M00482. Product Name: StablCal® Formazin Standard 4000 NTU. Product Catalog Number: 246102. HACH Company, Loveland, CO.

EPA Method 180.1, Determination of Turbidity By Nephelometry, Revision 2.0, August 1993, Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Model 2100N Laboratory Turbidimeter Instruction Manual, 1993. Hach Company, Loveland, CO.

23) Tables, Diagrams, Flowcharts and Validation Data

None.

QuikChem® Method 31-107-04-4-B

DETERMINATION OF TOTAL NITROGEN IN MANUAL PERSULFATE DIGESTS FOR SEAWATER AND BRACKISH WATERS

(LOW FLOW METHOD)

Written by Scott Tucker – Lachat Applications Group
and
Dennis Jones - North Dakota Department of Health

Revision Date:

16 June 2008

LACHAT INSTRUMENTS
5600 LINDBURGH DRIVE
LOVELAND, CO 80539 USA

APPENDIX 4
Lab Standard Operating Procedures

LACHAT INSTRUMENTS
A Hach Company Brand

QuikChem® Method 31-107-04-4-B

Determination of Total Nitrogen in Manual Persulfate Digests for Seawater and Brackish waters

0.02 to 5.00 mg N/L
1.00 to 40.0 mg N/L

– Principle –

This method utilizes an off-line digestion to convert all forms of nitrogen into nitrate using an alkaline and acidic persulfate digestion. In this digest, the potassium persulfate in an alkaline environment converts all forms of nitrogen containing compounds to the nitrate form. The oxygen for this oxidation comes from the reaction in equation one:

Eq.1. $K_2S_2O_8 + H_2O = 2KHSO_4 + 1/2O_2$

Eq.2. $KHSO_4 + NaOH = Na^+ + K^+ + SO_4^{2-} + H_2O$

The sodium hydroxide present must be sufficient to neutralize the hydrogen ions released in equation two and maintain an alkaline environment during the nitrogen digestion step. As one mole of potassium persulfate produces two moles of hydrogen ions; an approximately equal molar ratio of sodium hydroxide and potassium persulfate at the start of the digestion should produce an acid environment at the end of the digestion when all the sodium hydroxide is consumed.

Nitrate in the digestate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite thus produced is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water-soluble dye has a magenta color, which is read at 520 nm.

Note: This method utilizes a dual digestion, which allows both total nitrogen and total phosphorus to be analyzed from a single digest. (See method number 31-115-01-4-B for additional details on total phosphorus). This method is based upon Standard Methods 4500-N_{org} (Proposed).

– Interferences –

1. Residual chlorine can interfere by oxidizing the reductor column.
2. Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.
3. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.
4. Sample turbidity may interfere. Turbidity in digested samples can be removed by filtration through a 0.45 µm pore diameter membrane filter prior to analysis.

– Special Apparatus –

1. Block digester
2. Labware for Digestion:

   https://www1.fishersci.com/Coupon?catnum=028839E

   https://www1.fishersci.com/Coupon?gid=197330&cid=1328

   The tubes are Fisher number 14-932E and the replacement caps are Corning number 9999-241.
   Rubber, not Teflon seals are recommended.
   The tubes are made by Corning (They are Pyrex, NOT Kimax)

CONTENTS

APPENDIX 5

QuikChem® Method 31-107-04-4-B

DETERMINATION OF TOTAL NITROGEN IN MANUAL PERSULFATE DIGESTS FOR SEAWATER AND BRACKISH WATERS

1. SCOPE AND APPLICATION

1.1. The purpose of this method is to determine total nitrogen in persulfate digests for seawater and brackish samples.

1.2. The applicable range is 0.02 to 5.0 mg N/L for the low range and 1.0 to 40.0 mg N/L as for the high range. The statistical method detection limit is 0.0015 mg N/L for the low range and 0.07 mg N/L for the high range. However; due to the positive blank caused by the nitrogen in the persulfate digestion reagent, the blanks in the carry over study and the DIN calculation resulted in concentrations higher than the calculated statistical MDL. Therefore, the reported MDL for the low range is 0.0068 mg N/L, and the reported MDL for the high range is 0.111 mg N/L. These MDL values came from the average of the 15 blanks analyzed for the DIN calculation. The method throughput is 38 injections per hour.

1.3. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in section 11.

2. SUMMARY OF METHOD

2.1. This method utilizes an off-line digestion to convert all forms of nitrogen into nitrate using an alkaline and acidic persulfate digestion. In this digest, the potassium persulfate in an alkaline environment converts all forms of nitrogen containing compounds to the nitrate form. The oxygen for this oxidation comes from the reaction in equation one:

Eq.1. $K_2S_2O_8 + H_2O = 2KHSO_4 + 1/2O_2$
Eq.2. $KHSO_4 + NaOH = Na^+ + K^+ + SO_4^{2-} + H_2O$

The sodium hydroxide present must be sufficient to neutralize the hydrogen ions released in equation two and maintain an alkaline environment during the nitrogen digestion step. As one mole of potassium persulfate produces two moles of hydrogen ions; an approximately equal molar ratio of sodium hydroxide and potassium persulfate at the start of the digestion should produce an acid environment at the end of the digestion when all the sodium hydroxide is consumed.

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite thus produced is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm.

3. DEFINITIONS

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

3.1. ANALYTICAL BATCH -- The set of samples extracted/distilled/or digested at the same time to a maximum of 10 samples.

3.2. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

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

3.4. FIELD BLANK (FMB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all preanalysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

3.5. FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.6. LABORATORY BLANK (LRB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. Its purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibrating standard).

3.8. LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

3.9. QUALITY CONTROL CHECK SAMPLE (QCS) -- A sample containing analytes of interest at known concentrations (true values) of analytes. The QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

3.10. METHOD DETECTION LIMIT (MDL) -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

4. INTERFERENCES

4.1 Residual chlorine can interfere by oxidizing the reductor column.

4.2 Low results would be obtained for samples that contain high concentrations of iron,

copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.

4.3 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

4.4 Sample turbidity may interfere. Turbidity in the digested samples can be removed by filtration through a 0.45 µm pore diameter membrane filter prior to analysis.

5. SAFETY

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

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

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

5.3.1. Cadmium granules

5.3.2. Ammonium hydroxide

5.3.3. Sodium hydroxide

5.3.4. Phosphoric acid

5.3.5. Sulfanilamide

5.3.6. Sulfuric acid

5.3.7. Potassium persulfate

6. EQUIPMENT AND SUPPLIES

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

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1 Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.3.6. 10 mm, 80 µL, glass flow cell

6.3.7. 520 nm interference filter

6.4. Special Apparatus

6.4.1 Block Digester

6.4.2. Labware for Digestion:

6.4.2.1. Corning® Glass, Screw-capped Tubes for the Block Digester (Rubber cap liners) (25 x 150 mm for the BD-46)

7. REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized (10 megohm) water for all solutions. (See Standard Specification for Reagent Water D1193-77 for more information).

Degassing with helium:

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140kPa (20 lb/in^2) through a helium degassing tube (Lachat Part No. 50100.) Bubble He through the solution for one minute.

Reagent 1. 15 N Sodium Hydroxide

By Volume: In a **250 mL** volumetric flask add **150 g NaOH** to **50 mL or g of DI water**. **CAUTION**: The solution will get very hot! Swirl until dissolved. Dilute to mark. Cool and store in a plastic bottle.

Reagent 2. Ammonium Chloride buffer, pH 8.5

By Volume: In a **1 L** volumetric flask, dissolve **85.0 g ammonium chloride** (NH_4Cl) and **1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ($Na_2EDTA{\cdot}2H_2O$) in about **800 mL DI water.** Dilute to the mark and invert to mix. Adjust the **pH to 8.5** with **15 N sodium hydroxide solution.**

By Weight: To a tared **1 L** container, add **85.0 g ammonium chloride** (NH_4Cl), **1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ($Na_2EDTA{\cdot}2H_2O$) and **938 g DI water**. Shake or stir until dissolved. Then adjust the **pH** to **8.5** with **15 N sodium hydroxide solution.**

ACS grade ammonium chloride has been found occasionally to contain significant nitrate contamination. An alternative recipe for the ammonium chloride buffer is:

By Volume: CAUTION: Fumes!!! In a hood, to a **1 L** volumetric flask, add **500 mL DI water, 105 mL concentrated hydrochloric acid** (HCl), **95 mL ammonium hydroxide** (NH_4OH), and **1.0 g disodium EDTA.** Dissolve and dilute to the mark. Invert to mix. Adjust the **pH** to **8.5** with **HCl** or **15 N NaOH solution.**

By Weight: CAUTION: Fumes!!! In a hood, to a tared **1 L** container, add **800 g DI water, 126 g concentrated hydrochloric acid** (HCl), **85 g ammonium hydroxide** (NH_4OH) and **1.0 g disodium EDTA**. Stir until dissolved. Adjust the **pH** to **8.5** with **HCl** or **15 N NaOH.**

Reagent 3. Sulfanilamide color reagent

By Volume: In a **1 L** volumetric flask, add approximately **600 mL DI water**. Then add **100 mL 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide, and 1.0 g N-(1-naphthyl) ethylenediamine dihydrochloride** (NED). Shake to wet, and stir to dissolve for 30 minutes. Dilute to the mark, and invert to mix. Store in a dark bottle. This solution is stable for one month.

By Weight: To a **1 L** dark, tared container, add **876 g DI water, 170 g 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide, and 1.0 g N-(1-naphthyl) ethylenediamine dihydrochloride** (NED). Shake until wet and stir with stir bar for 30 minutes until dissolved. This solution is stable for one month.

Reagent 4. Stock 11N Sulfuric Acid

By Volume: To a **1L** Volumetric flask containing about **600 mL** of DI water, CAREFULLY add **305 mL (561.2g)** of **concentrated sulfuric acid** (H_2SO_4) CAUTION solution will be hot! Stir to mix, cool to room temperature and dilute to volume. Do not degas this reagent!

Reagent 5. Carrier: Sulfuric Acid, 0.231 M

By Volume: In a **1 L** volumetric flask, add **500 mL DI water** and **42 mL Reagent 4 (11N H_2SO_4)**. Dilute to the mark **DI water** and invert to mix. Degas daily. Prepare fresh weekly.

Reagent 6. 0.5 N Sodium Hydroxide

By Volume: To a 1L Volumetric flask containing about **600 mL** of DI water, add 20 g NaOH. Swirl until dissolved. Dilute to mark. Cool and store in a plastic bottle. To prepare 0.5 N NaOH solution from the 15 N NaOH (Reagent 1); in a 1L Volumetric flask containing about **600 mL** of DI water, add 33.33 mL of 15 N NaOH and dilute to mark with DI water. Store in plastic bottle.

Reagent 7. Basic Digestion Reagent (Digestion Reagent 1)

By Volume: In a **1 L** volumetric flask dissolve **10.48 g sodium hydroxide** (NaOH) and **63 g potassium persulfate** ($K_2S_2O_8$), in approximately **800 mL DI water**. Dilute to the mark and invert to mix. Prepare fresh monthly and store in plastic. Do not degas this reagent!

Reagent 8. Acidic Digestion Reagent (Digestion Reagent 2)

By Volume: In a **500 mL** volumetric flask, add **300 mL Reagent 4** (11N H_2SO_4) and **11.5g potassium persulfate ($K_2S_2O_8$).** Dilute to the mark with **Reagent 4** (11N H_2SO_4) and invert to mix. Prepare fresh weekly. Do not degas this reagent!

Note: The samples must be carried through the entire digestion procedure to be used with this method. This is true, even if phosphorus is not going to be measured.

7.2. PREPARATION OF STANDARDS

To prepare the stock and working standards, the following containers will be requires:

By Volume: Two, 1 L and seven, 250 mL volumetric flasks.

By Weight: Two, 1 L and seven, 250 mL containers.

NOTE: Standards are prepared in **DI water** and digested by the procedure in section 7.4.

Standard 1. Stock Nitrate Standard 500 mg N/L as NO_3^-

By Volume: In a **1 L** volumetric flask, dissolve **3.61 g potassium nitrate** (KNO_3) in about **600 mL DI water**. Dilute to the mark and invert to mix. This solution is stable for six months.

Standard 2. Working Stock Standard Solution 100 mg N/L – High Range

By Volume: In a **1L** volumetric flask, add about **550 mL DI water**, and **200.0 mL Stock Standard (Standard 1)**. Dilute to the mark with **DI water**. Invert to mix.

By Weight: To a tared **1L** container, add about **200 g Stock Standard (Standard 1).** Divide the actual weight of the solution added by 0.2 and make up to this resulting total weight with **DI water**. Invert to mix.

Standard 3. Working Stock Standard Solution 10 mg N/L – Low Range

By Volume: In a **1L** volumetric flask, add about **550 mL DI water**, and **20.0 mL Stock Standard (Standard 1)**. Dilute to the mark with **DI water**. Invert to mix.

By Weight: To a tared **1L** container, add about **20 g Stock Standard (Standard 1).** Divide the actual weight of the solution added by 0.02 and make up to this resulting total weight with **DI water**. Invert to mix.

Note: Standards are prepared in DI water and carried through the digestion procedure with the samples. Using simulated standards is not recommended, due to nitrogen and phosphorus present in the digestion reagents. **For the digested standards and samples to work with this method, they must be carried through the entire digestion procedure.**

If you are running both the TN and TP (31-115-01-4-B) methods together, it is recommended to use mixed standards of nitrate and phosphorus. This will allow you to only have to digest one set of standards for both analytes. See section 7.2 of the TP method, 31-115-01-4-B.

WORKING STANDARDS – LOW RANGE

These standards **will be** digested

Working Standards (Prepare Bi-Weekly) Concentration mg N/L	**A** 5.0	**B** 2.5	**C** 1.00	**D** 0.5	**E** 0.25	**F** 0.1	**G** 0.05	**H** 0.02	**I** 0.00

By Volume

Volume (mL) of **stock standard 3** diluted to 250 mL with **DI water**	125	62.5	25.0	12.5	6.25	2.5	---	---	---
Volume (mL) of Standard C diluted to 250 mL with **DI water**	---	---	---	---	---	---	10	4	---

By Weight

Weight (g) of **stock standard 3** diluted to final weight (~250 g) divided by **factor** below with **DI water**	125	62.5	25.0	12.5	6.25	2.5	---	---	---
Weight (g) of Standard C diluted to final weightv (~250g) with **DI water**	---	---	---	---	---	---	10	4	---
Division Factor Divide exact weight of the standard by this **factor** to give final weight	0.5	0.25	0.1	0.05	0.025	0.01	0.04	0.016	---

WORKING STANDARDS – HIGH RANGE

These standards **will be** digested

Working Standards (Prepare Bi-Weekly) Concentration mg N/L	**A** 40.0	**B** 20.0	**C** 10.0	**D** 5.00	**E** 2.50	**F** 1.00	**G** 0.00

By Volume

Volume (mL) of **stock standard 2** diluted to 250 mL with **DI water**	100	50.0	25.0	12.5	---	---	---
Volume (mL) of Standard C diluted to 250 mL with **DI water**	---	---	---	---	62.5	25	---

By Weight

Weight (g) of **stock standard 2** diluted to final weight (~250 g) divided by **factor** below with **DI water**	100	50.0	25.0	12.5	---	---	---
Weight (g) of Standard C diluted to final weightv (~250g) with **DI water**	---	---	---	---	62.5	25	---
Division Factor Divide exact weight of the standard by this **factor** to give final weight	0.4	0.2	0.1	0.05	0.25	0.1	---

7.3. PREPARATION OF DIGESTION CHECK STANDARDS

Stock Digestion Check Standards: 1000 mg N/L

In a **500 mL volumetric flask** dissolve **x.xx g** test compound (see table) in about **400 mL DI water**. Dilute to the mark and invert to mix.

Stock stds	Compound	g/500ml	Formula	FW
1	Ammonium p-toluenesulfonate	6.755	$C_7H_{11}O_3SN$	189.2
2	Nicotinic acid	4.394	$C_6H_5NO_2$	123.1
3	Glycine p-toluenesulfonate	8.827	$C_9H_{13}O_5SN$	247.3
4	DL-Glutamic acid monohydrate	5.895	$HO_2CCH_2CH_2CH(NH_2)$-CO_2HH_2O	165.2
5	Potassium Nitrite	3.038	KNO_2	85.1
6	Triethylamine	3.612	$(C_2H_5)_3N$	101.2
7	Ammonia sulfate	2.359	$(NH_4)_2SO_4$	132.1
8	Urea	1.072	H_2NCONH_2	60.1
9	Sulfanilamide	3.074	4-(H2N)C6H4SO2NH2	172.21
10	Disodium EDTA	6.644	$NaO_2CCH_2N(CH_2CO_2H)$ $CH_2CH_2N(CH_2CO_2Na)C$ $H_2CO_2H_2H_2O$	372.2

Digestion Check Standards (1 - 10) 20 mg N/L

By Volume: In a **500 ml** volumetric flask add **10 mL of Stock Check Standard # (1, 2, 3, 4, 5, 6, 7, 8, 9, or 10)** (1000 mg N/L). Dilute to the mark with DI water invert to mix. Prepare fresh weekly.

Working Check Standards

Working Standards (1-9)	**A**
Concentration mg N/L	2.00

By Volume

Volume (mL) of **check standard (1, 2...or 9)** diluted to 250 mL with DI water	25

By Weight

Weight (g) of **check standard (1, 2...or 9)** diluted to final weight (~250 g) divide by **factor** below with DI Water	25
Division Factor Divide exact weight of the standard by this **factor** to give final weight	0.10

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. Samples must be preserved with H_2SO_4 to a pH< 2 and cooled to 4°C at the time of collection.

8.2. Samples containing too much sulfuric acid will not be correctly neutralized during the digestion, and may not give accurate results.

8.3. **Do not preserve samples with mercuric chloride**, as this will rapidly degrade the cadmium reduction column. For long-term storage, samples should be frozen at -20°C. Samples should be frozen in plastic bottles, leaving about 30% headspace for expansion. Frozen samples may be stored for up to 30 days.

9. QUALITY CONTROL

9.1. Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.1.1. Analyses of matrix spike and matrix spike duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in section 9.3.

9.1.2. Analyses of laboratory blanks are required to demonstrate freedom from contamination.

9.1.3. The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control.

9.1.4. The laboratory should maintain records to define the quality of data that is generated.

9.2. INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. Method Detection Limit (MDL) --To establish the ability to detect the analyte, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this method. An MDL less than or equal to the MDL in section 1.2 must be achieved prior to the practice of this method.

9.2.2. Initial Precision and Recovery -- To establish the ability to generate acceptable precision results, the operator shall perform 10 replicates of a mid-range standard, according to the procedure beginning in Section 11.

9.2.2.1. Using the results of the replicates compute the average percent recovery (X) and the standard deviation (s) for the analyte. Use the following equation for the calculation of the standard deviation.

$$s = \sqrt{\frac{\sum x^2 - \frac{\left(\sum x\right)^2}{n}}{n-1}}$$

Where, n = Number of samples, x = concentration in each sample

9.2.2.2. Compare s and x results with the corresponding data in Section 17. If the results meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If however, s and x do not match the data in Section 17, system performance is unacceptable. In this event correct the problem, and repeat the test.

9.3. Matrix spikes- The laboratory must spike, in duplicate, a minimum of 10 percent of all samples (one sample in each batch of ten samples) from a given sampling site or if for compliance monitoring, from a given discharge. The two sample aliquots shall be spiked with the stock standard (section 7.2).

9.3.1. The concentration of the spike in the sample shall be determined as follows:

9.3.1.1. If, as in compliance monitoring, the concentration of the analyte in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at 1 to 5 times higher than the background concentration of the sample (determined in Section 9.3.2), which ever is higher.

9.3.1.2. If the concentration of the analyte in a sample is not being checked against a limit, the spike shall be at the concentration of the precision and recovery standard used in Section 9.2.5 or at 1 to 5 times higher than the background concentration, whichever concentration is higher.

9.3.2. Analyze one sample aliquot out of each set of ten samples from each site or discharge according to the procedure beginning in Section 11 to determine the background concentration of (B) of the analyte.

9.3.2.1. If necessary, prepare a standard solution appropriate to produce a level in the sample at the regulatory compliance limit or at 1 to 5 times the background concentration (per Section 9.3.1).

9.3.2.2. Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking (A)

9.3.3. Calculate the percent recovery (P) of the analyte in each aliquot using the following equation.

$$P = \frac{(A-B)100}{T}$$

Where, A = Measured concentration of analyte after spiking, B = measured background concentration of analyte, T = True concentration of the spike

9.3.4. The percent recovery of the analyte should meet current laboratory acceptance criteria.

9.3.4.1. If the results of the spike fail the acceptance criteria and the recovery of the QC standard in the ongoing precision and recovery test of the

analytical batch is within the current laboratory acceptance criteria, an interference is present. In this case, the results may not be reported for regulatory compliance purposes and the analyst must assess the potential cause for the interference. If the interference is attributable to sampling, the site or discharge should be resampled. If the interference is attributable to a method deficiency, the analyst must modify the method, repeat the test required in Section 9.1.2 and repeat the analysis of the sample and the matrix spike.

9.3.4.2. If the results of both the spike and ongoing precision and recovery test fail the acceptance criteria, the analytical system is judged to be out of control, and the problem shall be identified and corrected, and the sample reanalyzed.

9.3.5. Compute the relative percent difference (RPD) between two sample results using the following equation:

$$RPD = \frac{(D_1 - D_2)}{(D_1 + D_2)/2} \times 100$$

Where, D1 = Concentration of analyte in the sample, D2 = Concentration of analyte in the second (duplicate) sample.

9.3.6. The RPD for duplicates shall meet the current laboratory acceptance criteria. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

9.4 Laboratory blanks - Laboratory reagent water blanks are analyzed to demonstrate freedom from contamination.

9.4.1. Analyze a laboratory reagent water blank initially (with the test in Section 9.2) and with each analytical batch. The blank must be subjected to the same procedural steps as a sample.

9.4.2. If analyte is detected in the blank at a concentration greater than the Minimum Level (Section 1.6), analysis of the samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. All samples must be associated with an uncontaminated method blank before the results may be reported for regulatory compliance purposes.

9.5. Calibration Verification - Verify calibration using the procedure described in Section 10

9.6. On-going Precision and Recovery (OPR) - With every analytical batch, a midrange standard must be prepared using the procedure described in Section 11.

9.6.1. Compare the results with the current laboratory acceptance criteria. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

9.7. Quality Control Samples (QCS) - It is suggested that the laboratory obtain and/or prepare a quality control sample using a source different from the source routinely used in section 7. The QCS is used to verify the concentrations of the calibration standards.

9.8. Depending on the specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

10. DIGESTION PROCEDURE

This digestion allows the determination of both total nitrogen and total phosphorus from a single digest. The entire procedure must be carried out, even if both phosphorus and nitrogen will not be measured. **If the entire procedure is not carried out, the matrix will be incorrect for the final colorimetric analysis.** The combined digestion is performed as follows:

10.1. Digestion vessels must be compatible with the block digester in use. Digestion vessels are Corning™ glass, screw-capped tubes (for example, for the BD-46, 25 x 150 mm tubes are used). Caps should have rubber seals, as Teflon lined caps will not seal as tightly. Digestion vessels should be cleaned with 1.0 M HCl until the odor of chlorine is absent. This is then followed by several DI water rinses. Vessels are then dried and capped for future use.

10.2. Both the samples and standards must be carried through the digestion, as this will correct for background contamination of the digestion reagents. (i.e. there will be a blank peak).

10.3. If using a block digester, preheat to 150°C before beginning the digestion.

10.4. To each of the digestion vessels containing 20 mL of sample, add 5 mL of the basic digestion reagent (digestion reagent 1). Screw the caps on tightly. If smaller or larger tubes are used, scale volumes up or down accordingly. **Note**: If the liquid in the tubes boils, either the cap is not on tightly, or there is a chip in the rim of the tube.

10.5. Digest samples in the block digester for 15 minutes at 150°C. For more difficult organic nitrogen compounds, e.g. nicotinic acid, digestion of the sample for 30 during the first (alkaline) portion of the digestion will result in a more complete digestion. The digestion block should be preheated.

10.6. After the first part of the digestion is completed, even if phosphorus will not be measured, the second part of the digestion must be completed so the final sample matrix is correct for the measurement. Remove the tubes VERY CAREFULLY and cool until they can be comfortably handled. CAUTION: Internal pressure for hot tubes is about 20 psi. If dropped, they will explode!

10.7. When the tubes can be comfortably handled, add 0.5 mL of the acidic digestion reagent (11N H_2SO_4 containing persulfate, digestion reagent 2). If smaller or larger tubes have been used for the digestion, scale up or down accordingly.

10.8. Tightly recap the tube and shake to mix. Place the tubes back into the block at 150°C for another 30 minutes.

10.9. Remove the tubes, and cool. VERY CAREFULLY remove each tube from the block. CAUTION: Internal pressure for hot tubes is about 20 psi. If dropped, they will explode!

10.10. Shake the contents and decant into tubes for measurement.

10.11. Tubes may be stored for several days if capped tightly and kept refrigerated.

11. CALIBRATION AND STANDARDIZATION

11.1. Prepare reagents and standards as described in Section 7.

11.2. Set up manifold as shown in Section 17.

11.3. Input data system parameters as shown in Section 17.

11.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.5. Place standards in the sampler. Input the information required by the data system.

11.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the peak area for each standard to determine the calibration curve.

11.7. Verify calibration using a midrange calibration standard every ten samples or every analytical batch. Compute the percent recovery using the following equation:

$$\%recovery = \frac{D}{K} \times 100$$

Where, D = Determined concentration of analyte in the calibration standard, K = Actual concentration of the analyte in the calibration standard

11.8. If % recovery exceeds +/-10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed

12. DATA ANALYSIS AND CALCULATIONS

12.1. Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting peak area versus standard concentration. Sample concentration is calculated from the regression equation.

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

12.3 Report sample results for total nitrogen in mg N/L.

13. METHOD PERFORMANCE

13.1. The method support data are presented in section 17. This data was generated according to a Lachat Work Instruction during development of the method.

13.2. Although Lachat Instrument publishes method performance data, including MDL, precision, accuracy and carryover studies, we cannot guarantee that each laboratory will be capable of meeting such performance. Individual laboratory and instrument conditions, as well as laboratory technique, play a major role in determining method performance. The support data serves as a guide of the potential method performance. Some labs may not be able to reach this level of performance for various reasons, while other labs may exceed it. To achieve a lower detectable limit for the total nitrogen method, take special care to look for the ACS grade of the potassium persulfate used in the digestion solutions; there can be NO3 contamination in this reagent.

14. POLLUTION PREVENTION

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

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

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

15. WASTE MANAGEMENT

15.1. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operation. Compliance with all sewage discharge permits and regulations is also required.

15.2. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

16. REFERENCES

16.1. U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes Method 353.2

16.2. Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey.

16.3 Dennis Jones, North Dakota Department of Health, SOP I-1-46, Rev 3-20-03.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. DATA SYSTEM PARAMETERS FOR QUIKCHEM 8500/8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Low Range

Sample throughput: 38 samples/h, 95 s/sample
Pump Speed: 35
Cycle Period: 95

Analyte Data:

Concentration Units: mg N/L
Inject to Peak Start: 7 s
Peak Base Width: 95 s
Chemistry: Direct

Calibration Data:

Level	1	2	3	4	5	6	7	8	9
Concentration mg N/L	5.0	2.5	1.25	0.05	0.25	1.00	0.05	0.02	0.0

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: 30 s

Valve Timing:

Load Period: 24 s
Inject Period: 71 s

High Range

Sample throughput:	38 samples/h, 95 s/sample
Pump Speed:	35
Cycle Period:	95

Analyte Data:

Concentration Units:	mg P/L
Inject to Peak Start:	14 s
Peak Base Width:	77 s
Chemistry	Direct/Bipolar

Calibration Data:

Level	1	2	3	4	5	6	7
Concentration mg P/L	40	20	10	5.0	2.5	1.0	0.0

Calibration Rep Handling:	Average
Calibration Fit Type:	2nd Order Polynomial
Weighting Method:	1/X
Force through zero:	No

Sampler Timing:

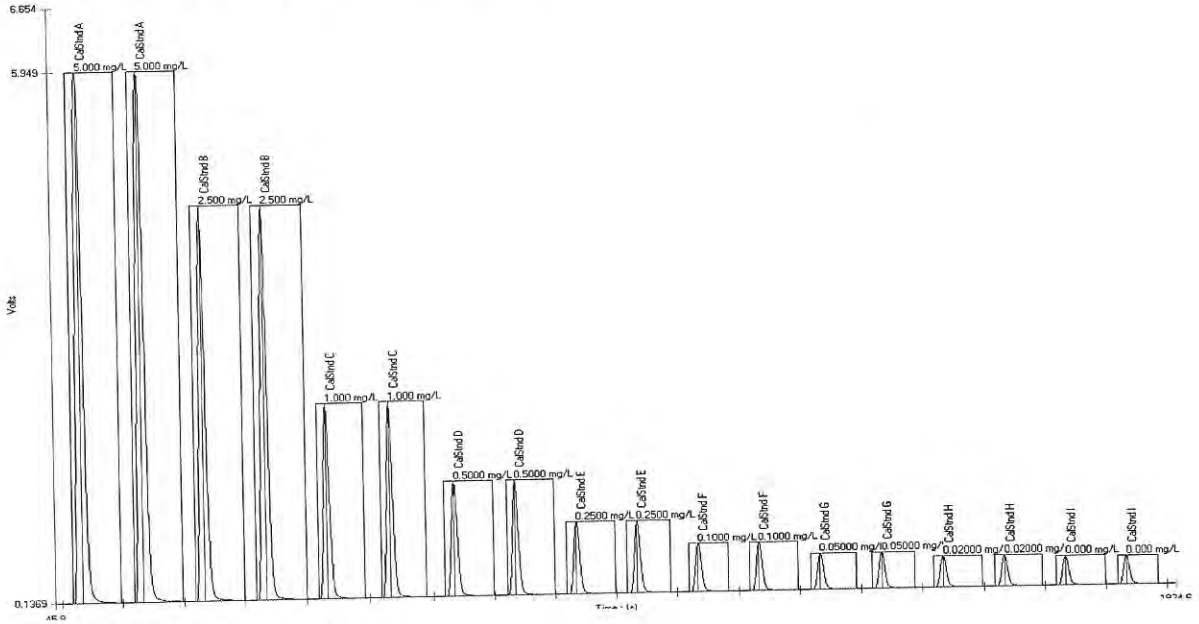
Min. Probe in Wash Period:	5 s
Probe in Sample Period:	25 s

Valve Timing:

Load Period:	10 s
Inject Period:	85 s

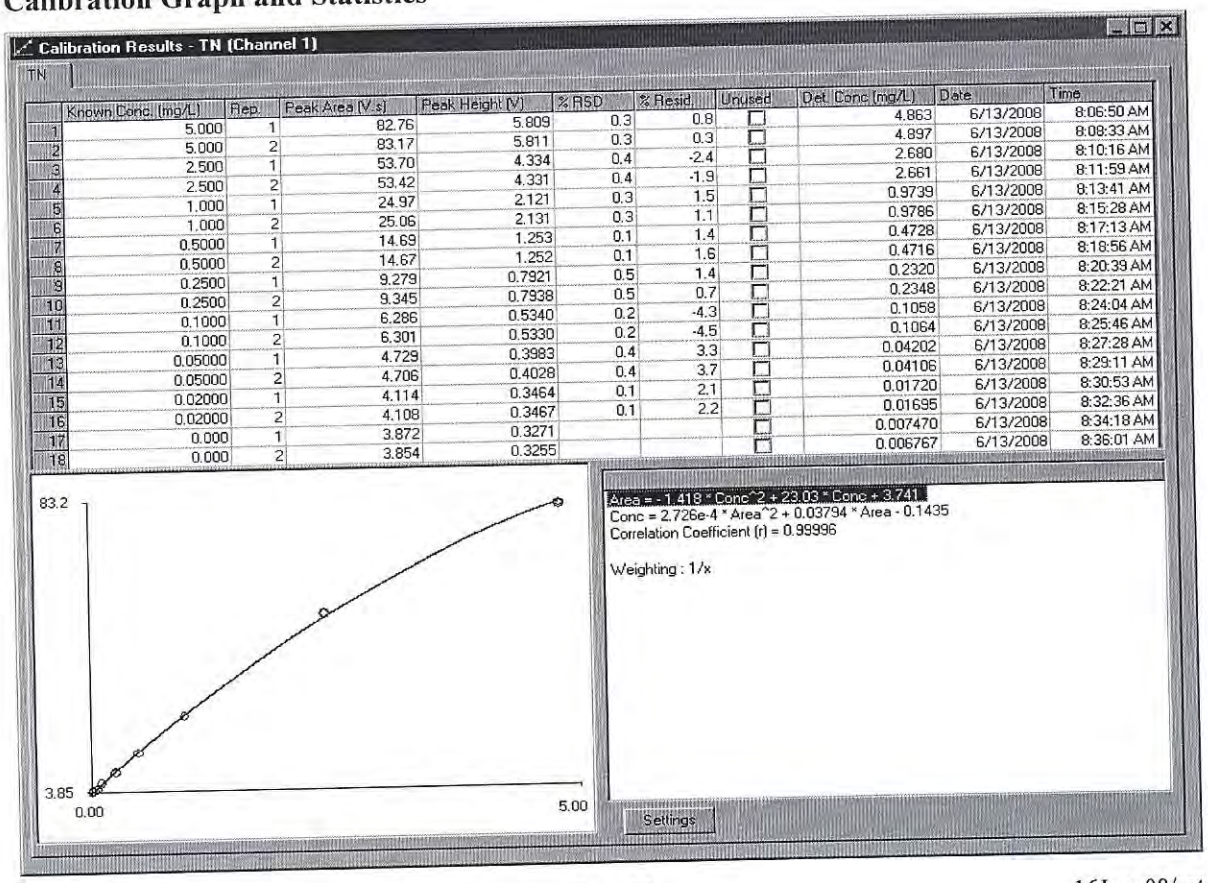
17.2. SUPPORT DATA FOR QUIKCHEM 8500/8000

Calibration Data for Low Range Nitrogen

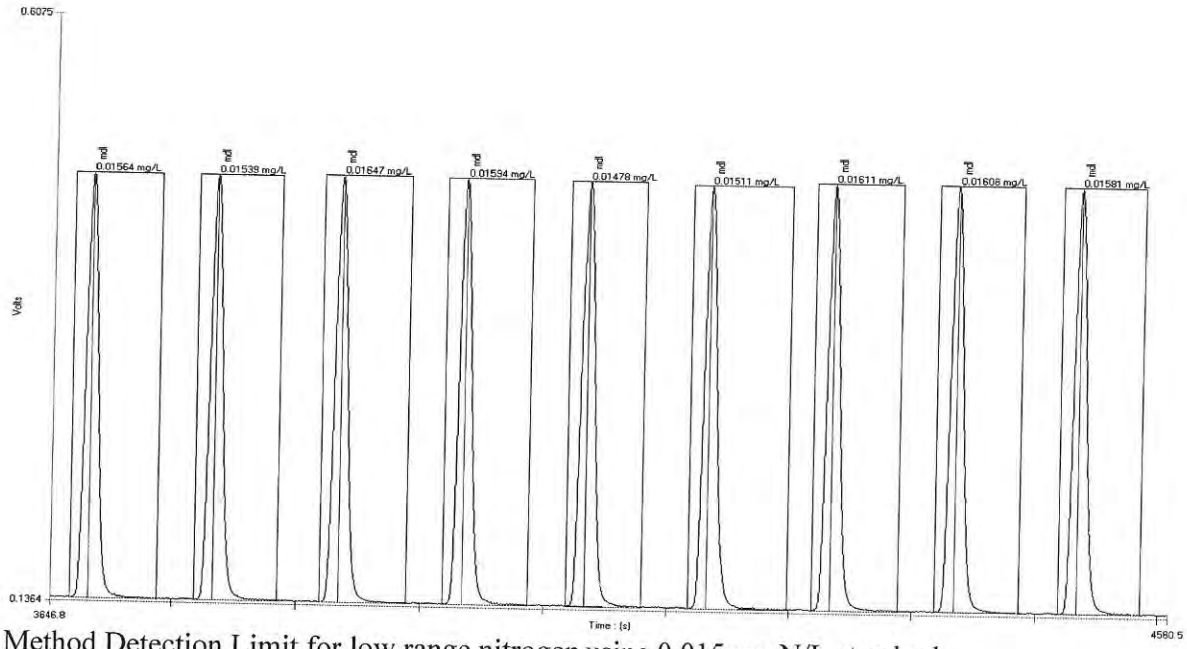


File Name: 6-13 LR cal support brack.omn
Acq. Date: 13 June 2008

Calibration Graph and Statistics



	Known Conc. (mg/L)	Rep.	Peak Area (V s)	Peak Height (V)	% RSD	% Resid	Unused	Det. Conc (mg/L)	Date	Time
1	5.000	1	82.76	5.809	0.3	0.8	☐	4.863	6/13/2008	8:06:50 AM
2	5.000	2	83.17	5.811	0.3	0.3	☐	4.897	6/13/2008	8:08:33 AM
3	2.500	1	53.70	4.334	0.4	-2.4	☐	2.680	6/13/2008	8:10:16 AM
4	2.500	2	53.42	4.331	0.4	-1.9	☐	2.661	6/13/2008	8:11:59 AM
5	1.000	1	24.97	2.121	0.3	1.5	☐	0.9739	6/13/2008	8:13:41 AM
6	1.000	2	25.06	2.131	0.3	1.1	☐	0.9786	6/13/2008	8:15:28 AM
7	0.5000	1	14.69	1.253	0.1	1.4	☐	0.4728	6/13/2008	8:17:13 AM
8	0.5000	2	14.67	1.252	0.1	1.6	☐	0.4716	6/13/2008	8:18:56 AM
9	0.2500	1	9.279	0.7921	0.5	1.4	☐	0.2320	6/13/2008	8:20:39 AM
10	0.2500	2	9.345	0.7938	0.5	0.7	☐	0.2348	6/13/2008	8:22:21 AM
11	0.1000	1	6.286	0.5340	0.2	-4.3	☐	0.1058	6/13/2008	8:24:04 AM
12	0.1000	2	6.301	0.5330	0.2	-4.5	☐	0.1064	6/13/2008	8:25:46 AM
13	0.05000	1	4.729	0.3983	0.4	3.3	☐	0.04202	6/13/2008	8:27:28 AM
14	0.05000	2	4.706	0.4028	0.4	3.7	☐	0.04106	6/13/2008	8:29:11 AM
15	0.02000	1	4.114	0.3464	0.1	2.1	☐	0.01720	6/13/2008	8:30:53 AM
16	0.02000	2	4.108	0.3467	0.1	2.2	☐	0.01695	6/13/2008	8:32:36 AM
17	0.000	1	3.872	0.3271			☐	0.007470	6/13/2008	8:34:18 AM
18	0.000	2	3.854	0.3255			☐	0.006767	6/13/2008	8:36:01 AM



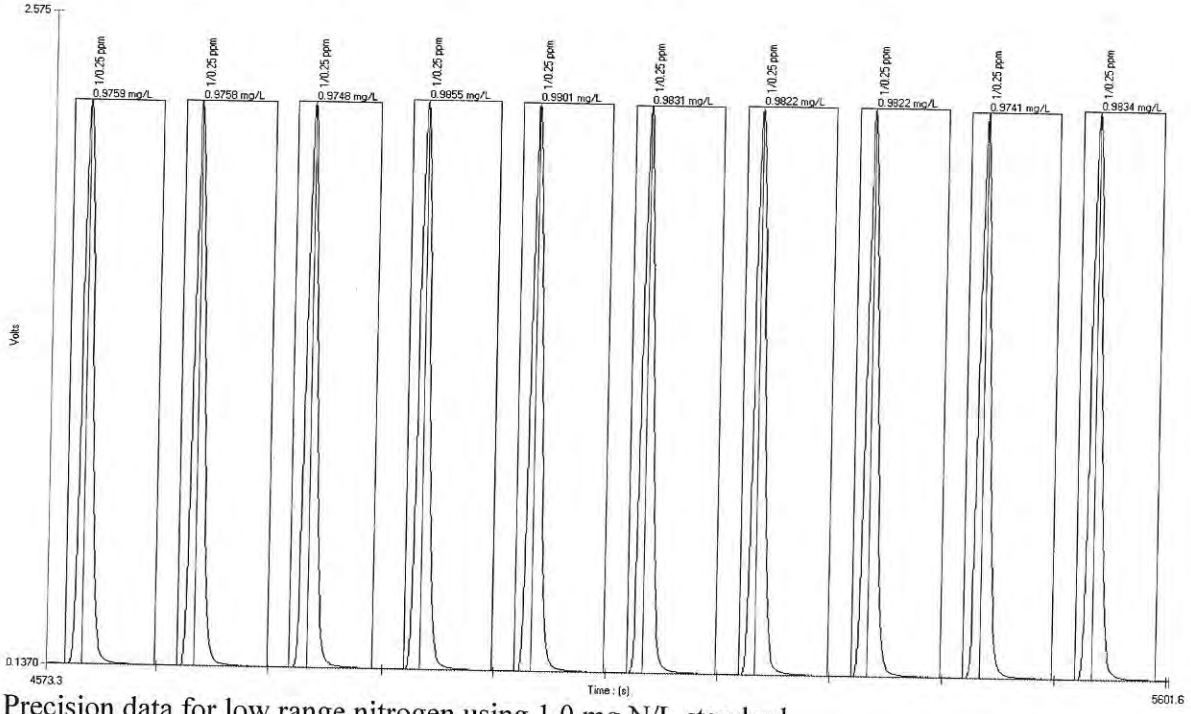
Method Detection Limit for low range nitrogen using 0.015 mg N/L standard

MDL= 0.0015 mg N/L

Standard Deviation (s) = 0.00053 mg N/L, Mean (x) = 0.0157 mg N/L, Known value = 0.015 mg N/L

File Name: 6-13 LR cal support brack.omn

Acq. Date: 13 June 2008



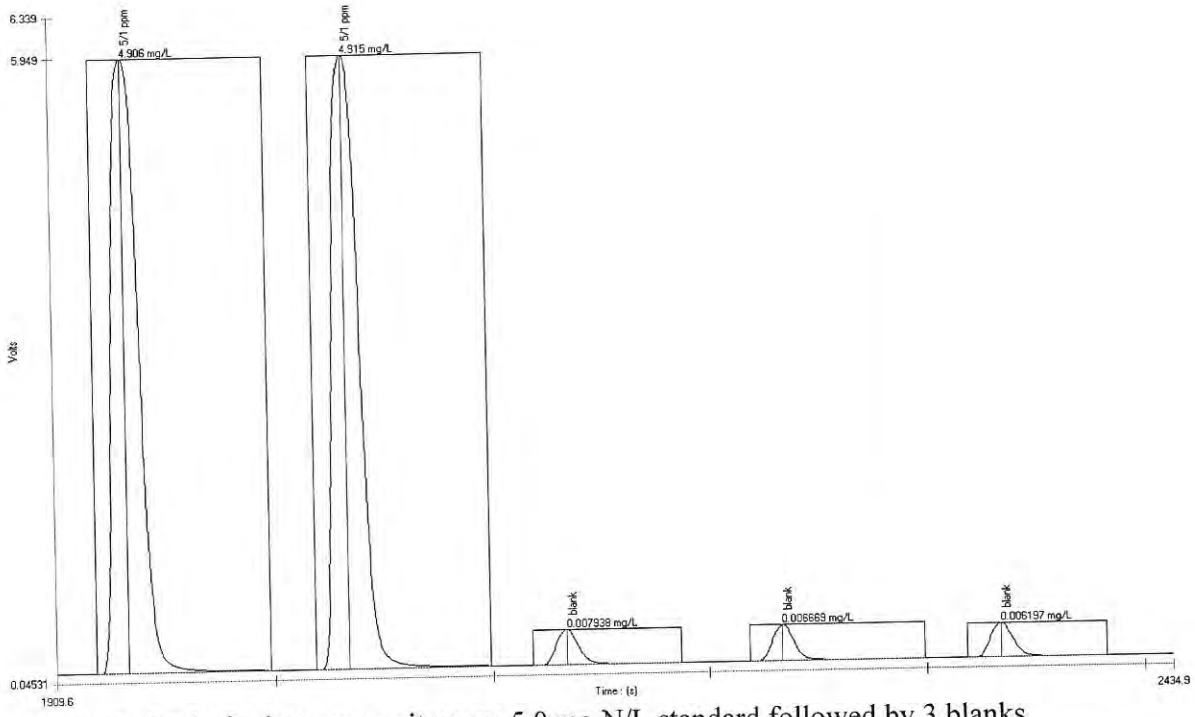
Precision data for low range nitrogen using 1.0 mg N/L standard

% RSD = 0.43

Standard Deviation (s) = 0.004 mg N/L, Mean (x) = 0.98 mg N/L, Known value = 1.0 mg N/L

File Name: 6-13 LR cal support brack.omn

Acq. Date: 13 June 2008

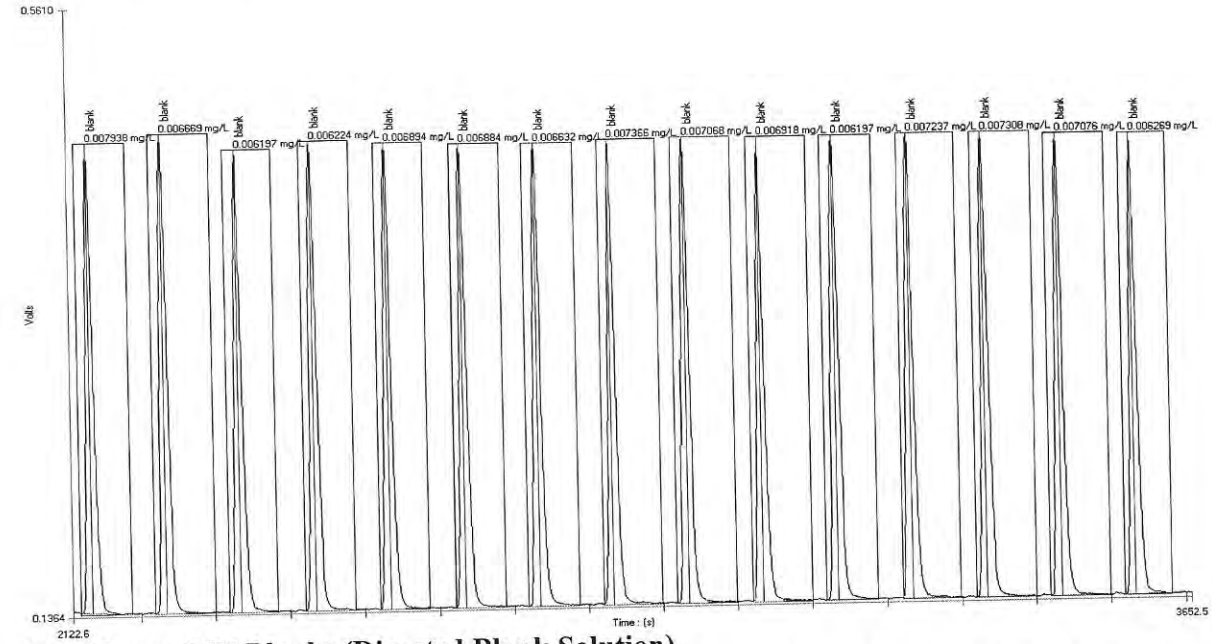


Carryover Study for low range nitrogen: 5.0 mg N/L standard followed by 3 blanks

The blank values are higher in concentration than the calculated statistical MDL, this is due to the nitrogen in the persulfate digestion reagent. Due to this interference, the reported MDL value will be 0.0068 mg N/L (the average of the 15 DIN blanks).

File Name: 6-13 LR cal support brack.omn

Acq. Date: 13 June 2008



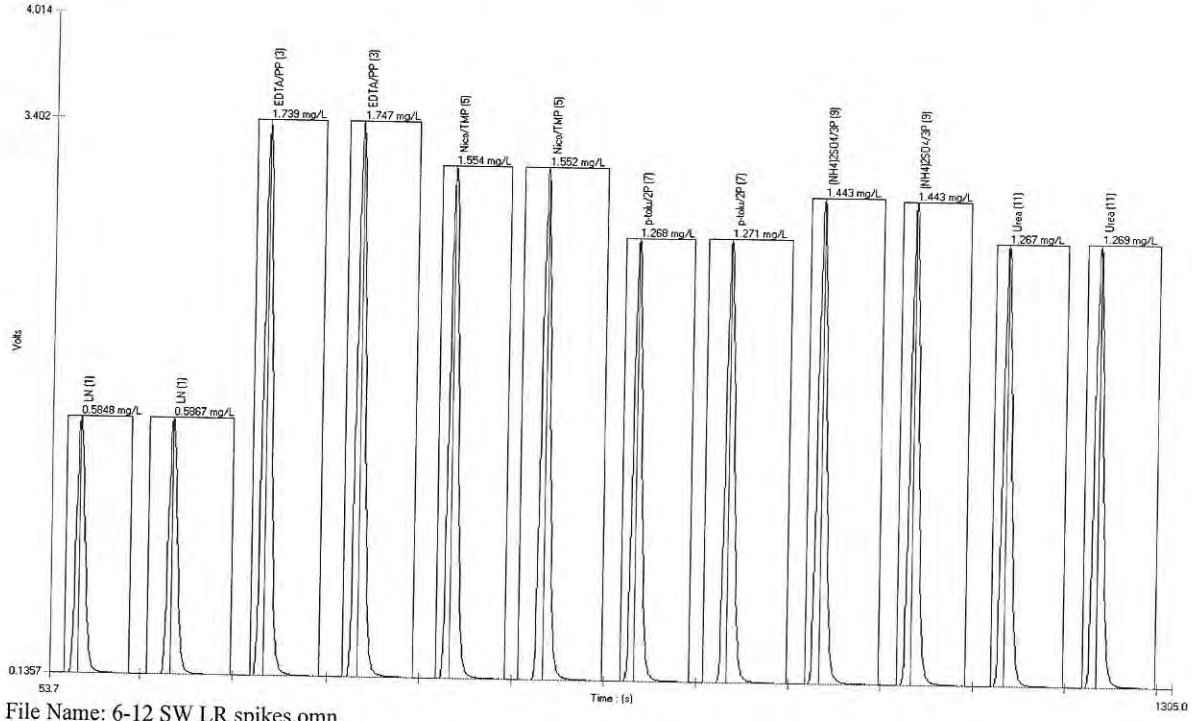
Low Range DIN Blanks (Digested Blank Solution)

Average: 0.0069 mg N/L, SD = 0.0005 mg N/L. Calculated DIN Limits: Detection Limit = 0.0015 mg N/L, Decision Limit = 0.003 mg N/L, Determination Limit = 0.0046 mg N/L;

File Name: 6-13 LR cal support brack.omn

Acq. Date: 13 June 2008

Low Range: Digested Low Nutrient Seawater Organic Nitrogen Compound Spike Recoveries

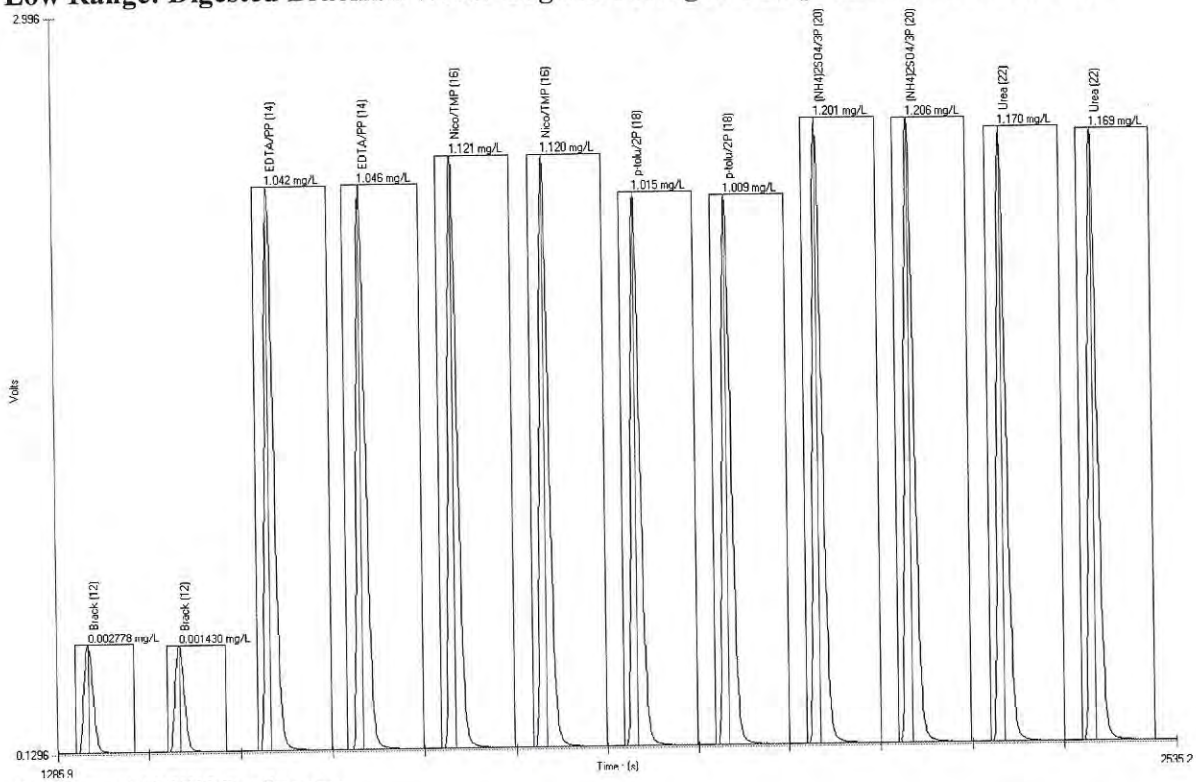


File Name: 6-12 SW LR spikes.omn
Acq. Date: 12 June 2008

Sample type	Initial (mg N/L)	Spiked (mg N/L)	Spike Level (mg N/L)	Spike Recovery
Disodium EDTA	0.586	1.743	1.00	115.7%
Nicotinic Acid	0.586	1.553	1.00	96.72%
Ammonium p-toluenesulfonate	0.586	1.270	1.00	68.38%
Ammonia sulfate	0.586	1.443	1.00	85.72%
Urea	0.586	1.268	1.00	68.22%

Conclusion: Ammonium p-toluenesulfonate and urea only recovered at approximately 70%.

Low Range: Digested Brackish Water Organic Nitrogen Compound Spike Recoveries

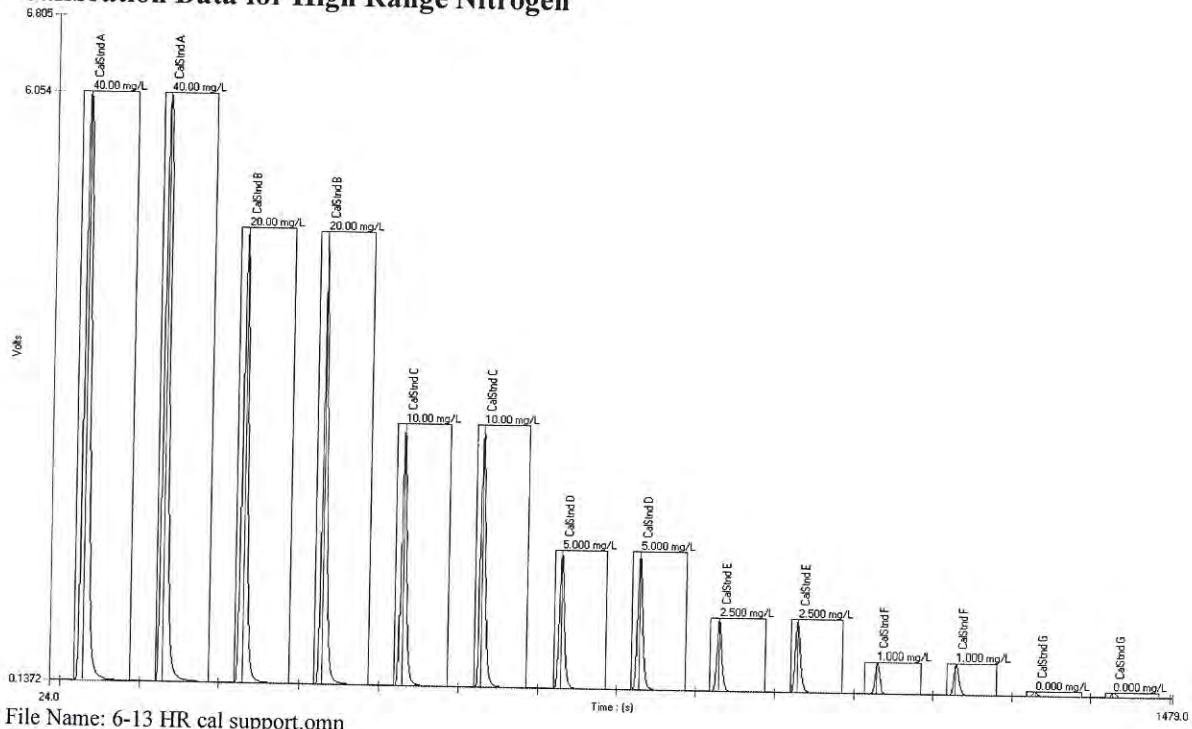


File Name: 6-12 SW LR spikes.omn
Acq. Date: 12 June 2008

Sample type	Initial (mg N/L)	Spiked (mg N/L)	Spike Level (mg N/L)	Spike Recovery
Disodium EDTA	0.002	1.044	1.00	104.19%
Nicotinic Acid	0.002	1.120	1.00	111.84%
Ammonium p-toluenesulfonate	0.002	1.012	1.00	100.99%
Ammonia sulfate	0.002	1.204	1.00	120.14%
Urea	0.002	1.170	1.00	116.74%

Conclusion: All of the organic nitrogen compounds tested recovered at above 100%.

Calibration Data for High Range Nitrogen



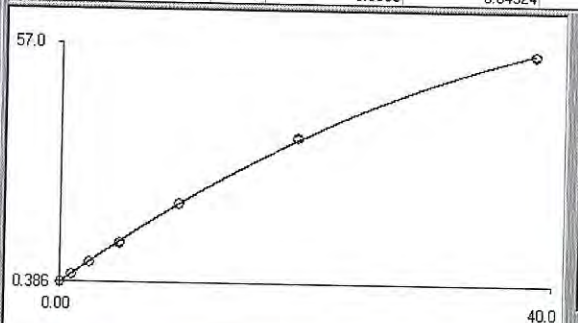
File Name: 6-13 HR cal support.omn
Acq. Date: 13 June 2008

Calibration Graph and Statistics

Calibration Results - TN (Channel 1)

TN

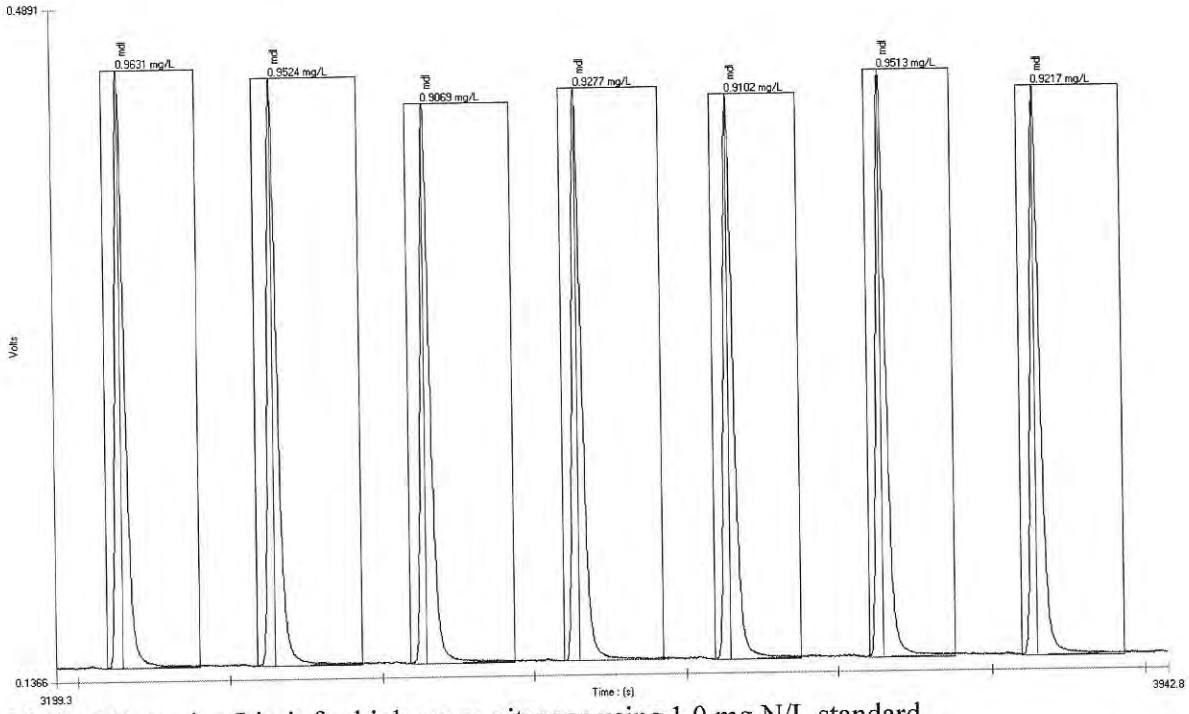
	Known Conc. (mg/L)	Rep.	Peak Area (V.s)	Peak Height (V)	% RSD	% Resid.	Unused	Det. Conc (mg/L)	Date	Time
1	40.00	1	57.01	5.912	0.3	0.2	☐	39.21	6/13/2008	9:55:45 AM
2	40.00	2	56.74	5.910	0.3	0.7	☐	38.96	6/13/2008	9:57:28 AM
3	20.00	1	36.21	4.563	0.5	-2.0	☐	21.34	6/13/2008	9:59:11 AM
4	20.00	2	35.98	4.542	0.5	-1.3	☐	21.16	6/13/2008	10:00:55 AM
5	10.00	1	19.67	2.628	0.1	-0.3	☐	10.03	6/13/2008	10:02:37 AM
6	10.00	2	19.63	2.621	0.1	-0.1	☐	10.01	6/13/2008	10:04:21 AM
7	5.000	1	10.17	1.380	0.3	2.0	☐	4.712	6/13/2008	10:06:03 AM
8	5.000	2	10.14	1.375	0.3	2.3	☐	4.693	6/13/2008	10:07:46 AM
9	2.500	1	5.338	0.7234	0.1	2.0	☐	2.328	6/13/2008	10:09:29 AM
10	2.500	2	5.329	0.7223	0.1	2.2	☐	2.324	6/13/2008	10:11:12 AM
11	1.000	1	2.370	0.3178	0.2	0.7	☐	0.9739	6/13/2008	10:12:54 AM
12	1.000	2	2.363	0.3175	0.2	1.0	☐	0.9707	6/13/2008	10:14:36 AM
13	0.000	1	0.3871	0.04910			☐	0.1153	6/13/2008	10:16:18 AM
14	0.000	2	0.3859	0.04924			☐	0.1148	6/13/2008	10:18:01 AM



Area = - 0.01697 * Conc^2 + 2.100 * Conc + 0.3033
Conc = 0.004713 * Area^2 + 0.4200 * Area - 0.04799
Correlation Coefficient (r) = 0.99997

Weighting : 1/x

Settings



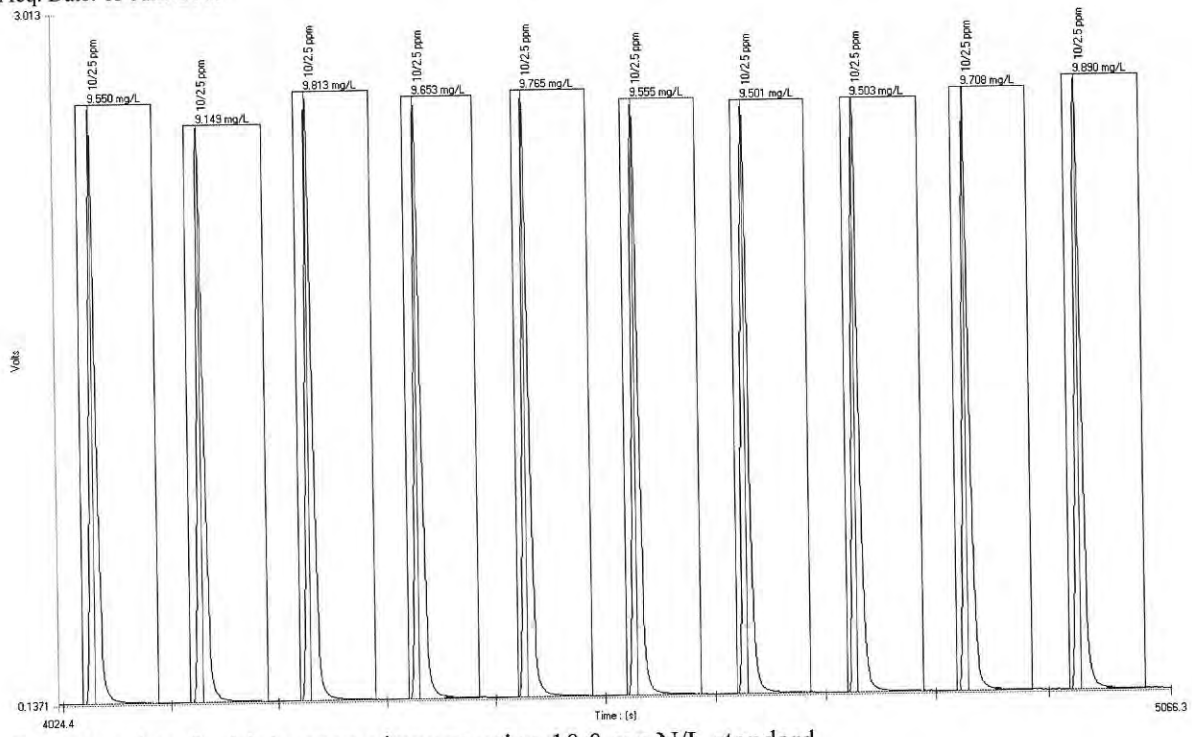
Method Detection Limit for high range nitrogen using 1.0 mg N/L standard

MDL= 0.070 mg N/L

Standard Deviation (s) = 0.0223 mg N/L, Mean (x) = 9.33 mg N/L, Known value = 1.0 mg N/L

File Name: 6-13 HR cal support.omn

Acq. Date: 13 June 2008



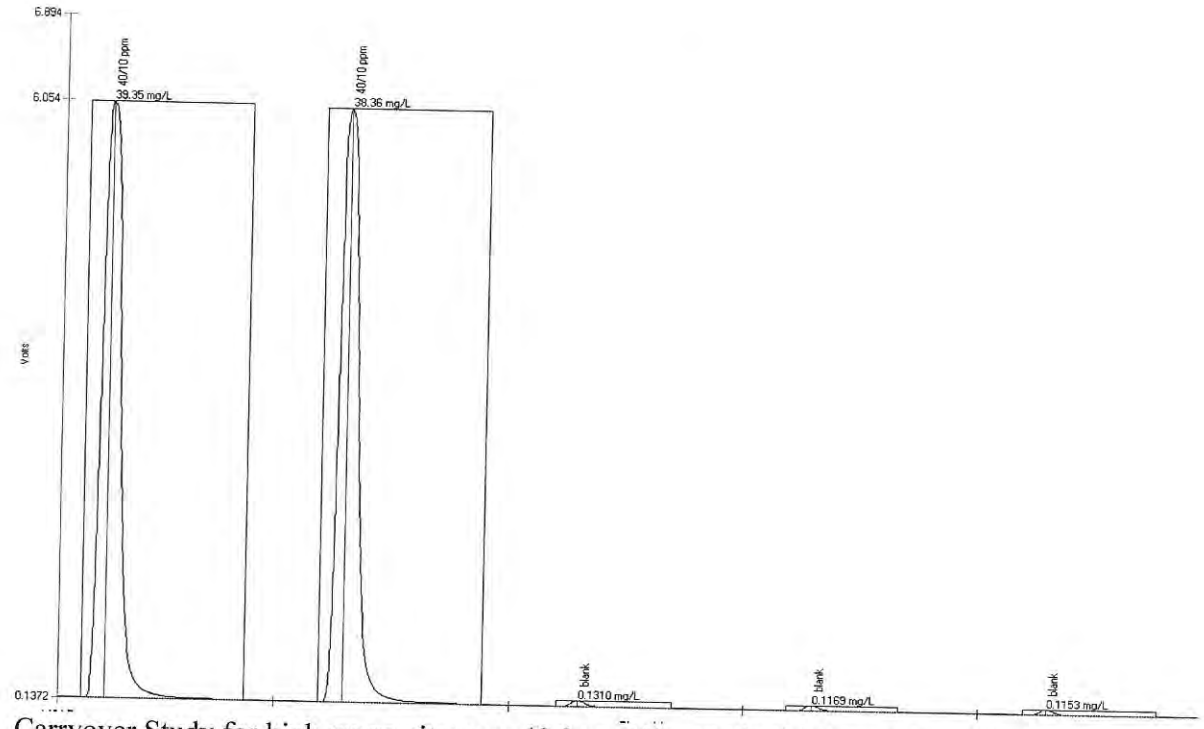
Precision data for high range nitrogen using 10.0 mg N/L standard

% RSD = 2.19

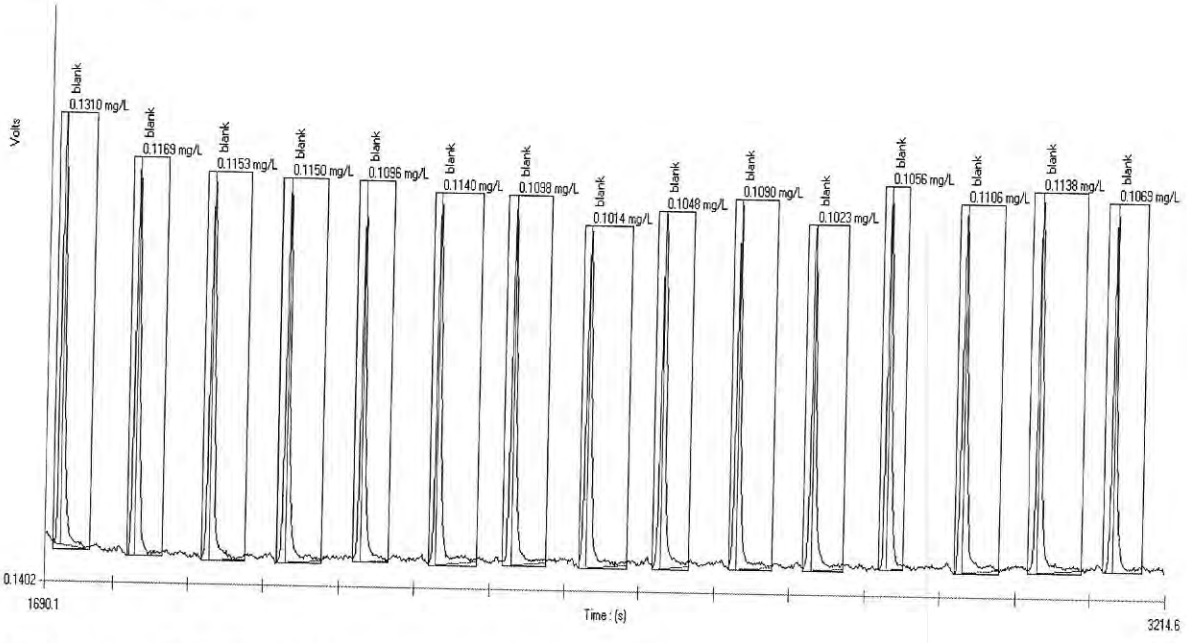
Standard Deviation (s) = 0.210 mg N/L, Mean (x) = 9.61 mg N/L, Known value = 10.0 mg N/L

File Name: 6-13 HR cal support.omn

Acq. Date: 13 June 2008



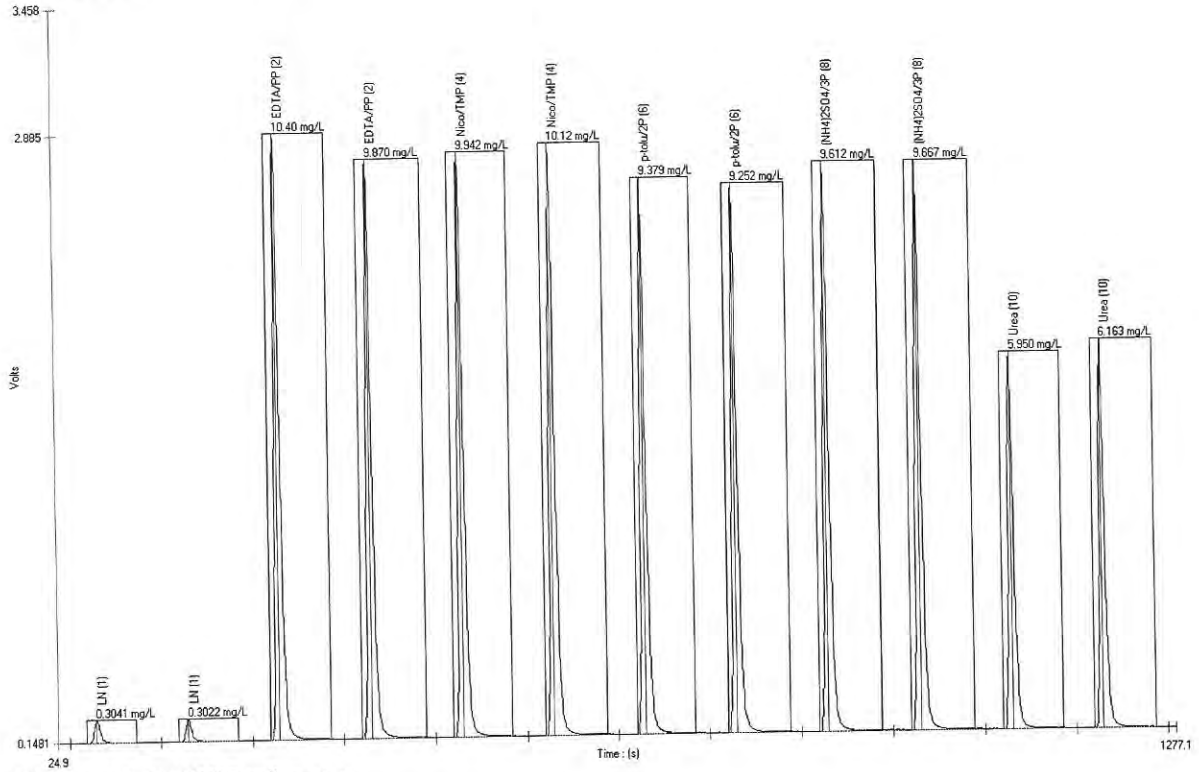
Carryover Study for high range nitrogen: 40.0 mg N/L standard followed by 3 blanks
The blank values are higher in concentration than the calculated statistical MDL, this is due to the nitrogen in the persulfate digestion reagent. Due to this interference, the reported MDL value will be the average of the 15 DIN blanks , **0.111 mg N/L.**
File Name: 6-13 HR cal support.omn
Acq. Date: 13 June 2008



High Range DIN Blanks (Digested Blank Solution)

Average: 0.111 mg N/L, SD = 0.0073 mg N/L. Calculated DIN Limits: Detection Limit = 0.0219 mg N/L, Decision Limit = 0.0438 mg N/L, Determination Limit = 0.0657 mg N/L;
File Name: 6-13 HR cal support.omn
Acq. Date: 13 June 2008

High Range: Digested Low Nutrient Seawater Organic Nitrogen Compound Spike Recoveries

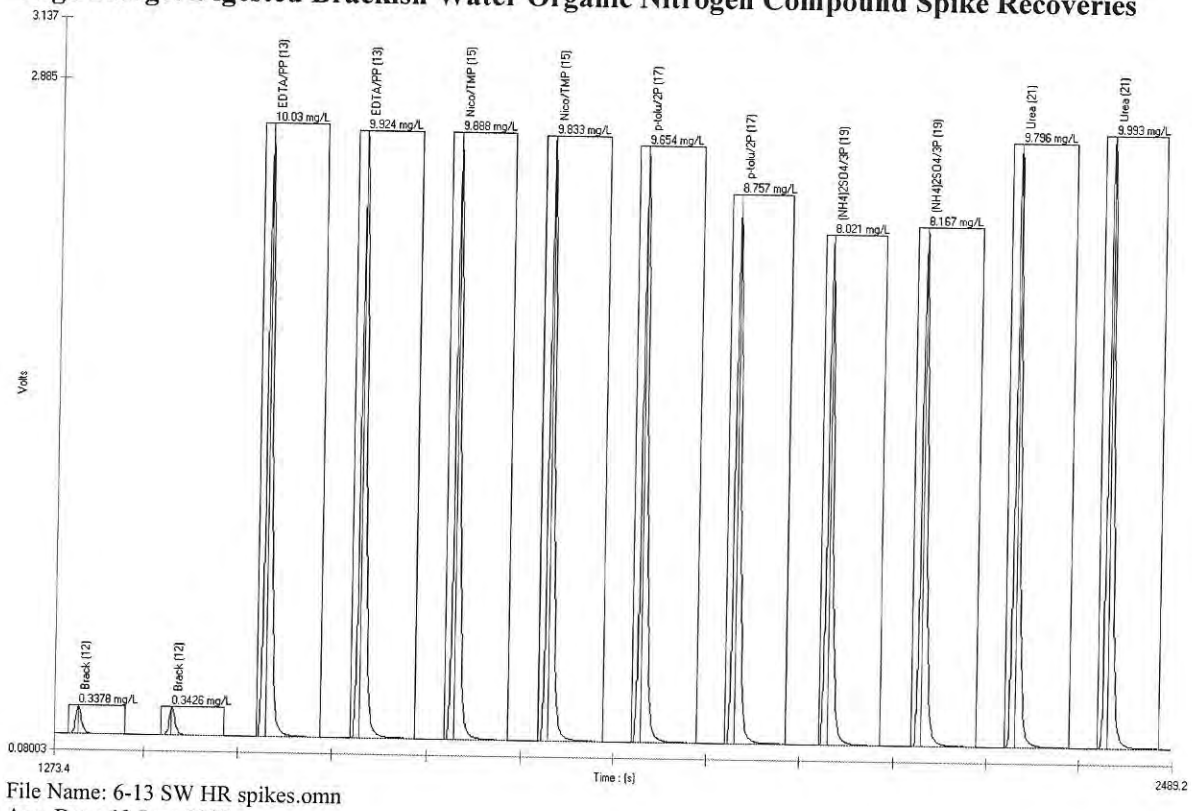


File Name: 6-13 SW HR spikes.omn
Acq. Date: 13 June 2008

Sample type	Initial (mg N/L)	Spiked (mg N/L)	Spike Level (mg N/L)	Spike Recovery
Disodium EDTA	0.303	10.14	10.0	98.32%
Nicotinic Acid	0.303	10.03	10.0	97.28%
Ammonium p-toluenesulfonate	0.303	9.32	10.0	90.12%
Ammonia sulfate	0.303	9.64	10.0	93.36%
Urea	0.303	6.06	10.0	57.53%

Conclusion: Urea only recovered at approximately 55%; the rest of the organic nitrogen compounds recovered above 90%.

High Range: Digested Brackish Water Organic Nitrogen Compound Spike Recoveries

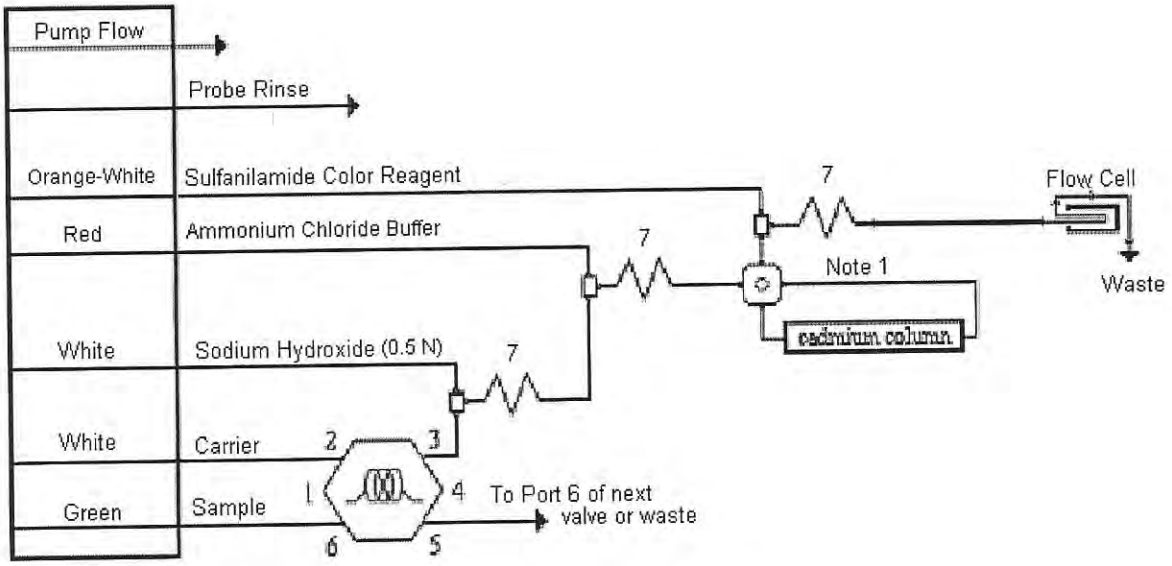


File Name: 6-13 SW HR spikes.omn
Acq. Date: 13 June 2008

Sample type	Initial (mg N/L)	Spiked (mg N/L)	Spike Level (mg N/L)	Spike Recovery
Disodium EDTA	0.34	9.98	10.0	96.37%
Nicotinic Acid	0.34	9.86	10.0	95.20%
Ammonium p-toluenesulfonate	0.34	9.21	10.0	88.65%
Ammonia sulfate	0.34	8.09	10.0	77.54%
Urea	0.34	9.89	10.0	95.54%

Conclusion: All of the organic nitrogen compounds tested recovered at near 90%, except ammonia sulfate that only recovered at 77%.

17.3. NITRATE/NITRITE MANIFOLD DIAGRAM

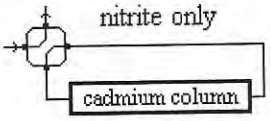


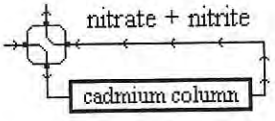
Carrier: **0.213 M sulfuric acid (Reagent 5)**
Manifold Tubing: **0.5 mm (0.022 in) i.d.**
QC8500 Sample Loop: 100 cm of 0.5 mm tubing for Low Range
Microloop (16 cm) for High Range (P/N 50091)
Interference Filter: 520 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

7: 135 cm of tubing on a 7 cm coil support

Note 1: This is a two state switching valve used to place the cadmium column in-line with the manifold





APPENDIX 5

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