Quality Assurance Project Plan (QAPP)

Hempstead Harbor Water-Quality Monitoring Program

Hempstead Harbor Protection Committee
Coalition to Save Hempstead Harbor

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Title and Approvals

Hempstead Harbor Protection Committee
Coalition to Save Hempstead Harbor
Water-Monitoring Program for Hempstead Harbor

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Region 2

Effective April __, 2014

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1 Interested-Parties List

The following agencies and municipalities will be notified of the availability of the approved Quality Assurance Project Plan (QAPP) (and any subsequent revisions) for the Water-Quality Monitoring Program for Hempstead Harbor. The QAPP will also be made available on the Hempstead Harbor Protection Committee website.

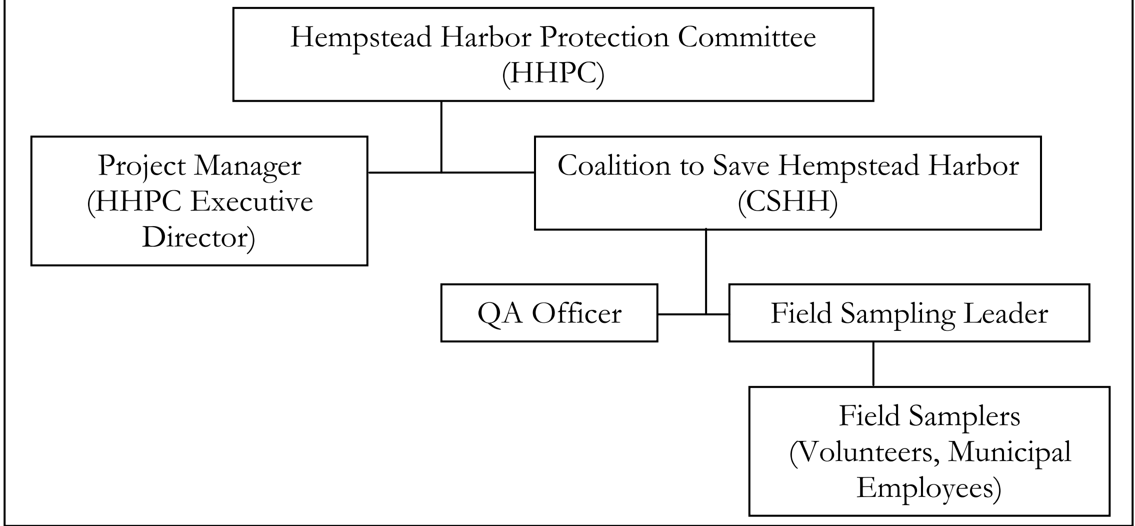
- United States Environmental Protection Agency (US EPA), Region 2
- US EPA Long Island Sound Study Office
- United States Fish and Wildlife Service (US FWS)
- New York State Department of Environmental Conservation (NYS DEC), Bureau of Marine Resources
- New York State Department of State (NYS DOS), Division of Coastal Resources
- Interstate Environmental Commission
- Connecticut Department of Environmental Conservation
- Nassau County Department of Health (NCDH)
- Nassau County Department of Public Works
- Town of North Hempstead, Department of Public Works
- Town of Oyster Bay, Department of Environmental Resources
- City of Glen Cove, Department of Public Works
- New York State Sea Grant
- Village of Flower Hill
- Village of Roslyn
- Village of Roslyn Harbor
- Village of Sands Point
- Village of Sea Cliff

2 Project and Task Organization

2.1 Project Organization and Personnel Responsibilities

The organizational chart prepared for the Water-Monitoring Program for Hempstead Harbor is presented in *Figure 1*. The Quality Assurance (QA) Officer and Field Sampling Leader are responsible for the implementation of the QAPP. *Table 1* presents the responsibilities of the personnel that are involved with the program. For purposes of this document, the Program Manager, Field Sampling Leader, and QA Officer positions are considered management personnel for the water-quality monitoring program and QAPP.

Figure 1: Hempstead Harbor Water-Monitoring Program Organizational Chart



2.2 Communication Pathways

Tasks to be accomplished during the monitoring events will be communicated between field personnel and managers following the Standard Operating Procedures presented in *Appendix A*. The QAPP will be reviewed by the Project Manager, Field Sampling Leader, and QA Officer at the beginning of each monitoring season. If issues arise during monitoring-program implementation, these personnel will discuss and institute any necessary changes. Issues pertaining to field activities or laboratory analyses will be addressed by the QA Officer or the Field Sampling Leader or both.

2.3 Modifications to QAPP

Modifications to this QAPP will be initiated by the Field Sampling Manager. When documenting amendments to the QAPP, the reasons for the changes will be outlined in a revision/modification log as will a description of how the changes are expected to affect the quality and usability of the data to be collected. Records of QAPP amendments will be maintained on-file at CSHH and HHPC offices. Proposed changes to the QAPP will be submitted to EPA for review and approval.

Table 1: Program Personnel Responsibilities

Title	Name	Affiliation	Responsibility
Advisory Board	Municipal Officials	Hempstead Harbor Protection Committee	1. Discusses and approves proposed changes in monitoring program 2. Reviews and approves budgets
Project Manager	Eric Swenson	Hempstead Harbor Protection Committee	1. Reviews and approves proposed changes to the QAPP 2. Maintains correspondence with other groups
Field Sampling Leader	Carol DiPaolo	Coalition to Save Hempstead Harbor	1. Organizes daily operation of monitoring program 2. Schedules activities related to monitoring program 3. Ensures that equipment is properly maintained and that consumables are available. 4. Trains volunteers and field samplers in the procedures described in this QAPP. 5. Procures analytical services. 6. Supervises sample handling. 7. Tracks samples to verify that they reach the laboratory. 8. Recommends changes to water-quality monitoring program. 9. Determines whether QAPP changes are necessary.
Quality Assurance Officer	David North	Coalition to Save Hempstead Harbor	1. Reviews the QAPP when necessary. 2. Reports data-quality deficiencies to Field Sampling Leader. 3. Oversees audits or data validation as mandated by this QAPP. 4. Assesses whether laboratory elements outlined in the QAPP are followed. 5. Oversees data verification activities.
Field Samplers	Vary	Coalition to Save Hempstead Harbor Volunteers and Municipal Employees	1. Assists the Field Sampling Leader as necessary. 2. Collects samples and collects and records field data. 3. Assists in maintaining field equipment.

3 Special Training Needs and Certification

3.1 Training for Program Managers

Managers for the Hempstead Harbor water-quality monitoring program are required to be familiar with this QAPP and the Standard Operating Procedures (SOPs) presented in *Appendix A*. Additionally, the QA Officer will be trained in the use of the data-verification procedures presented in *Section 14*. The Field Sampling Leader will be trained in the operation, calibration, and maintenance of field-data-collection equipment and will be familiar with appropriate field-sampling procedures. Training will be provided by an individual who is experienced with similar monitoring equipment and sampling techniques. Training provided by technicians from the sampling-equipment manufacturers, if available, is preferred. The QA Officer and Field Sampling Leader should have prior water-quality monitoring experience through this program, a similar program, or through work or education.

Program management will be evaluated during any cooperative monitoring events undertaken with similar water-quality monitoring groups or environmental-monitoring professionals. Deficiencies will be corrected with the procedures presented in *Section 3.3*.

3.2 Training for Field Samplers

Prospective Field Samplers (volunteers and/or municipal employees) will meet with program managers for information regarding the monitoring program. Interested individuals will be formally trained before participating in any water-quality monitoring. Training will include a discussion of this QAPP, the program's SOPs, and any other procedures that are necessary. Topics will typically include:

- Monitoring-program background and purpose.
- The QAPP and SOPs.
- Field-equipment care and maintenance, including:
  - Calibration
  - Checking the calibration
  - Checking items that may need replacement (e.g., DO probe membrane)
- Appropriate sample-collection procedures.
- Sample handling and labeling.
- Potential safety hazards.

Hands-on volunteer training will be provided during regularly scheduled sampling events. Field-sampler performance will be monitored informally by the Field Sampling Leader during sampling or during cooperative sampling events with members of other groups or environmental professionals. Deficiencies will be corrected with the procedures presented in *Section 3.3*.

The Field Sampling Leader will coordinate monitoring activities and will be assisted by field samplers. In instances where the Field Sampling Leader will not be present, experienced only field samplers who are familiar with this QAPP and the SOPs (and have demonstrated proficiency with all required procedures) may sample with no supervision, provided that the Field Sampling Leader reviews and approves documentation resulting from the monitoring activities (i.e., field data sheets and laboratory results)..

3.3 Corrective Procedures

Individuals requiring additional instruction will receive instruction in the field at the time of sampling or will receive additional training prior to the next sampling event in which they participate. Systematic (groupwide) deficiencies may require revision of the monitoring protocols, QAPP, Standard Operating Procedures, Data Quality Objectives (see *Section 8*), and other program documents. Deficiencies will be noted and the training program revised to improve future groupwide performance.

3.4 Laboratory Accreditations

Copies of accreditations for the Nassau County Department of Health Laboratory and Town of Oyster Bay Environmental Laboratory are presented in *Appendix B*. Accreditation for nitrate/nitrite analyses is not required for the Oyster Bay Town laboratory; this laboratory uses commercially available single-use reagents and vials, and the QA/QC procedures required by this QAPP are adequate to ensure reliability of these analyses. There are currently no Standard Methods or accreditation for the microbial source-tracking analysis performed by companies such as Source Molecular, the laboratory initially selected for the Hempstead Harbor water-monitoring program to provide microbial source-tracking services. However, Source Molecular implements SOPs and a Quality Policy Manual (available on the company website, www.sourcemolecular.com).

4 Problem Definition and Background

4.1 Problem Definition

The water-monitoring program for Hempstead Harbor (located on the north shore of Long Island) encompasses three components: a weekly in-harbor water-quality monitoring program and a prospective wet-weather outfall-monitoring program to identify other critical areas of pathogen loading to the harbor. Sampling begins in late April or early May and continues until November.

The monitoring data will be used by Hempstead Harbor Protection Committee, Coalition to Save Hempstead Harbor, Nassau County Department of Health, Nassau County Department of Public Works, the Interstate Environmental Commission, the New York State Department

of Environmental Conservation, the Connecticut Department of Environmental Protection, Long Island Sound Study, and the communities surrounding Hempstead Harbor. The data will be used to:

- Identify and study seasonal-scale trends in water quality.
- Monitor aquatic habitats.
- Identify causes for negative events (e.g., algal blooms and fish kills).
- Investigate long-term trends in water-quality parameter levels.
- Guide municipal and county-level environmental planning, policy, and compliance efforts (e.g., Phase II Stormwater Program and TMDL development).
- Measure progress towards meeting water-quality goals in the watershed.
- Determine whether the opening of additional shellfish-harvesting areas within the harbor is feasible.
- Identify pathogen sources for targeting pathogen-load reduction efforts.

4.2 Background

Hempstead Harbor is a dynamic hydrologic and ecologic system that is affected by both runoff from its watershed and tidal water from Long Island Sound. In the 1980s, beaches were frequently closed due to high bacteria levels. Low oxygen levels have also resulted in periodic fish kills. Once the most productive oystering harbor in New York, it is now mostly closed to shellfishing, although it is expected that by summer 2011 about 2,500 will be reclassified as certified for shellfish harvesting, due to dramatic improvements in water quality over the last 20 years.

Water-quality monitoring has been conducted in Hempstead Harbor over the past 30 years by various governmental and private organizations. The Hempstead Harbor water-monitoring program, initiated by the Coalition to Save Hempstead Harbor, is one of the most extensive and oldest programs of its kind on Long Island Sound. CSHH was founded in 1986 in response reports of continued degradation of the harbor on a number of fronts. Following county budget cuts that decimated Nassau County Department of Health's (NCDH) environmental program and ended midharbor sampling, CSHH developed the water-monitoring program to focus attention on the harbor, continue collecting midharbor samples (to be analyzed by NCDH) to monitor bacteria levels, and engage community residents in efforts to improve water quality.

In 1995, the Hempstead Harbor Protection Committee was created. HHPC is an intermunicipal organization comprising the nine municipalities that are situated in the Hempstead Harbor watershed and has been focused on reducing stormwater runoff into the harbor. Beginning in 2006, HHPC assumed financial responsibility for the water-quality monitoring program, CSHH continues to do field sampling, data collection, and reporting for the program. The monitoring program is necessary to continue beach monitoring and to track the impact of environmental policy in the surrounding communities.

To identify additional sources of pathogen loadings, an outfall pathogen-monitoring program was developed in 2010, to be implemented as funding for this aspect of the program became available. This component of the monitoring program was considered necessary to develop strategies to further reduce levels of pathogen contamination that prevent the opening of shellfish harvesting areas, result in occasional beach closures, and limit other recreational uses of the harbor.

4.3 Historical and Current Water Quality

Hempstead Harbor is a V-shaped harbor of approximately five miles from mouth to head, the harbor's average depth is 18 feet, and the maximum depth is about 40 feet. The harbor supports a number of uses including industry, primary and secondary contact recreation, and recreational fishing, and is classified by the New York State Department of State as a significant coastal fish and wildlife habitat. The industrial and commercial uses resulted in degraded water quality through oil spills, sewage spills, toxic contamination, air pollution, and industrial discharges. The greatest impacts of these discharges were noted in the mid-1980s. Restoration efforts in the harbor and its watershed had resulted in the closure of a landfill, two incinerators, and a sewage treatment plant. Remediation has been completed for some of the hazardous waste sites around the harbor but is ongoing for others.

During the last five years, the harbor has been characterized by dissolved oxygen levels (ranging from 0.1 to 14.4 mg/L during the monitoring season), salinity levels similar to Long Island Sound (19 to 28 parts per thousand), summer water temperatures averaging approximately 19ºC, and precipitation averaging 28 inches during the summer months.

5 Project and Task Description

5.1 In-Harbor Monitoring Program

CSHH monitors 18 CSHH locations weekly, generally from May to November. The principal CSHH stations that are sampled weekly during the monitoring season for all program parameters are located in the northern portion of the harbor, between the former Bar Beach sand spit (now part of the 36.2-acre North Hempstead Beach Park) and Long Island Sound, as well as stations in Glen Cove Creek. *Table 2* includes the latitude/longitude points for most of the monitoring stations, and a map showing the monitoring locations is presented as *Appendix C*.

Table 2. Latitude/Longitude Points for In-Harbor Monitoring Stations

Station ID	Latitude N		Longitude W	
	Degrees	Minutes	Degrees	Minutes
Upper-Harbor Stations				
CSHH #1, Beacon 11	40	49.540	73	39.120
CSHH #2, Bell 6	40	51.647	73	40.428
CSHH #3, Red Channel Marker	40	51.213	73	39.123
CSHH #8, Adjacent to STP Outfall Pipe	40	51.514	73	38.515
CSHH #9, 10 ft West of #8				
CSHH #10, 20 ft West of #8				
CSHH #11, 50 ft East of #8				
CSHH #12, 100 ft East of #8	40	51.561	73	38.430
CSHH #13, 60 ft from Mill Pond Weir	40	51.706	73	38.139
CSHH #15, about 50 yds from Scudders Pond Outfall, North of Tappen Beach pool area	40	50.109	73	39.247
CSHH #15A, at Outfall North of Tappen Pool				
CSHH #15B, at Sudder's Pond Weir				
Lower-Harbor Stations				
CSHH #4, East of North Hempstead Beach Park (formerly Bar Beach) Sand Spit	40	49.688	73	39.001
CSHH #5, Mott's Cove	40	49.317	73	38.770
CSHH #6, East of Pt. Washington transfer station	40	48.688	73	39.080
CSHH #7, West of Bryant Landing (formerly site of oil dock)	40	48.474	73	38.923
CSHH #14, about 50 yds from Powerhouse Drain outfall	40	49.706	73	38.916
CSHH #14A, at Powerhouse Drain Outfall				

In 2009, CSHH #15A (at outfall north of Tappen Pool) and #15B (Sudder's Pond weir) and in 2010, CSHH #14A (powerhouse drain outfall) were added to the program. These stations were established to monitor discharges prior to implementation/construction of stormwater strategies/structures at Scudder's Pond and the powerhouse drainage area.

CSHH monitors estuarine water quality by measuring a series of field parameters and collecting samples to be analyzed by a laboratory. Dissolved oxygen, temperature, pH, and salinity vertical profiles (1 meter intervals) are measured at each CSHH monitoring location. A surface grab sample is field analyzed for ammonia at monitoring location CSHH #1. If ammonia is detected at this location, samples collected at the other CSHH monitoring locations are field analyzed for ammonia as well. If ammonia is not detected at CSHH #1, then the only additional ammonia sample is collected near the sewage treatment plant outfall (CSHH #8). A Secchi-disk depth is recorded at each CSHH location. Turbidity is also field measured at two depths at each CSHH location – at a half-meter below the surface and at Secchi-disk depth. Samples are collected and analyzed for nitrate/nitrite, fecal coliform bacteria, and enterococci. *Table 3* summarizes the sampling program. The NCDH performs the bacterial analysis on CSHH station samples. Nitrate/nitrite is analyzed by the Oyster Bay Town lab.

Table 3: In-Harbor Water-Quality Monitoring

Parameter	Location	Analyzer or Method	Location of Analysis
Dissolved Oxygen	Vertical profiles at 1-meter intervals at CSHH #1-8 and 13-15	YSI Pro Plus	Field
Dissolved Oxygen	One location for electronic meter validation	LaMotte 7414	Field
Water Temperature	Vertical profiles at 1-meter intervals at CSHH #1-8, and 13-15	YSI Pro Plus	Field
Water Temperature	One station for electronic meter validation	Calibrated Thermometer	Field
Air Temperature	One measurement at each station during monitoring	Calibrated Thermometer	Field
Salinity	Vertical profiles at 1-meter intervals at CSHH #1-8 and 13-15	YSI Pro Plus	Field
pH	Vertical profile at 1-meter intervals at CSHH #1-8 and 13-15	YSI Pro Plus	Field
pH	One station for electronic meter validation	LaMotte 2218 reagent	Field
Turbidity	Two vertical locations (0.5 meter and Secchi depth) at CSHH #1-8, and 13-15	LaMotte 2020e (USEPA 180.1)	Field
Clarity	CSHH #1-8 and 13-15	LaMotte Secchi Disk	Field
Ammonia	CSHH #1, 8, and other stations when ammonia is detected at CSHH #1	LaMotte 4795 (Nessler Method)	Field
Ammonia	More refined method used at CSHH #1 and 8	LaMotte 3304 (Salicylate Method)	Field
Nitrate	Grab sample at half-meter depth at CSHH #1- 8 and 13-15	Hach 8192	Oyster Bay Town Lab
Nitrite	Grab sample at half-meter depth at CSHH #1-8 and 13-15	Hach 8507	Oyster Bay Town Lab
Fecal Coliform Bacteria	Grab sample half-meter depth at CSHH #1-13,14,14a, 15, 15a, 15b	Membrane Filter	Nassau County Department of Health
Enterococci	Grab sample at half meter depth at CSHH #1-13,14,14a, 15, 15a, 15b	Membrane Filter	Nassau County Department of Health
Precipitation	Village of Sea Cliff	Visually read rain gauge	Field

CSHH produces a publicly available Water-Quality Monitoring Report annually, summarizing findings and any changes to the program. The annual reports contain data collected during the preceding monitoring season as well as averages of certain parameters for comparison of previous years' conditions.

5.2 Outfall Pathogen Monitoring

The CSHH outfall pathogen-monitoring component of the water-monitoring program was developed as a multievent wet-weather-driven project. The goal for this segment of the water-monitoring program is to calculate actual pollutant loads and potential pathogen sources. Prior to monitoring the outfalls, CSHH will prioritize outfalls that have been surveyed and select those that pose the greatest risk of bacteria loading. To prioritize the outfalls, field surveys will be conducted consisting of visual inspection of the outfall condition, presence of flow (dry weather), and other indicators of potential pollution (examples surfactant and ammonia testing when available).

The study area for the program is defined by the Hempstead Harbor shoreline (and the associated upland drainage area) south of an east-west line starting at the mouth of Glen Cove Creek (also as reference, south of three DEC SGA #50 stations – DEC #10 [private dock], #11 [navigational marker C-A], and #12 [beyond mouth of Glen Cove Creek]). Based on results of shoreline surveys, including investigating upland drainage areas with the greatest potential pathogen loads and existing water-quality data, priority outfalls will be defined.

Outfall pathogen-monitoring will include the sampling of up to five outfall locations during three wet-weather events. Samples will be collected no more than 6 hours following the start of a precipitation event where more than 0.5 inches of precipitation is forecast and that occurred at least 72 hours after a previous storm event of 0.1 inch or greater (i.e., following a minimum 72-hour dry period).

Two types of parameters are monitored: those measured in-situ (field) and those analyzed at a laboratory including microbial source-tracking methods (also know as DNA fingerprinting, DNA source-tracking, or qPCR source-tracking), consisting of fecal Bacteroides analysis (identification and quantification of human Bacteroides and human enterococci for genetic fingerprinting). *Table 4* summarizes the outfall sampling program.

Bacteroides are growing in popularity for the identification of sources of fecal pollution. Unlike the traditional indicator organisms such as *E. coli* and enterococci, Bacteroides are strict anaerobes and their presence is indicative of recent fecal contamination when found in water systems. In addition, they are also more abundant in the feces of warm-blooded animals than *E.coli* and enterococci. Bacteroides analysis, in conjunction with enterococci analysis for genetic fingerprinting, can be useful for identifying contamination sources because distinct categories of Bacteroides have been shown to be detected predominately in humans rather than livestock or birds. The major value in using microbial source-tracking methods in this project is the ability to differentiate between human and nonhuman sources, which will help identify specific pathogen sources and better target controls to address those sources. EPA has been studying this identification method for over five years and its Office of Research and Development released a guidance document on the topic in 2005 entitled *Microbial Source Tracking Guide Document*.

Table 4: Outfall Pathogen Monitoring

Parameter	Location	Analyzer or Method	Location of Analysis	Sample Event
Water Temperature	All outfall locations	YSI Pro Plus	Field	All
Water Temperature	One outfall location for electronic meter validation	Calibrated Thermometer	Field	All
Air Temperature	One measurement at each outfall location during monitoring	Calibrated Thermometer	Field	All
Salinity	All outfall locations	YSI Pro Plus	Field	All
pH	All outfall locations	YSI Pro Plus	Field	All
pH	One outfall location for electronic meter validation	LaMotte 2218 reagent	Field	All
Ammonia	All outfall locations	LaMotte 3304 (Salicylate Method)	Field	All
Surfactants	All outfall locations	LaMotte 4508 reagent	Field	All
Fecal Coliform Bacteria	All outfall locations	Membrane Filter	Nassau County Department of Health	All
Enterococci	All outfall locations	Membrane Filter	Nassau County Department of Health	All
Human Enterococcus (microbial source tracking)	All outfall locations	Polymerase Chain Reaction (PCR)	Source Molecular[1]	1 - 3
Human Bacteroides (microbial source tracking)	All outfall locations	Polymerase Chain Reaction (PCR)	Source Molecular[1]	1 - 3
Precipitation	Village of Sea Cliff	Visually read rain gauge	Field	All

[1] *Source Molecular is the laboratory initially selected by CSHH to perform the analysis but a similar laboratory may be chosen when this program is initiated.*

6 Quality Objectives and Criteria for Measurement Data

6.1 Data Quality Objectives

Data-quality objectives (DQOs) specify the quality of environmental data required to support decision-making processes. The generation and use of quality data is important to the assessment of water quality within the harbor.

6.2 Measurement Performance Criteria

Data quality can be described in terms of precision, accuracy, completeness, representativeness, and comparability. Each of these terms is discussed in the following subsections.

6.2.1 Precision

Precision is defined as a measure of mutual agreement among individual measurements of the same type. In the case of laboratory analytical data, precision will be used to describe the reproducibility of the analytical data.

6.2.1.1 Sampling Measurement Systems

To assess precision in the field, a duplicate sample for all parameters to be analyzed by a laboratory will be collected nominally for every 20 samples per matrix for all parameters. The collection of field duplicates measures a combination of field and laboratory precision, thereby exhibiting more variability than a laboratory duplicate.

Additionally, duplicate samples for parameters to be field analyzed with chemical test kits (e.g., ammonia) will be collected and analyzed for every 20 samples. Dissolved oxygen and pH results from the YSI meter will be validated with a sample from one location per monitoring date and analyzed by the Winkler-titration method using a LaMotte field kit.

A calculation to determine Relative Percent Difference (RPD) between two corresponding sample results is performed. Relative Percent Difference (RPD) is used as a measure of precision. RPD limits are matrix and compound dependent. RPD is defined as follows:

$$RPD = \frac{|Conc(p) - Conc(d)|}{(1/2)(Conc(p) + Conc(d))} * 100$$

where:

Conc(p) = Primary Sample Concentration, the first sample collected at that location

Conc(d) = Duplicate Sample Concentration, the second sample collected at that location

Precision performance criteria are included in *Table 8*, *Table 9*, and *Table 10*. If a calculated RPD falls outside the criteria range, the discrepancy will be addressed on a case-by-case basis since the results are method, parameter, and matrix dependent.

6.2.1.2 Laboratory Measurement Systems

The objective concerning precision is to equal or exceed the precision demonstrated in the analytical methods on samples of a similar matrix. Relative Percent Difference (RPD) is used as a measure of precision. The laboratory will analyze matrix spikes/matrix spike duplicates for relative percent difference. RPD is defined as follows:

$$RPD = \frac{|MSR - MSDR|}{(1/2)(MSR + MSDR)} * 100$$

where:

MSR = matrix spike recovery

MSDR = matrix spike duplicate recovery

The absolute value of the recovery difference is used in the above equation.

Recovery limits are matrix and compound dependent. If necessary, corrective action by the laboratories will be performed according to the provisions of their Quality Assurance Plans or Quality Policy Manual as applicable. The Nassau County Health Department laboratory implement Quality Assurance Standard Operating Procedures (SOPs) presented in Standard Methods (See *Appendix E*). The Town of Oyster Bay lab will implement QA/QC procedures required by this QAPP. Source Molecular lab implements SOPs and a Quality Policy Manual (available on the company website, www.sourcemolecular.com).

6.2.2 Accuracy

Accuracy can be defined as the degree of agreement of a measurement with an accepted reference or true value. Accuracy is generally expressed as the ratio of the measured value to the true value, which gives a measure of bias inherent in the system. Accuracy can be assessed both in the field and in the laboratory.

6.2.2.1 Field Measurement Systems

Accuracy is measured for field activities to assess the performance of the monitoring equipment. Monthly, the YSI Pro Plus handheld multiparameter instrument is calibrated for dissolved oxygen, salinity, and pH. On the day of each monitoring event, the LaMotte 2020e meter is calibrated for turbidity and the YSI Pro Plus calibrations are checked for DO, salinity, and pH. The instruments are calibrated using the procedures outlined in the user manuals as presented in *Appendix D*. The calibrations are checked by:

- Comparing DO results from the YSI meter for one location to a result obtained via Winkler titration for the same location.
- Comparing pH results from the YSI meter for one location to a result obtained via LaMotte field kit for the same location.
- Checking the YSI meter salinity results via the method described in the operating manual.
- Checking the LaMotte turbidity meter results via the method described in the operating manual.

These checks will be performed on the boat during sampling. Calibration acceptance criteria, where applicable, are defined in *Table 9* in *Section 8.3.*

6.2.2.2 Laboratory Measurement Systems

Laboratory accuracy will be determined from laboratory control and surrogate samples, published historical data, method validation studies and experience with similar samples. The goal for spiked sample recoveries will be +/- 30%. These concentrations vary from one compound to another. Quality Assurance SOPs implemented by the NCDH laboratory is presented in *Appendix E*. Source Molecular implements SOPs and follows published methods for each analyte (SOPs are available on the company website, www.sourcemolecular.com).

6.2.3 Bias

Bias is the systematic or persistent distortion of a measurement process causing errors in one direction. Bias will be evaluated by considering factors associated with the sampling program design (e.g., time of sampling and choice of sampling sites) and through validation measurements using a modified Winkler titration method as described elsewhere in this plan.

6.2.4 Representativeness

In-Harbor Monitoring Locations

Six of the monitoring locations within Hempstead Harbor, including CSHH #1-3 and three of the stations south of sand spit that divides the harbor (CSHH #4-6) were selected to represent ambient estuarine water quality conditions. CSHH #1-3 are located near navigational markers north of the sand spit. These sites are away from the shore and less likely to be influenced by outfalls and other possible pollutant loadings. The sites are also well-spaced throughout the harbor, allowing monitoring results to reflect longitudinal gradients, if any.

CSHH #8-13, #14 and #14A, and #15 and #15A and #15B are located near four of the harbor’s significant freshwater and pollutant loading areas, Glen Cove Creek (CSHH #3) and the Glen Cove sewage treatment plant outfall, Glen Cove Creek, Mill Pond, the powerhouse drain, and Scudder's Pond. Constituent values recorded for these locations are thus not representative of ambient water quality and will only be used where inflows or loadings are discussed.

CSHH #9-12 are in the vicinity of the Glen Cove sewage treatment plant outfall (CSHH #8) and are used to track the frequency and source of unusual dry- and wet-weather flows that were noted at discharge points west of the STP outfall. CSHH #13 was established to monitor bacteria levels near the Mill Pond at the head of the Glen Cove Creek. Samples collected at CSHH #13 are used to indicate whether the restoration of Mill Pond is curtailing bacteria inputs to Glen Cove Creek.

Outfall Monitoring Locations

The outfall pathogen-monitoring component of the program focuses on identifying additional areas that may be significant contributors of pathogens and that likely contribute to shellfish-bed closures and create adverse impacts to aquatic habitat based. The outfalls with the greatest potential pathogen loads will be prioritized and selected for follow-up monitoring and bacteria-source investigations.

6.2.5 Data Comparability

Comparability is an expression of the confidence with which one data set can be compared to another. The comparability objective is to collect and analyze samples using methods which will demonstrate that current data are comparable to data collected in previous and future investigations for this study area. The comparability of data is addressed by using standard protocols for the collection of field samples and by using standard methodologies for analytical procedures which were used in past investigations. If, for instance, it is determined that the laboratory used a different method than one specified, an evaluation will occur and document whether this has compromised the comparability of the data.

6.2.6 Data Completeness

In-Harbor Monitoring Data

Data completeness is the fraction of the planned data that must be collected during a sampling program. As the results of the monitoring program are used for planning, research, and public education, not for legal or compliance purposes, CSHH will not be required to monitor all locations for an event to be considered complete. The completeness goal for each in-harbor monitoring event is 70% (e.g., at least 70% of the total number of stations must be sampled during each event). The overall completeness goal for the in-harbor monitoring program shall be 75% (e.g., only 25% of the samples proposed to be collected can be missed).

CSHH expects tidal conditions to prevent access to some or all of the sampling locations south of the sand spit of the Town of North Hempstead Beach Park on a regular basis, and it is important that failure to collect samples at these locations does not interfere with the usability of the data. Otherwise, CSHH will complete all sampling and field monitoring unless weather, tidal, safety issues, or other conditions interfere.

Outfall Monitoring Data

The completeness goal for each outfall pathogen-monitoring event is 70% (e.g., at least 70% of the total number of outfalls must be sampled each time). The overall completeness goal for the outfall monitoring program shall be 75% (e.g., only 25% of the samples proposed to be collected can be missed). This arrangement will allow for some flexibility if difficulties arise (i.e. tidal conditions, access issues) during a particular event while ensuring that an adequate number of samples are collected overall. However, uncollected or unanalyzed samples may not affect the goal of identifying potential pollutant sources (e.g., a high bacteria value at a particular outfall could still be useful as identifying a pollutant source or event even if the overall dataset does not meet the completeness criterion). As such, single-sample data may be used to identify potential pollutant sources without the dataset meeting the completeness requirement.

Because the selected monitoring locations will represent suspected pathogen contributors to the harbor, a complete sampling event (samples from all outfall locations, up to five) is intended to characterize the discharges to the harbor. Because the intended use of the data is to identify, quantify, and interpret pathogen inputs to the harbor, there is no specific representativeness requirement; e.g., if a sample is not collected during an event, the validity of the dataset will not be compromised.

6.2.7 Data Sensitivity

Sensitivity is the lowest detection limit of the method or instrument for each of the measurement parameters of interest. Laboratory analyses have preset limits of detection for the nitrogen analyses as well as the coliform bacteria and enterococci. Field sampling equipment has published specifications that include detection limits. *Table 8* presents detection limits for water quality parameters measured in this monitoring program.

7 Non-Direct Measurements

No additional data sources have been identified that could be used in the monitoring program reports and data analysis. If other data is identified, its usability and comparability will be assessed. Any such assessment will be included in this section of the QAPP.

8 Field-Monitoring Requirements

The monitoring program follows a judgment-based design intended to compare collected data with historical data and to provide a baseline for comparison with future sampling activities. A description of the monitoring locations, and the rational for the selection of those locations, is presented in *Section 6.2.4*. A map showing the monitoring locations is presented in *Appendix C.*

8.1 Monitoring-Process Design

The Hempstead Harbor aquatic system is affected by many factors including tidal, seasonal, and meteorological conditions, treated sanitary wastewater, power-plant water intake and discharges, nonpoint-source runoff, and recreational-uses. Therefore, the monitoring of CSHH stations is conducted once per week from May to November to document changes in ambient water-quality conditions and gain information on potential pollution sources. The outfall pathogen monitoring program will occur during three wet-weather events. Wet-weather samples will be collected no more than 6 hours following the start of a precipitation event where more than 0.5 inches of precipitation is forecast and that occurred at least 72 hours after a previous storm event of 0.1 inch or greater (i.e., following a minimum 72-hour antecedent dry period).

8.1.1 Addition of Monitoring Locations

If other locations of interest are identified (e.g., if an outfall reconnaissance survey identifies the potential for significant pollutant sources), they will be considered for inclusion in the in-harbor or outfall monitoring components of the program as appropriate. All additions must be submitted to EPA Region 2 with a short justification for review and approval.

8.1.2 Removal of Monitoring Locations

Program managers may consider removing in-harbor monitoring locations based on the changing needs of data users and the availability of funding. Outfall monitoring locations may be removed following the first monitoring event if the monitoring results for that location are at a significantly low level that the Field Sampling Leader deems this location not a likely source of pollutants. If a monitoring location is removed from the monitoring program, it must be noted in that year's annual report.

8.1.3 Sampling Parameters

Field parameters measured include dissolved oxygen, temperature, salinity, pH, clarity, and ammonia. Samples collected for laboratory analysis include those for nitrate/nitrite, fecal coliform, turbidity, enterococci and human enterococcus and human Bacteroides (microbial source tracking). *Table 5* and *Table 6* include a summary of the sampling programs. *Table 7* presents applicable New York State surface water quality standards.

Table 5: Sampling Design Logistics – Water-Quality Monitoring

Type of Sample	Parameter	Number of Locations	Sample Type	Sampling Frequency
Biological	Fecal Coliform Bacteria	CSHH Stations=18	Surface Grab	1 day/week
Biological	Enterococci	18	Surface Grab	1 day/week
Chemical	Ammonia Level	2 or more	Surface Grab	1 day/week

Type of Sample	Parameter	Number of Locations	Sample Type	Sampling Frequency
Chemical	Nitrate/Nitrite	11	Surface Grab	1 day/week
Chemical	Dissolved Oxygen	11	Vertical Profile	1 day/week
Chemical	Salinity	11	Vertical Profile	1 day/week
Chemical	pH	11	Surface Grab	1 day/week
Physical	Temperature	11	Vertical Profile	1 day/week
Physical	Water clarity	11	In-Situ (Secchi disk)	1 day/week
Physical	Turbidity	11	Surface Grab and at Secchi Depth	1 day/week

**Note: Number of locations may vary based on tidal conditions.*

Table 6: Sampling Design Logistics – Outfall Pathogen Monitoring

Type of Sample	Parameter	Number of Locations	Sample Type	Sampling Frequency
Biological	Fecal Coliform Bacteria	1 - 5	Outfall Discharge Grab	3- wet-weather events
Biological	Enterococci	1 - 5	Outfall Discharge Grab	3- wet weather events
Biological	Human Enterococcus and Human Bacteroides (microbial source tracking)	1 - 5	Outfall Discharge Grab	1-3 wet-weather events
Chemical	Ammonia	1 - 5	Outfall Discharge Grab	3- wet weather events
Chemical	Salinity	1 - 5	Outfall Discharge Grab	3- wet weather events
Chemical	pH	1 - 5	Outfall Discharge Grab	3- wet weather events
Chemical	Surfactants	1 - 5	Outfall Discharge Grab	3- wet weather events
Physical	Temperature	1 - 5	Outfall Discharge Grab	3- wet weather events

Table 7: New York State Water Quality Standards (6 NYCRR Part 703)

Parameter	Standard*
Fecal Coliform	NYS beach closure standards: 1,000 CFU/100 mL for fecal coliform; 200 Log AvgFC/100 mL
	NYS shellfish standards: geometric mean of 14 FC/100mL or 90th percentile values of 49 FC/100mL
Enterococci	NYS beach closure standards: 104 CFU/100 mL for enterococci; and 35 Log AvgEnt/100 mL
Ammonia plus Ammonium	Standard is pH and temperature dependent. Range from 0.0007 to 0.050 mg/L
Nitrate plus Nitrite	10 mg/L (Class SA)
Dissolved Oxygen	4.8 mg/L
pH	Normal range shall not be exceeded by more than 0.1 S.U.

**for SB Classified Water Body unless otherwise noted*

Dissolved oxygen is monitored because hypoxia is a common water-quality problem in Long Island Sound and Hempstead Harbor. DO is a significant indicator of estuarine health as it is required by marine fauna, and it is indirectly impacted by nutrient enrichment. In Hempstead Harbor, DO is an important indicator of the health of the fishery.

Monitoring temperature allows the YSI Pro Plus to determine the percent saturation of DO within the harbor. In addition to nutrient enrichment, increased temperatures reduce water's capacity for DO. Thus, monitoring temperature indicates whether low DO levels result from temperature or nutrient enrichment. Additionally, monitoring temperature helps to determine whether the water column is stratified. Density currents, caused by temperature differentials, can prevent mixing within the water column and can lead to hypoxia.

Monitoring salinity assists in determining whether the harbor is being influenced by tidal water or by freshwater from the watershed (i.e., whether any water quality problems result from stormwater, wastewater, other discharges, or from tidal backwater). Salinity measurements are also used by the YSI Pro Plus to determine the percent saturation of DO. pH is monitored to follow trends in aquatic life and water chemistry. Release of carbon dioxide (CO_2) by respiration and consumption via photosynthesis impact aquatic pH on small time scales (hours to days) whereas increasing atmospheric CO_2 may impact aquatic pH on the decadal time scale.

Water clarity is monitored through the use of a Secchi disk and LaMotte turbidity meter. The Secchi disk technique is used to determine the depth to which ambient light can penetrate the water column. In most productive waters, Secchi disk depth is limited by algal productivity, thus this monitoring tool is used to track the spatial and temporal occurrence of algal blooms.

Ammonia should only be present in significant quantities due to malfunctioning wastewater treatment systems, including septic tanks, cesspools, and publicly owned treatment works (POTWs), or from illicit stormwater discharges. Thus, ammonia is monitored weekly at CSHH #1, which is used to indicate the presence of ammonia distant from the harbor's inflows. If ammonia is detectable at this location, it is likely the result of an unusual inflow event, and ammonia levels are measured at the other locations. If ammonia is not detectable at CSHH #1, it is unlikely that ammonia will be detectable at other locations except CSHH #8, and CSHH #8 is the only additional location where ammonia analysis is performed. Ammonia will be quantified at all outfall pathogen-monitoring locations. The presence of ammonia in the harbor can indicate nutrient enrichment and can help to anticipate algal blooms and hypoxia.

Like ammonia, surfactants (e.g., detergents) can be an indicator of a failing wastewater treatment systems or illicit stormwater discharges, thus assisting in the diagnosis of the source(s) of elevated bacteria levels. The presents or absence of surfactants will be assessed at all outfall sampling locations with the use of a reagent.

Nitrate and nitrite occur in later stages of the nitrogen cycle and are expected to be present in the estuary. However, high concentrations indicate enrichment problems and can also be used to anticipate algal blooms and hypoxia. Thus, samples are collected at each CSHH location for nitrate/nitrite and are subsequently analyzed at the Town of Oyster Bay laboratory.

The Nassau County Department of Health and the New York State Department of Environmental Conservation use enterococci and coliform bacteria levels to open or close swimming beaches and shellfish beds, respectively. Coliform and enterococci are bacteria typically found in human and warm blooded animal feces. Enterococci are the preferred indicator for contamination of brackish and salt water environments. Monitoring for enterococci and coliform bacteria (the preferred indicator of fecal contamination for fresh water environments) in Hempstead Harbor, where salinity is variable, provides a comprehensive monitoring program for bacterial contamination.

Unlike the traditional indicator organisms such as *E.coli* and enterococci, Bacteroides are strict anaerobes and their presence is indicative of recent fecal contamination when found in water systems. In addition, they are also more abundant in the feces of warm-blooded animals than *E. coli* and enterococci. Bacteroides analysis, in conjunction with genetic fingerprinting of enterococci, can be useful for identifying contamination sources as distinct categories of Bacteroides have been shown to be predominately detected in humans. All microbial source tracking samples collected as part of outfall pathogen monitoring will be analyzed at Source Molecular Labs for identification, quantification, and interpretation.

All parameters will be measured in-situ or from grab samples. Quality-assurance controls for field-sampling systems, including field-measurement checks and duplicate sampling, are presented in *Section 6.2.1.1*.

Additional parameters and/or analyses are occasionally performed (e.g., for plankton samples) on a case-by-case basis or may be added to the monitoring program in the future. The QAPP does not currently address these analyses, but would be modified accordingly in the future for review and approval by EPA.

8.2 Monitoring Methods

The Hempstead Harbor water-quality monitoring program (including outfall pathogen-monitoring) Standard Operating Procedures, presented as *Appendix A*, contain methods for collecting field data and samples, operating field analytical equipment, and field decontamination procedures in addition to an equipment list. *Table 8* presents methods used by field equipment and the limitations of those methods.

Table 8: Analytical Methods and Limits for Field Parameters

Parameter	Method Basis	Sampling Equipment	Detection Range	Accuracy*
Temperature	Electrometric	YSI Pro Plus	-5 to 70 ºC	±0.2 ºC
Salinity	EPA 120.1 (electrometric)	YSI Pro Plus	0 to 70 ppt	Greater of ±1% or 0.1 ppt
Dissolved Oxygen	EPA 360.1 (electrometric)	YSI Pro Plus	0 to 50 mg/L	Greater of ±2% or 0.2 mg/L
Dissolved Oxygen	EPA 360.2 (Winkler)	LaMotte 7414	1 to 10 mg/L	0.2 mg/L
Water clarity	Secchi Disk	Secchi Disk	N/A	0.1 m
Turbidity	EPA 180.1 (nephelometric)	LaMotte 2020e	0 to 2000 NTU	±0.05 or ±2% of reading, whichever is greater, below 100 NTU ±3% of reading, above 100 NTU
Ammonia	USGS I-2522-90	LaMotte 3304	0 to 2.0 mg/L	0.0, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, 2.0 mg/L
pH	EPA 150.1 (electrometric)	YSI Pro Plus	0 to 14 S.U.	±0.2 S.U.
pH	N/A (colormetric)	LaMotte 2218 reagent	5 to 10 S.U.	5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0, 10.0 S.U.
Surfactants	N/A (follows EPA 1990 Fed Register)	LaMotte 4508 reagent	0 to 20 ppm (without dilution)	± 0.5 ppm

**A list of values indicates a test method that determines discrete values is used.*

8.3 Field Quality Control

Parameters monitored in the field are recorded on a copy of the field data sheet presented in *Appendix F*. Field equipment is maintained as discussed in the SOPs presented in *Appendix A*. *Table 9* presents a summary of field quality control requirements.

Table 9: Field Quality-Control Requirements

Analyte	Data Quality Indicator	Field QC Check	QC Action Frequency	Acceptance Criteria	Corrective Action
Coliform and Enterococci	Precision	Duplicate sample	Once per 20 samples	N/A, use ±30% for duplicates	Assess sampling technique
Coliform and Enterococci	Accuracy, Represent-ativeness	Temperature Control Sample	Each monitoring event	0 to 6ºC	Assess sample handling technique
Human Enterococcus and Human Bacteroides (microbial source tracking)	Precision	Duplicate sample	At least once monitoring event	N/A, use ±30% for duplicates	Assess sampling technique
Human Enterococcus and Human Bacteroides (microbial source tracking)	Accuracy, Represent-ativeness	Temperature Control Sample	Each monitoring event	0 to 6ºC	Assess sample handling technique
Ammonia (concentration)	Precision	Duplicate sample	Once per 20 samples	± 30%	Assess sampling and analysis technique
Nitrate/Nitrite	Precision	Duplicate sample	Once per 20 samples	± 30%	Assess sampling technique
Dissolved Oxygen	Accuracy	Winkler titration	Once per monitoring event	± 0.5 mg/L	Assess instrument calibration
Salinity	Accuracy	Check known reagent	Once per monitoring event	± 0.5 ppt	Assess instrument calibration
Temperature	Accuracy	Thermometer	Once per monitoring event	+/- 2 ºC	Assess instrument calibration
pH	Precision	Duplicate sample	Once per monitoring event	± 0.5 S.U.	Assess sampling and analysis technique
Surfactants	Precision	Duplicate sample	Once per monitoring event	± 0.5 ppm	Assess sampling and analysis technique
Water Clarity (by Secchi disk)	Precision	Duplicate measurement	Once per monitoring event	± 0.5 meter	Assess technique
Water Clarity (by Secchi disk)	Accuracy	Check rope for stretch	Approx. once per two months	± 0.02 meter/meter of line	Remark or replace rope

Analyte	Data Quality Indicator	Field QC Check	QC Action Frequency	Acceptance Criteria	Corrective Action
Turbidity (by turbidity meter)	Accuracy	Check known standard	Once per monitoring event	± 30%	Assess instrument calibration

9 Analytical Requirements

9.1 Analytical Methods

The Hempstead Harbor water quality monitoring program sends samples to be analyzed for nitrate/nitrite and microbiological parameters to laboratories for analysis. Quality-control procedures for laboratory analyses are included in this section. Quality control for other field parameters, including those measured with electrometric and field kit techniques, are discussed in *Section 14* and in the SOPs presented in *Appendix A* and/or available on the company's website. *Table 10* presents the analytical methods and DQOs used for parameters analyzed by the laboratories.

Table 10: Analytical Methods and Limits for Laboratory Parameters

Parameter	Method	Reporting Limit	Precision	Accuracy
Fecal Coliform	Standard Methods 9221E	<2 CFU/ 100mL	+/- 5%	+/- 20%
Enterococci	EPA 1600	<2 CFU/ 100mL	+/- 5%	+/- 20%
Human Enterococcus and Human Bacteroides (microbial source tracking)	Source Molecular SOPs and Quality Policy manual	<10 gene copies/ 100ml of water[1]	Duplication of results for 10% of samples	Consistent meeting of expected results
Nitrate	Hach 8192 WAH	0.01 mg/L	+/- 15% at 0.20 mg/L	+/- 30%
Nitrite	Hach 8507 WAH	0.002 mg/L	+/- 3% at 0.150 mg/L	+/- 20%

[1] *Reporting Limit for Source Molecular. Source Molecular is the laboratory initially selected by CSHH to perform the analysis. If a similar laboratory is chosen, this may vary slightly.*

9.2 Analytical Quality Control

9.2.1 Equipment Blanks

Dedicated sampling equipment will be used; therefore, no equipment blanks are required.

9.2.2 Trip Blanks

Volatile organic compounds are not constituents of concern; thus, no trip blanks are required.

9.2.3 Temperature-Control Blank

A temperature control blank will be obtained from the NCDH laboratory prior to each sampling event. The blank will be packed with the samples to be analyzed for bacterial parameters and the temperature checked upon delivery to the laboratory. The samples must be maintained in a cooler within the required temperature range (0°- 6°C). A temperature control blank is not required for samples being sent to Source Molecular as samples are checked upon arrival with an infrared thermometer to ensure proper temperature during shipment.

9.2.4 Field (Blind) Duplicate Samples

Duplicate samples will be collected to check the precision of the laboratory analysis and field-sampling procedures. Duplicate samples will be analyzed for the same parameters as the corresponding primary samples collected at the same time. The duplicate sample set will be assigned a different sample number than the original set so that the sample identity is blind to the laboratory. One duplicate sample will be collected nominally per 20 samples per matrix and submitted to the laboratory.

9.2.5 Fixed Laboratory QC

Quality-control samples that will be initiated by the laboratory (e.g., method blanks, instrument blanks, and MS/MSDs) will be analyzed in accordance with their quality assurance procedures and Laboratory Methods Manual.

10 Sample Handling and Custody Procedures

The majority of the measurements taken as part of the monitoring program are recorded in the field. Bacterial and nitrate/nitrite samples are labeled with a specific site identifier, the date, and the name of the sampler on a supplied data sheet. The samples are stored upright in a cooler with ice (for temperature control) during the monitoring event and are immediately transported to the laboratory once sampling is completed. A temperature-control vial is checked to assure the samples were maintained within the required temperature range (0°-6°C). If the temperature-control sample is out of range, the results are flagged and qualified. *Table 11* presents preservation and holding-time requirements for the analyses performed by laboratories. All other parameters are field measured and are not held or preserved.

A Chain of Custody (COC) document is completed to record the sample location/Site ID, data and time of sampling. This document remains with the field samples to document sample transfers. A field data sheet is completed on-site at the time of sampling.

Table 11: Sampling-Method Requirements

Parameter	Bottle Size/Type	Preservation	Type	Max Hold Time
Fecal Coliform	250 mL/ Plastic	Sodium Thiosulfate, Iced	Grab	8 hours
Enterococci	250 mL/ Plastic	Sodium Thiosulfate, Iced	Grab	8 hours
Human Enterococcus and Human Bacteroides (microbial source tracking)	500 mL/ Plastic	Sterile, Iced	Grab	48 hours
Nitrate	250 mL/ Plastic	Iced	Grab	28 days
Nitrite	250 mL/ Plastic	Iced	Grab	28 days

11 Testing, Inspection, Maintenance, and Calibration

11.1 Instrument/Equipment Testing, Inspection, and Maintenance

Equipment maintenance procedures are presented in the SOPs, included as *Appendix A* and/or available on the company's website, and in the equipment-specific operation manuals, presented in *Appendix D* and *Appendix H* and/or available on the company's website.

11.2 Instrument/Equipment Calibration and Frequency

Equipment calibration procedures are presented in the SOPs, included as *Appendix A* and/or available on the company's website, and in the equipment-specific operation manuals, presented in *Appendix D* and *Appendix H* and/or available on the company's website.

11.3 Inspection/Acceptance of Supplies and Consumables

Supplies needed for this monitoring program include sampling bottles, calibration solutions, and equipment replacement parts. Samples will be collected in bottles supplied by the laboratory scheduled to perform the analysis. Bottles will be inspected for signs of contamination (e.g., unexpected liquids and broken seals) and wear (e.g., cracks and scratched lid threads) before use. Calibration solutions and replacement parts will be obtained from the original manufacturer of the equipment.

12 Data Management

Field data is collected on a field data sheet during each sampling event (see *Appendix F*). Field data will be compiled electronically after each event. A sample of the electronic data repository is presented in *Appendix G*. The electronic file will be backed up periodically. The original field data sheets will be maintained on file for at least five years.

The Quality Assurance Officer will frequently (once per month) compare a sample of the field data sheets to the electronic file and edit any incorrectly entered data.

Records of QAPP amendments will be maintained at CSHH offices. A summary of changes and revisions from the previous version of the QAPP, along with a brief justification for the changes, will be appended to the front of the superseded QAPP in the file. A record of the EPA pertinent approvals shall be maintained with each version of the document.

13 Assessment and Response Actions

Volunteer training and review procedures are presented in *Section 3.2*. Management review procedures are presented in *Section 3.1*. Data review, verification, validation, and usability are discussed in *Section 14*. Data quality audits will be conducted at least once per season by the QA Officer or other program manager. Audits will consist of inspecting the field data sheets, laboratory QA/QC data, and field duplicate RPD calculation, if available. Any deficiencies will be reported to the QAPP Manager, who will oversee the resolution of deficiencies. Possible courses of action include revising the QAPP, seeking assistance from the laboratories and other groups, and marking previously accepted data as invalid or provisional.

The following is a list of possible occurrences that may require corrective action and the corresponding action that would likely occur:

- If any sample bottles break during transit such that insufficient sample is available to complete the analysis resampling may have to occur.
- If meters or other sampling equipment break or malfunction during sampling, efforts will be made to repair, recalibrate, or replace them with back-up equipment.
- If there are unusual changes in detection limits, resampling and reanalysis may have to occur.

14 Data Review, Verification, Validation, and Usability

The objectives of data verification are to:

- Assess and summarize the analytical quality and defensibility of data for the end user.
- Document factors contributing to analytical error that may affect data usability, such as: data discrepancies, poor laboratory practices that impact data quality, site locations for which samples were difficult to analyze.
- Document any "sampling error" that may be identified by the data verification process, such as contaminated trip or equipment blanks, incorrect storage or preservation techniques, improper sampling containers, and improper sampling techniques.

14.1 Data Review, Verification and Validation

During or soon after a monitoring event, monitoring and quality-control results will be reviewed by the Field Sampling Leader. Any unusual values will be flagged. Unusual values may include quality-control limits (DQOs) that are exceeded or not met, any changes in reporting or detection limits that are noted, unexpectedly large or small values that were recorded, any noted deviation from this QAPP, or any missing values. The QA Officer will compare manually entered electronic data with the original data sheets to ensure the data was entered correctly. Any errors found will be corrected.

The QA Officer will then examine and validate the reviewed data. Data that meets the data-quality objectives and that is collected following the procedures presented in this QAPP practice are considered valid. Data that is inconsistent with these standards (data that was flagged) will be examined by the Field Sampling Leader, QA Officer, (or both) to determine the cause of the deficiency and evaluate the usability of the affected data. This data may be accepted, marked as conditional, or discarded.

Depending on the outcome of the review, other actions may be taken. If equipment failure is suspected to be the reason for the problem, calibration or maintenance techniques will be reviewed and improved. If human error is suspected, team members will receive additional training as necessary. If data consistently violates DQOs, the SOPs and QAPP will be reviewed and revisions suggested to correct identified problems (e.g., due to more variability in the sampled system or site specific issues). Additionally, the DQOs will be evaluated and adjusted if they are unreasonably stringent. Any data discrepancies, DQO violations, or other conditions that are not anticipated by the QAPP will be resolved on a case-by-case basis. Pertinent program procedures and documents will be revised as necessary. EPA will be notified of modifications to the QAPP in order to approve changes.

CSHH will attempt to track the sources of any unexpected conditions encountered during monitoring, such as unusually high monitoring results or exceedance of water-quality standards. If appropriate, further investigation will be undertaken, or the situation will be referred to an appropriate state or local agency.

14.2 Data Usability

The purpose of this QAPP is to provide data that is acceptable to current users, including those identified in *Section 4.1*. Input from data users will be considered during any revisions and modifications that may be made to this QAPP. Possible input could include revising data quality objectives, changing calibration procedures, and adjusting data-verification techniques.

User requirements and data-quality problems will be considered on a case-by-case basis. For example, if the calculated relative percent difference (RPD) for a nitrate field duplicate and the corresponding sample is greater than 30%, the difference may result from variability in the sampled system, and the two results could be averaged. However, if the RPD for a laboratory matrix spike program is larger than 30%, equipment problems may be present and all results should be discarded. The lab, other monitoring groups, EPA guidance documents, and other information will be consulted to determine the usability of a conditional sample.

Collected data will be used for the intended purpose. For example, monitoring locations selected to monitor inflow concentrations of pollutants will not be included in evaluating ambient harbor water-quality conditions.

15 Reporting, Documentation, and Records

CSHH currently presents the data collected by this monitoring program in HHPC/CSHH Annual Water Quality Reports, periodically at Long Island Sound Citizen Advisory Committee meetings, and on the Hempstead Harbor Protection Committee's website.

Reporting, documentation, and record-keeping requirements are presented in *Section 12*.

Appendix A

Standard Operating Procedures

Standard Operating Procedures (SOPs)

Hempstead Harbor Water-Quality Monitoring Program

Hempstead Harbor Protection Committee
Coalition to Save Hempstead Harbor

July 2011
Revised April 2014

78 Interstate Drive
West Springfield, MA 01089

Project No. 20060249.B10

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1 Program Overview

The water-monitoring program for Hempstead Harbor encompasses two components: a weekly water-quality monitoring program and a wet-weather outfall monitoring program. Sampling begins in May and continues until November. Eighteen Coalition to Save Hempstead Harbor (CSHH) locations are monitored as well as five outfalls.

The outfall pathogen-monitoring program is planned as a multi-event wet-weather-driven project. The sampling of up to five priority outfalls will assess bacteria levels and include microbial source tracking methods to determine possible sources. Samples will be collected no more than 6 hours following the start of a precipitation event of 0.5 inches or more, and that occurred at least 72 hours after a previous storm event of 0.1 inch or greater (i.e., following a minimum 72-hour dry period). Microbial source tracking (fecal Bacteroides) analysis will be performed on samples from up to three wet weather events.

The data will be used by Hempstead Harbor Protection Committee (HHPC), CSHH, Nassau County Department of Health (NCDH), Nassau County Department of Public Works, the Interstate Environmental Commission, the NYS DEC, the Connecticut Department of Environmental Protection, Long Island Sound Study, and the communities surrounding Hempstead Harbor. The data will be used to:

- Identify and study seasonal-scale trends in water quality.
- Monitor aquatic habitats.
- Identify causes for negative events (e.g., algal blooms, and fish kills).
- Investigate long-term trends in water-quality parameter levels.
- Guide municipal and county-level environmental planning, policy, and compliance efforts (e.g., the Phase II Stormwater Program).
- Measure progress towards meeting water-quality goals in the watershed.
- Determine whether the opening of additional shellfish-harvesting areas within the harbor is feasible.
- Identify pathogen sources for targeting pathogen-load reduction efforts.

1.1 Sampling Parameters

Harbor water quality is monitored by measuring a series of parameters and collecting samples. Dissolved oxygen, temperature, pH, and salinity vertical profiles are field-measured with an electronic meter (YSI Professional Plus, or "Pro Plus," handheld multiparameter instrument) at 1-meter intervals at each water-body monitoring location. Surface samples are field analyzed for pH and the presence/absence of ammonia at certain locations. When the presence/absence test detects ammonia, the concentration is field-measured using a separate test method. All outfall samples will also be analyzed for ammonia concentration using the latter test method. A Secchi-disk depth is recorded at each water-body monitoring location. Turbidity is also field measured at two depths – at a half-meter below the surface and at Secchi-disk depth. Samples are collected and analyzed for nitrate/nitrite, fecal coliform bacteria, and enterococci. The NCDH lab performs the bacterial analysis. Nitrate/nitrite is analyzed by the Town of Oyster

Bay lab. Source Molecular (or other laboratory selected for this purpose) will analyze all microbial source-tracking samples (human Bacteroides and human enterococci).

Dissolved oxygen (DO) is monitored because hypoxia is a common water-quality problem in Long Island Sound and Hempstead Harbor. DO is a significant indicator of estuarine health as it is required by marine fauna, and it is indirectly impacted by nutrient enrichment. In Hempstead Harbor, DO is an important indicator of the health of the fishery.

Monitoring temperature allows the YSI Pro Plus to determine the percent saturation of DO. In addition to nutrient enrichment, increased temperatures reduce water's capacity for DO. Thus, monitoring temperature indicates whether low DO levels result from temperature or nutrient enrichment. Additionally, monitoring temperature helps to determine whether the water column is stratified. Density currents, caused by temperature differentials, can prevent mixing within the water column and can lead to hypoxia.

Monitoring salinity assists in determining whether the harbor is being influenced by tidal water or by freshwater from the watershed (i.e., whether any water quality problems result from stormwater, wastewater, other discharges, or from tidal backwater). Monitoring salinity also allows the YSI Pro Plus to determine the percent saturation of DO.

pH is monitored to follow trends in aquatic life and water chemistry. Release of carbon dioxide (CO_2) by respiration and consumption via photosynthesis impact aquatic pH on small time scales (hours to days) whereas increasing atmospheric CO_2 may impact aquatic pH on the decadal time scale.

In general, turbidity represents the clarity of the water. Water clarity is monitored both through the use of a LaMotte 2020e portable turbidity meter and a Secchi disk. The Secchi-disk technique is used to determine the depth to which ambient light can penetrate the water column. In most productive waters, Secchi-disk depth is limited by algal productivity, thus this monitoring tool is used to track the spatial and temporal occurrence of algal blooms.

Ammonia should only be present in significant quantities due to malfunctioning wastewater treatment systems, including septic tanks, cesspools, and publicly owned treatment works (POTWs). Thus, ammonia is monitored weekly at CSHH #1, which is used to indicate the presence of ammonia distant from the harbor's inflows. If ammonia is detectable at this location, it is likely the result of an unusual inflow event, and ammonia levels are measured at the other CSHH locations. If ammonia is not detectable at CSHH #1, it is unlikely that ammonia will be detectable at other locations except CSHH #8, and CSHH #8 is the only additional location where ammonia analysis is performed. Ammonia will be quantified at all outfall pathogen-monitoring locations. The presence of ammonia in the harbor can indicate nutrient enrichment and can help to anticipate algal blooms and hypoxia.

Like ammonia, surfactants (e.g., detergents) can be an indicator of a failing wastewater-treatment systems or illicit stormwater discharges and can thus assist in identifying the source(s) of elevated bacteria levels. The presents or absence of surfactants will be assessed at all outfall sampling locations with the use of a reagent.

Nitrate and nitrite occur in later stages of the nitrogen cycle and are expected to be present in the estuary. However, high concentrations indicate enrichment problems and can also be used to anticipate algal blooms and hypoxia. Thus, samples are collected at each CSHH location for nitrate/nitrite and are subsequently analyzed at the Town of Oyster Bay laboratory.

The Nassau County Department of Health and the New York State Department of Environmental Conservation use enterococci and coliform bacteria levels to open or close swimming beaches and shellfish beds, respectively. Coliform and enterococci are bacteria typically found in human and warm blooded animal feces. Enterococci are the preferred indicator for contamination of brackish and salt water environments. Monitoring for enterococci and coliform bacteria (the preferred indicator of fecal contamination for fresh-water environments) in Hempstead Harbor, where salinity is variable, provides a comprehensive monitoring program for bacterial contamination.

Unlike the traditional indicator organisms such as *E. coli* and enterococci, Bacteroides are strict anaerobes and their presence is indicative of recent fecal contamination when found in water systems. In addition, they are also more abundant in the feces of warm-blooded animals than *E. coli* and enterococci. Bacteroides analysis, in conjunction with genetic fingerprinting of enterococci, can be useful for identifying contamination sources as distinct categories of Bacteroides have been shown to be predominately detected in humans. All microbial source-tracking samples collected as part of outfall pathogen-monitoring will be analyzed at Source Molecular Laboratory (or another laboratory selected for this purpose) for identification, quantification, and interpretation.

1.2 Sampling Locations

CSHH monitors 18 CSHH locations. The principal CSHH stations that are sampled weekly during the monitoring season for all program parameters are located in the northern portion of the harbor, between the former Bar Beach sand spit (now part of the 36.2-acre North Hempstead Beach Park) and Long Island Sound, as well as stations in Glen Cove Creek. *Table 1* includes the latitude/longitude points for most of the monitoring stations and a map showing the monitoring locations is presented as *Appendix C* of the Quality Assurance Project Plan (QAPP) for the monitoring program.

Table 1. Latitude/Longitude Points for Monitoring Stations

Station ID	Latitude N		Longitude W	
	Degrees	Minutes	Degrees	Minutes
Upper-Harbor Stations				
CSHH #1, Beacon 11	40	49.540	73	39.120
CSHH #2, Bell 6	40	51.647	73	40.428
CSHH #3, Red Channel Marker	40	51.213	73	39.123
CSHH #8, Adjacent to STP Outfall Pipe	40	51.514	73	38.515
CSHH #9, 10 ft West of #8				
CSHH #10, 20 ft West of #8				
CSHH #11, 50 ft East of #8				
CSHH #12, 100 ft East of #8	40	51.561	73	38.430
CSHH #13, 60 ft from Mill Pond Weir	40	51.706	73	38.139
CSHH #15, about 50 yds from Scudders Pond Outfall, North of Tappen Beach pool area	40	50.109	73	39.247
CSHH #15A, at Outfall North of Tappen Pool				
CSHH #15B, at Sudder's Pond Weir				
Lower-Harbor Stations				
CSHH #4, East of North Hempstead Beach Park (formerly Bar Beach) Sand Spit	40	49.688	73	39.001
CSHH #5, Mott's Cove	40	49.317	73	38.770
CSHH #6, East of Pt. Washington transfer station	40	48.688	73	39.080
CSHH #7, West of Bryant Landing (formerly site of oil dock)	40	48.474	73	38.923
CSHH #14, about 50 yds from Powerhouse Drain outfall	40	49.706	73	38.916
CSHH #14A, at Powerhouse Drain Outfall				

The CSHH outfall pathogen-monitoring program is planned as a multievent wet-weather-driven project. The study area for the program is defined by the Hempstead Harbor shoreline (and the associated upland drainage area) south of an east-west line starting at the mouth of Glen Cove Creek (also as reference, south of three DEC SGA #50 stations – DEC #10 [private dock], #11 [navigational marker C-A], and #12 [beyond mouth of Glen Cove Creek]). Based on results of shoreline surveys, including investigating upland drainage areas with the greatest potential pathogen loads and existing water-quality data, priority outfalls will be defined.

2 Using Field Equipment

2.1 YSI Professional Plus (Pro Plus)

The YSI Professional Plus (Pro Plus) handheld multiparameter instrument is used to monitor DO, temperature, pH, and salinity within the water column.

2.1.1 Calibration Procedures

Detailed calibration procedures for the YSI Professional Plus handheld multiparameter instrument used by CSHH are presented in *Appendix D* of the QAPP. The YSI Pro Plus is calibrated weekly for DO and pH and monthly for salinity. At the start of each monitoring date, the Pro Plus DO reading obtained at the bottom of the water column for the first station tested is checked against the results of a Winkler titration for a grab sample collected at the corresponding location.

A quality-assurance test is also performed for pH using a LaMotte test kit– a wide-range indicator that uses a color comparator. *Attachment II* presents a calibration checklist for the YSI Pro Plus. This checklist should be stored with the instrument and completed during each monitoring event.

2.1.2 Maintenance

Sonde maintenance is performed according to the manufacturer's specifications. Procedures and maintenance items are presented in the YSI manual, maintained by CSHH. During routine operation, the following components of the sonde will be inspected:

- Battery charge.
- O-rings.
- Dampness within the sonde, cable connector port, and probe ports.
- DO membrane (replace at least every 30 days).
- Corrosion (blackness) on silver DO electrodes.
- Fluid openings on conductivity/temperature probe.
- Deposits on conductivity electrodes.

If maintenance procedures are unsuccessful, the sonde and/or datalogger is returned to the manufacturer or an authorized service center for maintenance or reconditioning.

2.1.3 Operation

While in the field, the probe is wrapped in a wet towel to avoid drying. At each monitoring location, the towel is removed and the sonde mounted on a platform constructed of PVC tubing. The platform prevents the probes from contacting the bottom surface of the harbor. The sonde and platform are lowered half a meter below the water surface and DO, salinity, pH, and temperature readings are recorded after the values on the datalogger stabilize. The process is repeated when the sonde is lowered to 1-meter followed by one-meter increments. When the platform touches the bottom, the depth and final readings are recorded after the values on the datalogger stabilize. This stabilization period allows disturbed sediment to settle.

The sonde must be properly stored between monitoring locations to prevent damage and drying of the probes. After use, the probe is rinsed with freshwater. The sensor tip is inserted into a plastic bottle containing a sponge saturated with freshwater.

2.2 LaMotte 2020e

The LaMotte 2020e portable meter is used to measure turbidity.

2.2.1 Calibration Procedures

Detailed calibration procedures for the LaMotte 2020e used by CSHH is presented in the *Appendix D* of the QAPP. The LaMotte 2020e is calibrated at the start of each monitoring event. *Attachment III* presents a calibration sheet for the LaMotte 2020e.

2.2.2 Maintenance

Meter maintenance is performed according to the manufacturer's specifications. Procedures and maintenance items are presented in the LaMotte 2020e manual maintained by CSHH and available on the LaMotte website (www.lamotte.com). During routine operation, the following components of the meter will be inspected:

- Battery charge.
- Dampness within the meter chamber or any other parts of the meter (including output port).
- Cleanliness of tubes (tubes must be clean and free from lint, fingerprints, dried spills and significant scratches, especially the central zone between the bottom and the sample line).

If maintenance procedures are unsuccessful, the meter is returned to the manufacturer or an authorized service center for maintenance or reconditioning.

2.2.3 Operation

Detailed operation procedures for the LaMotte 2020e used by CSHH is presented in the LaMotte 2020e manual maintained by CSHH and available on the LaMotte website (www.lamotte.com)

While in the field, samples are collected at a half-meter below the surface and at Secchi-disk depth. A sampling jar with siphon is used to collect the sample. Once the sampling tubes are filled with the sample and capped, the outsides of the tubes are cleaned with a clean, lint-free absorbent cloth until they are dry and smudge-free. The tubes are then placed in the meter chamber. Tubes are handled only by the cap to avoid problems from fingerprints.

After measurements have been recorded at each sampling location, sample tubes are rinsed twice with demineralized water and wiped with a clean lint-free absorbent cloth. At the end of the monitoring event, the sampling tubes are washed on the inside and outside with mild detergent to remove dirt and fingerprints. The tubes are allowed to air-dry in an inverted position to prevent dust from entering the tubes. All tubes are emptied and cleaned as soon as possible after reading a sample to prevent deposition of particulates on the inside of the tubes.

The meter and sampling tubes are properly stored between monitoring locations to prevent damage. Dry tubes are stored with the caps on to prevent contamination.

2.3 LaMotte Kits

LaMotte kits (including reagents) are used to measure surfactants and ammonia, and to validate results from the pH and DO probes. Instructions for using these kits are presented in *Appendix H* of the QAPP.

2.3.1 Calibration Procedures

No calibration is necessary for the LaMotte Field Kits.

2.3.2 Maintenance

The field kits use single-use chemical reagents that are replaced when expired or the supply is exhausted.

2.3.3 Operation

A half-meter below surface grab sample is collected at each applicable monitoring location for ammonia and pH; a bottom sample is grabbed for DO. Surfactant samples must be collected from exposed outfalls not in contact with salt or brackish waters (i.e., the outfall can not be partially or full submerged). The sample is collected in a sample collection bottle that was first decontaminated. As trace compounds are not being analyzed, decontamination means that the sample collection bottles will be washed with detergent once prior departure from the dock for a monitoring event. Bottles that will be reused will be rinsed twice with water from each sampling location prior to sampling at that location. The rinse water will be dumped away from the sampling location.

Wash water containing any chemicals or detergents will be collected in a dedicated container (see *Section 2.2.4* below) and discarded upon return to shore, in accordance with applicable regulations. Rinse water consisting of only native water will be dumped away from sampling locations (i.e., on the opposite side of the boat) where a sample has yet to be collected. When a duplicate sample is required, an initial sample will be collected and field-analyzed. The remaining contents will then be dumped away from the sampling location; a second sample will be collected from the sampling location but from a different position on the boat (away from where the contents of the previous sample had been dumped) and the water analyzed as a field duplicate. Samples that contacted chemical reagents will be deposited in the Sampling Refuse Container (See *Section 2.3.4*).

Where a field kit is to be used to validate results from the YSI meter, a sample will be collected as close as possible to the location where the meter recorded data immediately after the probe measurement is recorded. The sample will be field analyzed for the parameter of interest, and the results compared to the meter results via the data quality objectives (DQOs) presented in the QAPP.

2.3.4 Chemical Waste

Samples that have been mixed with reagent (after analysis), water used to rinse sample collection bottles, and other liquid wastes associated with parameter measurement are transferred to a container with a screw top lid labeled "SAMPLING REFUSE CONTAINER." Some of the compounds that may be dumped are considered hazardous.

The Field Sampling Leader should be aware of any hazardous compounds that may be present, label the dump container appropriately, and ensure that the dump container contents are disposed of in accordance with applicable regulations. The Field Sampling Leader should maintain a collection of Material Safety Data Sheets for hazardous compounds that are used.

2.4 Secchi Disk

Secchi disks are used to measure light attenuation within the water column. They are a simple indicator of algal blooms and sediment clouding.

2.4.1 Calibration Procedures

Markings on the Secchi-disk rope will be compared against a measuring tape approximately once every two months during the sampling season to ensure that the rope has not stretched. If the markings no longer allow for a precise measurement, the rope will be remarked or replaced.

2.4.2 Maintenance

The Secchi disk should be maintained as follows:

- Ensure that the rope is properly attached to the disk.
- Ensure that the disk corresponds to "zero meters" on the rope (i.e., the disk is the datum).
- Clean the surface of the disk periodically; any darkening of the disk face will reduce its reflectivity.
- Ensure that the paint has not chipped and that the disk is in good repair.

2.4.3 Operation

The Secchi disk is lowered into the water with the sun at the sampler's back. The sampler should not wear sunglasses and the disk should not enter the shadow of the boat. The disk is lowered until just after it disappears completely. This depth is recorded. The disk is raised until just after it becomes visible, and this depth is recorded. The average of these depths is the

Secchi-disk depth. The averaged value is recorded. At least once per monitoring event, another volunteer will repeat the process. Results from the two volunteers should deviate by less than 0.5 meters.

3 Sampling Techniques

The sample-collection procedures presented in this section reduce the likelihood of potential sample contamination. Samples are collected in bottles supplied by the laboratory. Sample preservative, if necessary, should be added to the bottles prior to sampling. See the Water Monitoring and Sampling Techniques document in *Attachment I* for additional detail.

3.1 Biological Parameters

Biological samples, including fecal coliform, enterococci, human Bacteroides, and human enterococci samples, are collected in 290-mL bottles supplied by the NCDH or Source Molecular laboratories. The volunteer, wearing an unused rubber glove, will collect the sample by attaching the sample jar to a collection pole and gently sweeping the water in a forward motion with the sample jar at half a meter below the surface. The bottle will be filled to the mark on the side of the bottle. The sampler will ensure that none of the sodium thiosulfate powder (a compound that neutralizes chlorine) present in the bottle will escape. The human Bacteroides and human enterococci samples do not require sodium thiosulfate for preservation.

It is important that the bottle be filled directly from the harbor or outfall and that a bucket, scoop, or other means is not used; bacterial samples are easily contaminated, and decontamination procedures would require the collection vessel to be acid washed. For monitoring locations where a duplicate sample is to be collected, a second sample bottle will be filled in the same manner as the first. The samples will be placed upright in a cooler on ice and transported to the laboratory or sent via overnight shipping as soon as feasible to meet the required hold time (e.g., 8 for coliform bacteria and 8 hours for enterococci, 48 hours for human Bacteroides and human enterococci). Each cooler will contain an additional container of freshwater to be used by laboratory personnel for measuring the sample temperature on arrival at the laboratory.

All human Bacteroides and human enterococci samples should be wrapped with a substantial amount paper towels and put individually into a leak proof zip lock bag(s). The same instructions should be followed for accompanying ice packs. If ice is used instead of ice packs, then the ice must go into a leak proof zip lock bag and that bag must be wrapped with abundant paper towels and inserted into another leak proof zip lock bag. Ice packs and/or ice (in bags) must not directly touch the samples (separate with additional packing material). As an added precaution, zip lock bags and packing material should be put in two overlapping garbage bags and wrapped tightly. All material should then be placed in a sturdy, leak proof disposable cooler.

3.2 Chemical Parameters

Samples for nitrate/nitrite analysis are collected in 250-mL bottles supplied by the laboratory. The volunteer, wearing an unused rubber glove, will collect the sample by attaching the sample jar to a collection pole and gently sweeping the water in a forward motion with the sample jar at half a meter below the surface. The collected sample is labeled and placed in a cooler for transport to the lab.

For monitoring locations where a duplicate sample is to be collected, a second sample bottle will be filled in the same manner as the first. The samples will be placed upright in a cooler on ice and transported to the laboratory as quickly as feasible.

For field-measured parameter such as pH and ammonia, a decontaminated sample-collection jar is rinsed with sample water and then immersed at half a meter below the surface and filled. The sample water is poured directly to cleaned sample vials included in the test kits. After each monitoring event, the sample jar and vials are washed with detergent and rinsed thoroughly.

3.3 Flow Measurement

An estimate of flow will be made from each outfall, if practical. Methods for estimating flow are presented in *Attachment I* to these SOPs.

4 Equipment Checklist

The Field Sampling Leader is responsible for ensuring that the sampling group is properly prepared for each sampling outing. This section includes a list of equipment that will be present during each monitoring event. This list is not intended to be comprehensive; the list should be augmented or revised as necessary.

Group Safety Equipment – To Be Supplied by Town of Oyster Bay

- Personal Floatation Devices (PFD) for each individual present on the boat
- Fire Extinguisher
- Sound-Producing Device
- Visual Distress Signals
    - Flares for night
    - Red or orange flags for daylight
- Anchor and Anchor Line
- Alternate Propulsion (paddle etc.)
- Dewatering Device
- First-Aid Kit
- Cellular Phone
- Rubber Gloves

Personal Equipment – To Be Supplied by Each Field Sampler

- Appropriate Footwear

- Hat
- Rain Gear
- Cold-Weather Gear
- Sunblock
- Insect Repellant
- Personal Identification
- Emergency Contact Information

Monitoring Equipment—To Be Supplied by the Field Sampling Leader

- Copy of this Standard Operating Procedures Manual
- YSI Professional Plus
- LaMotte 2020e Portable Turbidity Meter
- LaMotte pH, Surfactants, and Ammonia Kits/Reagents
- LaMotte Kit for Winkler Titration
- Secchi Disk and Line
- PVC Platform and Line for Sonde
- Data Sheets and Spares
- 250-mL Bacterial Bottles and Spares
- 250-mL Nitrate/Nitrite Bottles and Spares
- Bottle for Field Sample Analysis
- Distilled Water
- Calibration Solutions
- Cooler with Ice for Storage and Transport of Bacteria Samples.
- Writing Utensils
- "Sharpie" Permanent Markers for Labeling Sample Bottles
- Gauge for Determining Wind Speed And Direction
- Dump Container for Storage of Liquid Waste Materials
- Electronic Thermometer for Air Temperature
- Thermometer for Sample Jar
- Spare Batteries

Attachment I

Water Monitoring and Sampling Techniques

INTRODUCTION

This Standard Operating Procedure is applicable to the collection of representative liquid samples, both aqueous and non-aqueous from streams, rivers, lakes, ponds, outfall pipes, and surface impoundments.

SURFACE WATER FLOW RATE ESTIMATION

Area-Rate Method

To estimate the flow rate of flowing surface water using the Area-Rate method, a stop watch, float and tape measure will be used. The rate will be calculated using the equation:

$$q = \frac{W \times d \times a \times l}{t}$$

Where:

q	flow (cubic feet/second)
W	the average width (feet) of the stream section
d	the average depth (feet) of the stream section
a	a constant of 0.8 for a gravely stream bed, or 0.9 for a smooth stream bed
l	length (feet) of the stream section
t	time (seconds) required for the float to travel a measured section of the stream

This test should be performed several times. The average value for q will be recorded on the Surface Water Field Data Sheet.

Volume/Time Method

To calculate the flow rate using the Volume/Time method, a bucket of known volume and a stopwatch will be used. The rate will be calculated using the equation:

$$q = \frac{V}{t * 7.481}$$

Where:

q	flow (cubic feet/second)
V	volume of bucket that is filled with the discharging water (gallons)
t	time required for the bucket to fill to mark of known volume (seconds)
7.481	conversion factor from gallons to cubic feet

This test should be performed several times. The average value for q will be recorded on the Surface Water Field Data Sheet.

SURFACE WATER SAMPLING

When the sample location is easily accessible by foot, grab samples will be collected by submerging the sample container directly into the surface water, or filling directly from the outfall pipe discharge. Dedicated sample containers may also be used for sample collection in these circumstances. Disturbance of sediment at the sample location should be prevented.

In areas where access is limited or difficult, sampling may be conducted with a long-handled scoop. This is often the case at lakes or large stream locations where sampling away from the bank is necessary to achieve representative surface water samples. When such an intermediate container is utilized, it will be constructed of an inert material and decontaminated or replaced between samples.

The gloves worn by field personnel during surface water sample collection will be dedicated to that operation. Samples will be collected in order of decreasing volatility.

Sampling techniques for flowing and standing surface water are outlined in the following procedures.

SAMPLING FLOWING SURFACE WATER

For surface water samples collected from flowing water, the sample will be collected at mid-stream to ensure that the water is not stagnant. The sample will be collected upstream of the sampler, so as not to disturb the sample during collection. The downstream samples will be collected before upstream samples. The remainder of the samples will be collected as field personnel move upstream. Disturbance of sediment at the sample location should be prevented.

BOTTLE IMMERSION TECHNIQUE

Surface water samples can be collected from flowing water by direct bottle submersion or by using a scoop or dipper. It is important to ensure that sampling personnel do not place fingers in the sampling bottles, to avoid sample contamination and chemical burns. The protocol for collecting a surface water sample from flowing water using direct bottle submersion is as follows:

a. Uncap the sample bottle.

b. Lower the lip of the sample bottle just below the water surface.

c. Allow the bottle to fill slowly with the water running down the sidewalls to prevent splashing.

d. Cap the sample bottle.

e. Label the sample bottles and place into an iced cooler.

SCOOP SAMPLING TECHNIQUE

The protocol for collecting a surface water sample from flowing water using a scoop or dipper is outlined below.

a. Uncap the sample bottle.

b. Reach the dipper out above the water. Lower the lip of the dipper to just below the water surface.

c. Allow the dipper to fill slowly with the water running down the sidewalls to prevent splashing.

f. Cap the sample bottle.

g. Label the sample bottles and place into an iced cooler.

SUSPENDED OUTFALL PIPE TECHNIQUE

The protocol for collecting a sample from an outfall pipe that is suspended above the receiving water surface is outlined below.

a. Uncap the sample bottles.

b. Reach the bottle or dipper toward the nappe (free-falling sheet of water) of the discharge. Fill the bottle or dipper directly from the nappe.

c. Allow the dipper to fill, or fill the bottle directly. Do not allow the water to overflow the bottle if it contains preservatives.

d. Cap the bottles.

e. Label each sample bottle and place into a cooler with ice.

PARTIALLY SUBMERGED OUTFALL PIPE TECHNIQUE

For collecting a sample from an outfall pipe where the end is partially submerged, follow the bottle immersion or scoop sampling technique, above (the latter is preferred) while reaching up the outfall pipe as far as possible.

Attachment II

Field Procedure Sheets

PROTOCOLS FOR USING YSI PROFESSIONAL PLUS FOR WEEKLY WATER-MONITORING

- Just before going into the field, remove the probe from the plastic calibration cup. Put metal guard on sonde. Wrap in a wet white towel— make sure bottom is covered. Power on the display unit and let warm up for 5 to 15 minutes.

- After arriving at the first sampling station, remove the towel from the probe and attached the probe to the PVC platform. Secure a rope to the top of the platform. Lower the probe on the platform to a half meter below surface and then to every meter in the water column. When you reach bottom, record the specific depth on the data sheet.

- When pulling probe back up to boat, use the rope rather than the cable.

- When the probe is back on boat, wrap it in the wet towel while it is attached to the platform and you are on your way to the next station.

- Repeat the steps in the three preceding boxes for subsequent sampling stations.

- At final station, after monitoring is completed, remove probe from platform, replace in vinyl sleeve with wet sponge.

- Back at the office, rinse the sonde with tap water, rinse the vinyl sleeve and sponge, remove metal guard on sonde, and place sonde in plastic calibration cup with about 1/8 inch of water.

Attachment III

Calibration Sheets

Coalition to Save Hempstead Harbor
Citizens Water-Monitoring Program – YSI Professional Plus Calibration Checklist

Date	DO Calibration	Membrane checked for bubbles, rips, or tears	Check Contact Points on DO Probe	Depth Calibration	Salinity Calibration	Two-point pH Calibration		Any Errors Noted?
						7.0	10.0	

Appendix B

Laboratory Accreditations and Evaluations

NEW YORK STATE DEPARTMENT OF HEALTH
WADSWORTH CENTER

Expires 12:01 AM April 01, 2012
Issued April 01, 2011

CERTIFICATE OF APPROVAL FOR LABORATORY SERVICE

Issued in accordance with and pursuant to section 502 Public Health Law of New York State

DR. RODGER SILLETTI
NASSAU COUNTY DEPT OF HEALTH
209 MAIN STREET
HEMPSTEAD, NY 11550

NY Lab Id No: 10339
EPA Lab Code: NY00010

is hereby APPROVED as an Environmental Laboratory in conformance with the National Environmental Laboratory Accreditation Conference Standards for the category
ENVIRONMENTAL ANALYSES NON POTABLE WATER
All approved analytes are listed below:

Bacteriology

Analyte	Method
Coliform, Fecal	SM 18-21 9221E (99)
	SM 18-21 9222D (97)
Coliform, Total	SM 18-21 9221B (99)
	SM 18-21 9222B (97)
Enterococci	EPA 1600

Chlorinated Hydrocarbon Pesticides

Analyte	Method
4,4'-DDD	EPA 8270C
4,4'-DDE	EPA 8270C
4,4'-DDT	EPA 8270C
Aldrin	EPA 8270C
alpha-BHC	EPA 8270C
beta-BHC	EPA 8270C
Captan	EPA 8270C
Chlordane Total	EPA 8081A
delta-BHC	EPA 8270C
Dichloran	EPA 8270C
Dieldrin	EPA 8270C
Endosulfan I	EPA 8270C
Endosulfan sulfate	EPA 8270C
Endrin	EPA 8270C
Endrin aldehyde	EPA 8270C
Heptachlor	EPA 8270C
Heptachlor epoxide	EPA 8270C
Isodrin	EPA 8270C
Lindane	EPA 8270C

Chlorinated Hydrocarbon Pesticides

Analyte	Method
Methoxychlor	EPA 8270C
Mirex	EPA 8270C
Toxaphene	EPA 8081A
Trifluralin	EPA 8270C

Chlorinated Hydrocarbons

Analyte	Method
1,2,4,5-Tetrachlorobenzene	EPA 8270C
2-Chloronaphthalene	EPA 8270C
Hexachlorobenzene	EPA 8270C
Hexachlorobutadiene	EPA 8270C
Hexachlorocyclopentadiene	EPA 8270C
Hexachloroethane	EPA 8270C

Demand

Analyte	Method
Biochemical Oxygen Demand	SM 18-21 5210B (01)
Carbonaceous BOD	SM 18-21 5210B (01)

Fuel Oxygenates

Analyte	Method
Methyl tert-butyl ether	EPA 8260B

Mineral

Analyte	Method
Alkalinity	SM 18-21 2320B (97)
Calcium Hardness	EPA 200.7 Rev. 4.4
Chloride	EPA 300.0 Rev. 2.1
Fluoride, Total	EPA 300.0 Rev. 2.1
Hardness, Total	EPA 200.7 Rev. 4.4
Sulfate (as SO4)	EPA 300.0 Rev. 2.1

Serial No.: 43829

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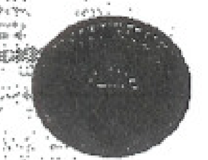

NEW YORK STATE DEPARTMENT OF HEALTH
WADSWORTH CENTER
RICHARD F. DAINES, M.D.

Expires 12:01 AM April 01, 2011
Issued April 01, 2010
Revised October 06, 2010

CERTIFICATE OF APPROVAL FOR LABORATORY SERVICE
Issued in accordance with and pursuant to section 502 Public Health Law of New York State

MR. LIENHSING HUANG
TOWN OF OYSTER BAY ENVIRONMENTAL LABORATORY
101 BETHPAGE SWEETHOLLOW ROAD
OLD BETHPAGE, NY 11804

NY Lab Id No: 11734
EPA Lab Code:

is hereby APPROVED as an Environmental Laboratory in conformance with the National Environmental Laboratory Accreditation Conference Standards for the category ENVIRONMENTAL ANALYSES NON POTABLE WATER All approved analytes are listed below:

Purgeable Aromatics

1,3-Dichlorobenzene	SM 20-21 6200C (97)
Benzene	SM 20-21 6200C (97)
Ethyl benzene	SM 20-21 6200C (97)

Purgeable Halocarbons

1,1,2,2-Tetrachloroethane	SM 20-21 6200C (97)
1,1,2-Trichloroethane	SM 20-21 6200C (97)
1,2-Dichloroethane	SM 20-21 6200C (97)
1,2-Dichloropropane	SM 20-21 6200C (97)
2-Chloroethylvinyl ether	EPA 601
Bromodichloromethane	SM 20-21 6200C (97)
Bromoform	SM 20-21 6200C (97)
Bromomethane	SM 20-21 6200C (97)
Chloroethane	SM 20-21 6200C (97)
Chloroform	SM 20-21 6200C (97)
Chloromethane	SM 20-21 6200C (97)
cis-1,3-Dichloropropene	SM 20-21 6200C (97)
Dibromochloromethane	SM 20-21 6200C (97)
Dichlorodifluoromethane	SM 20-21 6200C (97)
trans-1,3-Dichloropropene	SM 20-21 6200C (97)
Trichlorofluoromethane	SM 20-21 6200C (97)
Vinyl chloride	SM 20-21 6200C (97)

Serial No.: 43089

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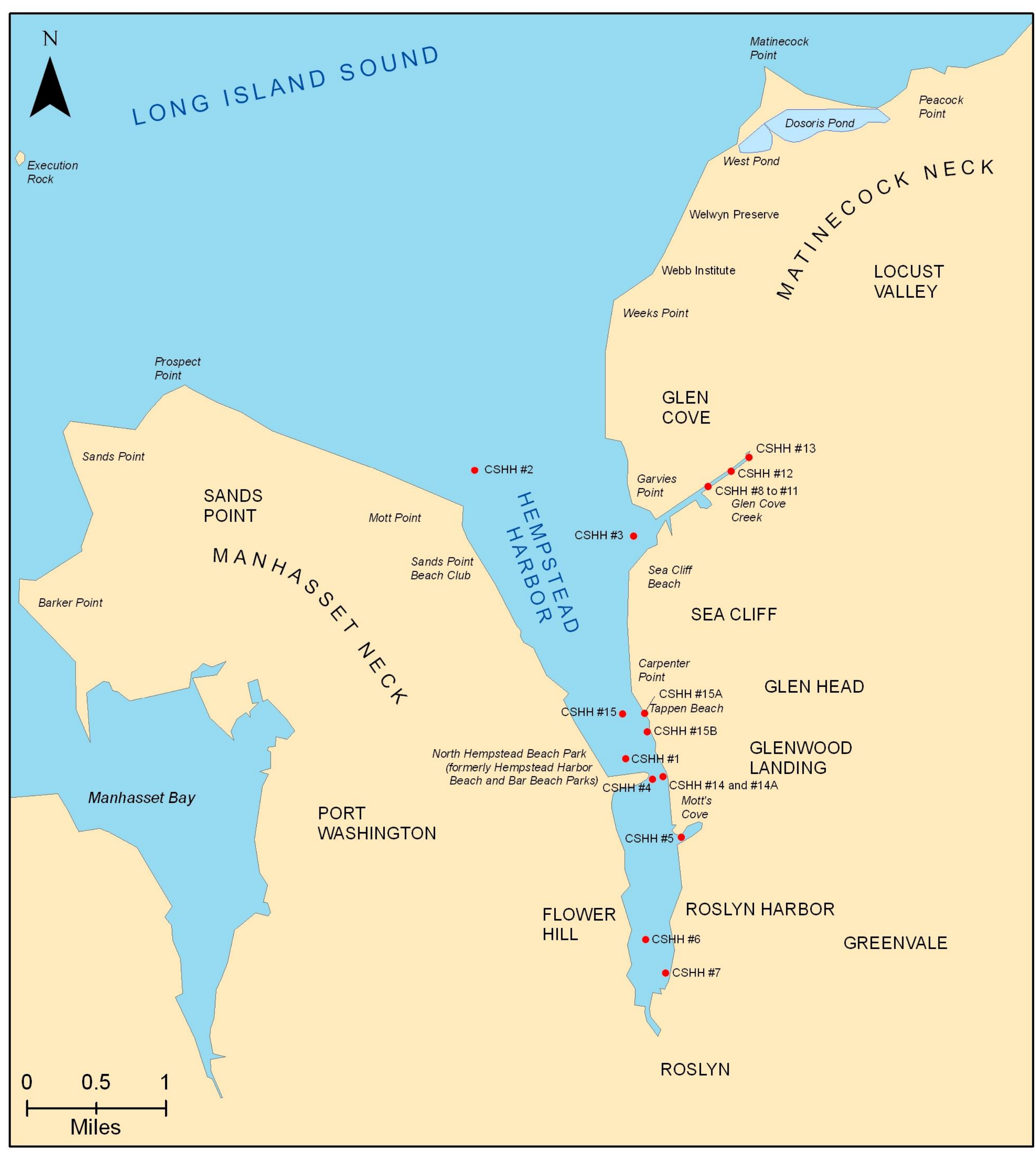
Appendix C

Monitoring Locations Map



N
LONG ISLAND SOUND
Execution Rock
Matinecock Point
Peacock Point
Dosoris Pond
West Pond
MATINECOCK NECK
Welwyn Preserve
Webb Institute
LOCUST VALLEY
Weeks Point
Prospect Point
GLEN COVE
CSHH #13
CSHH #12
CSHH #8 to #11
Glen Cove Creek
Garvies Point
CSHH #2
Sands Point
SANDS POINT
Mott Point
HEMPSTEAD HARBOR
CSHH #3
MANHASSET NECK
Sands Point Beach Club
Sea Cliff Beach
Barker Point
SEA CLIFF
Carpenter Point
GLEN HEAD
CSHH #15A
Tappen Beach
CSHH #15
CSHH #15B
North Hempstead Beach Park (formerly Hempstead Harbor Beach and Bar Beach Parks)
CSHH #1
GLENWOOD LANDING
CSHH #4
CSHH #14 and #14A
Manhasset Bay
Mott's Cove
PORT WASHINGTON
CSHH #5
ROSLYN HARBOR
FLOWER HILL
CSHH #6
GREENVALE
CSHH #7
ROSLYN
0
0.5
1
Miles

Appendix D

Meter Operation Manuals (portions)

YSI PROFESSIONAL PLUS (PRO PLUS)

Handheld Multiparameter Instrument

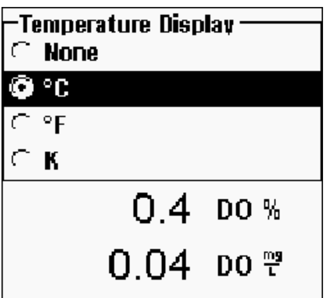
PARAMETERS: SETUP, DISPLAY, AUTO STABLE, AND CALIBRATION

The following section is separated by parameter and will discuss sensor setup, display options, auto stable features, and calibration procedures for each parameter. The sections are separated by parameter due to the versatility of the Pro Plus. You may focus solely on the parameters of your choice.

For the highest accuracy, calibrate or verify each sensor regularly. For your convenience, YSI offers 5580 Confidence Solution® which allows you to check the accuracy of pH, conductivity, and ORP readings to help determine if a sensor calibration is necessary.

If you receive an error message during a calibration that indicates questionable results, you have the option to either accept or decline the calibration. YSI recommends that you decline a questionable calibration since accepting it may result in erroneous data. After declining a questionable calibration, ensure the sensor is clean, the calibration solution is good, the calibration vessel is clean, and that you are entering the correct calibration value if entering manually. Then, try to recalibrate the sensor. If you continue to have problems, see the Troubleshooting section of this manual.

TEMPERATURE



All probe/cable assemblies, except the Quatro, have a built-in temperature sensor. The Quatro cable ships with a Conductivity/Temperature sensor that must be installed on the cable. Temperature calibration is not required nor is it available.

To set the units, press Sensor, highlight Display and press enter. Highlight Temperature and press enter. Highlight the desired

temperature units of °F, °C, or K and press enter to confirm the selection. Only one temperature unit may be displayed at a time. You may also choose not to display temperature. If you choose not to display temperature, other parameters that require a temperature reading will still be temperature compensated.

DISSOLVED OXYGEN (DO)

DO sensors can be used on 60520-X, 6051020-X, 6052030-X, and Quatro cables.

PREPARING THE DO SENSOR FOR THE FIRST TIME

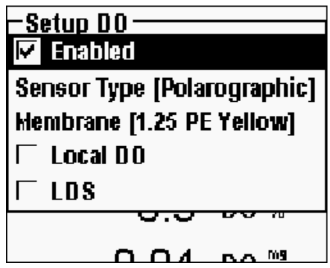
The dissolved oxygen sensor is shipped with a dry, protective red cap that will need to be removed before using. It is very important to put a new membrane with electrolyte solution on the sensor after removing the red cap.

Prepare the membrane solution according to the instructions on the bottle. After mixing, allow the solution to sit for 1 hour. This will help prevent air bubbles from later developing under the membrane. Ensure you are using the correct electrolyte solution for the correct sensor. Galvanic sensors utilize electrolyte with a light blue label and Polarographic sensors utilize electrolyte with a white label. The dissolved oxygen sensor is supplied with cap membranes specific to the sensor type ordered (Polarographic or Galvanic). 5912, 5913, and 5914 membrane kits are for Galvanic sensors and the 5906, 5908, and 5909 membrane kits are for Polarographic sensors. See the Setup - Dissolved Oxygen section of this manual for more information on the different types of membranes available from YSI.

Remove the red cap by pulling it straight off the sensor tip. Discard or save for later use during long term storage. Thoroughly rinse the sensor tip with distilled or deionized water. Fill the cap membrane 3⁄4 full of electrolyte solution, then tap the cap with a finger to release any trapped air. Be careful not to touch the membrane portion of the cap. Thread the membrane cap onto the sensor, moderately tight. Do not use a tool. It’s typical for some of the electrolyte solution to spill over. For detailed instructions on changing a membrane cap, see the Care, Maintenance, and Storage section of this manual.

SETUP - DISSOLVED OXYGEN

Press Sensor , highlight Setup and press enter. Next, highlight DO and press enter.



Enabled allows you to enable or disable the Dissolved Oxygen function. Highlight Enabled and press enter to activate(Q) or deactivate(L1) dissolved oxygen. Disable dissolved oxygen if you do not have a dissolved oxygen sensor connected to the instrument.

If a sensor is Enabled that isn't connected to the instrument, the display will show an unstable, false reading, ?????, or -- next to the units.

Sensor Type sets the type of oxygen sensor being used: either Polarographic (black) or Galvanic (grey). Highlight Sensor Type and press enter. Highlight the correct sensor type installed on the cable and press enter to confirm.

If using a ProBOD sensor/cable assembly, the sensor type should be set to polarographic.

The Pro Plus has two compatible sensors for use with a field cable:

Polarographic – This sensor has a black sensor body and is engraved with the model number 2003.

Galvanic – This sensor has a grey sensor body and is engraved with the model number 2002.

In terms of physical configuration, membrane material, and general performance, YSI Professional Series Galvanic dissolved oxygen sensors are exactly like the Professional Series Polarographic sensors. The advantage of using Galvanic sensors is convenience. Galvanic sensors provide for an instant-on sensor without the need for warm-up time but this affects the life of the sensor. Polarographic sensors last longer and have a longer warranty but require a 5-15 minute warm-up time before use or calibration.

IMPORTANT – *The instrument default setting is Galvanic. Please change the Sensor Type to match the correct sensor. If you observe readings very close to 0 or extremely high readings (i.e. 600%), your Sensor Type setting (Polarographic or Galvanic) may be set incorrectly and you should immediately ensure it matches the sensor installed on your cable.*

Membrane sets the type of membrane used on the dissolved oxygen sensor. Highlight **Membrane** and press enter. Highlight the correct membrane type installed on the sensor and press enter to confirm. The DO sensor is supplied with membranes specific to the sensor type ordered and are color coded as described in the following tables.

Galvanic membrane kits:

Item	*Color*	*Material*	*Description*
5912	Black	1 mil Teflon®	Traditional membrane material
5913	Yellow	1.25 mil polyethylene	Improved response time and less flow dependence than Teflon® Ships standard with the sensor.
5914	Blue	2 mil polyethylene	Less flow dependence than 1.25 mil but somewhat slower response

Polarographic membrane kits:

Item	*Color*	*Material*	*Description*
5906	Black	1 mil Teflon®	Traditional membrane material
5908	Yellow	1.25 mil polyethylene	Improved response time and less flow dependence than Teflon® Ships standard with the sensor.
5909	Blue	2 mil polyethylene	Less flow dependence than 1.25 mil but somewhat slower response

Selecting a Dissolved Oxygen Membrane:

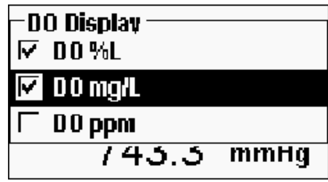
Membrane Type	*Flow Dependence After 4 Minutes*	*Typical Response Time - 95%*
5912, 5906 - Black	60%	18 seconds
5913, 5908 - Yellow	25%	8 seconds
5914, 5909 - Blue	18%	17 seconds

Local DO allows for localized DO% measurements. This sets the calibration value to 100% regardless of the altitude or barometric pressure. Highlight Local DO and press enter to enable (Q) or disable (P) this function. Local DO is a method for the Pro Plus to factor in the barometric pressure on each DO measurement. In essence, if the barometric pressure changes you wouldn't notice the difference in the DO% readings in air-saturated water or water-saturated air. Local DO is ideal for EU compliance. When Local DO is enabled, an L will appear next to DO% on the run screen. DO mg/L readings are unaffected by the selection of DO Local.

LDS (Last Digit Suppression) rounds the DO value to the nearest tenth; i.e. 8.27 mg/L becomes 8.3 mg/L. Highlight LDS and press enter to enable (Q) or disable (P) this function.

DISPLAY - DISSOLVED OXYGEN

Press Sensor , highlight Display and press enter. Highlight DO and press enter. All DO units can be displayed simultaneously. Highlight the unit(s) and press enter to activate (Q) or deactivate (P) units from the run screen. Note - You will not be able to display dissolved oxygen unless it is Enabled in the Sensor Setup menu first, see previous section.



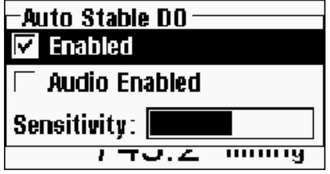
DO % will show DO readings in a percent scale from 0 to 500%.

DO mg/L will show DO readings in milligrams per liter (equivalent to ppm) on a scale from 0 to 50 mg/L.

DO ppm will show DO readings in parts per million (equivalent to mg/L) on a scale from 0 to 50 ppm.

AUTO STABLE - DISSOLVED OXYGEN

Auto Stable indicates when a reading is stable.When Auto Stable is enabled, AS will blink next to the parameter until it is stable. Once the parameter is stable, AS will stop blinking.



To enable Auto Stable, press Sensor , highlight Auto Stable and press enter. Highlight DO and press enter.

Highlight Enabled and/or Audio Enabled (instrument will beep when the stability

is achieved) and press enter to confirm. The Auto Stable Sensitivity can be decreased or increased. Highlight Sensitivity and use the left and right arrow keys to slide the bar. The more sensitive you make it (larger black bar) the harder it is to achieve stability in a changing environment.

The Auto Stable system works by examining the previous 5 readings, computing the percent change in the data and comparing that change against a % threshold value. The % threshold value is determined by the Sensitivity bar setting. The following chart can be used as a guide when setting the Sensitivity bar.

Sensitivity selected by User	*% Data Variance Threshold*
100 - Most Sensitive, Sensitivity bar is set to the far right	0.05%
75	0.62525%
50	1.275%
25	1.8875%
0 - Least Sensitive, Sensitivity bar is set to the far left	2.5%

Example:

The instrument obtained the following data:

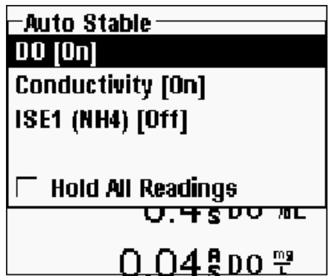
Reading #1 95.5 DO%
Reading #2 95.7 DO%
Reading #3 95.8 DO%
Reading #4 96.1 DO%
Reading #5 95.3 DO%

The instrument is programmed to determine the minimum and maximum data value over the previous 5 samples, and to compute the percent difference between those values. In this example, that gives a percent change of:

% Change = 100 * ((96.1 – 95.3) / 95.3)
% Change = 0.83%

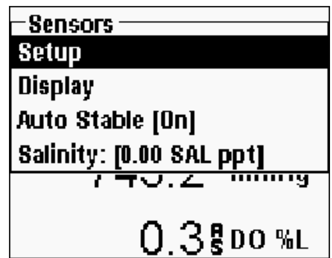
In this example, if the Sensitivity bar is set to the far right, the Auto Stable requirement would not be met and AS would continue to blink. However, if the sensitivity bar is set to the median threshold (1.275%), the Auto Stable requirement would be met and AS would display steadily on the display.

Within the Auto Stable menu, you can also choose to Hold All Readings for as many parameters as you set for Auto Stable. For instance, if DO and pH have



Auto Stable and Hold All Readings enabled, then the display will hold the readings once DO and pH have both reached their Auto Stable settings. You must press the Esc key to “release” the held display in order to take subsequent readings Hold All Readings must be reactivated after each use!

SALINITY CORRECTION



The last feature in the Sensor menu is the Salinity correction value which is used to calculate the dissolved oxygen mg/L and ammonia readings when a conductivity sensor is not in use.
Press Sensor , highlight Salinity, and press enter. Then, use the numeric entry screen to enter the Salinity value of the water you will be testing from 0 to 70 ppt.

If using a cable with a conductivity sensor, the salinity measured by the conductivity sensor will be used in the DO and ammonia mg/L calculations and ‘As Measured’ will be displayed next to Salinity in the Sensor menu.

As the salinity of water increases, its ability to dissolve oxygen decreases. For example, fully oxygenated 20 °C water at sea level with zero salinity will hold 9.092 mg/L of dissolved oxygen. If that same sample had a salinity value of 9 ppt, then it would hold 8.621 mg/L of dissolved oxygen. Therefore, to obtain accurate mg/L readings, it is important to know the salinity of the water you will be testing and to input that value into the instrument. The salinity of fresh water is typically 0-0.5 ppt and seawater is typically 35 ppt. You will also have the opportunity to enter or modify the Salinity correction value during DO calibration.

CALIBRATION - DISSOLVED OXYGEN

The Pro Plus offers several options for calibrating dissolved oxygen: DO% in water saturated air, DO mg/L and DO ppm in a solution of known dissolved oxygen determined by a Winkler Titration, and a Zero point. If performing a zero point calibration, you must also perform a %, mg/L, or ppm calibration following the zero calibration. For both ease of use and accuracy, YSI recommends performing the following 1-point DO % water saturated air calibration:

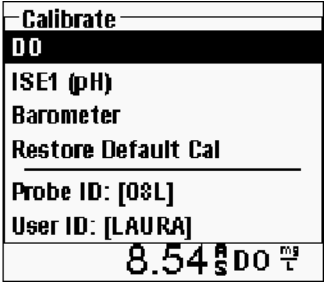
It is not necessary to calibrate in both % and mg/L or ppm. Calibrating in % will simultaneously calibrate mg/L and ppm and vice versa.

Calibrating DO % in Water Saturated Air:
1-Point Calibration

The supplied sensor storage container (a grey sleeve for a single port cable or a screw on plastic cup for the dual-port and Quatro cables) can be used for DO calibration purposes.

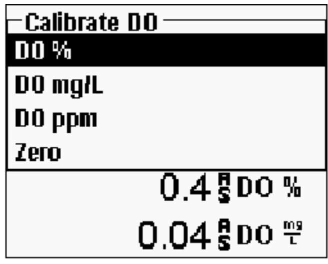
Moisten the sponge in the storage sleeve or plastic cup with a small amount of clean water. The sponge should be clean since bacterial growth may consume oxygen and interfere with the calibration. If using the cup and you no longer have the sponge, place a small amount of clean water (1/8 inch) in the plastic storage cup instead.

Make sure there are no water droplets on the DO membrane or temperature sensor. Then install the storage sleeve or cup over the sensors. The storage sleeve ensures venting to the atmosphere. If using the cup, screw it on the cable and then disengage one or two threads to ensure atmospheric venting. Make sure the DO and temperature sensors are not immersed in water. Turn the instrument on and wait approximately 5 to 15 minutes for the storage container to become completely saturated and to allow the sensors to stabilize.



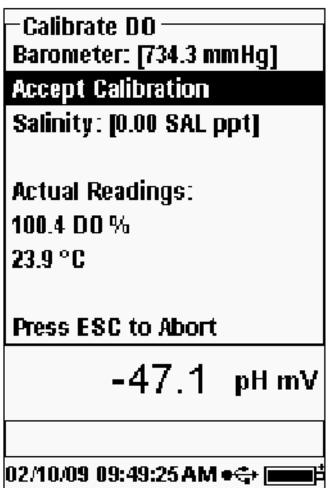
Press Cal . Highlight Probe ID or User ID if you wish to add, select, edit, or delete an ID. Probe ID must be enabled in the System GLP menu to appear in the Calibrate menu. User ID will appear automatically. Select ‘None’ if you do not want a User ID stored with the calibration. When enabled, these IDs are stored with each calibration record in the GLP file.

After selecting your User ID and/or Probe ID if appropriate, highlight DO and press enter.



Highlight **DO %** and press enter to confirm.

The instrument will use the internal barometer during calibration and will display this value in brackets at the top of the display. Highlight **Barometer** and press enter to adjust it if needed. If the barometer reading is incorrect, it is recommended that you calibrate the barometer. Note - the barometer should be reading "true" barometric pressure (see Barometer section for more information on "true" barometric pressure). If the value is acceptable, there is no need to change it or perform a barometer calibration.



The Salinity value displayed near the top of the screen is either the salinity correction value entered in the Sensor menu or the Salinity value as measured by the conductivity sensor in use and enabled. If you are not using a conductivity sensor, the Salinity correction value should be the salinity of the water you will be testing. Highlight **Salinity** and press enter to modify this setting if necessary. See the **Salinity Correction** section of this manual for more information.

Wait for the temperature and DO% values under "Actual Readings" to stabilize, then highlight **Accept Calibration** and press enter to calibrate. Or, press Esc to cancel the calibration. If User Field 1 or 2 are enabled in the GLP menu, you will be prompted to select these inputs and then press Cal to complete the calibration. The message line at the bottom of the screen will display "Calibrating Channel..." and then "Saving Configuration...".

Calibrating DO% in Water Saturated Air: 2-Point Calibration with Zero Solution

Place the sensor in a solution of zero DO.

A zero DO solution can be made by dissolving approximately 8 - 10 grams of sodium sulfite (Na_2SO_3) into 500 mL tap water or DI water. Mix the solution thoroughly. It may take the solution 60 minutes to be oxygen-free.

Press Cal . Highlight Probe ID or User ID if you wish to add, select, edit, or delete an ID. Probe ID must be enabled in the System GLP menu to appear in the Calibrate menu. When enabled, these IDs are stored with each calibration record in the GLP file.

After selecting the Probe ID and/or User ID if appropriate, highlight DO and press enter. Highlight Zero and press enter. Wait for the temperature and DO% values under "Actual Readings" to stabilize, then press enter to Accept Calibration. If User Field 1 or 2 are enabled, you will be prompted to select the fields and then press Cal to complete the calibration. The screen will then prompt for a follow-up second point calibration.

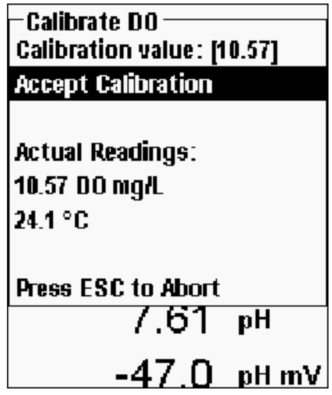
Highlight DO% and press enter to continue with the next calibration point. Rinse the sensor of any zero oxygen solution using clean water. Then follow the steps under Calibrating DO % in Water Saturated Air to complete the second point.

Calibrating in mg/L or ppm as a Titration: 1-Point Calibration

Place the sensor into an adequately stirred sample that has been titrated to determine the dissolved oxygen concentration. Allow the sensor to stabilize.

Press Cal . Highlight Probe ID or User ID if you wish to add, select, edit, or delete an ID. Probe ID must be enabled in the System GLP menu to appear in the Calibrate menu. When enabled, these IDs are stored with each calibration record in the GLP file.

After selecting the Probe ID and/or User ID if appropriate, highlight DO and press enter. Highlight DO mg/L or ppm and press enter.



Highlight Calibration value and press enter to manually input the sample's dissolved oxygen value. Highlight Accept Calibration and press enter once the temperature and Dissolved Oxygen readings stabilize. Or, press Esc to cancel the calibration. If User Field 1 or 2 are enabled in the GLP menu, you will be prompted to select the fields after selecting Accept Calibration. After making your selection, press Cal to complete the calibration. After completing the calibration, the message line will display "Calibrating Channel..." and then "Saving Configuration...".

Calibrating in mg/L or ppm as a Titration: 2-Point Calibration with Zero Solution

Place the sensor in a solution of zero DO.

A zero DO solution can be made by dissolving approximately 8 - 10 grams of sodium sulfite (Na_2SO_3) into 500 mL tap water. Mix the solution thoroughly. It may take the solution 60 minutes to be oxygen-free.

Press Cal . Highlight Probe ID or User ID if you wish to add, select, edit, or delete an ID. Probe ID must be enabled in the System GLP menu to appear in the Calibrate menu. When enabled, these IDs are stored with each calibration record in the GLP file.

After selecting the Probe ID and/or User ID if appropriate, highlight DO and press enter. Highlight Zero and press enter. Wait for the temperature and DO% values under "Actual Readings" to stabilize, then press enter to Accept Calibration. If User Field 1 or 2 are enabled, you will be prompted to select the fields and then Press Cal to complete the calibration. The screen will then prompt for a follow-up second point calibration.

Highlight the desired calibration units (mg/L or ppm) and press enter to continue with the next point. Rinse the sensor of any zero oxygen solution using clean water. To complete the second calibration point, follow the steps under Calibrating in mg/L or ppm as a Titration: 1-Point Calibration.

BAROMETER

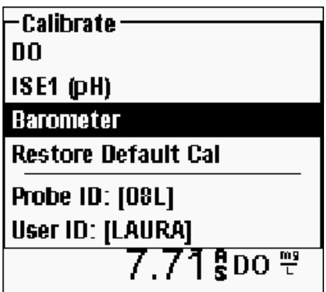
All Professional Plus instruments contain an internal barometer.

DISPLAY - BAROMETER

Press Sensor , highlight Display and press enter. Highlight Barometer and press enter. The measurement unit options are: mmHg, inHg, mBar, PSI, kPa, or Atm. Only one unit can be displayed at a time. Select None if you do not want to display a barometric pressure reading.

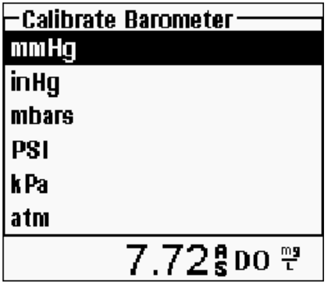
Whether or not you choose to display the barometer reading, the barometric pressure will still be used for calibrating DO% and for compensating for pressure changes if Local DO is enabled.

CALIBRATION - BAROMETER



The barometer in the instrument is calibrated at the factory. If the barometer requires calibration, press Cal . Highlight Probe ID or User ID if you wish to add, select, edit, or delete an ID. Probe ID must be enabled in the System GLP menu to appear in the Calibrate menu. When enabled, these IDs are stored with each calibration record in the GLP file.

After selecting the Probe ID and/or User ID if appropriate, highlight Barometer and press enter.



Highlight the desired unit and press enter.

Calibrate Barometer
Calibration value: [733.2]
Accept Calibration

Actual Readings:
733.2 mmHg
26.1 °C

Press ESC to Abort

Highlight Calibration Value and press enter to manually enter the correct "true" barometric pressure. Next, highlight Accept Calibration, and press enter. If User Field 1 or 2 are enabled, you will be prompted to select the fields and then press Cal to complete the calibration or press Esc to cancel the calibration.

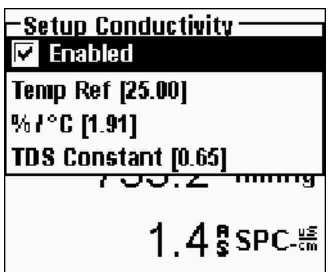
> ⓘ *Laboratory barometer readings are usually "true" (uncorrected) values of air pressure and can be used "as is" for barometer calibration. Weather service readings are usually not "true", i.e., they are corrected to sea level, and therefore cannot be used until they are "uncorrected". An approximate formula for this "uncorrection" is below:*

True BP = [Corrected BP] – [2.5 * (Local Altitude in ft. above sea level/100)]

CONDUCTIVITY

Conductivity sensors are supplied with 60530-X, 6051030-X, 6052030-X , and Quatro cables. Conductivity sensors are built into the 60530-X, 6051030-X, and 6052030-X cables and are not replaceable. Conductivity/Temperature sensors are shipped with the Quatro cable, must be installed, and are replaceable.

SETUP - CONDUCTIVITY



Press Sensor , highlight Setup, and press enter. Highlight Conductivity, press enter.

Enabled allows you to enable or disable the conductivity measurement. Highlight Enabled and press enter to activate (Q) or deactivate (P) conductivity. Disable conductivity if you do not have a conductivity sensor connected to the instrument.

> *If a sensor is Enabled that isn't connected to the instrument, the display will show an unstable, false reading next to the units.*

Temp Ref (Temperature Reference) is the reference temperature used for calculating temperature compensated Specific Conductance. This will be the

temperature all Specific Conductance values are compensated to. The default is 25 °C. To change the Reference Temperature, highlight Temp Ref and press enter. Use the numeric entry screen to enter a new value between 15.00 and 25.00 °C. Next, highlight <<<ENTER>>> at the bottom of the screen and press enter on the keypad to confirm.

%/°C (Percent per Degree Celsius) is the temperature coefficient used to calculate temperature compensated Specific Conductance. The default is 1.91% which is based on KCl standards. To change the temperature coefficient, highlight %/°C and press enter. Use the numeric entry screen to enter a new value between 0 and 4%. Next, highlight <<<ENTER>>> at the bottom of the screen and press Enter on the keypad to confirm.

TDS Constant is a multiplier used to calculate an estimated TDS (Total Dissolved Solids) value from conductivity. The multiplier is used to convert Specific Conductance in mS/cm to TDS in g/L. The default value is 0.65. This multiplier is highly dependent on the nature of the ionic species present in the water sample. To be assured of moderate accuracy for the conversion, you must determine a multiplier for the water at your sampling site. Use the following procedure to determine the multiplier for a specific sample:

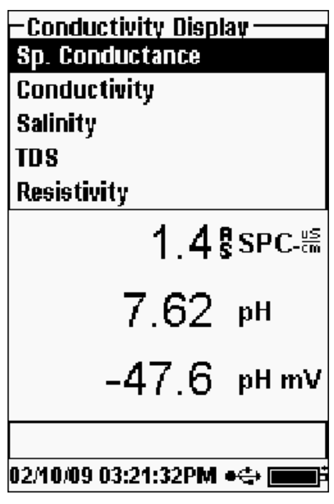
1. Determine the specific conductance of a water sample from the site;
2. Filter a portion of water from the site;
3. Completely evaporate the water from a carefully measured volume of the filtered sample to yield a dry solid;
4. Accurately weigh the remaining solid;
5. Divide the weight of the solid (in grams) by the volume of water used (in liters) to yield the TDS value in g/L for this site; Divide the TDS value in g/L by the specific conductance of the water in mS/cm to yield the conversion multiplier. Be certain to use the correct units.

If the nature of the ionic species at the site changes between sampling studies, the TDS values will be in error. TDS cannot be calculated accurately from specific conductance unless the make-up of the chemical species in the water remains constant.

To change the multiplier, highlight TDS Constant and press enter. Use the numeric entry screen to enter a new value between 0 and 0.99. Highlight <<<ENTER>>> at the bottom of the screen and press Enter on the keypad to confirm.

DISPLAY - CONDUCTIVITY

Press Sensor , highlight Display and press enter. Highlight Conductivity and press enter. Highlight Sp. Conductance (Specific Conductance), Conductivity, Salinity, TDS, or Resistivity, and press enter to select the reporting units for each parameter. One reporting unit per parameter may be enabled. To disable a parameter, select None. You will not be able to display any of these parameters unless the Conductivity sensor is Enabled in the Sensor Setup menu first.



Sp. Conductance can be displayed in us/cm or ms/cm. Specific conductance is temperature compensated conductivity.

Conductivity can be displayed in uS/cm or mS/ cm. Conductivity is the measure of a solution's ability to conduct an electrical current. Unlike specific conductance, conductivity is a direct reading without any temperature compensation.

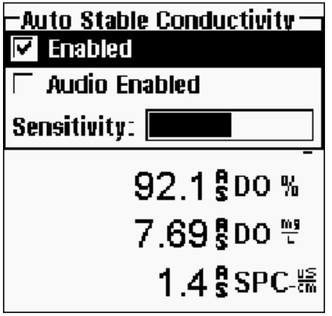
Salinity can be displayed in ppt (parts per thousand) or PSU (practical salinity units). The units are equivalent as both use the Practical Salinity Scale for calculation.

TDS can be displayed in mg/L (milligrams per liter), g/L (grams per liter), or kg/L (kilograms per liter).

Resistivity can be displayed in ohm-cm (ohms per centimeter), kohm-cm (kilo ohms per centimeter), or Mohm-cm (mega ohms per centimeter).

AUTO STABLE - CONDUCTIVITY

Press Sensor , highlight Auto Stable and press enter. Highlight Conductivity and press enter.

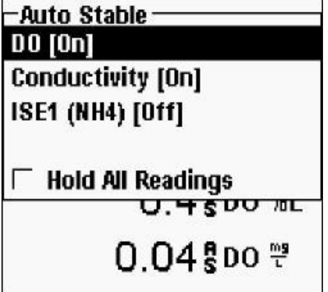


Auto Stable indicates when a reading is stable. Highlight Enabled and/or Audio Enabled (instrument will beep when the stability is achieved) and press enter enable (Q) or disable (P). When Auto Stable is enabled, AS will blink next to the parameter until it is stable. Once the parameter is stable, AS will stop blinking.

The Auto Stable Sensitivity can be decreased or increased. Highlight Sensitivity and use the left and right arrow keys to slide the bar. The more sensitive you make it (larger black bar) the harder it is to achieve stability in a changing environment.

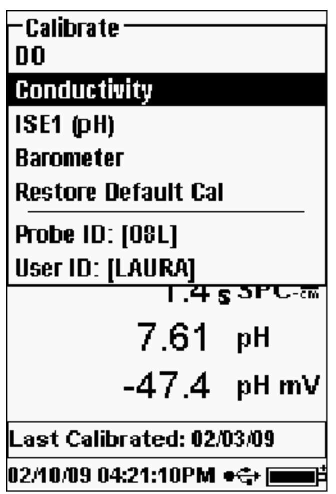
The Auto Stable system works by examining the previous 5 readings, computing the percent change in the data and comparing that change against a % threshold value. The % threshold value is determined by the Sensitivity bar setting. The following chart can be used as a guide when setting the Sensitivity bar.

Sensitivity selected by User	*% Data Variance Threshold*
100 - Most Sensitive, Sensitivity bar is set to the far right	0.025%
75	0.39375%
50	0.7625%
25	1.13125%
0 - Least Sensitive, Sensitivity bar is set to the far left	1.5%



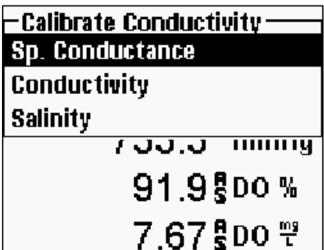
Within the Auto Stable menu, you can also choose to Hold All Readings for as many parameters as you set for Auto Stable. For instance, if conductivity and DO have Auto Stable and Hold All Readings enabled, then the display will hold the readings once conductivity and DO have both reached their Auto Stable settings. You must press the Esc key to “release” the held display in order to take subsequent readings. Hold All Readings must be reactivated after each use!

The 6051030 ISE/conductivity cable has a specialized calibration container that resembles a large test tube. This calibration chamber can be used to calibrate the conductivity sensor with an ISE sensor installed. A ring-stand should be used to support this chamber.



Press Cal . Highlight Probe ID or User ID if you wish to add, select, edit, or delete an ID. Probe ID must be enabled in the System GLP menu to appear in the Calibrate menu. User ID will appear automatically. Select 'None' if you do not want a User ID stored with the calibration. When enabled, these IDs are stored with each calibration record in the GLP file.

After selecting the User ID and/or Probe ID if appropriate, highlight Conductivity and press enter.



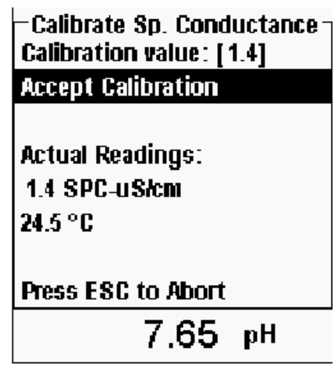
Highlight the desired calibration method; Sp. Conductance, Conductivity, or Salinity and press enter. YSI recommends calibrating in specific conductance for greatest ease.

Calibrating in Specific (Sp.) Conductance or Conductivity

Place the sensor into a fresh, traceable conductivity calibration solution. The solution must cover the holes of the conductivity sensor that are closest to the cable. Ensure the entire conductivity sensor is submerged in the solution or the instrument will read approximately of half the expected value!

Calibrate Sp. Conductance
SPC-uS/cm
SPC-mS/cm
733.2 mmHg
91.8 DO %

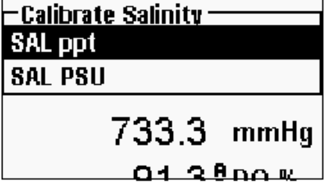
Choose the units in either SPC-us/cm, C-us/cm or SPC-ms/cm, C-ms/cm and press enter.



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. If User Field 1 or 2 are enabled in the GLP menu, you will be prompted to select the fields and then press Cal to complete the calibration. After completing the calibration, the message line at the bottom of the screen will display "Calibrating Channel..." and then "Saving Configuration...".

Calibrating in Salinity

Place the sensor into a salinity calibration solution. The solution must cover the holes of the conductivity sensor that are closest to the cable. Ensure the entire conductivity sensor is submerged in the solution or the instrument will read approximately of half the expected value!



Select SAL ppt or SAL PSU and press enter.

Calibrate Salinity
Calibration value: [0.00]
Accept Calibration
Actual Readings:
0.00 SAL ppt
24.6 °C
Press ESC to Abort

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. If User Field 1 or 2 are enabled, you will be prompted to select the fields and then press Cal to complete the calibration.

pH

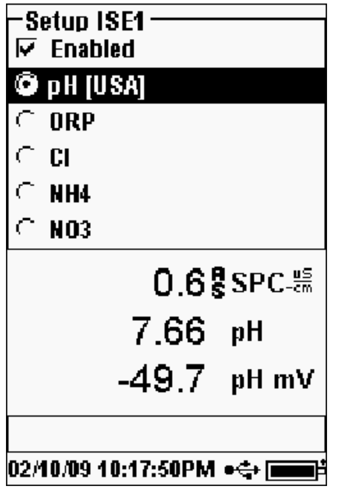
pH sensors can be used on 60510-X, 6051020-X, 6051030-X, 6051010-X, and Quatro cables.

If using a 605103 pH/oRP combination sensor on a 6051020 or 6051030 cable you can report both pH and oRP by configuring ISE1 as pH and ISE2 as oRP in the Sensor Setup menu.

The 605103 pH/oRP combination sensor is not recommended for use on a 6051010 or Quatro cable. If used on one of these cable, only pH will be reported and oRP will not be measured.

SETUP - pH

Press Sensor , highlight Setup, press enter. Highlight ISE1 if using a 60510, 6051020, or 6051030 cable. If using a 6051010 or Quatro cable, highlight ISE1 if the pH sensor is installed in port 1 or highlight ISE2 if the pH sensor is installed in port 2(a sensor must be installed in port 1 for port 2 to operate). Press enter.



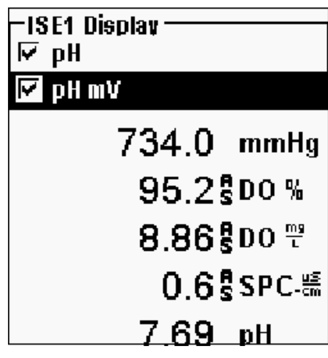
Enabled allows you to enable or disable the ISE function and select which ISE sensor is installed on the cable. Highlight Enabled and press enter to enable (Q) or disable (P) the ISE you selected previously (either ISE1 or ISE2). Disable the ISE function(s) if you do not have a ISE sensor connected to the instrument.

After enabling the ISE function, ensure that it is set to pH as shown in the left screen shot. If necessary, highlight pH and press enter to set the ISE to pH.

Highlighting pH[USA] and pressing enter will also allow you to select the values for auto buffer recognition which are used during calibration. The buffer options are USA (4, 7, 10), NIST (4.01, 6.86, 9.18), and User-Defined. The selected option will be displayed in [brackets].

If a sensor is Enabled that isn't connected to the instrument, the display will show an unstable false reading, ?????, or - next to the units.

DISPLAY - pH



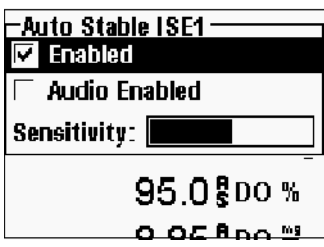
Press Sensor , highlight Display and press enter.

Highlight ISE (pH) and press enter. You will not be able to Display the sensor unless it is Enabled in the Sensor Setup menu.

Highlight pH and/or pH mV, press enter to enable (Q) or disable (LI). Both can be reported at the same time.

AUTO STABLE - pH

Press Sensor , highlight Auto Stable and press enter. Highlight ISE (pH) and press enter.

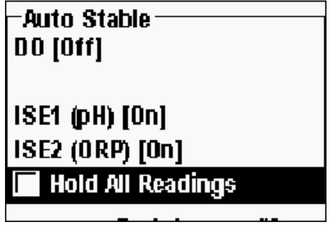


Auto Stable indicates when a reading is stable. Highlight Enabled and/or Audio Enabled (instrument will beep when the stability is achieved) and press enter enable (Q) or disable (LI). When Auto Stable is enabled, AS will blink next to the parameter until it is stable. Once the parameter is stable, AS will stop blinking.

The Auto Stable Sensitivity can be decreased or increased. Highlight Sensitivity and use the left and right arrow keys to slide the bar. The more sensitive you make it (larger black bar) the harder it is to achieve stability in a changing environment.

The Auto Stable system works by examining the previous 5 readings, computing the percent change in the data and comparing that change against a % threshold value. The % threshold value is determined by the Sensitivity bar setting. The following chart can be used as a guide when setting the Sensitivity bar.

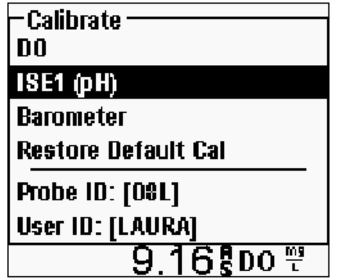
Sensitivity selected by User	*% Data Variance Threshold*
100 - Most Sensitive, Sensitivity bar is set to the far right	0.025%
75	0.39375%
50	1.5%
25	1.13125%
0 - Least Sensitive, Sensitivity bar is set to the far left	0.15%



Within the Auto Stable menu, you can also choose to Hold All Readings for as many parameters as you set for Auto Stable. For instance, if ORP and pH have Auto Stable enabled and Hold All Readings is enabled, then the display will hold the readings once ORP and pH have both reached their Auto Stable settings. You must press the Esc key to “release” the held display in order to take subsequent readings. Hold All Readings must be reactivated after each use!

CALIBRATION - pH

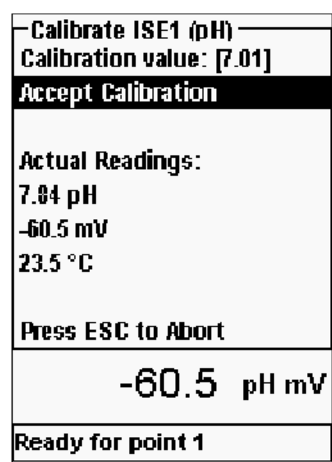
Calibration can be accomplished in any buffer order. pH 7 buffer should be used regardless of how many calibration points you use but it does not have to be used first.



Press Cal . Highlight Probe ID or User ID if you wish to add, select, edit, or delete an ID. Probe ID must be enabled in the System GLP menu to appear in the Calibrate menu. User ID will appear automatically. Select ‘None’ if you do not want a User ID stored with the calibration. When enabled, these IDs are stored with each calibration record in the GLP file.

After selecting your User ID and/or Probe ID if appropriate, highlight ISE (pH) and press enter. The message line will show the instrument is “Ready for point 1”. The pH calibration allows up to six calibration points.

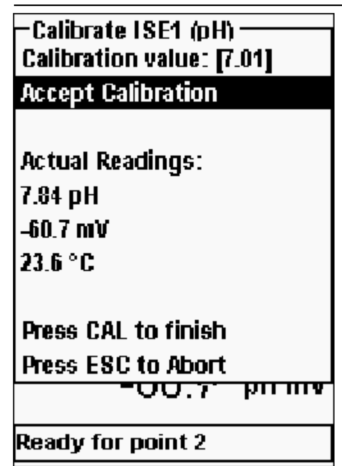
Place the sensor in a traceable pH buffer solution. The instrument should automatically recognize the buffer value and display it at the top of the calibration



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. The message line will then display "Ready for point 2".

If you do not wish to perform a second point, press Cal to finalize the calibration. Or, press Esc to cancel the calibration. If User Field 1 or 2 are enabled, you will be prompted to select these fields and then press Cal to finalize the calibration.



To continue with the 2nd point, place the sensor in the second buffer solution. The instrument should automatically recognize the second buffer value and display it at the top of the 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. The message line will then display 'Ready for point 3" and you can continue with the 3rd calibration point if desired.

If you do not wish to perform a 3rd calibration point, press Cal to complete the calibration. If User Field 1 or 2 are enabled, you will be prompted to select these fields and then press Cal to finalize the calibration.

Continue in this fashion until the desired number of calibration points is achieved (up to six).

Once you've achieved the desired number of cal points you must press Cal *to finalize the calibration and to allow the instrument to update the pH offset and slope. The instrument will not take these cal values into account until Cal has been pressed.*

The actual readings displayed during the calibration will NOT reflect the updated calibration information. These values will not change until Cal is pressed to finalize the calibration and to update the instrument.

TAKING MEASUREMENTS

To obtain the most accurate readings, be sure the instrument is calibrated before taking measurements.

DISSOLVED OXYGEN

Turn the instrument on and wait 5-15 minutes if using a polarographic sensor. If using a field cable/sensor, install the sensor guard to protect the sensor and membrane. Place 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. Next, stir the probe in the sample to overcome the stirring dependence of the dissolved oxygen sensor. You must provide at least 3 inches per second for 2.0 PE membranes, 6 inches per second for 1.25 PE membranes, and 12 inches per second for Teflon® membranes. Once the values plateau and stabilize, you may record the measurement and/or log the data set. The dissolved oxygen reading will drop over time if stirring is ceased.

If placing the DO sensor into a stream or fast flowing waters it is best to place it perpendicular to the flow and NOT facing into the flow.

If using the DO sensor in an aeration tank/basin, it is helpful to make sure bubbles do not burst on the membrane since this may cause unstable readings. You should be able to prevent this by pointing the sensor upwards so it's facing the sky and then twist tying, zip tying, or rubber banding the bulkhead to the cable. Making a simple curve to the cable without bending or breaking the cable will allow you to lower the sensor into the aeration tank while the sensor points skyward so the bubbles are no longer bursting on the membrane surface.

CONDUCTIVITY

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. Immerse the probe into the sample so the sensors are completely submerged and then shake the probe to release any air bubbles. Occasional cleaning of the sensor may be necessary to maintain accuracy and increase the responsiveness. To clean the sensor, use the conductivity cleaning brush with a mild detergent.

pH

pH readings are typically quick and accurate. However, it may take the sensors a little longer to stabilize if they become coated or fouled. To improve the response time of a sensor, follow the cleaning steps in the Maintenance section of this manual.

CARE, MAINTENANCE, AND STORAGE

This section describes the proper procedures for care, maintenance and storage of the sensors. The goal is to maximize their lifetime and minimize down-time associated with improper sensor usage.

UPDATING INSTRUMENT FIRMWARE

The instrument's firmware can be updated via www.ysi.com. There you will find the new firmware file and instructions on how to update the instrument. There is no need to send the instrument back to the factory for upgrades.

GENERAL MAINTENANCE

GENERAL MAINTENANCE - O-RINGS

The instrument utilizes o-rings as seals to prevent water from entering the battery compartment and sensor ports. Following the recommended procedures will help keep your instrument functioning properly.

If the o-rings and sealing surfaces are not maintained properly, it is possible that water can enter the battery compartment and/or sensor ports of the instrument. If water enters these areas, it can severely damage the battery terminals or sensor ports causing loss of battery power, false readings, and corrosion to the sensors or battery terminals. Therefore, when the battery compartment lid is removed, the o-ring that provides the seal should be carefully inspected for contamination (e.g. debris, grit, etc.) and cleaned if necessary.

The same inspection should be made of the o-rings associated with the sensor connectors when they are removed. If no dirt or damage to the o-rings is evident, then they should be lightly greased without removal from their groove. However, if there is any indication of damage, the o-ring should be replaced with an identical o-ring. At the time of o-ring replacement, the entire o-ring assembly should be cleaned.

To remove the o-rings:
Use a small, flat-bladed screwdriver or similar blunt-tipped tool to remove the o-ring from its groove. Check the o-ring and the groove for any excess grease or contamination. If contamination is evident, clean the o-ring and nearby plastic parts with lens cleaning tissue or equivalent lint-free cloth. Alcohol can be used to clean the plastic parts, but use only water and mild detergent on the o-ring itself. Also, inspect the o-rings for nicks and imperfections.

Using alcohol on o-rings may cause a loss of elasticity and may promote cracking. Do not use a sharp object to remove the o-rings. Damage to the o-ring or the groove may result.

Before re-installing the o-rings, make sure to use a clean workspace, clean hands, and avoid contact with anything that may leave fibers on the o-ring or grooves. Even a very small amount of contamination (hair, grit, etc.) may cause a leak.

To re-install the o-rings:
Place a small amount of o-ring grease between your thumb and index finger. (More grease is NOT BETTER!)

Draw the o-ring through the grease while pressing the fingers together to place a very light covering of grease to the o-ring. Place the o-ring into its groove making sure that it does not twist or roll.

Use your grease-coated finger to once again lightly go over the mating surface of

Do not over-grease the o-rings. The excess grease may collect grit particles that can compromise the seal. Excess grease can also cause the waterproofing capabilities of the o-ring to diminish, potentially causing leaks. If excess grease is present, remove it using a lens cloth or lint-free cloth.

GENERAL MAINTENANCE - SENSOR PORTS

It is important that the entire sensor connector end be dry when installing, removing or replacing. This will prevent water from entering the port. Once a sensor is removed, examine the connector inside the port. If any moisture is present, use compressed air to completely dry the connector or place directly in front of a steady flow of fresh air. If the connector is corroded, return the cable to your dealer or directly to an YSI Repair Center.

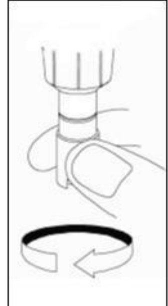
Remove sensors upside down (facing the ground) to help prevent water from entering the port upon removal.

SENSOR MAINTENANCE

SENSOR MAINTENANCE - DISSOLVED OXYGEN

Membrane Cap Installation

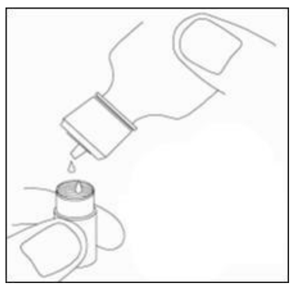
The DO sensor (Polarographic and Galvanic) is shipped with a dry, protective red cap that will need to be removed before using. Remove the protective cap or used membrane cap and replace it with a new membrane cap following these instructions:



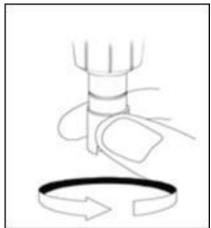
Remove the sensor guard to access the sensor tip.

Unscrew and remove any old membrane cap by holding the sensor when unscrewing the membrane cap and discard.

Thoroughly rinse the sensor tip with distilled or DI water.



Fill a new membrane cap with O2 sensor electrolyte solution that has been prepared according to the directions on the bottle. Be very careful not to touch the membrane surface. Lightly tap the side of the membrane cap to release bubbles that may be trapped.



Thread the membrane cap onto the sensor. It is normal for a small amount of electrolyte to overflow.

Polarographic Sensors - Model # 605203

The KCl (potassium chloride) solution and the membrane cap should be changed at least once every 30 days during regular use. In addition, the KCl solution and membrane should be changed if (a) bubbles are visible under the membrane; (b) significant deposits of dried electrolyte are visible on the membrane; and (c) if the sensor shows unstable readings or other sensor-related symptoms.

During membrane changes, examine the gold cathode at the tip of the sensor and the silver anode along the shaft of the sensor. If either the silver anode is black in color or the gold cathode is dull, the sensor may need resurfaced using the fine sanding disks included in the membrane kit. Do not sand the electrode every membrane change as this is not routine maintenance. In fact, visually, the anode may appear tarnished and operate just fine. YSI recommends using the 400 grit wet/dry sanding disks to resurface the electrodes if the sensor has difficulty stabilizing or calibrating after a membrane change.

To resurface the sensor using the fine sanding disk, follow the instructions below.

Gold Cathode:

For correct sensor operation, the gold cathode must be textured properly. It can become tarnished or plated with silver after extended use. Never use chemicals or abrasives not recommended or supplied by YSI.

First dry the sensor tip completely with lens cleaning tissue. Wet a sanding disk with a small amount of clean water and place it face up in the palm of your hand. Next, with your free hand, hold the sensor in a vertical position, tip down. Place the sensor tip directly down on the sanding disk and twist it in a circular motion to sand the gold cathode. The goal is to sand off any build-up and to lightly scratch the cathode to provide a larger surface area for the o2 solution under the membrane. Usually, 3 to 4 twists of the sanding disk are sufficient to remove deposits and for the gold to appear to have a matte finish. Rinse thoroughly and wipe the gold cathode with a wet paper towel before putting on a new membrane

cap. If the cathode remains tarnished, contact YSI Technical Support or the Authorized dealer where you purchased the instrument.

Silver Anode

After extended use, a thick layer of Silver Chloride (AgCl) builds up on the silver anode reducing the sensitivity of the sensor. The anode must be cleaned to remove this layer and restore proper performance. The cleaning can be chemical or mechanical:

Chemical cleaning: Remove the membrane cap and rinse the electrodes with deionized or distilled water. Soak the sensing anode section of the sensor in a 14% ammonium hydroxide solution for 2 to 3 minutes or in a 3% ammonia solution overnight for 8-12 hours (most household ammonia cleaners are typically around 3%). Rinse heavily in cool tap water followed by a thorough rinsing with distilled or deionized water. The anode should then be thoroughly wiped with a wet paper towel to remove the residual layer from the anode. You can smell the tip of the sensor to help ensure all the ammonia has been rinsed off. Trapping residual ammonia under the new membrane cap can quickly tarnish the electrode and/or give false readings.

Chemical cleaning should be performed as infrequently as possible. First attempt a membrane change and recalibrate. If a new membrane does not resolve the problem, then proceed with cleaning.

Mechanical cleaning: In order to sand the silver anode along the shaft of the sensor, simply hold the sensor in a vertical position. Wet the sanding disk with a small amount of clean water then gently wrap it around the sensor shaft and twist it a few times to lightly sand the anode (the goal is to simply sand off any build-up without scratching or removing layers of the anode itself). Usually, 3 to 4 twists of the sanding disk are sufficient to remove deposits. However, in extreme cases, more sanding may be required to regenerate the original silver surface.

After completing the sanding procedure, repeatedly rinse the electrode with clean water and wipe with lens cleaning tissue to remove any grit left by the sanding disk. Thoroughly rinse the entire tip of the sensor with distilled or deionized water and install a new membrane.

IMPORTANT: *Be sure to: (1) Use only the fine sanding disks provided and (2) Sand as mentioned in the above procedures. Not adhering to either of these instructions can damage the electrodes. If this procedure is unsuccessful, as indicated by improper electrode performance, contact YSI Technical Support or the Authorized dealer where you purchased the instrument.*

SENSOR MAINTENANCE - CONDUCTIVITY

The openings that allow sample access to the conductivity electrodes should be cleaned regularly. The small cleaning brush included in the Maintenance Kit is ideal for this purpose. Dip the brush in clean water and insert it into each hole 10 to 12 times. In the event that deposits have formed on the electrodes, it may be necessary to use a mild detergent (laboratory grade soap or bathroom foaming tile cleaner) with the brush. Rinse thoroughly with clean water, then check the response and accuracy of the conductivity cell with a calibration standard.

If this procedure is unsuccessful, as indicated by improper electrode performance, contact YSI Technical Support or the Authorized dealer where you purchased the instrument.

SENSOR MAINTENANCE - TEMPERATURE

You must keep the temperature portion of the sensor free of build up. Other than that, the sensor requires no maintenance. The conductivity cleaning brush can be used to scrub the temperature sensor if needed. Alternatively, you can use a toothbrush to clean the sensor.

SENSOR MAINTENANCE - pH, ORP AND COMBINATION pH/ORP

Typical working life for pH and ORP sensors is approximately 12-24 months depending on usage, storage, and maintenance. Proper storage and maintenance generally extends the sensor's working life.

Cleaning is required whenever deposits or contaminants appear on the glass and/or platinum surfaces or when the sensor's response slows. The cleaning can be chemical and/or mechanical.

Removing the sensor from the cable may make cleaning easier. Initially, use clean water and a soft clean cloth, lens cleaning tissue, or cotton swab to remove all foreign material from the glass bulb and/or platinum button. Then use a moistened cotton swab to carefully remove any material that may be blocking the reference electrode junction of the sensor.

CAUTION*: When using a cotton swab, be careful NOT to wedge the swab between the guard and the glass sensor. If necessary, remove cotton from the swab tip, so that the cotton can reach all parts of the sensor tip without stress. You can also use a pipe cleaner for this operation if more convenient.*

If good pH and/or ORP response is not restored, perform the following additional procedure:

1. Soak the sensor for 10-15 minutes in clean water containing a few drops of commercial dishwashing liquid.
2. GENTLY clean the glass bulb and platinum button by rubbing with a cotton swab soaked in the cleaning solution.
3. Rinse the sensor in clean water, wipe with a cotton swab saturated with clean water, and then rerinse with clean water.

If good pH and/or ORP response is still not restored, perform the following additional procedure:

1. Soak the sensor for 30-60 minutes in one molar (1 M) hydrochloric acid (HCl). This reagent can be purchased from most lab supply distributors. Be sure to follow the safety instructions included with the acid.
2. Rinse the sensor in clean water, wipe with a cotton swab saturated with clean water (not DI water), and then rerinse with clean water. To be certain that all traces of the acid are removed from the sensor crevices, soak the sensor in clean water for about an hour with occasional stirring.

If biological contamination of the reference junction is suspected or if good response is not restored by the above procedures, perform the following additional cleaning step:

1. Soak the sensor for approximately 1 hour in a 1:1 dilution of commercially-available chlorine bleach.
2. Rinse the sensor with clean water and then soak for at least 1 hour in clean water with occasional stirring to remove residual bleach from the junction. (If possible, soak the sensor for a period of time longer than 1 hour in order to be certain that all traces of chlorine bleach are removed.) Then rerinse the sensor with clean water and retest.

SENSOR STORAGE

SHORT-TERM STORAGE

The cable assembly is supplied with a sensor storage container, or sleeve, that attaches to the cable. The container is used for short-term storage (less than 30 days). Be sure to keep a small amount of moisture (tap water) in the container during storage. This is done to maintain a 100% saturated air environment which is ideal for short-term sensor storage. The sensors should not be submersed in water. The intent is to create a humid air storage environment.

LONG-TERM STORAGE

Long-term Storage - Temperature

No special storage is required. The temperature sensor can be stored dry or wet as long as solutions in contact with the thermistor are not corrosive (for example, chlorine bleach).

Long-term Storage Temperature: *-5 to 70°C (23 to 158°F)*

Long-term Storage - Conductivity

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). However, it is recommended that the sensor be cleaned with the provided brush prior to and after long term storage.

Long-term Storage Temperature: *-5 to 70°C (23 to 158°F)*

Long-term Storage - Dissolved Oxygen

Dissolved oxygen sensors (Polarographic and Galvanic) should be stored in a dry state for long term storage First, remove the membrane cap and thoroughly rinse the sensor with clean water. Next, either blow it dry with compressed air or allow to air dry completely. Install a clean, dry new membrane cap over the sensor to keep it dry and to protect the electrodes.

After storing the sensor for a long period of time, it is necessary to “condition” the sensor by putting a new membrane with electrolyte solution on the sensor and then turning the instrument on to allow the sensor sufficient time to stabilize.

Long-term Storage Temperature: *-5 to 70°C (23 to 158°F)*

Long-term Storage - pH

The key to pH sensor storage, short or long-term, is to make certain that the sensor does not dry out. Sensors which have been allowed to dry out due to improper storage procedures may be irreparably damaged by the dehydration and will require replacement. You can try to rehydrate the sensor by soaking it (preferably overnight) in a potassium chloride solution or a pH 4 buffer before attempting to calibrate.

To store the sensor, remove it from the cable and seal the vacant port with a port plug. Fill the original shipping/storage vessel (plastic boot or bottle) with buffer 4 solution and then submerge the sensor into the solution. The sensor should remain submerged in the solution during the storage period; therefore, make

certain that the vessel is sealed to prevent evaporation and periodically check the vessel to ensure the sensor does not dry out.

Long-term Storage Temperature: *0 to 30°C (32 to 86°F)*

It is important not to store the pH sensor in distilled or deionized water as the glass sensor may be damaged by exposure to this medium.

2020e/i Turbidity Meter

2020e • Code 1979-EPA

2020i • Code 1979-ISO

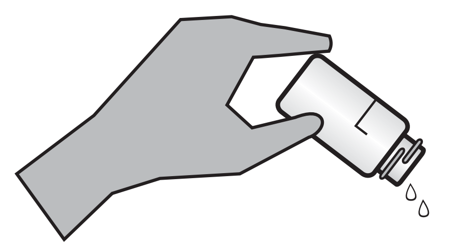
Version 1.5 • Code 1979-MN • 12-07

Turbidity Standards

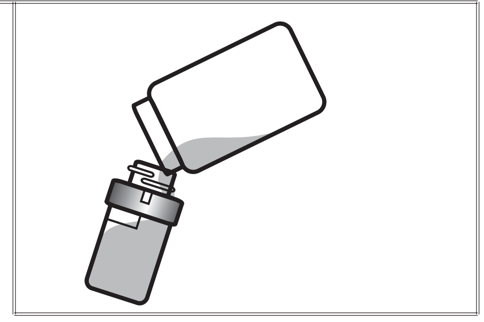
Only use AMCO or formazin standards with the 2020. StablCal® standards below 50 NTU should not be used to calibrate the 2020. The diluent used in StablCal® standards has a different refractive index than traditional formazin standards and will affect the results. The concentration of the calibration standard should be similar to the expected concentration of samples that will be tested. The following standards are available from LaMotte Company:

1480	0 NTU Standard, 60 mL (EPA and ISO)
1484	1 NTU Standard, 60 mL (EPA)
1481	1 NTU Standard, 60 mL (ISO)
1485	10 NTU Standard, 60 mL (EPA)
1482	10 NTU Standard, 60 mL (ISO)
1486	100 NTU Standard, 60 mL (EPA)
1483	100 NTU Standard, 60 mL (ISO)

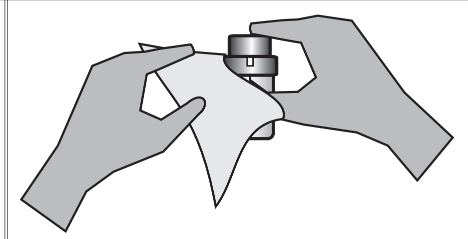
CALIBRATION PROCEDURE

1. Press ***ION** to turn the meter on.	1.3
2. Press ***IOK** to select **Measure**.	Main Menu * Measure Data Logging Options 16:02:19 01/04/05
3. Rinse a clean tube (0290) three times with the blank. Below 1 NTU – The meter should be blanked with a 0 NTU Primary Standard or prepared turbidity-free (<0.1 NTU) water. For the most accurate results, use the same tube for the blank and the sample.	

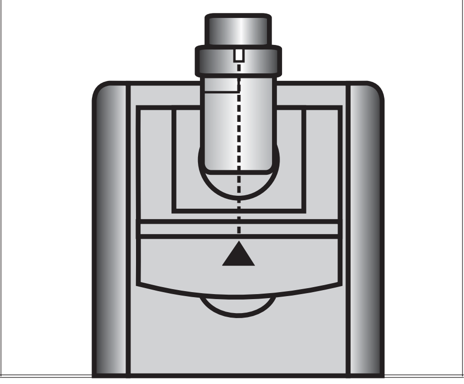
4. Fill the tube to the fill line with the blank. Pour the blank down the inside of the tube to avoid creating bubbles. Cap the tube.



5. Dry the tube with a lint-free cloth. Put on a dry positioning ring. Cap the tube. Wipe the tube thoroughly again with a lint-free cloth.



6. Open the meter lid. Insert the tube into the chamber. Align the index notch on the positioning ring with the index arrow on the meter. Close the lid.

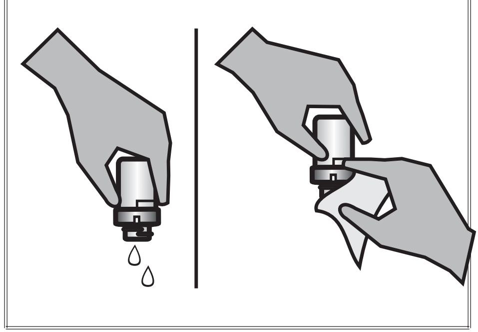


7. Press *IOK to select **Scan Blank**. Remove the tube.

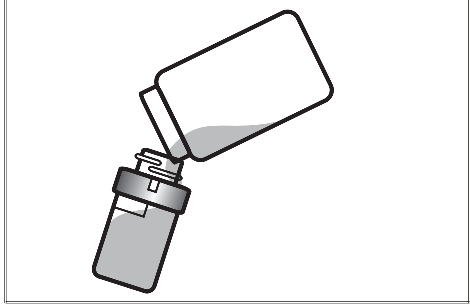
   NOTE: For the best accuracy, especially at low level turbidity, see Tip 17 on page 29.

Turbidity	(F)
* Scan Blank	
16:02:19	01/04/05

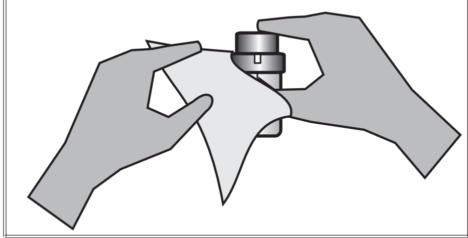
8. Rinse a clean tube (0290), or the same tube, three times with the standard. Avoid spilling standard on the outside of the tube. **IMPORTANT:** While the tube is inverted, wipe the lip of the tube to remove droplets of liquid that may be present. This will prevent liquid from being trapped under the ring when the tube is returned to an upright position.



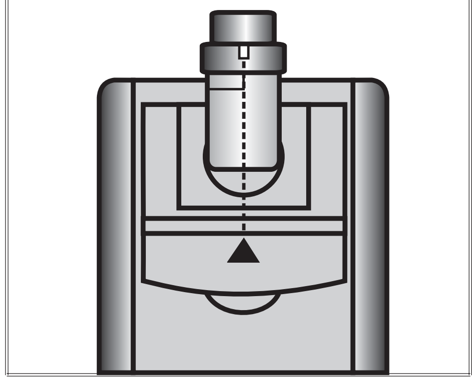
9. Fill the tube to the fill line with the standard. Pour the standard down the inside of the tube to avoid creating bubbles. Cap the tube.



10. Wipe the tube thoroughly again with a lint-free cloth.



11. Open the meter lid. Insert the tube into the chamber. Align the index notch on the positioning ring with the index arrow on the meter. Close the lid.

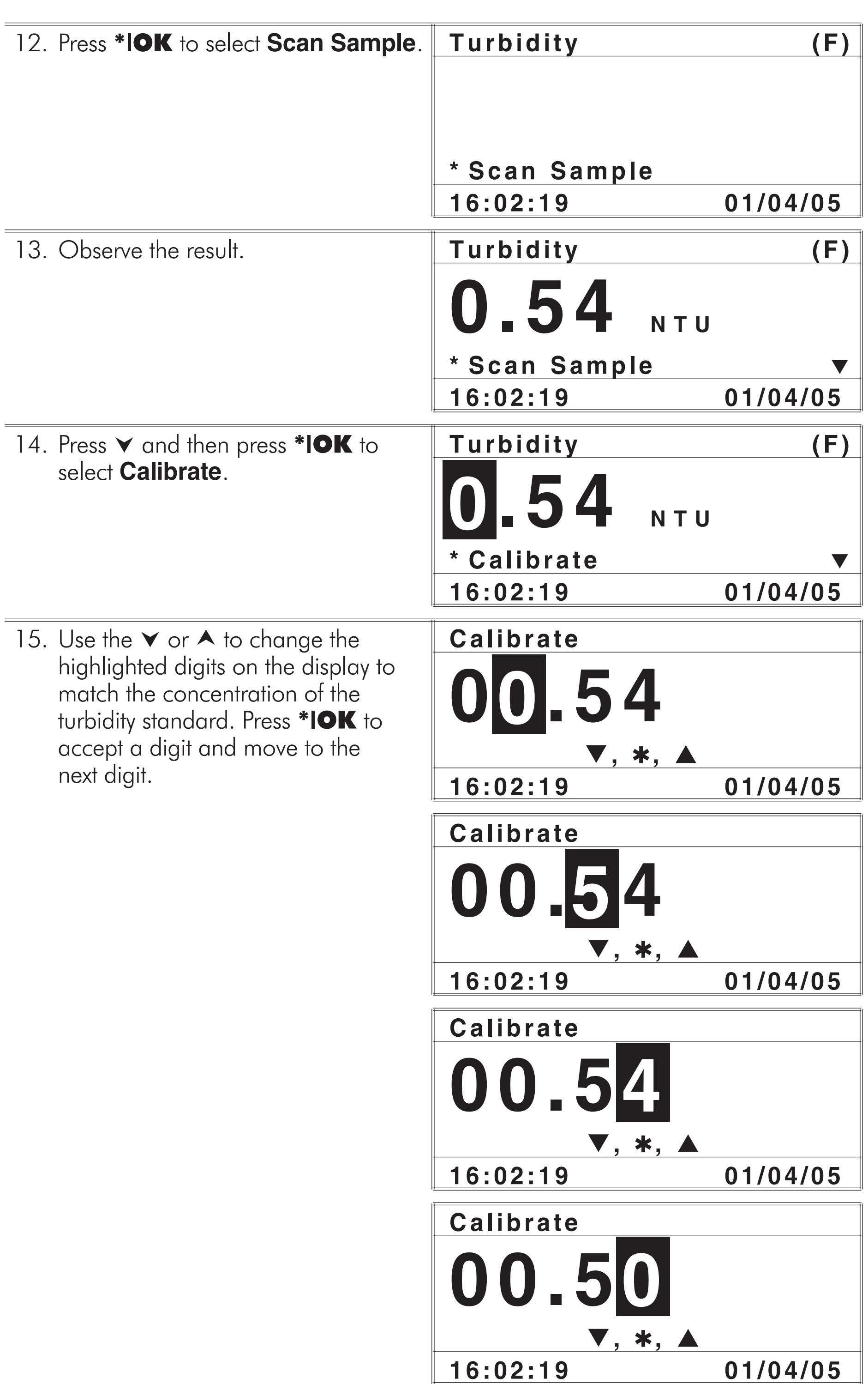


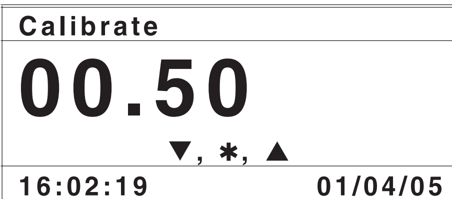
12. Press ***OK** to select **Scan Sample**.

13. Observe the result.

14. Press ∨ and then press ***OK** to select **Calibrate**.

15. Use the ∨ or ∧ to change the highlighted digits on the display to match the concentration of the turbidity standard. Press ***OK** to accept a digit and move to the next digit.

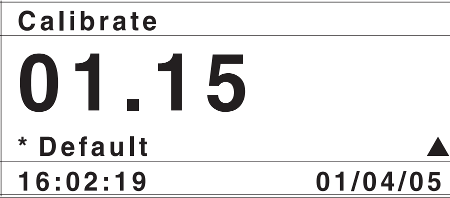




16. When the value on the display matches the concentration of the turbidity standard, press the ***|OK** to select **Set**.

Calibrate
00.50
* Set ▼
16:02:19 01/04/05

Or press ⌄ press ***|OK** to return the meter to the default setting.



17. Press ***|OK** to proceed to Turbidity analysis. Press **OFF** to turn the meter off or press ‹ to exit to a previous menu or make another menu selection.

Note: The meter will remember the last scanned blank reading. It is not necessary to scan a blank each time the test is performed. To use the previous blank reading, instead of scanning a new one, scroll to Scan Sample and proceed. For the most accurate results, the meter should be blanked before each test and the same tube with tube positioning ring should be used for the blank and the reacted sample.

continue next page...

General Operating Information

The operation of the 2020 is controlled by the menu driven software and user interface. A menu is a list of choices. This allows a selection of various tasks for the 2020 to perform, such as, scan blank and scan sample. The keypad is used to make menu selections that are viewed on the display.

The Keypad

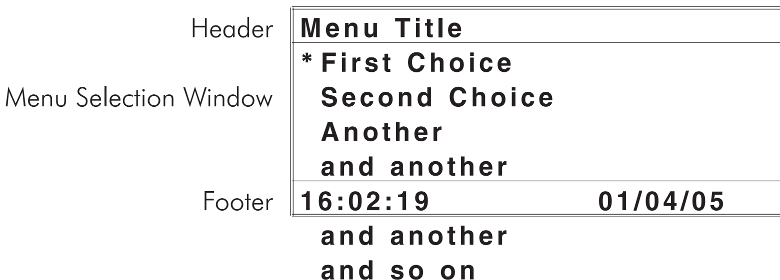
⮟	This button will scroll down through a list of menu selections.
***|OK**	This button is used to select menu choices adjacent to the * in a menu viewed in the display
⮝	This button will scroll up through a list of menu selections.
OFF	This button turns the 2020 off.
⮜	This button is an exit or escape button. When pressed, the display will exit the current menu and go to the previous menu.
ON	This button is used to turn on the 2020.

The Display & Menus

The display allows menu selections to be viewed and selected. These selections instruct the 2020 to perform specific tasks. The menus are viewed in the display using two general formats that are followed from one menu to the next. Each menu is a list of choices or selections.

The display has a header line at the top and a footer line at the bottom. The header displays the title of the current menu. The footer line displays the time and the date. The menu selection window is in the middle of the display between the header and the footer.

The menu selection window displays information in two general formats. In the first format only menu selections are displayed. Up to 4 lines of menu selections may be displayed. If more selections are available they can be viewed by pressing the arrow buttons (⮝ or ⮟) to scroll the other menu selections into the menu selection window. Think of the menu selections as a vertical list in the display that moves up or down each time an arrow button (⮝ or ⮟) is pressed. All menus in the 2020 are looping menus. The top and bottom menu choices are connected in a loop. Scrolling down past the bottom of the menu will lead to the top of the menu. Scrolling up past the top of the menu will lead to the bottom of the menu



An asterisk, *****, will start in the far left position of the top line in the menu choice window. To move the ***** press the up or down arrow buttons (⮝ or ⮟) to scroll through the menu selections. The ***** in the display corresponds with the ***|OK** button. Pushing the ***|OK** button selects the menu choice which is adjacent to the ***** in the menu selection window.

In the second format the menu choice window takes advantage of the graphical capabilities of the display. Large format graphic information, such as test results or error messages or the LaMotte logo is displayed. The top three lines of the display are used to display information in a large, easy to read format. The menus work in the same way as previously described but only one line of the menu is visible at the bottom of the display. On the lower right side of the display small up and down arrows (⮝ or ⮟) indicate that other menu selections are available above or below the one visible lines of the menu.

Header	**Menu Title**
Message or Result Window	**Message or Result**
Menu Selection Window	*** First Choice** ▲▼
Footer	**16:02:19 01/04/05**

Second Choice
another
and so on

As described previously, the < button allows an exit or escape from the current menu and a return to the previous menu. This allows a rapid exit from an inner menu to the main menu by repeatedly pushing the < button. Pushing **OFF** at any time will turn the 2020 off.

The display may show the following messages:

Err1 to Err7	Error messages. See Trouble Shooting Guide. (page 30)
low battery	Low battery
>	Over range indicator.
▲▼	More choices are available and can be viewed by scrolling up and/or down through the display.
Header	Identifies the current menu and information on units and reagent systems if applicable. In the data logging mode the number of the data point is displayed and the total number of data points in the memory will be shown.
Footer	Shows current time and date.

Negative Results

There are always small variations in readings with analytical instruments. Often these variations can be observed by taking multiple readings of the same sample. These variations will fall above and below an average reading. Repeated readings on a 0.00 sample might give readings above and below 0.00. Therefore, negative readings are possible and expected on samples with concentrations at or near zero. This does not mean there is a negative concentration in the sample. It means the sample reading was less that the blank reading. Small negative readings can indicate that the sample was at or near the detection limit. This is a normal variation that results in a negative reading. A large negative reading, however, is not normal and indicates a problem. Some instruments are designed to display negative readings as zero. In this type of instrument, if the meter displayed zero when the result was actually a large negative number there would be no indication that a problem existed. For this reason, the 2020 displays negative numbers.

Tubes

The 2020 uses one type of tube (Code 0290) for the turbidity test. There is no need for a special turbidity tube.

The handling of the tubes is of utmost importance. Tubes must be clean and free from lint, fingerprints, dried spills and significant scratches, especially the central zone between the bottom and the sample line.

Scratches, fingerprints and water droplets on the tube can cause stray light interference leading to inaccurate results when measuring turbidity. Scratches and abrasions will affect the accuracy of the readings. Tubes that have been scratched in the light zone through excessive use should be discarded and replaced with new ones.

Tubes should always be washed on the inside and outside with mild detergent prior to use to remove dirt or fingerprints. The tubes should be allowed to air-dry in an inverted position to prevent dust from entering the tubes. To prevent introducing moisture into the meter chamber, tube positioning rings should be removed before washing tubes. If tubes are washed with tube positioning rings in place, the rings should be removed and thoroughly dried before replacing them on the dry tubes. Dry tubes should be stored with the caps on to prevent contamination.

After a tube has been filled and capped, it should be held by the cap and the outside surface should be wiped with a clean, lint-free absorbent cloth until it is dry and smudge-free. Handling the tube only by the cap will avoid problems from fingerprints. Always set the clean tube aside on a clean surface that will not contaminate the tube. It is imperative that the tubes and light chamber be clean and dry. The outside of the tubes should be dried with a clean, lint-free cloth or disposable wipe before they are placed in the meter chamber.

Tubes should be emptied and cleaned as soon as possible after reading a sample to prevent deposition of particulates on the inside of the tubes. When highly accurate results are required, reduce error by designating tubes to be used only for very low turbidity and very high turbidity testing.

Variability in the geometry of the glassware and technique are the predominate causes of variability in results. Slight variations in wall thickness and the diameter of the tubes may lead to slight variations in the test results. To eliminate this error the tubes should be placed in the chamber with the same orientation each time. The orientation of the tubes in the chamber is controlled by use of a tube positioning ring. For improved accuracy and precision, especially at low concentrations, the tubes should always be used with a positioning ring. (See page 32)

Cleaning

Clean meter with a damp, lint-free cloth.

DO NOT ALLOW WATER TO ENTER THE METER CHAMBER OR ANY OTHER PARTS OF THE METER.

Clean meter chamber and lenses over LEDs with a lint-free cloth slightly dampened with alcohol.

Repairs

Should it be necessary to return the meter for repair or servicing, pack the meter carefully in a suitable container with adequate packing material. A return authorization number must be obtained from LaMotte Company by calling 800-344-3100 (US only) or 410-778-3100, faxing 410-778-6394, or emailing tech@lamotte.com. Often a problem can be resolved over the phone or by email. If a return of the meter is necessary, attach a letter with the return authorization number, meter serial number, a brief description of problem and contact information including phone and FAX numbers to the shipping carton. This information will enable the service department to make the required repairs more efficiently.

Meter Disposal

Waste Electrical and Electronic Equipment (WEEE)

Natural resources were used in the production of this equipment. This equipment may contain materials that are hazardous to health and the environment. To avoid harm to the environment and natural resources, the use of appropriate take-back systems is recommended. The crossed out wheeled bin symbol on the meter encourages you to use these systems when disposing of this equipment.

Take-back systems will allow the materials to be reused or recycled in a way that will not harm the environment. For more information on approved collection, reuse, and recycling systems contact your local or regional wase administration or recycling service.

Appendix E

Laboratory Standard Operating Procedures

9020 QUALITY ASSURANCE/QUALITY CONTROL*

9020 A. Introduction

1. General Considerations

The emphasis on microorganisms in water quality standards and enforcement activities and their continuing role in research, process control, and compliance monitoring require the establishment, documentation, and effective operation of a quality system (QS). The QS establishes an environmental testing and management operation describing both a quality assurance (QA) policy or program and quality control (QC) operational techniques and practices. These are designed to substantiate the validity of analytical data and ensure compliance with regulatory requirements, customer requirements, and applicable standards of accreditation or certification.

The laboratory practices set forth in Section 9020 are not mandatory, but represent practices that should be followed. Each laboratory must develop its own QS suitable for its needs and, in some cases, as required by regulatory agencies, standard-setting organizations, and laboratory certification or accreditation programs.

A laboratory documents its quality system's policies and objectives in a quality management plan or quality manual. The document denotes the laboratory's commitment to the QA program for integration of intra- and inter-laboratory QC activities, standardization of laboratory operating procedures, and management practices. It also clearly defines responsibilities and duties to ensure that the data are of the type, quality, and quantity required.

The program must be practical and require only a reasonable amount of time or it will be bypassed. Once a QA program is established, about 15% of overall laboratory time should be spent on different aspects of the program. However, additional time may be needed for more important analytical data, e.g., data for enforcement actions. When properly administered, a balanced, conscientiously applied quality system will optimize data quality, identify problems early, and increase satisfaction with the analytical results without adversely affecting laboratory productivity.

Because microbiological analyses measure constantly changing living organisms, they are inherently variable. Quality control tools available to microbiologists are different from those used by chemists because many of the measurements made by microbiologists involve discrete variables rather than continuous variables used by the analytical chemists. Discrete variables have only integer values, whereas continuous variables are not limited to particular values but only by the accuracy of the measuring tool used. Therefore, different statistics and probability distributions are used to evaluate data.

Documented quality systems will vary among laboratories as a result of differences in organizational mission, responsibilities, and objectives; laboratory size, capabilities, and facilities; and staff skills and training.

2. Guidelines for a Quality System

The laboratory must develop, document, and initiate its processes to result in controlled experimental conditions that meet its specific needs and the planned use of the data.

a. Management responsibilities: Management must evaluate the risks associated with errors, recognize and actively support the need for the QS, involve staff in development and operation of the program, commit monetary and personnel resources, and assume a leadership role. Management should meet with the laboratory supervisor and staff to develop and maintain a comprehensive program, to establish specific responsibilities for management, supervisors, and analysts, and to maintain awareness of conditions through periodic and systematic review of laboratory functions. Upper management has overall responsibility to the end customer for the QA/QC program and activities performed by the laboratory analyst. The QA officer, the laboratory supervisor, and the laboratory analyst can be delegated responsibilities to carry out a function of their individual job duties by upper management; however, upper management is ultimately responsible for the QA program and cannot avoid their managerial responsibilities by delegation to a lower authority in the organization.

b. Quality assurance officer/quality manager: In large laboratories, a QA officer has the authority and oversight responsibility for implementing the QA program. Ideally, this person has a staff position reporting directly to upper management and thus has operational independence. The QA officer must have a technical background that includes courses in microbiology, be acquainted with all aspects of laboratory work, and be aware of and familiar with the QA program and QC practices, and statistical techniques for data evaluation. The QA officer is responsible for initiating the QA program, convincing management and staff of its value, and providing necessary technical support and training. Once the QA program is functioning, the QA officer should conduct frequent (weekly to monthly) reviews with the laboratory management and staff to determine conformance to the program and to identify and resolve problems. The QA officer also reports periodically to management to secure backing in actions necessary to correct problems that threaten data quality. In small laboratories these responsibilities will be assigned to one or more of the staff on a part-time basis or the staff may form a QA unit.

c. Staff: Laboratory and field staffs should participate with management in planning the QA program, preparing standard operating procedures, and most importantly, implementing the QA program and QC activities in their daily tasks of collecting samples, conducting analyses, performing quality control

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* Approved by Standard Methods Committee, 2005.
Joint Task Group: Margo Hunt (chair), Ellen Braun-Howland, Gil Dichter, Nancy Hall, Stephanie Harris.

checks, and calculating and reporting results. Staff members are the first to identify potential problems and should work with the QA officer and management/supervisor to correct and prevent them. It is critical to the success of the QA program that staff members understand what is expected of them and actively support the QA program.

3. Quality System Objectives

The objectives of a QS include providing data of known quality, ensuring a high quality of laboratory performance, maintaining continuing assessment of laboratory operations, identifying weaknesses in laboratory operations, detecting training needs, improving documentation and recordkeeping, developing adequate and clear reporting systems, and assuring compliance with regulations and client's requirements.

4. Elements of a Quality System Manual

Each laboratory implements a QS and develops a written management plan or manual describing the laboratory's policies and plans for ensuring the quality of their work for their clients. Updated routinely, the plan is signed by both upper management and the QA officer to indicate their approval. For a small laboratory, the owner/operator will sign the plan.

Having the upper management and the QA officer sign off on a written management plan or manual describing laboratory policies and activities makes upper management responsible. This means that personnel support, analytical instruments, and materials are ultimately the responsibility of upper management and cannot be disposed of through delegation to lesser authority, such as the QA officer.

The plan should address the following basic common aspects:

a. Quality policy statement, describing the specific objectives and commitment of the laboratory and its management to quality and data integrity. An ethics statement may be included.

b. Organization and management structure, describing the functional aspects of the laboratory and its management responsibilities with an accompanying organizational chart.

c. Personnel policies, indicating specific qualification and training requirements and job responsibilities for supervisors and analysts.

d. Equipment and instrument requirements, listing critical equipment and instruments available, noting the laboratory's requirements and frequency for calibration procedures and preventative maintenance, and ensuring acceptable functionality before equipment is put into service.

e. Specifications for supplies, noting procedures to ensure that reagents and supplies are of sufficient quality and acceptable for use.

f. Specifications for subcontracting of tests and calibrations, establishing standards for the laboratory's oversight and acceptance of products.

g. Sampling procedures (if performed by the laboratory) and sample acceptance criteria, describing procedures for collecting, handling (e.g., time and temperature), accepting, and tracking submitted samples, and procedures for chain-of-custody required if data may be subjected to litigation.

h. Analytical methods, listing the laboratory's scope for testing, and denoting the accreditation/certification status for individual methods and, for nonstandard or new methods, the laboratory's validation procedures.

i. Analytical quality control measures, stating the laboratory's requirements for measurement assurance, e.g., method verification and documentation, error prevention, and analytical checks as replicate analyses, positive and negative controls, sterility checks, and verification tests, as well as statistical methods to be used.

j. Standard operating procedures (SOPs), listing all generic laboratory processes and specific routine laboratory operations, documented and signed by management, which are available to clients upon request and readily accessible to staff.

k. Documentation control and record-keeping requirements, identifying recordkeeping formats, e.g., hard-copy, e-notebooks, and computer files, and procedures to ensure data review, traceability, and accountability; noting procedures to ensure customer confidentiality, where applicable, and other requirements, such as control, security, storage, record retention time, and disposal of laboratory records. When confidentiality and security allow, a backup copy of the records should be stored offsite.

l. Assessments, describing the laboratory's processes to monitor and report on the effectiveness of its QA program.

1) Internal audits of the laboratory operations, performed on a routine basis, at least annually, by the QA officer and supervisor. For a small laboratory, an outside expert may be needed. These audits should involve all aspects of the laboratory including, e.g., analyses conducted, data manipulations, and reporting.

2) On-site evaluations by outside experts to ensure that the laboratory and its personnel are following an acceptable QA program. This is a required component for laboratory certification or accreditation. For laboratories not seeking such recognition, this activity is a suggestion.

3) Proficiency test (PT) studies, in which the laboratory participates. These collaborative studies should confirm the capability of a laboratory to generate acceptable data comparable to the reference laboratory and other laboratories and to identify potential problems. PT studies are generally conducted once or twice a year.

m. Corrective and preventive activities, identifying procedures used to determine the causes of identified problems and to record, correct, and prevent their recurrence.

n. Service to the customer, describing the laboratory's commitment to and activities for responding to customer requests and complaints, and for ensuring customer confidentiality and proprietary rights.

The QC guidelines discussed in 9020B and 9020C are recommended as useful source material of elements that need to be addressed in developing policies for a QA program and QC activities. Additional information is available from several standards-setting organizations such as the American Association for Laboratory Accreditation (A2LA), AOAC International Inc., International Organization for Standardization (ISO), National Environmental Laboratory Accreditation Conference (NELAC), Institute for National Environmental Laboratory Accreditation (INELA), and the United States Environmental Protection Agency (USEPA).

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9020 B. Intralaboratory Quality Control Guidelines

Quality control (QC) practices are designed to ensure that the laboratory's processes are in control. All laboratories have some intralaboratory QC practices that have evolved from common sense and the principles of controlled experimentation to indicate method efficiency and laboratory performance. A laboratory's QS sets in place the QA policies or program and QC activities necessary to minimize systematic and random errors resulting from variations in personnel, instrumentation, equipment, reagents, supplies, sampling and analytical methods, data handling, and data reporting. It is especially important that laboratories performing only a limited amount of microbiological testing exercise strict QC. A listing of key QC practices is given in Table 9020:I and is discussed in ¶ 5 below. Additional sources of information about laboratory QC practices are available.[1-10] Laboratories should address all of the QC guidelines discussed herein, but the depth and details may differ for each laboratory. Many items mentioned here are also applicable to other laboratories such as chemical and radiological laboratories. For those microbiology laboratories testing under Good Manufacturing Practices (GMP)/Good Laboratory Practices (GLP) regulations, certain QC practices will be different from those listed here.

1. Personnel

Microbiological testing should be performed by a professional microbiologist or technician with an appropriate level of education, training, and experience in general microbiological techniques. If not, a professional microbiologist should provide close supervision to guide and train the analyst in basic microbiological laboratory procedures to perform their assigned functions. The supervisor routinely should evaluate and document the technician's skills. Sample collection (if performed by the laboratory), sample handling, media and glassware preparation, sterilization, clean room gowning and access requirements, aseptic techniques, routine analytical testing, counting, data handling, and QC techniques to identify and eliminate problems should be closely monitored. Management should assist laboratory personnel in obtaining additional training and course work to enhance their technical skills and advance their careers. An employee training record and performance score obtained by analyzing single-blind samples should be maintained. Initial demonstration of capability prior to generating data, and an ongoing demonstration of capability for each analytical method conducted should be recorded.

2. Biosafety Criteria

Biosafety is of concern to all microbiological laboratories to protect laboratory personnel and others who may be potentially exposed. There are three elements to be considered: laboratory practices, safety equipment, and facility design. The risk assessment of the work to be done with each specific biological agent will determine the appropriate combination of these elements necessary to the individual laboratory.

The Centers for Disease Control (CDC) and Prevention, U.S. Public Health Service, classifies laboratories handling potential hazardous biological agents into four biosafety levels. The four biosafety levels (BSLs 1, 2, 3, and 4) consist of a combination of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the suspected routes of transmission of the infectious agents, and the laboratory function or activity.

The following is a brief discussion of the four different biosafety levels. Indigenous, dangerous, or exotic agents that may cause serious or potentially lethal disease are not described in *Standard Methods*; therefore, detailed information

TABLE 9020:I. KEY QUALITY CONTROL PRACTICES

Item	Action	Frequency	Further Information in Section 9020B,¶
Reagent water	Monitor quality	See Table 9020:II	
Air in workplace	Monitor bacterial density	Monthly	3*e*
Temperature devices:			4*a*
Working units	Check accuracy	Annually	
Reference units	Recertify	Every 5 years	
Balances	Check zero	Each use	4*b*
	Check accuracy	Monthly/each use preferably	
	Service and recalibrate	Annually	
Weights:			4*b*
Working	Check with reference weights	Annually	
Reference	Recertify	Every 5 years	
pH meter	Standardize	Each use	4*c*
	Determine slope	Monthly	
Media-dispensing apparatus	Check volume dispense accuracy	Each use	4*f*
Hot-air sterilizing oven	Check performance	Monthly	4*g*
Autoclave	Check temperature with max-registering thermometer	Weekly	4*h*
	Check performance with bioindicator	Monthly	
Timer:			
Autoclave	Check timing with stopwatch	Quarterly	4*h*
Stopwatch	Check against National Time Signal	Annually	
Refrigerator	Check temperature	Daily	4*i*
Freezer	Check temperature	Daily	4*j*
	Defrost	Annually	
Membrane filtration equipment	Check for leaks and surface scratches	Each use	4*k*
	Check sterility	Pre- and post-test	
	100-mL volume check	Initially	
UV lamps (shortwave)	Test with UV meter or perform plate count check	Quarterly	4*l*
Biosafety cabinet	Inspect for airflow	Each use	4*m*
	Have certified	Annually	
Incubator	Check temperature	Twice daily	4*n* and *o*
Microscope	Clean optics and stage, check alignment	Each use	4*p*
Conductivity meter	Calibrate	Monthly	4*q*
Micropipettors	Check dispense accuracy and precision	Quarterly or more frequently if heavily used	4*s*
	Calibrate	Annually	
Glassware	Inspect for cleanliness, chips, and etching	Each use	5*a*
	Check pH with bromthymol blue	Each wash batch	
	Conduct inhibitory residue test	Initial use and new washing procedure (also may be annual)	
	Check for autofluorescence if used for testing	Each batch or lot	
Dilution water bottles	Check sterility, pH, and volume	Each batch or lot	5*c* and 9050C.1*a*
Sample bottles	Check sterility	Each batch or lot	5*d*
	Check dechlorination agent efficacy	Each batch or lot	
	Check 100 mL line	Each lot	
	Check for autofluorescence if also used for testing	Each lot	
Multi-well sealer	Check performance	Monthly	5*e*
Membrane filters	Check sterility and properties	Each new lot	5*i*
Media	Check sterility, pH and appearance	Each batch or lot	5*j*
	Check recovery of new vs. old media	Before first use	
	Check performance with + and − culture controls	Each batch or lot	
Plate counts	Perform duplicate analyses	Monthly	9*a*
	Repeat counts	Monthly	

on special practices, containment, and facilities for BSLs 3 and 4 are not included here. For further information on all BSLs, review CDC's protocols.[11]

a. Biosafety level 1 (BSL 1): As noted by CDC, BSL 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adults and of minimal potential hazard to laboratory personnel and the environment. Work is generally conducted on open bench tops using standard microbiological practices. The agents listed in *Standard Methods* that should be handled under BSL 1 practices are total and

thermotolerant (fecal) coliform bacteria, *E. coli*, enterococci, iron and sulfur bacteria, actinomycetes, and other nonpathogenic microorganisms. It is under the discretion of the laboratory director what biosafety practices are to be adhered to depending on the practices involved. The standard practices and safety equipment for this level are as follows:

1) Access to the laboratory is limited or restricted at the discretion of the laboratory director by posting a sign, e.g., "Restricted Area–Biohazards – Laboratory Personnel Only" when experiments or work with samples are in progress. Ensure that doorways and windows are closed when aseptic work is progressing.

2) Personnel wash hands thoroughly with soap and water after they handle viable materials, after removing gloves, and before leaving the laboratory.

3) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in work areas.

4) Mouth pipetting is prohibited.

5) Policies for the safe handling of sharp items are instituted.

6) Work surfaces are decontaminated before and after each use and after any spill of viable material.

7) All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving and that information is recorded.

8) An insect and rodent control program is in effect.

It is recommended that laboratory coats, gown, or uniforms be worn to prevent contamination or soiling of street clothes. Gloves should be worn if skin on the hands is broken or if a rash is present. All procedures should be performed so that no aerosols or splashes occur.

b. Biosafety level 2 (BSL 2): BSL 2 builds upon BSL 1 practices and involves work with agents of moderate potential hazard to personnel and the environment. The agents listed in *Standard Methods* that require BSL 2 practices are the pathogenic microorganisms described in Sections 9260, 9510, 9610, and 9711. This level differs from BSL 1 in that: laboratory personnel have specific training in handling pathogenic agents; access to the laboratory is limited when work is in progress; extreme precautions are taken with contaminated sharp items; and certain procedures in which infectious aerosols may be created are conducted in biological safety cabinets (BSC). Appropriate immunizations should be given if available.

The standard practices for this level include all those listed for BSL 1 and additional special practices, including the following:

1. A high degree of precaution is always taken with any contaminated sharp items, including needles and syringes, slides, pipets, capillary tubes, and scalpels.

2. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material, by using disinfectants that are effective against the agents of concern.

3. Cultures or potentially infectious wastes are placed in a container labeled "Biohazardous Waste" with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.

4. Biological safety cabinets, preferably Class II, or other appropriate personal protective equipment. are used whenever procedures with a potential for creating infectious aerosols or splashes are conducted and high concentrations or large volumes of infectious agents are used.

5. Face protection is used for anticipated splashes or sprays of infectious materials to the face whenever the microorganism must be manipulated outside the BSC.

6. Protective laboratory coats, gowns, or uniforms, and safety glasses designated for laboratory use are worn while in the laboratory and removed and left in the laboratory before leaving for nonlaboratory areas.

7. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces, or equipment.

c. Biosafety levels 3 and 4: BSLs 3 and 4 involve working with indigenous, dangerous, or exotic agents that may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Because agents in these categories are not described in *Standard Methods*, special practices, containment, and facilities for these levels are described only briefly here.

Personnel must be trained in handling infectious materials. All preceding safety practices must be followed. Access must be limited and areas secured. Work must be conducted within biological safety cabinets by personnel wearing appropriate protective clothing and devices. No one with open lesions should enter the laboratory. A passage area where personnel can change into protective clothing should be available between the entrances from the outside hallway and the inner laboratory. Prevent both doors from opening at the same time. All potentially contaminated material such as gloves, laboratory coats, etc., must be decontaminated before disposal or reuse.

BSL 4, as noted above for BSL 3, involves biological agents, often exotic, that are extremely hazardous both to personnel and/or the environment. All preceding safety practices must be followed. Access to the laboratory must be strictly controlled and situated in an area clearly marked and removed from normal operations or in a separate building. Personnel must completely disrobe and put on laboratory clothing prior to entering the test areas and must be decontaminated before leaving.

3. Facilities

Develop an environmental control policy to ensure that environmental conditions do not invalidate results, affect the required quality of the measurements, nor adversely affect personnel.[12] Factors to be considered and monitoring to be performed are described below. Much of this information applies to any laboratory facility.

a. Ventilation: Plan well-ventilated laboratories that can be maintained free of dust, drafts, and extreme temperature changes. Install air conditioning and temperature- and humidity-control systems to reduce contamination, permit more stable operation of incubators, and decrease moisture problems with media and instrumentation. Adjust air system vents so that air flow does not blow directly on the working surface areas. Where feasible, air flow should be negative into the laboratory (so that airflow is always into, rather than out of, the laboratory) to avoid risk of contamination of the exterior.

b. Space utilization: To ensure test and sample integrity and minimize potential contamination, design and operate the laboratory to minimize through traffic and visitors. Do not obstruct access or egress points.

Ensure that there is sufficient work space available for the volume of work to be performed. For example, maintain separate work areas for sample receipt; preparation and sterilization; decontamination of media, glassware, and equipment; testing and culturing; and data handling and storage. Maintain heat-generating equipment, such as autoclaves, in a room separate from incubators. Use of a hood or biological safety cabinet for dispensing and preparing sterile media, transferring microbial cultures, or working with pathogenic materials is recommended. In smaller laboratories it may be necessary, although undesirable, to carry out these activities in the same room. However, do not perform these activities near open doorways or open windows. Have sufficient storage space available within the laboratory so that materials can be stored appropriately.

c. Laboratory bench areas: Provide at least 2 m of linear bench space per analyst and additional areas for preparation and support activities. Bench height should be reasonable and comfortable for the technicians. For stand-up work, typical bench dimensions may range from 90 to 97 cm high and 70 to 76 cm deep and for sit-down activities such as microscopy and plate counting, benches may range from 75 to 80 cm high. Specify bench tops of stainless steel, epoxy plastic, or other smooth, impervious surfaces that are inert and corrosion-resistant with a minimum number of seams and free of cracks and crevices. Install even, glare-free lighting with about 1000 lux (100 ft-c) intensity at the working surface. Test using a photometer.

d. Walls and floors: Assure that walls are covered with a smooth finish that is easily cleaned and disinfected. Specify floors of smooth concrete, vinyl, asphalt tile, or other impervious, sealed washable surfaces. Specify ceiling surfaces that are smooth, nonfibrous, and with recessed lights.

e. Work-area: Maintain high standards of cleanliness in work areas. Disinfect surfaces before and after testing. Institute a regular preventive maintenance policy for work areas and equipment such as incubators and refrigerators. Sterilize contaminated supplies and media promptly after use. Avoid buildup of water in pan underneath refrigerator and clean all vent filters.

Develop an environmental monitoring program to monitor air quality routinely, at least monthly or more frequently if area is heavily used or biocontamination risk analysis indicates the need for more frequent monitoring. Use air density settling plates where aseptic work is conducted. This is a passive sampling process wherein particles can settle on the agar surface. Use active air samplers if risk assessment indicates possible aerosol conditions.[4] RODAC (replicate organism detection and counting) contact plates or the swab method[1] can be used weekly or more frequently to monitor bench surface contamination.

Average results obtained from tests over a period of time to set normal limits, i.e., set a baseline for that location. Although uniform limits for bacterial density have not been set, each laboratory can use these tests to establish a baseline for specific work areas, evaluate trends, establish alert and action levels, and take appropriate action when necessary. The number of colonies on the air density plate should not exceed 160/m^2/15 min exposure (15 colonies/plate/15 min). In addition to this surveillance system, the laboratory may wish to identify contaminants recovered with commercially available automated identification systems.

Prevent any adverse sound and vibration levels within the laboratory. Install easy-to-clean sun shades on large glass windows to prevent heat buildup.

f. Laboratory cleanliness: Regularly clean laboratory rooms and wash benches, shelves, floors, windows, overhead lights, and exposed pipe surfaces. Wet-mop floors and treat with a disinfectant solution weekly; do not sweep or dry-mop. Wipe bench tops and treat with a disinfectant at least daily, or more frequently depending on the biosafety level required for the work being done (see 9020B.2). Do not permit laboratory to become cluttered. Store supplies and paperwork away from bench tops. Eliminate or cover any overhead pipes that cannot be cleaned routinely. Have liquid hand soap in a gravity-fed dispenser and paper towels available at laboratory sinks. Do not allow smoking or consumption of food or drink in the laboratory.

g. Electricity: Ensure a stable electric source, a sufficient number of outlets, circuit breaker (GFCI) protected where needed, and the placement of surge protectors. An emergency power backup and alarm system may be necessary where the work is critical.

4. Laboratory Equipment and Instrumentation

Have procedures in place to verify that each identified item of equipment is installed properly and is operating in a consistent and satisfactory manner.[13] Verify by constant monitoring, routine maintenance, and a regular calibration schedule that each piece of equipment or instrument meets the user's needs for precision and minimization of bias. Provide written procedures on the use, operation, calibration, and maintenance of relevant equipment and instruments (see 9020B.6) and keep manufacturers' manuals available for easy retrieval. Perform equipment calibration using reference standards and equipment maintenance on a regular basis as recommended by the manufacturer or obtain preventive maintenance contracts on autoclaves, balances, microscopes, and other critical equipment. Directly record all quality control checks in permanent log books and maintain documentation. Develop a system for "flagging" problems and actions needed for correction.

Ensure that the laboratory has all equipment and supplies required for the performance of environmental tests and calibration. Have available sufficient equipment and supplies where needed so that they are not routinely moved from one laboratory area to another. Where equipment is available only off-site, document how the laboratory will ensure that the quality will be satisfactory. For molecular testing, the laboratory's equipment and supplies need to be dedicated to specific rooms.[9] Maintain all documentation showing determination of acceptability for equipment, instruments, and supplies, as well as all analytical analyses. Keep the records in a permanent record format such as a bound notebook, e-notebook, or computer file.

Use the following quality control procedures for the basic applied as well as the research laboratory (equipment needed for specialized testing may not be listed here):

a. Temperature-sensing and recording devices: Annually or, preferably, semiannually check accuracy of all working temperature-sensing devices, such as liquid-in-glass thermometers, thermocouples, and temperature-recording instruments at the use temperature against a certified National Institute of Standards and Technology (NIST) thermometer or one traceable to NIST

and conforming to NIST specifications. Record calibration results, along with the date and the technician's signature, in a quality control logbook. Mark the necessary calibration correction factor on each temperature measuring device so that only calibrated-corrected temperature values are recorded. Verify accuracy of the reference certified thermometer as specified on the certificate of calibration or at least every 5 years. Some accreditation organizations or federal or state agencies may require more frequent calibration.

For general purposes use thermometers graduated in increments of 0.5°C or less. Maintain bulb in water or glycerol for air incubators and refrigerators. For example, for a 44.5 ± 0.2°C water bath, use a total immersion thermometer, e.g., short range and length, graduated to 0.1°C. For air convection incubators, use thermometers, e.g., short range and length, with bulbs immersed in glycerol sealed in a flask or test tube having equivalent volume to containers being used in incubators. Record the calibration-corrected temperature reading in a quality control log. Where possible, equip incubators and water baths with temperature-recording instruments that provide a continuous record of operating temperature.

Abstain, where possible, from using mercury-filled thermometers to avoid potential release of mercury into the environment when the thermometer is broken.

b. Balances: Locate balances in areas without rapid air movement and level balances on firm, even surfaces to prevent vibrations. Relevel balance each time it is moved to a new location. Follow manufacturer's instructions for operation and routine maintenance of analytical and top-loading balances. Service balances annually or more often as conditions change or problems occur.

Before each use wipe balance with a soft brush and make sure it is at zero weight when empty. If it is necessary to zero display, press tare button. Use weigh paper or boats and tare weight before adding reagents. Place item to be weighed on the pan and read the weight after the stability indicator symbol (if available) appears in display. Clean balance pans after use and wipe spills up immediately with a laboratory tissue. Replace weights if corroded or dropped. Use only a plastic-tip forceps to handle weights. Check balance routinely, preferably daily before use, with at least two working weights that bracket the normal usage range. Check working weights monthly against a set of reference weights of known tolerance[14] (e.g., ANSI/ASTM Class 1 or NIST Class S accompanied by appropriate certificate) for accuracy, precision, and linearity. Record results along with date and technician's initials. Recertify reference weights as specified in the certificate of calibration or at least every 5 years.[15,16]

Note that some regulatory agencies or accreditation organizations may require more frequent recertification of reference weights.

c. pH meter: Use a meter, graduated in 0.1 pH units or less, that includes temperature compensation, because the electrode pH response is temperature-dependent. Use digital meters, commercial buffer solutions, and electrodes suitable for a wide temperature range. A flat-head electrode can be used to measure solid agar media. Calibrate pH meter with at least two certified pH buffers that bracket the pH of sample being measured. The most desired temperature range for determining pH is 25° ± 5°C. Take pH measurement of test solution close to the temperature used to calibrate the meter.

Record calibration results, date, and technician's initials. Date buffer solutions on bottle and in logbook when opened and check monthly against another pH meter, if possible. Immediately after use, discard buffer solutions or single-use/ready-to-use pH solution packets used to calibrate meter. After 1 d, discard all buffer solutions made from packets. Replace pH buffer supply containers by the expiration date. Store electrode immersed in solution recommended by manufacturer. Do not allow electrode to dry out.

Measure and record pH meter slope after calibration at least once a month, and preferably after each use, to see if meter is malfunctioning. If the pH meter does not have a feature that automatically calculates the slope, but can provide the pH in millivolts (mV), use the following formula to calculate the slope: Slope, as % = (mV at pH 7 − mV at pH 4) × 100/177. If the slope is below 95% or above 105%, the electrode or meter may need maintenance.

For full details of pH meter use and maintenance, see Section 4500-H^+ or follow manufacturer's instructions.

d. Water purification system: Commercial systems are available that include some combination of prefiltration, activated carbon, mixed-bed resins, and reverse-osmosis with final filtration to produce reagent-grade water. Such systems tend to produce the same quality water until resins or activated carbon are near exhaustion and quality abruptly becomes unacceptable. Some deionization components that automatically regenerate the ion exchange resins are now available. Do not store reagent water unless a commercial UV irradiation device is installed and is confirmed to maintain sterility.

Monitor reagent water continuously or every day of use with a calibrated conductivity meter and analyze at least annually for trace metals. Monthly determination of heterotrophic bacteria may indicate potential problems before other test parameters. Increasing numbers of bacteria in the system can affect bacterial tests as they represent nutrient sources for bacteria being isolated. The water quality test should be performed annually and when there is a repair or change in water supply system. This bacteriological quality test is not needed for Type II water or better as defined in *Standard Methods* (18th and 19th Editions), Section 1080C, or medium-quality water or better, as defined in *Standard Methods* (20th, 21st, and Online Editions), Section 1080C, or as defined by other widely accepted standards.[17] Most systems used today meet or exceed these standards.

Replace cartridges at intervals recommended by the manufacturer based on the estimated usage and source water quality. Do not wait for column failure. If bacteria-free water is desired, and a UV irradiation device is not available, include aseptic final filtration with a 0.2-μm-pore membrane filter and collect in a sterile container. Monitor treated water for contamination and replace filter as necessary.

e. Water still: Stills produce water of a good grade that characteristically deteriorates slowly over time as corrosion, leaching, and fouling occur. These conditions can be controlled with proper maintenance and cleaning. Stills efficiently remove dissolved substances but not dissolved gases or volatile organic chemicals. Freshly distilled water may contain combined chlorine and ammonia (NH_3). On storage, additional NH_3 and CO_2 are absorbed from the air. Use softened water as the source water to reduce frequency of cleaning the still. Drain and clean still and reservoir according to manufacturer's instructions and usage.

f. Mechanical media dispensing apparatus: Check volume by dispensing into a graduated cylinder at start of each volume change and periodically throughout extended runs; record results. Flush with a small volume of medium before dispensing and pump hot reagent-grade water through the unit to rinse between runs. Correct leaks, loose connections, or malfunctions immediately. At the end of the work day, break apparatus down into parts, wash, rinse with reagent-grade water, and dry. Lubricate parts according to manufacturer's instructions or at least once per month.

g. Hot-air sterilizing oven: Test performance monthly with commercially available biological spore strips of a spore-forming microorganism such as *Bacillus atrophaeus*, preferably having a minimum spore density of 1×10^6 and placed in glassware similar to items being sterilized. Use a thermometer, bulb placed in sand, accurate in the 160 to 180°C range to measure temperature, or a thermocouple-type probe, or a continuous-read temperature recorder. Record results and contents when in use. Use heat-indicating tape to identify supplies and materials that have been exposed to sterilization temperatures.

h. Autoclave: Record items sterilized and sterilization temperature along with total run-time (exposure to heat), actual time period at sterilization temperature, set and actual pressure readings, and initials of responsible person for each run cycle.[18] New units may print out most of this information on tape automatically. For older units use of a recording thermometer chart is highly recommended.

For new autoclaves an initial temperature profile can be conducted to determine differences in the various locations within the autoclave. For routine use, verify the autoclave temperature weekly by using a maximum registering thermometer (MRT) to confirm that 121°C has been reached.

Test monthly for sterilization efficacy, using normal sterilization time and temperature for media, with a biological such as commercially available *Geobacillus stearothermophilus* in spore strips, suspensions, or capsules, preferably at a 1×10^6 concentration and placed into glassware containing a liquid to simulate actual autoclave sterilization performance on media.[19] With changing standards, some biological indicators may require a longer time period at sterilization temperature than is used for most carbohydrate media. If a problem is noted, use biological indicators for autoclave runs that exceed 20 min, e.g., dilution water and contaminated materials.

The additional use of a chemical steam indicator for each cycle is a practical and quick method to show if minimum exposure conditions were met. Use heat-indicating tape to identify supplies and materials that have been sterilized. Check timing quarterly by using a calibrated timer or by national time signal. Keep autoclave clean and free of debris by checking both trap and seals.

i. Refrigerator: Maintain temperature at 2 to 8°C with thermometer bulb in distilled water or glycerol solution. An initial temperature profile is suggested. Check and record calibration-corrected temperature daily when in use and clean annually or more frequently if needed. Identify and date materials stored. Defrost as required and discard outdated materials monthly. Frost-free units may result in faster dehydration of stored media. Refrigerators and freezers should be explosion-proof if they are used for storing flammable materials.

j. Freezer: Freezer temperature range will be determined by analytical need, e.g., the standard laboratory freezer may range from −10 to −20 ± 5°C to an ultra-cold freezer which may range from −70 to −90°C. Check and record temperature daily. A recording thermometer and alarm system are highly desirable. Identify and date materials stored. Defrost and clean at least annually (semiannually if needed); discard outdated materials.

k. Membrane filtration equipment: Before initial use, assemble filtration units and check for leaks. Discard units if inside surfaces are scratched. Wash and rinse filtration assemblies thoroughly after use, wrap in nontoxic paper or foil, and sterilize. When volumetric graduation marks are used to measure sample volumes, check accuracy of graduation marks initially using a Class A graduated cylinder or volumetric pipet. Record results. For presterilized single-use funnels check one per lot or use a set percentage, e.g., 1 to 4%, for accuracy of volumetric graduation mark.

l. Ultraviolet lamps: When used, disconnect lamps monthly and clean bulbs with a soft cloth moistened with ethanol, 70% methanol/30% reagent-grade water, or use spectroscopic grade 2-propanol where baked-on material may be collecting. Test lamps quarterly with an appropriate (short-wave) UV light meter* and replace bulbs if output is less than 70% of the original. Alternatively, expose plate count agar spread plates containing 200 to 300 colony forming units (CFU) /mL of a selected bacterial suspension, for 2 min. Incubate plates at 35°C for 48 h and count colonies. Replace bulb if colony count is not reduced 99%.

CAUTION: *Although short-wave (254-nm) UV light is known to be more dangerous than long-wave UV (365-nm, used to detect fluorescence), both types of UV light can damage eyes and skin and potentially are carcinogenic.[20] Protect eyes and skin from exposure to UV light. Consider a lockout mechanism so that laboratory lights cannot be turned on without turning off overhead UV lights if used. (See Section 1090B.)*

m. Biohazard safety cabinet (BSC): Properly maintained Class I and II BSCs, when used in conjunction with good microbiological techniques, provide an effective containment system for safe manipulation of moderate and high-risk microorganisms (Biosafety Level 2 and 3 agents). Both Class I and II BSCs have inward face velocities (75 to 100 linear ft/min) that provide comparable levels of containment to protect laboratory workers and the immediate environment from infectious aerosols generated within the cabinet. Class II BSCs also protect the material itself through high-efficiency particulate air filtration (HEPA filtration) of the air flow down across the work surface (vertical laminar flow). Standard operating procedures are as follows:

1) Before use and after use, purge air for 10 to 15 min and wipe down unit with disinfectant. Ensure inward air flow with a piece of tissue.

2) Enter straight into cabinet and perform work in a slow methodical manner. Place material well within cabinet — not on front grill — and do not disrupt or block laminar air flow. Place discard pan within cabinet.

* Fisher Scientific, short-wave meter (Cat. No. 11-924-54) and long-wave meter (Cat. No. 11-984-53), Pittsburgh, PA 15219-4785, or equivalent.

3) Decontaminate interior of BSC after completion of work and before removal. Allow cabinet to run for 10 to 15 min and then shut off.[21]

Provide for testing and certification of Class I and II BSCs *in situ* at the time of installation, at any time the BSC is moved, and at least annually thereafter. Maintain cabinets as directed by the manufacturer.

n. Water bath incubator: Verify that water bath incubators maintain the set temperature, such as 35 ± 0.5°C or 44.5 ± 0.2°C. Use a total immersion thermometer (¶ 4*a*, above) having the appropriate increments required for test incubation temperatures. When incubator is in use, monitor and record calibration-corrected temperature twice daily.

Fill unit only with reagent-quality water. Maintain water level so that it is above the upper level of the medium either in tubes or flasks. For optimum operation, equip water bath with a gable cover to prevent evaporation and with a circulating pump to maintain even temperature distribution. Use only stainless steel, plastic-coated, or other corrosion-proof racks. Use screens or weights to keep materials from floating. Empty and clean bath as needed to prevent buildup of salts and microbial growth and disinfect before refilling.

o. Incubator (air, water-jacketed, or aluminum block): Measure and establish that incubators maintain appropriate and uniform spatial test temperatures. Allow sufficient space between items to permit unobstructed airflow. Do not overload nor stack petri dishes more than four plates high. Verify initially that cold sample test media are incubated at the test temperature for the required time. Note that static air incubators will take longer to reach set incubation temperature. Bring all cold samples in media to room temperature before insertion and use incubators of sufficient size to avoid overfilling incubators with cold samples. During usage periods check and record calibration-corrected temperature twice daily (morning and afternoon, separated by at least 4 h) on the shelves in use, or at least one on the top shelf and one on the bottom shelf, to ensure and record temperature consistency throughout unit. If a glass thermometer is used, submerge bulb and stem in water or glycerin to the immersion mark. For best results use a recording thermometer and alarm system. Place incubator in an area where room temperature is maintained between 16 and 27°C (60 to 80°F). Alternatively, use well-insulated walk-in incubator rooms with forced air circulation. Clean and then sanitize incubators routinely.

p. Microscopes: Check Kohler illumination each time the microscope is put to use. Clean optics and stage after each use with lens paper and cover microscope when not in use. Further information is available in Section 9030 and elsewhere.[22]

Permit only trained technicians to use fluorescence microscope and light source. Monitor fluorescence lamp and replace when a significant loss in fluorescence is observed, when manufacturer recommends replacement, or when a rule or laboratory guidance document specifies maximum hour usage, whichever occurs first. Record lamp operation time/usage, efficiency, and alignment. Always realign lamp after bulb has been replaced. Use known positive fluorescence slides as controls.

q. Conductivity meter: Conductivity measurements are temperature dependent and the effect of temperature will vary with different solutions. Therefore, calibrate meter monthly using certified low-level standard at 25°C or determine cell constant using certified low-level standard at 25°C. When solutions must be measured at a different temperature, use a meter with automatic temperature compensation or take temperature of solution, record reading, and then correct reading to 25°C using the formulae in Section 2510B.5*b* (usually 2%/°C).

r. Microwave: Units vary in power and acceptable placement of material; however, microwaves have been used successfully to melt presterilized agar media. Use at minimum time and power setting position. Check unit for quality performance and compare to standardized melting procedures by performing comparison study.

s. Micropipettors:[23,24] Micropipettors are high-precision laboratory instruments for dispensing extremely small volumes. Use with precision tips supplied by manufacturer and securely fix to the nose cone to ensure a tight seal. Maintain consistency in pipetting action such as pre-wetting, release of plunger, and tip immersion depth (between 1 and 3 mm). Operate only in a vertical position and have both sample and equipment at equivalent temperature. Avoid over-dialing the recommended range of the micropipettor to avoid mechanical damage. Follow manufacturer's instructions to perform routine maintenance such as cleaning, seal replacement, and re-lubrication and have each pipet operator check accuracy and precision of volume dispensed at a frequency related to its usage, e.g., quarterly or sooner if pipettor is showing overt signs that it is inaccurate or if tip manufacturer changes. Calibrate at least annually either in-house or send to the manufacturer. Note that pipettor is calibrated with water; changes in liquid viscosity can result in a change in volume dispensed. Maintain documentation.

5. Laboratory Supplies

Retain records and manufacturer certificates of analysis, purity, or tolerance level, if supplied, for all laboratory supplies.

a. Glassware: The term "glassware" refers to both borosilicate glass and heat-resistant plastic materials. Volumetric glassware, pipets, graduated cylinders, and beakers with calibration marks should be accurate to the specified volumetric tolerances. See established standards[25] for calibration of laboratory volumetric apparatus. Volumetric glassware is generally either Class A or Class B (undesignated). Class A is the more precise volumetric glassware. Determine tolerance once per lot or at a set percentage, e.g., 1 to 4%.

Before each use, examine glassware and discard items with chipped edges or etched inner surfaces. Particularly examine screw-capped dilution bottles and flasks for chipped edges that could leak and contaminate the sample, analyst, and area. Inspect glassware after washing for excessive water beading, stains, and cloudiness and rewash if necessary. Replace glassware with excessive writing if markings cannot be removed. Either cover glassware or store glassware with its bottom up to prevent dust from settling inside it.

Perform the following tests for clean glassware:

1) pH check—Because some cleaning solutions are difficult to remove completely, spot check batches of clean glassware for pH reaction, especially if soaked in alkali or acid. To test clean glassware for an alkaline or acid residue add a few drops of 0.04% bromthymol blue (BTB) or other pH indicator and observe the color reaction. BTB should be blue-green (in the acceptable neutral range).

To prepare 0.04% bromthymol blue indicator solution, add 16 mL 0.01*N* NaOH to 0.1 g BTB and dilute to 250 mL with reagent water.

2) Test for inhibitory residues on glassware and plasticware—Certain wetting agents or detergents used in washing glassware may contain bacteriostatic, inhibitory, or stimulatory substances that require 6 to 12 rinses to remove all traces and insure freedom from residual bacteriostatic action. As long as the bromthymol blue test is being done on each batch of glassware, run this test before initial use of a washing compound and whenever a new washing procedure is used. If the bromthymol blue test is not done consistently also run the toxicity test on an annual basis. Record results. Although the following procedure describes testing of petri dishes for inhibitory residue, it is applicable to other glass or plasticware.

a) Procedure—Wash and rinse six petri dishes according to usual laboratory practice and designate as Group A. Wash six petri dishes as above, rinse 12 times with successive portions of reagent water, and designate as Group B. Rinse six petri dishes with detergent wash water (in use concentration), air-dry without further rinsing, and designate as Group C.

Sterilize dishes in Groups A, B, and C by the usual procedure. For presterilized plasticware, set up six plastic petri dishes and designate as Group D.

Prepare and sterilize 200 mL plate count agar and hold in a 44 to 46°C water bath.

Prepare a culture of *Enterobacter aerogenes* known to contain 50 to 150 colony-forming units/mL. Preliminary testing may be necessary to achieve this count range. Inoculate three dishes from each test group with 0.1 mL and the other three dishes from each group with 1 mL culture.

Follow the heterotrophic plate count method (Section 9215B) for all inoculated plates and incubate at 35°C for 48 h. Count plates with 30 to 300 colonies and record results as CFU/mL.

b) Interpretation of results—Difference in averaged counts on plates in Groups A through D should be less than 15% if there are no toxic or inhibitory effects.

Differences in averaged counts of less than 15% between Groups A and B and greater than 15% between Groups A and C indicate that the cleaning detergent has inhibitory properties that are eliminated during routine washing. Differences between B and D greater than 15% indicate an inhibitory residue is present and plasticware must not be used for microbiological analyses. A new washing procedure, equipment, or detergent supply may be needed.

b. Utensils and containers for media preparation: Use utensils and containers of borosilicate glass, stainless steel, aluminum, or other corrosion-resistant material (see Section 9030). Do not use copper utensils.

c. Dilution water bottles: Use bottles scribed at 99 mL and made of nonreactive borosilicate glass or plastic with screw caps equipped with inert liners. Clean before use. Bottles prefilled with dilution water available commercially are acceptable. Before use of each batch or lot conduct sterility test, check one per lot or a set percentage, e.g., 1 to 4%, for pH and volume (99 ± 2 mL), and examine dilution water bottles for a precipitate; discard if present. Reclean bottles with acid if necessary, and remake the dilution water. If precipitate repeats, procure a different source of bottles. Recheck volume at regular intervals to determine volume loss rate under holding conditions. Discard by expiration date.

d. Sample bottles: Use wide-mouth nonreactive borosilicate glass or plastic bottles with screw caps (which should contain liners) or commercially prepared sterilized plastic bags with ties of sufficient size to collect the needed sample and still have an adequate headspace to allow shaking of the sample in the container. Clean and sterilize bottles before use and, depending upon use, add sufficient dechlorination agent to neutralize residual chlorine (9060A.2). Minimally test for sterility one sample bottle per batch sterilized in the laboratory or one sample bottle per lot purchased as presterilized, or at a set percentage such as 1 to 4%. Document results. Resterilize entire batch or lot if growth occurs. Check and record efficacy of dechlorination agent, one per batch or lot. Also, check accuracy of 100-mL mark (if present) and auto-fluorescence properties (if used for fluorescence testing), one per lot. Record results.

e. Multi-well trays† *and sealers:* When used for growth studies, check sterility of multi-well trays one per lot by aseptically adding 100 mL of tryptic soy broth or other non-selective medium, seal, and incubate at 35 ± 0.5°C for up to 48 h. No growth indicates sterility. Note that if the wells become very turbid (indicating nonsterile condition), there could be gas production and concomitant blowout between wells.

Evaluate sealing performance of heat sealer unit monthly by adding one to two drops of a food-color dye to 100 mL deionized water sample, run through sealer, and visually check each well for leakage. Perform cleaning and preventive maintenance on sealer annually or more frequently if needed.

Microtiter plates are used in a variety of analytical procedures, e.g., DNA hybridization and immunoassay studies, and may contain >96 wells. The laboratory should examine the tray wells for consistency and run appropriate controls. The laboratory may need to detoxify the plates if their use requires this.

f. Reagent-grade water: Use reagent-grade water for preparation of solutions and media and for final rinsing of glassware. The water must be proven to be free from inhibitory and bactericidal substance. The quality of water obtainable from a water purification system differs with the system used and its maintenance. See ¶s 4*d* and *e* above. Recommended limits for reagent water quality for the microbiology laboratory are given in Table 9020:II. If these limits are not met, investigate and correct or change water source. Although pH measurement of reagent water is characterized by drift, extreme readings are indicative of chemical contamination.

1) Test for bacteriological quality—This test, also known as the water suitability test, is based on the growth of *Enterobacter aerogenes* in a chemically defined minimal-growth medium. The presence of a toxic agent or a growth-promoting substance will alter the 24-h population by an increase or decrease of 20% or more when compared to a control. Perform the test at least annually, when the source of reagent water is changed, and when an analytical problem occurs. This bacteriological quality test is not needed for Type II water or better, as defined in *Standard Methods* (18th and 19th Editions), Section 1080C, or medium-quality water or better, as defined in *Standard Methods* (20th,

† For example, Quanti-Tray® or Quanti-Tray®/2000, available from IDEXX Laboratories, Inc., Westbrook, ME, 04092, or equivalent.

TABLE 9020:II. QUALITY OF REAGENT WATER USED IN MICROBIOLOGY TESTING

Test	Monitoring Frequency	Maximum Acceptable Limit
Chemical tests:		
Conductivity	Monthly*	<2 μmhos/cm (μmsiemens/cm) at 25°C
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
Total chlorine residual	Monthly or with each use	<0.1 mg/L
Bacteriological tests:		
Heterotrophic plate count (See Section 9215)	Monthly	$<$ 500 CFU/mL
Use test [(see 5*f*2)]	For a new source	Student's $t \leq 2.78$
Water quality test [see 5*f*1)]‡	Annually	0.8–3.0 ratio

* Monthly, if meter is in-line or has a resistivity indicator light; otherwise with each new batch of reagent water.
† Or more frequently if there is a problem.
‡ This bacteriological quality test is not needed for Type II water or better, as defined in *Standard Methods* (18th and 19th Editions), Section 1080C, or medium-quality water or better, as defined in *Standard Methods* (20th, 21st, and Online Editions), Section 1080C.

21st, and Online Editions), Section 1080C. Test to ensure continued quality of this water to meet these alternative standards.

The test is complex, requires skill and experience, and is not easily done on an infrequent basis. It requires work over 4 d, an ultrapure water from an independent source as a control, high-purity reagents, and extreme cleanliness of culture flasks, petri dishes, test tubes, pipets, and other equipment.

a) Apparatus and material—Use borosilicate glassware for all steps, although presterilized plastic petri dishes may be used in plating steps. Rinse it in water freshly redistilled from a glass still and then sterilize it with dry heat; steam sterilization will recontaminate these specially cleaned items. Test sensitivity and reproducibility depend in part on cleanliness of sample containers, flask, tubes, and pipets. It often is convenient to set aside new glassware for exclusive use in this test. Use any strain of coliform with IMViC type − − + + (*E. aerogenes*) obtained from an ambient water or wastewater sample or reference culture.

b) Reagents—Use only reagents and chemicals of ACS grade. Test sensitivity is controlled in part by the reagent purity. Prepare reagents in water freshly redistilled from a glass still as follows:

Sodium citrate solution: Dissolve 0.29 g sodium citrate, $Na_3C_6H_6O_7 \cdot 2H_2O$, in 500 mL water.

Ammonium sulfate solution: Dissolve 0.26 g $(NH_4)_2SO_4$ in 500 mL water.

Salt-mixture solution: Dissolve 0.26 g magnesium sulfate, $MgSO_4 \cdot 7H_2O$; 0.17 g calcium chloride, $CaCl_2 \cdot 2H_2O$; 0.23 g ferrous sulfate, $FeSO_4 \cdot 7H_2O$; and 2.50 g sodium chloride, NaCl, in 500 mL water.

Phosphate buffer solution/dilution water: Dilute stock phosphate buffer solution (9050C.1*a*) 1:25 in water.

Boil all reagent solutions 1 to 2 min to kill vegetative cells. Store solutions in sterilized glass-stoppered bottles in the dark at 5°C for up to several months provided that they are tested for sterility before each use. Because the salt-mixture solution will develop a slight turbidity within 3 to 5 d as the ferrous salt converts to the ferric state, prepare the salt-mixture solution without $FeSO_4$ for long-term storage. To use the mixture, add an appropriate amount of freshly prepared and freshly boiled iron salt. Discard solutions with a heavy turbidity and prepare a new solution. Discard if solution becomes turbid.

c) Samples—To prepare test samples collect 150 to 200 mL laboratory reagent water and control (redistilled) water in sterile borosilicate glass flasks and boil for 1 to 2 min. Avoid longer boiling to prevent chemical changes.

d) Procedure—Label five flasks or tubes, A, B, C, D, and E. Add water samples, media reagents, and redistilled water to each

TABLE 9020:III. REAGENT ADDITIONS FOR WATER QUALITY TEST

	Control Test *mL*		Optional Tests *mL*		
Media Reagents	Control A	Test Water B	Carbon/Nitrogen Available C	Nitrogen Source D	Carbon Source E
Sodium citrate solution	2.5	2.5	—	2.5	—
Ammonium sulfate solution	2.5	2.5	—	—	2.5
Salt-mixture solution	2.5	2.5	2.5	2.5	2.5
Phosphate buffer (7.3 ± 0.1)	1.5	1.5	1.5	1.5	1.5
Unknown water	—	21.0	21.0	21.0	21.0
Redistilled water	21.0	—	5.0	2.5	2.5
Total volume	30.0	30.0	30.0	30.0	30.0

flask as indicated in Table 9020:III. Add a suspension of *E. aerogenes* (IMViC type − − + +) of such density that each flask will contain 30 to 80 cells/mL, prepared as directed below. Cell densities below this range result in inconsistent ratios while densities above 100 cells/mL result in decreased sensitivity to nutrients in the test water.

e) Preparation of bacterial suspension—On the day before making the distilled-water suitability test, inoculate a strain of *E. aerogenes* onto a nutrient agar slant with a slope approximately 6.3 cm long contained in a 125- × 16-mm screw-cap tube. Streak entire agar surface to develop a continuous-growth film and incubate 18 to 24 h at 35°C.

f) Harvesting of viable cells—Pipet 1 to 2 mL sterile dilution water from a 99-mL water blank onto the 18- to 24-h culture. Emulsify growth on slant by vibrating; then pipet suspension back into original 99-mL water blank.

g) Dilution of bacterial suspension—Make a 1:100 dilution of original bottle into a second water blank, a further 1:100 dilution of second bottle into a third water blank, and a 1:10 dilution of a third bottle into a fourth water blank, shaking vigorously after each transfer. Pipet 1.0 mL of the fourth dilution ($1{:}10^5$) into each of Flasks A, B, C, D, and E. This procedure should produce a final dilution of the organisms to a range of 30 to 80 viable cells per milliliter of test solution.

h) Verification of bacterial density—Variations among strains of the same organism, different organisms, media, and surface area of agar slopes possibly will necessitate adjustment of the dilution procedure to arrive at a specific density range between 30 to 80 viable cells. To establish the growth range numerically for a specific organism and medium, make a series of plate counts from the third dilution to determine bacterial density. Choose proper volume from this third dilution, which when diluted by the 30 mL in Flasks A, B, C, D, and E, will contain 30 to 80 viable cells/mL. If the procedures are standardized as to slant surface area and laboratory technique, it is possible to reproduce results on repeated experiments with the same strain of microorganism.

i) Procedural difficulties—Problems in this method may be due to: storage of test water sample in soft-glass containers or in glass containers without liners for metal caps; use of chemicals in reagent preparation not of analytical-reagent grade or not of recent manufacture; contamination of reagent by distilled water with a bacterial background (to avoid this, make a heterotrophic plate count on all media and reagents before starting the suitability test, as a check on stock solution contamination); failure to obtain bacterial density or incorrect choice of dilution used to obtain 24-h plate count; delay in pouring plates; and prolongation of incubation time beyond 26-h limit, resulting in desensitized growth response.

j) Calculation—For growth-inhibiting substances:

$$\text{Ratio} = \frac{\text{colony count/mL, Flask B}}{\text{colony count/mL, Flask A}}$$

A ratio of 0.8 to 1.2 (inclusive) shows no toxic substances; a ratio of less than 0.8 shows growth-inhibiting substances in the water sample. For nitrogen and carbon sources that promote growth:

$$\text{Ratio} = \frac{\text{colony count/mL, Flask C}}{\text{colony count/mL, Flask A}}$$

For nitrogen sources that promote growth:

$$\text{Ratio} = \frac{\text{colony count/mL, Flask D}}{\text{colony count/mL, Flask A}}$$

For carbon sources that promote bacterial growth:

$$\text{Ratio} = \frac{\text{colony count/mL, Flask E}}{\text{colony count/mL, Flask A}}$$

Do not calculate the last three ratios when the first ratio indicates a toxic reaction. For these ratios a value above 1.2 indicates an available source for bacterial growth.

k) Interpretation of results—The colony count from Flask A after 20 to 24 h at 35°C will depend on number of organisms initially planted in Flask A and strain of *E. aerogenes* used. For this reason, run the control, Flask A, for each individual series of tests. However, for a given strain of *E. aerogenes* under identical environmental conditions, the terminal count should be reasonably constant when the initial plant is the same. The difference in initial plant of 30 to 80 will be about threefold larger for the 80 organisms initially inoculated in Flask A, provided that the growth rate remains constant. Thus, it is essential that initial colony counts on Flasks A and B be approximately equal.

When the ratio exceeds 1.2, assume that growth-stimulating substances are present. However, this procedure is extremely sensitive and ratios up to 3.0 have little significance in actual practice. Therefore, if the ratio is between 1.2 and 3.0, do not make Tests C, D, and E, except in special circumstances.

Usually Flask C will be very low and Flasks D and E will have a ratio of less than 1.2 when the ratio of Flask B to Flask A is between 0.8 and 1.2. Limiting growth factors in Flask A are nitrogen and organic carbon. An extremely large amount of ammonia nitrogen with no organic carbon could increase the ratio in Flask D above 1.2, or absence of nitrogen with high carbon concentration could give ratios above 1.2 in Flask E, with a B:A ratio between 0.8 and 1.2.

A ratio below 0.8 suggests that the water contains toxic substances, and this ratio includes all allowable tolerances. As indicated in the preceding paragraph, the ratio could go as high as 3.0 from 1.2 without any undesirable consequences.

Specific corrective measures cannot be recommended for every instance of defective distillation apparatus. However, make a careful inspection of the distillation equipment and review production and handling of distilled water to help locate and correct the cause of difficulty.

Feedwater to a still often is passed through a deionizing column and a carbon filter. If these columns are well maintained, most inorganic and organic contaminants will be removed. If maintenance is poor, input water may be degraded to a quality lower than that of raw tap water.

The best distillation system is made of quartz or high-silica-content borosilicate glass with special thermal endurance. Tin-lined stills are not recommended. For connecting plumbing, use stainless steel, borosilicate glass, or special plastic pipes made of polyvinyl chloride (PVC). Protect storage reservoirs from dust.

l) Test sensitivity—Taking copper as one relative measurement of distilled water toxicity, maximum test sensitivity is 0.05 mg Cu/L in a distilled water sample.

2) Use test for evaluation of reagent water, media, and membranes—When a new source of reagent-grade water or a new lot of culture medium or membrane filters is used, checking product equivalency by testing the current lot in use (reference lot) against the test lot using reference culture is recommended. It is not possible always to conduct the use test on new reagent-grade water sources, because the previous system may no longer be available.

a) Procedure—Use a single batch of control water (redistilled or distilled water polished by deionization), glassware, membrane filters, or other needed materials to control all variables except the one factor under study. Perform replicate pour or spread plate or membrane filter plate tests on reference lot and test lot, according to procedures in Sections 9215 and 9222. At a minimum, make single analyses on five different water samples positive for the target organism or culture controls of known density. Replicate analyses and additional samples can be tested to increase the sensitivity of detecting differences between reference and test lots.

When conducting the use test on reagent water, perform the quantitative bacterial tests in parallel using known high-quality water as control water. Prepare dilution/rinse water and media with new source of reagent and control water. Test water for all uses (dilution, rinse, media preparation, etc.).

b) Counting and calculations—After incubation, compare bacterial colonies from the two lots for size and appearance. If colonies on the test lot plates are atypical or noticeably smaller than colonies on the reference lot plates, record the evidence of inhibition or other problem, regardless of count differences. Count plates and calculate the individual count per 1 mL or per 100 mL. Transform the count to logarithms and enter the log-transformed results for the two lots in parallel columns. Calculate the difference, d, between the two transformed results for each sample, including the + or − sign, the mean, $\bar{d}$, and the standard deviation s_d of these differences (see Section 1010B).

Calculate Student's t statistic, using the number of samples as n:

$$t = \frac{\bar{d}}{s_d/\sqrt{n}}$$

These calculations may be made with various statistical software packages available for personal computers.

c) Interpretation—Use the critical t value from a Student's t table for comparison against the calculated value. At the 0.05 significance level this value is 2.78 for five samples (four degrees of freedom). If the calculated t value does not exceed 2.78, the lots do not produce significantly different results and the test lot is acceptable. If the calculated t value exceeds 2.78, the lots produce significantly different results and the test lot is unacceptable. Software packages are available for use on personal computers for these calculations.

If the colonies are atypical or noticeably smaller on the test lot or the Student's t exceeds 2.78, review test conditions, repeat the test, and/or reject the test lot and obtain another one.

g. Reagents:[26] Because reagents are an integral part of microbiological analyses, their quality must be assured. Use only chemicals of ACS or equivalent grade because impurities can inhibit bacterial growth, provide nutrients, or fail to produce the desired reaction. Maintain any Material Safety Data Sheets (MSDS) provided with reagents or standards and have them available to all personnel.

Date chemicals and reagents when received and when first opened for use. Maintain records for receipt, expiration, and subsequent preparation. During preparation bring all reagents to room temperature, make reagents to volume in volumetric flasks, and transfer for storage to good-quality inert plastic or borosilicate glass bottles with borosilicate, polyethylene, or other plastic stoppers or caps. Label prepared reagents with name, concentration, date prepared, name of preparer, and expiration date if known. Store under proper conditions and discard by expiration date. Include positive and negative control cultures with each series of cultural or biochemical tests.

h. Dyes and stains: In microbiological analyses, organic chemicals are used as selective agents (e.g., brilliant green), indicators (e.g., phenol red), and stains (e.g., Gram stain). Dyes from commercial suppliers vary from lot to lot in percent dye, dye complex, insolubles, and inert materials. Because dyes for microbiology must be of strength and stability to produce correct reactions, use only dyes certified by the Biological Stain Commission. Check bacteriological stains before use with at least one positive and one negative control culture and record results. For fluorescent stains, test for positive and negative reactivity each day of use.

i. Membrane filters and pads: The quality and performance of membrane filters vary with the manufacturer, type, brand, and lot as a result of differences in manufacturing methods, materials, quality control, storage conditions, and application.[27]

1) Specifications—Manufacturers of membrane filters and pads for water analyses must meet standard specifications for retention, recovery, extractables, and flow-rate characteristics.[28] Some manufacturers provide information beyond that required by specifications and certify that their membranes are satisfactory for water analysis. They report retention, pore size, flow rate, sterility, pH, percent recovery, and limits for specific inorganic and organic chemical extractables. Although the standard membrane filter evaluation tests were developed for the manufacturers, a laboratory can conduct its own tests, if desired.

2) Use test—Each new lot of membrane filters should perform satisfactorily in the use test to assure that it does not yield low recoveries, poor differentiation, or malformation of colonies due to toxicity, chemical composition, or structural defects. For procedure, see ¶ *f*2) above.

3) Standardized tests—To maintain quality control inspect each lot of membranes before use and during testing to insure they are round and pliable. Critically check for brittleness if lot is held for one or more years. Discard lots showing brittleness. Record lot number and date received to maintain record of length of time in laboratory. Confirm sterility by absence of growth when a membrane filter is placed on a pad saturated with tryptone glucose extract broth (or equivalent non-selective broth or agar) and incubated at 35 ± 0.5°C for 24 h or by running a sterility control for each analytical test run.

After sample incubation, colonies should be well-developed with appropriate color and shape as defined by the test procedure. The gridline ink should not channel growth along the ink line nor restrict colony development. Colonies should be distrib-

uted evenly across the membrane surface. Reject membrane lot if these criteria are not met and inform manufacturer.

j. Culture media: Because cultural methods depend on properly prepared media, use the best available materials and consistent techniques in media preparation, storage, and application, and prepare the correct medium for the intended application. For control of quality, use commercially prepared media whenever available but note that such media may vary in quality among manufacturers and even from lot to lot from the same manufacturer. For this reason, a use test is recommended to confirm that the new batch of media is equivalent to the older media. It is also the responsibility of the laboratory to ensure that the microbiological media meet growth promotion requirements by running both positive and negative culture controls having an estimated density on both the old media lot and the new media lot. Maintain any MSDS.

Order media in quantities to last no longer than 1 year, preferably no longer than 6 months after opening. Order commercially prepared media in quantities such that it is used by the manufacturer's expiration date. Use media on a first-in, first-out basis. When practical, order media in small quantities, e.g., 0.25-lb or 125-g, rather than 1-lb or 500-g bottles, to keep the supply sealed as long as possible. Record type, amount, and appearance of media received, lot number, expiration date, and dates received and opened in a logbook or computer file; also place date of expiration and date opened on container. Check inventory quarterly for reordering.

Store all media under controlled conditions to ensure quality until expiration date is reached. Store dehydrated media in a tightly closed container in a cool (15 to 25°C), dry, controlled-temperature room or desiccator away from direct sunlight. Discard media that cake, discolor, or show other signs of deterioration. Discard unused media by manufacturer's expiration date. A conservative time limit for unopened bottles is 2 years at room temperature. Use of expired media is not recommended.

Compare growth recovery of newly purchased lots of media against proven lots, using reference cultures, preferably, or recent pure-culture isolates, or natural samples [see ¶ *f*2) above], because lot-to-lot variability may occur.

Use opened bottles of media within 6 months. Dehydrated media are hygroscopic; avoid excessive humidity. Close bottles as tightly as possible, immediately after use. If caking or discoloration of media occurs, discard media. Store opened bottles in desiccator if available.

1) Preparation of media—Prepare media in clean containers that are at least twice the volume of the medium being prepared. Prepare media using reagent-grade water. Measure water volumes and media with graduates or pipets conforming to NIST and APHA standards, respectively. Do not use blow-out pipets. Use TD (to deliver) pipets. Stir media, particularly agars, while heating. Avoid scorching or boil-over by using a boiling water bath for small batches of media and by continually attending to larger volumes heated on a hot plate or gas burner. Preferably use hot plate-magnetic stirrer combinations. Label and date prepared media.

Check and record pH of a portion of each medium after sterilization. This is the actual pH required for adequate growth. Adjustment of pH will seldom be necessary when commercially available media are used. If needed, make minor adjustments to the pH specified in the formulation with filter-sterilized 1*N* NaOH or 1*N* HCl solutions. If the pH difference is larger than 0.5 units, discard the batch and check preparation instructions and pH of reagent water to resolve the problem. If medium is known as requiring pH adjustment, adjust pH appropriately prior to sterilization and record final pH. Incorrect pH values may be due to reagent water quality, deterioration of medium, or improper preparation. Review instructions for preparation and check water pH. If water pH is unsatisfactory, prepare a new batch of medium using water from a new source (see ¶s 4*d* and *e*). If water is satisfactory, remake medium and check pH; if pH is again incorrect, prepare medium using a different lot or source. Certain specific isolation media prepared with organic or fatty acids will demonstrate marked changes in pH following sterilization.

Document preparation activities such as name of medium, volume produced, format, final pH, date prepared, and name of preparer. Record pH problems in the media record book and inform the manufacturer if the medium is indicated as the source of error. Examine prepared media for unusual color, darkening, or precipitation, and record observations. Consider variations of sterilization time and temperature as possible causes for problems. If any of the above occurs, discard the medium.

2) Sterilization—Sterilize media at 121°C maximum for minimum time specified. Follow manufacturer's directions for sterilization of specific media. The required exposure time varies with form and type of material, type of medium, presence of carbohydrates, and volume. Table 9020:IV gives guidelines for typical items. Do not expose media containing carbohydrates to the elevated temperatures for more than 45 min. Exposure time is defined as the period from initial exposure to heat to removal from the autoclave. Overheating of media can result in nutrient degradation. Maintain printout records.

TABLE 9020:IV. TIME AND TEMPERATURE FOR AUTOCLAVE STERILIZATION*

Material	Time at 121°C *min*
Membrane filters and pads	10
Carbohydrate-containing media (lauryl tryptose, BGB broth, etc.)	12–15†
Contaminated materials and discarded cultures	30
Membrane filter assemblies (wrapped), sample collection bottles (empty)	15
Buffered dilution water, 99 mL in screw-cap bottle	15
Rinse water, volume > 100 mL	Adjust for volume

* Except for media, times are guidelines.
† Certain media may require different sterilization conditions.

Remove sterilized media from autoclave as soon as chamber pressure reaches zero or, if a fully automatic model is used, as soon as the door opens. Use extreme care to avoid boiling over due to superheated liquids. Do not reautoclave media.

Sterilize heat-sensitive solutions or media by filtration through a 0.2-μm-pore-diam filter in a sterile filtration and receiving apparatus. Filter and dispense medium in a laminar-flow hood or laminar-flow safety cabinet if available. Sterilize glassware (pipets, petri dishes, sample bottles) in an autoclave or in a hot-air

TABLE 9020:V. HOLDING TIMES FOR PREPARED MEDIA

Medium	Holding Time
Broth in screw-cap flasks*	96 h
Poured agar in plates with tight-fitting covers*	2 weeks
Agar or broth in loose-cap tubes*	2 weeks
Agar or broth in tightly closed screw-cap tubes†	3 months
Poured agar plates with loose-fitting covers in sealed plastic bags*	2 weeks
Large volume of agar in tightly closed screw-cap flask or bottle*	3 months

* Hold under refrigerated conditions 2–8°C.
† Hold at <30°C.

sterilizing oven (170 ±10°C for a minimum of 2 h). Sterilize equipment, supplies, and other solid or dry materials that are heat-sensitive, by exposing to ethylene oxide in a gas sterilizer. Use commercially available spore strips or suspensions to check dry heat and ethylene oxide sterilization.

3) Use of agars and broths—Temper melted agars in a water bath at <50°C, preferably 44 to 46°C, until used but not for longer than 3 h. To monitor agar temperature, expose a bottle of water or medium to the same heating and cooling conditions as the agar. Insert a thermometer in the monitoring bottle to determine when the temperature is suitable for use in pour plates. Add heat-sensitive solutions, e.g., antibiotics, to tempered agar. Preparation of media at least 2 d before tests is recommended to allow sufficient time for sterility and positive and negative control culture testing to be performed and read. If agar medium is not poured but allowed to solidify to use later, re-melt agar media in boiling water, flowing steam, or low-wattage microwave, use, and then discard any remainder. Agar may be remelted only once.

The volume dispensed will change relative to the size of the petri dish and its intended use. Invert plates as soon as poured medium has solidified.

Handle tubes of sterile fermentation media carefully to avoid entrapping air in durham (inner) tubes, thereby producing false positive reactions. Examine freshly prepared tubes to determine that gas bubbles are absent in the durham tubes.

4) Storage of media—Prepare media in amounts that will be used within holding time limits given in Table 9020:V. Fresh medium is required to ensure proper isolation of the target microorganisms, especially for bacteria stressed or injured through the disinfection process.

For prepared ready-to-use media with a manufacturer's expiration date greater than that noted in the table, have the manufacturer supply evidence of media quality for that extended period of time. Verify usability weekly by testing recoveries with known densities of culture controls that will also meet QC check requirements.

Control of moisture content is important because recovery and selectivity may be altered with prolonged storage. When media are used for research purposes, establish appropriate media expiration dates and document results. Protect laboratory-prepared and purchased-prepared media containing dyes from light; if color changes occur, discard the media. Refrigerate poured agar plates not used on the day of preparation. To prevent dehydration, seal agar plates in plastic bags or other sealed container if they will be held more than 2 d. Store plates inverted so as to prevent condensation from falling on medium. In cases where condensate has formed, consider placing plates briefly in a 35-37°C incubator. For media in test tubes tighten caps before storage. Weigh plates or mark liquid level in several tubes (10% of each batch) after sterilization and monitor for loss of liquid by weight or volume stored for more than 2 weeks. If loss is 10% or more, discard the batch. Discard all petri dishes with solid media that have been stored for longer than 2 weeks; discard them earlier if they are dried out, e.g., wrinkled, cracked, or pitted.

If media are refrigerated, bring to room temperature before use and reject the batch if growth or false positive responses are present. Prepared sterile broths and agars available from commercial sources may offer advantages when analyses are done intermittently, when staff is not available for preparation work, or when cost can be balanced against other factors of laboratory operation. Check performance of these media as described in ¶s 5) through 7) below.

5) Use test—Subject both laboratory-prepared and purchased media to the use test. For procedure, see ¶ *f*2) above.

6) Quality control of laboratory-prepared media—Maintain in a bound book a complete record of each batch of laboratory-prepared medium with date and name of preparer, name and lot number of medium, amount of medium weighed, volume of medium prepared, sterilization time and temperature, pH adjustments needed, final pH, and preparations of labile components. Compare quantitative recoveries of new lots with previously acceptable ones [¶ 5) above] with the microorganism of concern. Include media sterility checks and positive and negative control culture checks to determine specificity on all media as described below. Culture controls can be used to detect growth promotion and medium selectivity, and to monitor analyst technique.

A good laboratory practice is to periodically challenge prepared media with low numbers of an appropriate microorganism. Growth would be affected by media quality and media preparation, sterilization, storage time, and storage conditions.

7) Quality control of purchased-prepared media—Shipment of ready-to-use media should not invalidate any of the media holding times or conditions described above. The manufacturer should supply validation information if shipment conditions are otherwise. Record dates of receipt and expiration, lot number, and then measure and record medium. Store as directed by manufacturer and discard by expiration date. Comparison of quantitative recoveries, as directed in ¶ 5) above, is recommended. Test each new lot for sterility and with positive and negative control culture checks. For purchased-prepared media which have a longer shelf-life than those prepared in the laboratory, perform these tests more frequently.

6. Standard Operating Procedures (SOPs)[29-31]

Generic and specific SOPs are the operational backbone of an analytical laboratory and are designed to prevent deviations resulting from a misinterpretation of a process or method. Each specific SOP describes in a step-by-step fashion the details of a task or procedure performed on a routine basis, tailored to the laboratory's own equipment, instrumentation, and sample types. These laboratory operations include preparation of reagents,

reagent water, standards, culture media, proper use of balances, sterilization practices, dishwashing procedures, and disposal of contaminated material, as well as methods of sampling, sample analysis, chain of custody, record-keeping, and procedures for quality control. Simple citation of a published analytical method is not a SOP, although that information can be consolidated into the laboratory's own SOP.

SOPs are unique to the laboratory and are written by the person who is doing the work and signed as approved by the supervisor, with the effective date indicated. Follow the SOPs as written and keep them current through routine reviews and accessible to all necessary personnel. When changes are needed, document them and have the SOP re-signed. Retain outdated SOPs in files for possible future reference. Consistent use of SOPs helps to ensure uniform operations. They also provide a solid training tool and a means for determining competency when conducting an assessment.

7. Sampling

The laboratory generally is not involved with actual sample collection but personnel need to be knowledgeable about the different aspects of the sample collection process.[30]

a. Planning: Microbiologists should participate in the planning of monitoring programs that will include microbial analyses. They can provide valuable expertise on the selection of sampling sites, sampling depth, number of samples and analyses needed, workload, and supplies. For natural waters, knowledge of the probable microbial densities, and the impact of season, weather, tide and wind patterns, known sources of pollution, and other variables, are needed to formulate the most effective sampling plan. In addition, the microbiologist can indicate when replicate samples will be needed, e.g., when a new water source is being tested or a sample is being collected from a different area of the same locale. For compliance monitoring, the sampling plan must be approved by the state.

b. Methods: Sampling plans must be specific for each sampling site and based on appropriate statistical sampling designs. Prior sampling guidance can be only general in nature, addressing the factors that must be considered for each site. Sampling SOPs describe sampling equipment, techniques, frequency, holding times and conditions, safety rules, etc., that will be used under different conditions for different sites to ensure sample integrity and representativeness. From the information in these SOPs sampling plans can be drawn up.

c. Sample acceptance: The laboratory must determine whether sample integrity, holding conditions and time, and accompanying documentation are acceptable for the intended use of the resulting data.

8. Analytical Methods

a. Method selection: Media, temperature, time at incubation temperature, and minor variations in techniques are factors that need to be applied consistently for appropriate microbial recovery for qualitative and quantitative determinations. To avoid significant changes in results, microbiological methods must be standardized so that uniform data result from multiple laboratories. Select analytical methods appropriate for the sample type from *Standard Methods* or other sources of standardized methods and ensure that methods have been properly validated in a multi-laboratory study with the sample types of interest. The laboratory should validate any new method or nonstandard method to be used in the laboratory and any method being used for a matrix not specified by the method.

b. Data objectives: Review available methods and determine which best produce data meeting the program's needs for precision, bias, specificity, selectivity, detection limit, and recovery efficiency under actual test conditions. Methods that are rapid, inexpensive, and less labor-intensive are desirable, but not if verification steps are time-consuming or if the data produced will not meet the program's or customer's needs.

c. Internal QC: Written analytical methods contain the required QC checks to assure data quality, such as the use of positive and negative control cultures, sterility method blanks, replicate analyses (precision), and bacterial cultures having a known density level for quantitative methods.

d. Method SOPs: As part of the series of SOPs, provide each analyst with a copy of the analytical procedures written in stepwise fashion exactly as they are to be performed and specific to the sample type, equipment, and instrumentation used in the laboratory.

9. Analytical Quality Control Procedures for Established Methods[6-8,18,32]

a. General quality control procedures:

1) Analyst colony counting variability—For routine performance evaluation, repeat counts on one or more positive samples at least monthly, record results, and compare the counts with those of other analysts testing the same samples. Replicate counts for the same analyst should agree within 5% (within analyst repeatability of counting) and those between analysts should agree within 10% (between analysts reproducibility of counting). If they do not agree, initiate investigation and any necessary corrective action. See 9020B.13*b* for a statistical calculation of data precision.

2) Positive and negative control cultures—Use certified reference cultures. For each lot of medium received, each laboratory prepared batch of medium, and each lot of purchased prepared medium, verify appropriate response by testing with known positive and negative control cultures for the organism(s) under test. Record results. Obtain certified reference cultures from nationally or internationally recognized sources or reference cultures impregnated onto discs or strips from established commercial sources. From reference culture, subculture to develop one or more primary working stocks.[33] Minimize subsequent transfers, i.e., transfer to a fresh medium to promote growth, to ensure that cultures maintain phenotypic and genotypic identity and to reduce potential contamination. Test periodically to ensure viability and performance. For each lot of medium, check analytical procedures by testing with known positive and negative control cultures for the organism(s) under test. Record results. See Table 9020:VI for examples of test cultures. For a wastewater treatment laboratory without the facilities to maintain a pure culture, use single-use culture strips or submit to another laboratory for testing.

3) Duplicate analyses[34,35]—Precision of quantitative analytical results when counting plate colonies is evaluated through replicate analyses. Perform duplicate analyses at least monthly or

TABLE 9020:VI. SUGGESTED CONTROL CULTURES FOR MICROBIOLOGICAL TESTS*

Group	Control Culture	
	Positive	Negative
Total coliforms	*Escherichia coli* *Enterobacter aerogenes*‡ *Klebsiella pneumoniae* (ATTC 4352)	*Staphylococcus aureus*† *Proteus vulgaris*§ *Pseudomonas aeruginosa*†
Fecal coliforms	*Escherichia coli* *Klebsiella pneumoniae* (thermotolerant)‖	*Enterobacter aerogenes*
Escherichia coli	*Escherichia coli* (MUG-positive s strain)	*Enterobacter aerogenes* *Klebsiella pneumoniae* (thermotolerant)
Enterococci#	*Enterococcus faecalis* *Enterococcus faecium*	*Staphylococcus aureus*** *Escherichia coli*††

* Use appropriate ATCC strains.
† *S. aureus, P. aeruginosa* - not lactose fermenter.
‡ *E. aerogenes* — ferments lactose, but is not typically thermotolerant.
§ *P. vulgaris* — not lactose fermenter, uses hydrolyzed lactose, indicating “overcooked” medium.
‖ *K. pneumoniae* — ferments lactose, but does not hydrolyze MUG.
\# Do not use closely related strains from genus *Streptococcus* as a positive control.
** *S. aureus* — sensitive to sodium nalidixic acid medium.
†† *E. coli* — sensitive to sodium azide in medium.

more frequency as needed, e.g., 10% of samples when required by the analytical method or regulations, one sample per test run, or one sample per week for a laboratory that conducts less than 10 tests/week. A test run is defined as an uninterrupted series of analyses. Evaluate and record results. An adequate sample volume is essential. Balance frequency of replicate analyses against the time, effort, and expense incurred. When the laboratory or analyst is first initiating a method or for a method or matrix in which considerable variability in results is expected, greater effort will need to be expended in performing replicate analyses. Replicate analyses of environmental samples can result in widely different counts and can be considered estimates only.

4) Sterility checks—Test media sterility before first use. Incubate minimally one per lot or a set percentage, e.g., 1 to 4%, of laboratory-prepared and ready-to-use medium, broth, or agar, at an appropriate temperature for the amount of time the test would be performed, e.g., 48 h for coliforms, and observe for growth. For enzyme defined substrate tests, check for sterility by adding media packet to 100 mL sterile deionized water and incubating at 35°C for 18 to 24 h. Certain granulated ready-to-use enzyme-substrate media may not be sterile but only free of coliforms; use of nonselective broth could result in growth and turbidity but should not produce a positive reaction.

Check each new batch (or lot, if commercially prepared) of buffered water for sterility before first use by adding 50 mL of the water to 50 mL of a double-strength broth (e.g., tryptic soy, trypticase soy, or tryptose broth). Alternatively, aseptically pass 100 mL or more dilution water through a membrane filter and place filter on nonselective medium. Incubate at 35 ± 0.5°C for 24 h and observe for growth.

Record results. If any contamination is indicated, discard medium, invalidate any data associated with that batch, and check for contamination source. Request immediate resampling.

For membrane filter tests, check the sterility of the entire process by using sterile reagent or dilution water as the sample at the beginning and end of each filtration series of samples and test for growth. With a processing interruption of more than 30 min use new sterilized funnels and repeat sterility test. Record results. If contamination is indicated, invalidate data associated with that batch and check for source. Request immediate resampling and reanalyze.

For multiple-tube and presence-absence procedures, check sterility of prepared media and dilution water as outlined above. If any contamination is indicated, determine the cause and reject analytical data from samples tested with these materials. Request immediate resampling and reanalyze.

For pour plate procedures check sterility by pouring at least one uninoculated plate per batch or lot of media and record results. If any contamination is indicated, determine the cause. Document both problem and corrective action and request resampling.

Laboratories interested in contaminant identification can use either standardized phenotypic testing systems or genotypic procedures.

5) Precision of quantitative methods[33, 34]—Calculate precision of replicate analyses for each different type of sample examined, for example, drinking water, ambient water, or wastewater, according to the following procedure and record results:

Perform duplicate analyses on first 15 positive samples of each matrix type, with each set of duplicates analyzed by a single analyst. If there is more than one analyst, include all analysts regularly running the tests, with each analyst performing an approximately equal number of tests. Record duplicate analyses as D_1 and D_2. Calculate the logarithm of each result. If either of a set of duplicate results is <1, add 1 to both values before calculating the logarithms. Calculate the range (R) for each pair of transformed duplicates as the mean ($\bar{R}$) of these ranges. See sample calculation in Table 9020:VII.

Thereafter, analyze 10% of routine samples in duplicate or one per test run. Transform the duplicates and calculate their range as above. If the range is greater than $3.27\bar{R}$, there is greater than 99% probability that the laboratory variability is excessive; in such a case, discard all analytical results since the last precision

TABLE 9020:VII. CALCULATION OF PRECISION CRITERION

Sample No.	Duplicate Analyses D_1	D_2	Logarithms of Counts L_1	L_2	Range of Logarithms (R_{log}) ($L_1 - L_2$)
1	89	71	1.9494	1.8513	0.0981
2	38	34	1.5798	1.5315	0.0483
3	58	67	1.7634	1.8261	0.0627
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
14	7	6	0.8451	0.7782	0.0669
15	110	121	2.0414	2.0828	0.0414

Calculations:
Σ of $R_{log} = 0.0981 + 0.0483 + 0.0627 + \ldots + 0.0669 + 0.0414 = 0.718\ 89$
$\bar{R} = \frac{\Sigma R_{log}}{n} = \frac{0.71889}{15} = 0.0479$
Precision criterion $= 3.27\ \bar{R} = 3.27\ (0.0479) = 0.1566$

check (see Table 9020:VIII). Identify and resolve the analytical problem before making further analyses.

Update by periodically repeating the procedures using the most recent sets of 15 duplicate results.

10. Verification

Verification is a general process used to determine whether the microbiological analytical method is performing as expected to provide reliable data. If a laboratory finds a low percentage of verification with a certain water supply or matrix, another test method must be chosen. For the most part, the confirmation/verification procedures for drinking water differ from those for other waters because of specific regulatory requirements. The following is a brief summary; further information may be found in the appropriate discussions of the specific microorganism or microbial group.

a. Multiple-tube fermentation (MTF) methods:

1) Total coliform procedure (9221B)

a) Drinking water—Carry tests through confirmed phase only. The Completed Test is not required.

For QC purposes, if normally there are no positive results within a quarter, analyze at least one positive source water sample to confirm that the media and laboratory procedures and equipment produce appropriate responses. For samples with a history of heavy growth without gas in presumptive-phase tubes, carry the tubes through the confirmed phase to check for false negative responses for coliform bacteria. Verify any positives for thermotolerant (fecal) coliforms or *E. coli*.

b) Other water types—Verification can be achieved by performing the completed phase at a frequency established by the laboratory, such as 10% of positive samples, or one sample per test run, or a certain percentage depending on normal laboratory work load. For large laboratories analyzing a significant number of samples daily, 10% of positive samples may result in an unnecessary burden and a lower percentage value may be used.

2) Fecal streptococci procedure—Verification can be performed as outlined in 9230C.5 at a frequency established by the laboratory. Growth of catalase-negative, gram-positive cocci appearing as brownish-black colonies with brown halos on bile esculin agar at 35°C and in brain-heart infusion broth at 45°C verifies the organisms as fecal streptococci. Growth also in 6.5% NaCl broth and in brain-heart infusion broth at 10°C indicates that the streptococci are members of the *Enterococcus* group.

b. Membrane filter methods:

1) Total coliform procedures

a) Drinking water—Swab entire membrane or pick up five typical and five atypical (nonsheen) colonies from positive samples on M-Endo or LES-Endo agar medium and verify as in 9222B.5*f*. Also verify any positives for thermotolerant coliforms. If there are no positive samples, test at least one known positive source water sample quarterly.

b) Other water types—Verify positives monthly by picking at least 10 typical and atypical colonies from a positive water

TABLE 9020:VIII. DAILY CHECKS ON PRECISION OF DUPLICATE COUNTS*

Analyses	Duplicate Analyses D_1	D_2	Logarithms of Counts L_1	L_2†	Range of Logarithms	Acceptance of Range†
8/29	71	65	1.8513	1.8129	0.0384	A
8/30	110	121	2.0414	2.0828	0.0414	A
8/31	73	50	1.8633	1.6990	0.1643	U

* Precision criterion $= (3.27\ \bar{R}) = 0.1566$
†A = acceptable; U = unacceptable.

sample as in 9222B.5*f.* Adjust counts based on percent verification.

c) To determine false negatives, pick representative atypical colonies of different morphological types and verify as in 9222B.5*f.*

2) Thermotolerant (fecal) coliform procedure—Verify positives monthly by picking at least 10 blue colonies from one positive sample using lauryl tryptose broth and EC broth as in 9221E.1*b*. Adjust counts based on percent verification. To determine false negatives, pick representative atypical colonies of different morphological types and verify as in Presumptive Phase, 9221B.

3) *Escherichia coli* procedure

a) Drinking water—Verification is not required.

b) Other water types—Verify one positive sample monthly by picking from well-isolated colonies while taking care not to pick up medium, which can cause a false positive response. Perform the citrate test and the indole test as described in 9225D, or other equivalent identification procedures or systems. Incubate indole test at 44.5°C. *E. coli* are indole-positive and yield no growth on citrate. Adjust counts according to percentage of verification.

c) To determine false negatives, pick representative atypical colonies of different morphological types and verify as in ¶ b) above.

4) Fecal streptococci procedure—Pick to verify monthly at least 10 isolated red colonies from m-Enterococcus agar to brain heart infusion (BHI) media and proceed as described in 9230C. Adjust counts according to percentage of verification.

5) *Enterococci* procedures—Pick to verify monthly at least 10 well-isolated pink to red colonies with black or reddish-brown precipitate from EIA agar. Transfer to BHI media as described in 9230C. Adjust counts according to percentage of verification.

c. Enzyme defined substrate tests:

1) Total coliform test Method 9223

a) Drinking water—Verification is not required.

b) Other water types—No confirmation/verification step is required. Enzyme substrate tests use a defined substrate in which noncoliform bacterial growth is inhibited. The following is a brief description for those who desire to conduct verification testing.

For total coliform analyses aseptically transfer material from a certain percentage (e.g., 5%) of ONPG or CPRG-positive wells and ONPG or CPRG-negative wells to mEndo or Levine EMB or other suitable media. Streak for isolation. Test for lactose fermentation (a number of coliforms can be either slow lactose fermenters or may not ferment lactose at all) or for β-D galactopyranosidase by *o*-nitrophenyl-β-D galactopyranoside (ONPG) test and indophenol cytochrome oxidase (CO) test or organism identification. See 9225D for test descriptions or use other equivalent identification procedures or systems.

2) *E. coli*—For *E. coli* analyses verification, if desired, can be accomplished by aseptically transferring material from a certain percentage (e.g., 5%) MUG-positive and MUG-negative wells to MacConkey or Levine EMB or other suitable media. Streak for isolation. Verify by confirmation of MUG reaction using EC+MUG or NA+MUG media or *E. coli* biochemical identification as described in 9225D or other equivalent identification procedure or system. Adjust counts according to percentage of verification.

3) Enterococci —Verify colonies by selecting 10 typical colonies (positives) and 10 atypical colonies (negative) once per month or 1 typical and 1 atypical colony from 10% of positive samples, whichever is greater.[36]

11. Validation of New or Nonstandard Methods[37-43]

All nonstandard methods, laboratory-developed methods, and standard methods used under different test conditions, e.g., matrix, must be validated by the laboratory before gathering data with these methods. Validation involves establishing and demonstrating that the performance criteria of a method or process provide accurate and reliable data for its intended use. The term "validation" has been applied historically to the field of chemistry. Validation is now applied to microbiology, using the same terms used in chemistry. The main difference is that, where discrete variables are used, i.e., plate counts, different statistics are applied and different probability distributions are used.

For the culture-based microbiologist, validation focuses on the suitability of the test method or process to detect and/or quantify a specific microorganism or group of microorganisms having set characteristics in the matrix of concern. For the culture-independent methods, such as immunoassays and molecular genetic techniques, the same need exists to demonstrate process control and confidence in the reliability of the information. This is essentially a proof of concept.

For standard compliance methods obtain validation data from the manufacturer and/or the regulatory agency. Before a method is adopted by the laboratory, conduct parallel tests with the standard or reference procedure to determine comparability to the stated performance criteria of the standard and its suitability for use. Obtain at least 30 positive data points over the year to allow a statistical determination of equivalence to the established or standard method before replacement with the new method for routine use. This can be called a secondary or cross validation.

For methods in development, such as research methods, establish confidence in the analytical method or process by conducting full intralaboratory validation studies on a statistically significant number of samples to ensure reliability before final determination of usability. Conduct interlaboratory studies (also called collaborative studies) to validate the method for wider use. The following is a brief discussion of microbial method validation and desired quality performance criteria to be ascertained. Review the cited references for further information and for programs involved with microbial method validation.

To determine the effect of matrix on recoveries add a known concentration set at an anticipated ambient level to a field sample collected from the same site as the original. Use commercial‡ or laboratory-prepared suspensions of the target microorganism.

a. Qualitative test methods: Validation of presence or absence (growth versus no-growth) methods involve establishing method performance characteristics in the matrix of choice such as:

1) Accuracy and precision (repeatability and reproducibility)—For qualitative tests, the number of replicates would need to be extremely large to reach a statistical evaluation of comparability. Therefore these data quality indicators generally are not determined.

‡ Bioball™, BTF Pty LTD, Australia, or equivalent.

2) Specificity/selectivity—the ability of the test method or process to preferentially select or distinguish the target organisms from the nontarget species in the matrix of choice under normal laboratory sample analysis conditions, i.e., fitness for use. For qualitative methods growth of the target organism is the indicator. It is determined by verifying all responses, e.g., by microbial identification testing.

3) Detection limit—the lowest microbial density that can be determined under the stated conditions. Determine by using dilutions of reference cultures and measurement of recovery among replicates of each dilution.

4) Robustness—the measure of how well a test method can perform under changing conditions. This test is conducted by the initial developer of the method and is determined by changing variables such as sample holding time or conditions, incubation temperature, medium pH, and incubation time.

5) Repeatability—the degree of agreement between replicate analyses or measurements conducted under the same conditions, e.g., laboratory, technician, and equipment. Use a target microorganism or microbial group density such that at least 75% will be positive, i.e., growth, so that a sufficient number of responses can be detected[44] for either a quantitative or qualitative test. This can serve as one measure of uncertainty.

b. Quantitative test methods: Validation of a method or process concerned with numerical determinations, e.g., count per unit volume, involves ascertaining the method's performance characteristics as noted above, in addition to the following:

1) Accuracy—the degree of agreement, or lack of uncertainty, between the observed and the true value. It is estimated by using known reference cultures at the anticipated range of environmental densities and comparing the test method results to that of the reference or standard method. It is usually expressed as the percentage of recovery.

2) Precision/repeatability—the degree of agreement between replicate analyses or measurements conducted under the same conditions, e.g., laboratory, technician, and equipment. Use a target microorganism or microbial group density such that at least 75% will be positive, so that a sufficient number of responses can be detected[44] for either a quantitative or qualitative test. This can serve as one measure of uncertainty.

3) Precision/reproducibility—the degree of variability when the same method or process is conducted under changed conditions, e.g., more than one analyst following the method or procedure in another area or room in the laboratory and/or using different equipment. This serves as another measure of uncertainty.

4) Recovery/sensitivity—the capability of a test method to recognize or detect the target microorganism or component thereof in the matrix of choice. Determine by analyzing a sufficient number of samples using at least two added suspension levels of the target microorganism or by increasing or decreasing the sample volume or dilution analyzed, followed then by determination of statistical confidence.

5) Detection limit—the lowest microbial density that can be determined. Determine by using dilutions of reference cultures and measurement of recovery among replicates of each dilution.

6) Upper counting limit—the level at which quantitative measurements become unreliable, e.g., due to overcrowding on an agar plate. Determine as above.

7) Range—the interval between the upper and lower detection limits determined as above.

12. Documentation and Recordkeeping

a. QA Plan: The laboratory's QA Plan or Quality Manual documents management's commitment to a QA policy and sets forth the requirements needed to support program objectives. The plan describes overall policies, organization, objectives, and functional responsibilities for achieving the quality goals and specifies the QC activities required to achieve the data representativeness, completeness, comparability, and compatibility. In addition, the QA plan includes the laboratory's implementation plan to ensure maximum coordination and integration of QC activities within the overall program (sampling, analyses, and data handling) and indicates compliance with federal, state, and local regulations and accreditation requirements where applicable.

b. Sampling records: A written SOP for sample handling records the laboratory's procedures for sample collection, acceptance, transfer, storage, analyses, and disposal. The sampling record is most easily kept in a computer file or on a series of printed forms that prompt the user to provide all the necessary information. It is especially critical that this record be exact and complete if there is any chance that litigation may occur. Such record systems are called "chain-of-custody" and may be required by certain federal or state programs to ensure integrity of the samples. Because laboratories do not always know whether analytical results will be used in future litigation, some maintain chain-of-custody on all samples. Details on chain-of-custody are available in Section 1060B and elsewhere.[1] A laboratory system that uniquely identifies samples within the laboratory and that is tied to the field sample number will ensure that samples cannot be confused.

c. Recordkeeping: An acceptable recordkeeping system provides needed information on sample collection and preservation, analytical methods, raw data, calculations through reported results, and a record of persons responsible for sampling, sample acceptance, and analyses. Choose a format agreeable to both the laboratory and the customer (the data user). Use preprinted forms if available. Ensure that all data sheets are signed and dated by the analyst and the supervisor. The preferable record form is a bound and page-numbered notebook, with entries in ink and a single line drawn through any change with the correction, as well as the initials of the correction recorder entered next to it, or in a computer file, e.g., an e-notebook.

Keep records of microbiological analyses for at least 5 years in a secure location. Off-site storage is recommended as backup for all records. Data expected to become part of a legal action must be maintained for a longer period of time. Actual laboratory reports may be kept, or data may be transferred to tabular summaries, provided that the following information is included: date, place, and time of sampling; name of sample collector; identification of sample; date and time of sample receipt; condition and temperature of received sample; dates of sample analysis start and completion; person(s) responsible for performing analysis; analytical method used; the raw data; and the calculated results of analysis. Verify that each result was entered correctly from the bench sheet and initialed by the analyst.

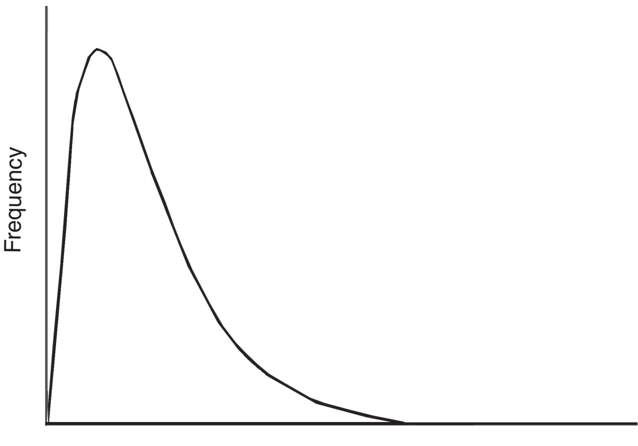

Figure 9020:1. Frequency curve (positively skewed distribution).

When a laboratory information management system (LIMS) is used, verify the software input and output and arithmetic computations. Back up all laboratory data on disk or hardcopy system to meet the customer and laboratory needs for both data management and reporting. Verify data on the printouts. Always back up electronic data by protected tape or disk or hard copy.[44] If the system (hardware or software) is changed, transfer old data to the new system so that it remains retrievable within the specified period of time. Data expected to become part of a legal action must be maintained for a longer period of time; check with the laboratory's legal counsel. Further guidance is available.[45-47]

13. Data Handling

a. Distribution of bacterial populations: In most chemical analyses the distribution of analytical results follows a normal (Gaussian) curve, which has symmetrical distribution of values about the mean (see Section 1010B). Microbial distributions are not necessarily symmetrical and rarely fit a normal distribution curve. Bacterial counts often are characterized as having a skewed distribution because of many low values and a few high ones. These characteristics lead to an arithmetic mean that is considerably higher than the median. The frequency curve of this distribution has a long right tail, such as that shown in Figure 9020:1, and is said to display positive skewness. Natural random variation in the distribution of microorganisms within a sample may be unique to the sample and matrix, and not a function of laboratory performance.[48] In addition, the microbial counts obtained represent colony-forming units (CFUs), which may have resulted from one cell or multiples thereof,[49] resulting in variation in colony count numbers in replicate plating or multiple dilutions.

Application of the more common statistical techniques requires the assumption of symmetry such as the normal distribution. Therefore it usually is necessary to convert skewed data so that a symmetrical distribution resembling the normal distribution results. An approximately normal distribution may be obtained from positively skewed data by converting numbers to their logarithms, as shown in Table 9020:IX. Comparison of the frequency tables for the original data (Table 9020:X) and their logarithms (Table 9020:XI) shows that the logarithms approximate a symmetrical distribution.

TABLE 9020:IX. COLIFORM COUNTS AND THEIR LOGARITHMS

MPN Coliform Count *No./100 mL*	log MPN
11	1.041
27	1.431
36	1.556
48	1.681
80	1.903
85	1.929
120	2.079
130	2.114
136	2.134
161	2.207
317	2.501
601	2.779
760	2.881
1020	3.009
3100	3.491
$\bar{x} = 442$	$\bar{x}_g$ = antilog 2.1825 = 152

b. Central tendency measures of skewed distribution: The best estimate of central tendency of log-normal data is the geometric mean. The term "mean" in geometric mean is misleading. What is being determined is the maximum likelihood estimate, which is based on the mode or maximum frequency of the distribution curve. The probability of observing results by multiple dilutions is expressed by a multiple Poisson distribution.[50,51] The curve generated appears skewed to the right; the characteristic is similar to that of a log-normal distribution curve. This results from the fact that colony counts under the envelope of the log-normal curve result from multiple Poisson distribution curves for numerous other organisms that are not of interest and cause the distribution curve to be further

TABLE 9020:X. COMPARISON OF FREQUENCY OF MPN DATA

Class Interval	Frequency (MPN)
0–400	11
400–800	2
800–1200	1
1200–1600	0
1600–2000	0
2000–2400	0
2400–2800	0
2800–3200	0

TABLE 9020:XI. COMPARISON OF FREQUENCY OF LOG MPN DATA

Class Interval	Frequency (log MPN)
1.000–1.300	1
1.300–1.600	2
1.600–1.900	1
1.900–2.200	5
2.200–2.500	1
2.500–2.800	2
2.800–3.100	2
3.100–3.400	0
3.400–3.700	1

skewed. When the maximum likelihood approach[52,53] is used the maxima of these organisms are spread out under the curve because different organisms respond differently to the nutrients and media. This process is affected by temperature, pH, and time of incubation. The process of analyzing the data for the maximum frequency insures that the correct organism is selected for colony count.

When the MPN curves for 1, 2, 3, and 4 positive tubes out of 5 total tubes incubated are examined, the log-normal probability graph is close to being linear (thus indicating approximate normality) but it bows upward and could indicate possible kurtosis, a sharpness, brought about by measuring the cumulative probability on the low and high ends of the distribution curve. The error is in the extreme values of the tails of the distribution because measurement is difficult at the extreme values of the log-normal distribution curve.

The log-normal probability assumption is confirmed when the log of values is plotted against colony count MPN (maximum probable number) on log-normal − cumulative probability graph paper.

The use of the geometric mean,[54] calculated as the *n*th root of the product of all the data values, is based upon the likelihood of a probability distribution. The likelihood estimate is based on both frequency of *n* observations and the count of a random sample on *n* observations.

When the likelihood ratio is observed before and after the log transformation of the variable, x, it can be shown that the ratios are the same.[55] By means of the log-likelihood ratio, product properties are converted into summation properties, which are easy to understand and deal with.

The likelihood approach differs from ordinary arithmetic averages in that both frequency and variable colony count are considered, rather than only the arithmetic average of colony counts.

The geometric average is the log of the inverse of the average log of likelihoods of a parameter being measured. This is quite different from the average of MPNs and will generally give a lower possible number than an arithmetic average.[56] The geometric average of the maximum likelihood estimates is a better estimate than the arithmetic average for living organisms.

In the derivation of the Maximum Likelihood[56] for a Poisson Probability Distribution, the log of the products of MPN can be shown to be a function of the log of frequency. Thus, geometric averages are justified as the method of obtaining a maximum likelihood estimate of multiple MPN determinations.

c. "Less than" (<) values: There has always been uncertainty as to the proper way to include "less than" values in calculation and evaluation of microbiological data because such values cannot be treated statistically without modification. Proposed modifications involve changing such numbers to zero, choosing values halfway between zero and the "less than" value, or assigning the "less than" value itself, i.e., changing <1 values to 1, 1/2, or 0.[57,58]

There are valid reasons for not including "less than" values, whether modified or not. If the database is fairly large with just a few such values, the influence of these uncertain values will be minimal and of no benefit. If the database is small or has a relatively large number of "less than" values, inclusion of modified forms of such values would exert an undue influence on the final results and could result in an artificial negative or positive bias. Including "less than" values is particularly inappropriate if the values are <100, <1000, or higher because the unknown true values could be anywhere from 0 to 99, 0 to 999, etc. When such values are first noted, adjust or expand test volumes. The only exception to this caution would be regulatory testing with defined compliance limits, such as the <1/100 mL values reported for drinking water systems where the 100-mL volume is required.

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9020 C. Interlaboratory Quality Control

1. Background

Interlaboratory QC programs are a means of establishing an agreed-upon, common performance criteria system that will assure an acceptable level of data quality and comparability among laboratories with similar interests and/or needs. A number of different publications[1-4] and organizations* are concerned with interlaboratory programs.

A certification program is one in which an independent authority issues a written assurance or certificate that the laboratory's management is compliant to that authority's standards. An accreditation program is one in which a specialized accreditation body sets standards and a certification body then determines whether the laboratory exhibits competence in following the standards. The laboratory then receives formal recognition. Often the term "accreditation" is used interchangeably with "certification."

Usually, interlaboratory QA programs have three elements: uniform criteria for laboratory operations, external review of the program, and external proficiency testing. These programs assist the laboratories in addressing continual improvement efforts.

2. Uniform Criteria

Interlaboratory quality control programs begin as a voluntary or mandatory means of establishing uniform laboratory standards for a specific purpose. The participants may be from one organization or a group of organizations having common interests or falling under common regulations. Often one group or person may agree to draft the criteria. If under regulation, the regulating authority may set the criteria for compliance-monitoring analyses.

Uniform sampling and analytical methods and quality control criteria for personnel, facilities, equipment, instrumentation, supplies, and data handling and reporting are proposed, discussed, reviewed, modified if necessary, and approved by the group for common use. Criteria identified as necessary for acceptable data quality should be mandatory. A formal document is prepared and provided to all participants.

The QA/QC responsibilities of management, supervisors, and technical staff are described in 9020A. In large laboratories, a QA officer is assigned as a staff position but may be the supervisor or other senior person in smaller laboratories.

After incorporation into laboratory operations and confirmation that the QA program has been adapted and is in routine use, the laboratory supervisor and the QA officer conduct an internal program review of all operations and records for acceptability, to identify possible problems and assist in their resolution. If this is done properly, there should be little concern that subsequent external reviews will find major problems.

3. External Program Review

Once a laboratory has a QA program in place, management informs the certifying or accrediting organization and an external quality assessment is requested. The type of assessment and the organization performing the assessment will depend on a number of variables, such as the request for accreditation and whether the samples to be tested will be for compliance purposes. An experienced external QA professional or team then arranges an on-site visit to evaluate the QA program for acceptability and to work with the laboratory to solve any problems. Laboratories applying for review will have their laboratory documentation and procedures reviewed. An acceptable rating confirms that the laboratory's QA program is operating properly and that the laboratory has the capability of generating valid defensible data. Such on-site evaluations are repeated and may be announced or unannounced.

4. External Proficiency Testing

Laboratories applying for certification or accreditation are required to participate in routine proficiency testing for those analytical, technological, or matrix-specific procedures the laboratory intends to use. Challenge samples are prepared and sent as unknowns on a set schedule for analyses and reporting of results. The proficiency test samples are to be processed as routine samples by the analyst routinely running the method being reviewed. The reported data are coded for confidentiality and evaluated according to an agreed-upon scheme. The results are summarized for all laboratories and individual laboratory reports are sent to participants. Results of such studies indicate the quality of routine analyses of each laboratory as compared to group performance. Also, results of the group as a whole characterize the performance that can be expected for the analytical methods tested. Failure to successfully evaluate the proficiency test sample can result in loss of recognition.

For those laboratories not applying for certification or accreditation, control samples or proficiency test samples can be purchased.

5. Maintenance

The laboratory needs to undergo an external evaluation and successfully pass a set number of proficiency test samples. Upon successful completion of both, the laboratory will receive formal notification. To maintain this recognition the laboratory must successfully complete annual or semiannual proficiency test samples at a rate set by the authority and to pass an on-site assessment about once every 3 years.

6. Example Program

In the Federal Drinking Water Laboratory Certification Program, public water supply laboratories must be certified according to minimal criteria and procedures and quality assurance described in the EPA manual on certification: Criteria are established for laboratory operations and methodology; on-site inspections are required by the certifying state agency or its surrogate to verify minimal standards; annually, laboratories are required to perform acceptably on unknown samples in formal studies, as samples are available; and the responsible authority follows up on problems identified in

* American Association for Laboratory Accreditation, www.a2la.net, and National Cooperation for Laboratory Accreditation, www.nacla.net.

the on-site inspection or performance evaluation and requires corrections within a set period of time. Individual state programs may exceed the federal criteria.

To maintain accreditation by the National Environmental Laboratory Accreditation Conference, acceptable performance on two of the last three Proficiency Tests, as well as successful on-site assessments conducted on a routine basis, are required.

On-site inspections of laboratories in the present certification program show that primary causes for discrepancies in drinking water laboratories have been inadequate equipment, improperly prepared media, incorrect analytical procedures, and insufficiently trained personnel.

7. References

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9030 LABORATORY APPARATUS*

9030 A. Introduction

This section contains specifications for basic microbiological laboratory equipment. For testing and maintenance procedures related to quality control, see Section 9020. Additional equipment needed for determination of specific microorganisms is discussed in the sections pertaining to those organisms. Ensure that all laboratory equipment is calibrated and its performance verified before initial use and on a routine basis thereafter. Conduct maintenance at least annually, either in-house or by service contract, following manufacturer's and analytical requirements.

9030 B. Equipment Specifications

1. Incubators

Use incubators of sufficient size to prevent internal crowding and capable of maintaining a uniform and constant temperature at all times in all areas (i.e., temperatures must not vary more than is allowed by the analytical equipment). Obtain such accuracy and sensitivity by using a water-jacketed or anhydric-type incubator with thermostatically controlled low-temperature electric heating units properly insulated and located in or adjacent to the walls or floor of the chamber and preferably equipped with mechanical means of circulating air.

Incubators equipped with high-temperature heating units are unsatisfactory because such sources of heat, when improperly placed, frequently cause localized overheating and excessive dehydration of media, with consequent inhibition of bacterial growth. Incubators so heated may be operated satisfactorily by replacing high-temperature units with suitable wiring arranged to operate at a lower temperature and by installing mechanical air-circulation devices. It is desirable, where ordinary room temperatures vary excessively, to keep laboratory incubators in special rooms maintained at a few degrees below the recommended incubator temperature.

Alternatively, use special well-insulated incubating rooms equipped with properly distributed heating units, forced air circulation, and air exchange ports, provided that they conform to desired temperature limits. When such rooms are used, record the daily temperature range in areas where plates or tubes are incubated. Use open metal wire or perforated shelves so spaced as to assure temperature uniformity throughout the chamber. Leave a 2.5-cm space between walls and stacks of dishes or baskets of tubes.

Use a water-bath incubator, of sufficient size for workloads, with a gabled cover to reduce water and heat loss and a circulating pump to maintain set temperature. Keep water depth in the incubator sufficient to immerse tubes to upper level of media. Use weights or screens on plastic bags containing plated media to permit total immersion. Water baths may also be used for such purposes as tempering agar.

Calibrate heat block incubators and determine their stability before initial use; calibrate the built-in thermostat annually thereafter with a NIST-traceable thermometer.

2. Hot-Air Sterilizing Ovens

Use hot-air sterilizing ovens of sufficient size to prevent internal crowding; constructed to give uniform and adequate sterilizing temperatures of $\geq 170 \pm 10°C$; and equipped with suitable thermometers, inserted where possible in sand. Optionally use a calibrated temperature-recording instrument. These units may also be used at lower temperatures to dry glassware.

3. Autoclaves/Steam Sterilizers

Use autoclaves of sufficient size to prevent internal crowding; constructed to provide uniform temperatures within the chambers (up to and including the sterilizing temperature of 121°C); and capable of reaching the desired temperature within 15 min. Autoclaves should be equipped with an accurate thermometer, the bulb of which is located properly in the exhaust line so as to register minimum temperature within the sterilizing chambers (temperature-recording instrument is optional); a pressure gauge; and properly adjusted safety valves directly connected either to saturated-steam supply lines equipped with appropriate filters to remove particulates and oil droplets or to a suitable special steam generator. Do not use steam from a boiler treated with amines for corrosion control.

Some currently available autoclave models are automatic and include features such as vertical sliding, self-sealing and opening doors, programmable sterilization cycles, and continuous multipoint monitoring of chamber temperature and pressure. These units also may incorporate solution cooling and vapor removal features. When sterilizer design includes heat exchangers and solution cooling features as part of a factory-programmed liquid cycle, strict adherence to the 45-min total elapsed time in the autoclave is not necessary provided that printout records verify normal cycle operation and chamber cooling during exhaust and vapor removal.

* Approved by Standard Methods Committee, 2006.
Joint Task Group: Margo E. Hunt (chair), Ellen B. Braun-Howland, Terry C. Covert, Gil Dichter, Nancy Hall, Robin Oshiro.

Use of a vertical autoclave or pressure cooker is not recommended because of difficulty in adjusting and maintaining sterilization temperature and the potential hazard. If a pressure cooker is used in emergency or special circumstances, equip it with an efficient pressure gauge and a thermometer the bulb of which is 2.5 cm above the water level.

4. Gas Sterilizers

Use a sterilizer equipped with automatic controls capable of carrying out a complete sterilization cycle and a sterilizing gas such as ethylene oxide diluted to 10 to 12% with an inert gas. Provide an automatic control cycle to evacuate sterilizing chamber to at least 0.06 kPa, to hold the vacuum for 30 min, to adjust humidity and temperature, to charge with the ethylene oxide mixture to a pressure dependent on mixture used, to hold such pressure for at least 4 h, to vent gas, to evacuate to 0.06 kPa, and finally, to bring to atmospheric pressure with sterile air. The humidity, temperature, pressure, and time of sterilizing cycle depend on the gas mixture used. A wet gauze or paper towel may be needed to achieve the correct humidity for proper sterilization. CAUTION: *Ethylene oxide is toxic—avoid inhalation, ingestion, and contact with the skin. Also, ethylene oxide forms an explosive mixture with air at 3 to 80% proportion and provides a much narrower margin of safety than sterilization by steam. Malfunctions can occur that are not easily detected.*

Store overnight sample bottles with loosened caps that were sterilized by gas, to allow last traces of gas mixture to dissipate. Incubate overnight media sterilized by gas, to insure dissipation of gas. An aerator may also be used to remove traces of gas.

In general, mixtures of ethylene oxide with chlorinated hydrocarbons such as trichlorotrifluoroethane are harmful to plastics, although at temperatures below 55°C, gas pressure not over 35 kPa, and time of sterilization less than 6 h, the effect is minimal. If carbon dioxide is used as a diluent of ethylene oxide, increase exposure time and pressure, depending on temperature and humidity that can be used.

Determine proper cycle and gas mixture for objects to be sterilized and confirm by sterility tests.

5. Optical Counting Equipment

a. Pour and spread plates: Use a dark-field model colony counter providing 1.5 times normal magnification with satisfactory visibility and adequate lighting.

b. Membrane filters: Use a binocular or zoom microscope with magnification of 10 to 15×. Provide daylight fluorescent light source at angle of 60 to 80° above the colonies; use low-angle lighting for non-pigmented colonies.

c. Tally equipment: Do not use automatic colony counters when determining count for compliance purposes or when enumerating pour plates, which typically have submerged pinpoint colonies in clear-colored medium. When automatic colony counters are used, have units calibrated when installed and check performance using conventional counting methods.

When counting colonies, either a mechanical hand tally or an electronic tally unit that touches the agar surface can be used, (the latter after any colony culture transfer, if needed, has occurred). Test unit before initial use and annually thereafter for accuracy.

6. pH Equipment

Use electrometric pH meters accurate to at least 0.1 pH units for determining pH values of media and preferably having automatic temperature compensation. Use appropriate probes to determine pH of liquid and solid agar media.

7. Balances

Use balances providing a sensitivity of at least 0.1 g at a load of 150 g, with weights traceable to appropriate national standards. Use an analytical balance having a sensitivity of 1 mg under a load of 10 g for weighing small quantities (less than 2 g) of materials. Single-pan rapid-weigh balances are most convenient. Place balances on solid surfaces to avoid vibrations and in locations where drafts and humidity levels are reduced.

8. Media Preparation Utensils

Use borosilicate glass or other suitable noncorrosive equipment such as stainless steel. Use glassware that is clean and free of residues, dried agar, or other foreign materials that may contaminate media and washed as noted in Section 9040.

9. Pipets, Micropipets, and Graduated Cylinders

Use pipets of any convenient size, provided that they deliver the required volume accurately and quickly. The error of calibration for a given manufacturer's lot must not exceed 2.5%. Use pipets having graduations distinctly marked and with unbroken tips. Bacteriological transfer pipets or pipets conforming to APHA standards may be used. *Do not pipet by mouth; use a pipet aid.*

Calibrate and maintain adjustable micropipets according to manufacturer's instructions because differences will exist between different units. Use sterile tips specific to the micropipettor and their intended use. For example, for PCR analyses these units may contain aerosol-resistant tips and should be certified as RNase-free, DNase-free, and pyrogen free.

Use graduated cylinders meeting ASTM Standards (D-86 and D-216) and with accuracy limits established by NIST where appropriate.

10. Pipet Containers

Use boxes of aluminum or stainless steel, end measurement 5 to 7.5 cm, cylindrical or rectangular, and length about 40 cm. When these are not available, paper wrappings for individual pipets may be substituted. To avoid excessive charring during sterilization, use best-quality sulfate pulp (kraft) paper. *Do not use copper or copper alloy cans or boxes as pipet containers.*

11. Refrigerator and Freezers

Use a refrigerator capable of maintaining a temperature of 2 to 8°C to store samples, media, reagents, etc. Do not store volatile solvents, food, or beverages in a refrigerator with media or cultures. Frost-free refrigerators may cause excessive media dehydration on storage longer than 1 week and should not be used if this is of concern.

Freezer temperature range will be determined by analytical need, e.g., storage of cultures. Standard laboratory freezers may range from –10 to –20°C ± 5°C to ultra-low temperatures such as –70 to –90°C. Monitor temperature of these units.

12. Temperature-Monitoring Devices

Use glass or metal thermometers graduated in increments to monitor required analytical temperature range for incubators and refrigerators. For example, use thermometers graduated to 0.1°C for incubators operated above 40°C. Continuous recording devices or thermocouples may be used in lieu of glass or metal thermometers. Annually, calibrate and record accuracy of all temperature-monitoring devices by comparison with a NIST-certified thermometer, or equivalent.

13. Dilution Bottles or Tubes

Use bottles or tubes of resistant glass, preferably borosilicate glass, closed with glass stoppers or screw caps equipped with liners that do not produce toxic or bacteriostatic compounds on sterilization. Do not use cotton plugs as closures. Mark graduation levels indelibly on side of dilution bottle or tube. Confirm that the mark is within required level of accuracy. Plastic bottles of nontoxic material and acceptable size may be substituted for glass provided that they can be sterilized properly. Discard any items with chips, cracks, or scratches.

14. Petri Dishes

For the plate count and environmental monitoring, use glass or plastic petri dishes about 100 × 15 mm or 150 × 20 mm. Use dishes having bottoms that are free from bubbles and scratches and that are flat so that the medium will be of uniform thickness throughout the plate. For the membrane filter technique use loose-lid glass or plastic dishes, 60 × 15 mm, or tight-lid dishes, 50 × 12 mm. Sterilize glass petri dishes and store in metal cans (aluminum or stainless steel, but not copper), or wrap in paper—preferably best-quality sulfate pulp (kraft)—before sterilizing.

15. Multi-Well Trays

A variety of different trays for most-probable-number determination is available. Use trays sterilized by the manufacturer. Seal with unit supplied by the manufacturer. Quality control information is discussed in 9020B.5*e*.

16. Membrane Filtration Equipment

Use filter funnel and membrane holders made of seamless stainless steel, glass, or autoclavable plastic, that do not leak and are not scratched or subject to corrosion. Replace metal grids if they are broken. Field laboratory kits are acceptable for in-field use only. In the laboratory use standard laboratory filtration equipment and procedures.

17. Fermentation Tubes and Vials

Use fermentation tubes of any type, if their design permits conforming to medium and volume requirements for concentration of nutritive ingredients as described subsequently. Where tubes are used for a test of gas production, enclose an inverted Durham tube or vial. Use tube and vial of such size that the vial will be filled completely with medium, at least partly submerged in the tube, and large enough to make gas bubbles easily visible.

18. Inoculating Equipment

Use wire loops made of 22- or 24-gauge nickel alloy* or platinum-iridium for flame sterilization. Use loops at least 3 mm in diameter. Single-service hardwood or plastic applicators, 0.2 to 0.3 cm in diameter and at least 2.5 cm longer than the fermentation tube, also may be used. Sterilize wooden applicators by dry heat and plastic applicators by autoclave, while stored in glass or other nontoxic containers. Prepackaged sterile disposable plastic loops also are available for ready use.

19. Sample Bottles

If preparing sample bottles in-house, use reusable bottles of glass or plastic made of nontoxic materials such as polypropylene. Commercial sampling bottles are single-use. Use bottles of suitable size and shape that can hold a sufficient volume of sample for all required tests, have an adequate air space to permit proper mixing, and can be capped to keep samples uncontaminated until examinations are completed. Wide-mouthed bottles are recommended. Presterilized disposable plastic bags, with or without dechlorinating agent, are available commercially. Most plastic containers eliminate the possibility of breakage during shipment and reduce shipping weight.

Metal or plastic screw-cap closures with liners may be used on sample bottles provided that no toxic compounds are produced on sterilization.

Before sterilization, cover tops and necks of sample bottles having glass closures with aluminum foil or heavy kraft paper.

20. Microscopes

Microscope properties such as magnification (ocular and objective lenses) and lighting components depend on the needs of the laboratory. Most basic microbiology laboratories will have a compound brightfield light microscope.

Stage micrometer calibration and lens quality and focus should be checked by manufacturer. Follow manufacturer's recommendations for adjustment of lighting and focusing of eyepieces, and check alignment routinely. Follow Kohler illumination procedures for each objective used. Clean the body of the microscope as well as the lens after each use. Use immersion oil recommended by manufacturer for oil immersion lenses only. Blow off dust using pressurized air or rubber bulb; do not blow onto lenses. Use only optically safe tissues and cleaning solutions designed for microscopes. Cover microscope when not in use and store in areas where temperature and humidity levels are consistently low. For some microscopes, e.g., fluorescent microscopes, record light usage times and limit technician time at the microscope.

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* Chromel, nichrome, or equivalent.

21. Centrifuges

Centrifuges should have speed capabilities that meet the criteria needed for the analytical method. Follow manufacturer's instructions for usage, maintenance, and calibration. Use equipment designed for that centrifuge and capable of withstanding speeds to be used. Ensure centrifuge tube loads are balanced. Clean units when contamination is suspected or at least monthly using appropriate disinfectant solutions, e.g., with a freshly prepared 0.5% sodium hypochlorite solution, followed by an ethanol (70%) rinse to remove residual bleach and avoid metal pitting.

22. Laminar-Flow Hoods/Biological Safety Cabinets

Purchase units designed for analytical needs. Laminar-flow hoods duct to the outside and unidirectional air flow blows sterile air towards the operator. These units do not protect the environment unless filtered exhausts are installed.

Biological Safety Cabinets (BSCs) are classified according to the degree of protection each affords to the technician, the microbiological activity being conducted, and the environment. Class I cabinets have a filtered inward air-flow system and a filtered exhaust. The remaining BSCs have both HEPA air intake and exhaust filter systems; however, Class III requires a larger air intake and has a completely closed front with integrated gloves.

Sanitize all units after each use, check air flow monthly, and have units serviced annually. Maintain certificates.

23. Ultraviolet Lights

UV lights (254-nm) can be used for sanitation purposes and to decrease nucleic acid contamination. Long-wave UV lights (365–366-nm) are used to detect fluorescence for enzymatic methods. QC and safety considerations are discussed in 9020B.4*l*.

24. Spectrophotometer

A spectrophotometer measures light absorption and can be used, for example, to determine turbidity/growth of microbial cultures. Have unit calibrated at installation and check performance each day of use with a method-specific blank and an appropriate calibration standard obtained from an outside source.

25. Homogenization Apparatus

Blenders and stomachers are used to extract bacteria from materials in which they may be found or attached to, such as filters. Select units of appropriate capacity and variable speed for intended use. Vortex mixers and rotary shakers are designed to resuspend bacteria in a solution. Rotary shakers also are used in incubator rooms to shake cultures. Determine required stroke measurement and revolutions per minute initially, following manufacturer's instructions, and quarterly thereafter.

26. Media and Solution Dispensers

Follow manufacturer's instructions to verify the performance, i.e., delivery volume accuracy and precision, of automatic diluters, micropipettors, and media dispensing apparatus at initial use and when volume amounts dispensed are changed. If volume delivery amounts do not change, verify continued capability at least quarterly. This can be determined by taking replicate mass/volume measurements.

27. Bibliography

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9040 WASHING AND STERILIZATION*

Sterilize all contaminated laboratory ware before cleaning to prevent potential contamination to personnel handling contaminated material. Remove all markings before initiating washing sequence. If material has dried on glassware, a pre-soaking may be needed. If mechanical laboratory washers are used, equip them with influent plumbing of stainless steel or other nontoxic material. Do not use units designed for home use. Ensure that the spray from water jets reaches all parts of deep vessels. Do not use copper plumbing to distribute water. Use stainless steel or other nontoxic material for the rinse-water system. Consistently follow the same washing procedures whether using an automated system or washing by hand.

Cleanse all glassware and plasticware thoroughly with a suitable detergent and warm water. To remove all traces of residual washing compound, rinse five to ten times with cold water after bubbles/foam are gone. In addition, rinse two to three times with reagent-grade water.

If desired, dry glassware before use by placing in a drying oven at 100°C for 10 to 15 min.

Glassware may be sterilized, except when in metal containers, by dry heat for 2 to 4 h at a temperature of 170°C. Longer times are needed to kill cryptosporidia and denature prions. Alternatively, add a small amount of distilled water (to prevent airlock) and autoclave at 121°C for at least 30 min. Longer sterilization times are needed to kill spores and very resistant microorganisms. Sterilize glassware in metal containers at 170°C for not less than 2 h. For all bottles, loosen caps before autoclaving. If desired, moisture present in empty sterile containers after autoclaving may be removed by placing items in a drying oven at 100°C for 10 to 15 min.

As long as the bromthymol blue test is being done on *each* batch of washed glassware, run the toxicity test, ¶ 9020B.5*a*2), before initial use of a washing compound and whenever a new formulation or washing procedure is used. If the bromthymol blue test is not done consistently, run the toxicity test on a per-lot or annual basis, whichever is more frequent. Review Section 9020B for quality control checks on autoclaves and sterilized bottles.

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* Approved by Standard Methods Committee, 2006.
Joint Task Group: Margo E. Hunt (chair), Ellen B. Braun-Howland, Terry C. Covert, Gil Dichter, Nancy Hall, Robin Oshiro.

9050 PREPARATION OF CULTURE MEDIA*

9050 A. General Procedures

1. Storage of Culture Media

Store dehydrated media (powders) in tightly closed bottles in the dark at less than 30°C in an atmosphere of low humidity, e.g., desiccator. Do not use them if they are discolored or caked, or if the character of a free-flowing powder is lost. Use stocks of dehydrated media before their expiration date or use within a year of purchase if dehydrated media contain selective agents such as sodium azide, bile salts, antibiotics, sulfur-containing amino acids, etc., so as to maintain optimum selectivity. Review manufacturer's instructions, product Material Safety Data Sheets (MSDS), and analytical methods before preparing media. Use commercially prepared media wherever available. Avoid preparing media from essential ingredients unless necessary. See also Section 9020B.5*j*.

Prepare culture media in batches that will be used in less than 2 weeks unless otherwise specified by the method. However, if the media are contained in screw-capped tubes they may be stored for up to 3 months. See Table 9020:V for specific details. Store media out of direct sunlight and avoid excessive evaporation. Place prepared petri dishes in airtight containers or plastic bags, close with twist-ties, and store under refrigerated conditions. Invert petri dishes to prevent moisture condensation on agar.

If refrigerated, liquid media in fermentation tubes may dissolve sufficient air to produce, upon incubation at 35°C, a bubble of air in the tube. Bring all media (especially fermentation or carbohydrate broth) to room temperature before use and discard tubes containing air bubble.

Fermentation tubes may be stored at approximately 25°C; but because evaporation may proceed rapidly under these conditions—resulting in marked changes in concentration of the ingredients—do not store at this temperature for more than 2 weeks. Discard tubes with growth due to contamination or an evaporation loss of more than 1 mL. A 1-mL or greater loss from the initial 10 mL can affect most-probable-number calculations. Selective agents also may break down after prolonged incubation or storage.

2. pH Adjustment

Determine and record pH of medium after sterilization. The required final pH is given in the directions for preparing each medium. If a specific pH is not prescribed, adjustment is unnecessary. The decrease in pH during sterilization will vary slightly with the individual sterilizer in use, and the initial pH required to obtain the correct final reaction will have to be determined. The decrease in pH usually will be 0.1 to 0.2 but occasionally may be as great as 0.3 in double-strength media. When buffers are present in the media the decrease in pH value will be negligible.

For determination of final pH, cool medium to 44 to 46°C, aseptically remove a small quantity, and determine pH after setting the meter for the higher temperature if not done automatically. Alternatively, cool a sample of the medium completely to room temperature before determining final pH. If pH adjustment is necessary, use sterile stir bar and pipet a sufficient quantity of filter-sterilized 0.1*N* NaOH or 0.1*N* HCl into the bulk medium to reach the proper pH.

The pH of reconstituted dehydrated media seldom will require adjustment if made according to directions. Errors in weighing dehydrated medium, overheating reconstituted medium, or a problem with the medium itself may produce an unacceptable final pH. If pH values of prepared media are consistently outside the allowed pH range, determine cause. Adjustment of pH before sterilization may be necessary.

3. Sterilization

After rehydrating a medium, dispense promptly to culture vessels and sterilize within 2 h. Do not store nonsterile media.

Sterilize media in an autoclave at 121°C. The required exposure time will vary with form and type of material, medium, presence of carbohydrates, and volume. For example, sterilize carbohydrate broths at 121°C for only 12 to 15 min. after the temperature has reached 121°C. When the pressure reaches zero, remove medium from autoclave and cool quickly to avoid decomposition of sugars by prolonged exposure to heat. To permit uniform heating and rapid cooling, pack materials loosely and in small containers. The maximum elapsed time for exposure of carbohydrate broths to any heat (from time of closing loaded autoclave to unloading) is 45 min. Preferably use a double-walled autoclave to permit preheating before loading to reduce total heating time to within the 45-min. or less limit. Adjust autoclave times as volumes/loads increase. Presterilized media may be available commercially.

4. Quality Control

See Section 9020B.5*j*.

5. Bibliography

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* Approved by Standard Methods Committee, 2006.
Joint Task Group: Margo E. Hunt (chair), Ellen B. Braun-Howland, Terry C. Covert, Gil Dichter, Nancy Hall, Robin Oshiro.

9050 B. Water

1. Specifications

To prepare culture media and reagents, use only distilled or demineralized reagent-grade water that has been tested and found free from traces of dissolved metals and bactericidal or inhibitory compounds. Toxicity in distilled water may be derived from fluoridated water high in silica. Other sources of toxicity are silver, lead, and various unidentified organic complexes. Where condensate return is used as feed for a still, toxic amines or other boiler compounds may be present in distilled water. Residual chlorine or chloramines also may be found in distilled water prepared from chlorinated water supplies. If chlorine compounds are found in distilled water, neutralize them by adding an equivalent amount of sodium thiosulfate or sodium sulfite.

Distilled water also should be free of contaminating nutrients. Such contamination may be derived from flashover of organics during distillation, continued use of exhausted carbon filter beds, deionizing columns in need of recharging, solder flux residues in new piping, dust and chemical fumes, and storage of water in unclean bottles.

Store distilled water out of direct sunlight to prevent growth of algae. Aged distilled water may contain toxic volatile organic compounds absorbed from the atmosphere if stored for prolonged periods in unsealed containers. Good housekeeping practices that minimize the presence of airborne particulates usually will eliminate nutrient contamination.

See Section 9020B and Table 9020:II.

2. Bibliography

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9050 C. Media Specifications

The need for uniformity dictates the use of dehydrated media. Never prepare media from basic ingredients when suitable commercially prepared dehydrated media are available. Follow manufacturer's directions for rehydration and sterilization. Commercially prepared media in liquid form (sterile ampule or other) also may be used if known to give equivalent results. See Section 9020B.5*j* for quality-control specifications.

The terms used for protein source in most media, for example, peptone, tryptone, tryptose, were coined by the developers of the media and may reflect commercial products rather than clearly defined entities. It is not intended to preclude the use of alternative materials provided that they produce equivalent results.

NOTE—The term "percent solution" as used in these directions is to be understood to mean "grams of solute per 100 mL solution."

1. Dilution Water

Various dilution water solutions can be prepared. The following represent two of the most commonly used solutions in the basic water microbiology laboratory.

a. Buffered water:

1) Stock phosphate buffer solution—Dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 mL reagent-grade water, adjust to pH 7.2 ± 0.5 with 1*N* sodium hydroxide (NaOH), and dilute to 1 L with reagent-grade water. Sterilize by filtration or autoclave. Store stock solution under refrigerated conditions and discard if turbidity develops.

2) Magnesium chloride stock solution—Add magnesium chloride (38 g/L $MgCl_2$ or 81.1 g $MgCl_2 \cdot 6H_2O$) to 1 L reagent-grade water. Sterilize and store stock solution under refrigerated conditions, discarding if solution becomes turbid.

3) Working solution—Add 1.25 mL stock phosphate buffer solution and 5.0 mL magnesium chloride stock solution to 1 L reagent-grade water. Dispense in amounts that will provide 99 ± 2.0 mL or 9 ± 0.2 mL after autoclaving for 15 min. Final pH should be 7.2 ±0.1. Note that pH values will change with time. Store under refrigerated conditions after opening and discard if turbidity develops. Use within 6 months.

b. Peptone water, 0.1%: Prepare by adding 1 g peptone to 1 L reagent water. Final pH should be 7.0 ± 0.2 after sterilization.

Dispense in amounts to provide 99 ± 2.0 mL or 9 ± 0.2 mL after autoclaving for 15 min. Store as above.

Do not suspend a sample in any dilution water for more than 30 min at room temperature because injury, death, or multiplication may occur.

2. Culture Media

Specifications for individual media are included in subsequent sections. Details are provided where use of a medium is first described.

3. Bibliography

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1978. Microbiological Methods for Monitoring the Environment, Water and Wastes. EPA-600/8-78-017, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Appendix F

Field Data Sheets

Water-Monitoring Data Sheet

Collection Date : ______________________ **Time :** ______________________
Monitor Name : Carol DiPaolo, Mark Ring, Tony Alfieri, Jim Moriarty
Site Name : CSHH #1, Beacon 11 **Location :** Hempstead Harbor

Weather : ☐ fog/haze ☐ drizzle ☐ intermittent rain ☐ rain ☐ snow ☐ clear ☐ partly cloudy

% Cloud Cover : ☐ 0% ☐ 25% ☐ 50% ☐ 75% ☐ 100% other ________

Wind Direction : ☐ N ☐ NE ☐ NW ☐ S ☐ SE ☐ SW ☐ E ☐ W **Velocity :** ______ kt (mph)

Rainfall :			Date	Amount
Previous 24 hrs accumulation	____.__	mm	________	________
Previous 48 hrs accumulation	____.__	mm	________	________
Previous week's accumulation	____.__	mm	________	________

Tidal Stage : ☐ incoming ☐ outgoing hours to high tide : ____________

Water Surface : ☐ calm ☐ ripple ☐ waves ☐ whitecaps

Water Color : ☐ normal : ☐ brown ☐ green other ________
☐ abnormal : ☐ brown ☐ green other ________

Water Observations : ☐ jelly fish ☐ dead fish ☐ dead crabs ☐ algal bloom
☐ odors ☐ sea weed ☐ bubbles ☐ foam
☐ oil slick ☐ floatables ☐ ice
☐ submerged aquatic vegetation (SAV) ☐ turbidity (suspended particles)

Comments ____

Plankton count ________ type ________ sample taken : ☐ surface ☐ below surface

Human Activities

☐ Barges/tugs, Pt. W. gravel op.____________ Gladsky____________ Raison____________
DiNapoli____________ Global/fuel____________ other____________
☐ Boats, power____________ sailboats____________ kayaks____________ crew____________
☐ Anglers, at beaches____________ at piers____________
☐ Other____________

Floatables Observations (type, approximate number…)

☐ Bottles, glass________ plastic________ ☐ Cans________ ☐Paper________ ☐Plastic bags/pieces________
☐ Styrofoam, cups________ pieces________ ☐Wood, boards________ pieces________
☐ Other____________

Cal. Constants ____________

Water Monitoring Data Sheet

Air Temperature : ________ **°C**

Station : **Time :** ______________ **Date:** ____________

Wind ______

Air °C ______

DO cal. ck. ______ % sat.

Depth (meters)	Temp °C	Salinity (ppt)	DO (ppm)	pH	Secchi (meters)	Nitrogen (ppm)		
						NO_2	NO_3	NH_3
Surface								
0.5								
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Station : **Time :** ______________

Wind ______

Air °C ______

DO cal. ck. ______ % sat.

Depth (meters)	Temp °C	Salinity (ppt)	DO (ppm)	pH	Secchi (meters)	Nitrogen (ppm)		
						NO_2	NO_3	NH_3
Surface								
0.5								
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Station : **Time :** ______________

Wind ______

Air °C ______

DO cal. ck. ______ % sat.

Depth (meters)	Temp °C	Salinity (ppt)	DO (ppm)	pH	Secchi (meters)	Nitrogen (ppm)		
						NO_2	NO_3	NH_3
Surface								
0.5								
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Water-Monitoring Data Sheet

Wildlife Observations

Date ___________

BIRDS

	Upper Harbor		Lower Harbor	
☐ Cormorants				
☐ Ducks, mallards		ducklings		ducklings
☐ Egrets, great				
snowy				
☐ Geese, Canada		goslings		goslings
brandts				
☐ Gulls, hooded				
☐ Herons, blue				
night, green				
☐ Kingfisher, belted				
☐ Ospreys				chicks
☐ Plover-type, killdeer				
☐ Swans, mute		cygnets		cygnets
☐ Other				

JELLIES/JELLYFISH

☐ Comb, sea walnuts: CSHH stations ☐#1_____ ☐#2_____ ☐#3_____ ☐#4_____ ☐#5_____ ☐#6____ ☐#7____

☐#8-10_____ ☐#11____ ☐#12___ ☐#13____________

sea gooseberries: CSHH stations ☐#1_____ ☐#2_____ ☐#3_____ ☐#4_____ ☐#5_____ ☐#6____ ☐#7____

☐#8-10_____ ☐#11____ ☐#12____ ☐#13____________

☐ Lion's mane: CSHH stations ☐#1_____ ☐#2_____ ☐#3_____ ☐#4_____ ☐#5_____ ☐#6____ ☐#7________

☐ Moon: CSHH stations ☐#1_____ ☐#2_____ ☐#3_____ ☐#4_____ ☐#5_____ ☐#6____ ☐#7________

FISH

☐ Baitfish ____________

☐ Blue ____________

☐ Bunker ____________

☐ Striped bass ____________

☐ Small shrimp ____________

CRABS

☐ Asian shore ____________

☐ Blue-claw ____________

☐ Horseshoe ____________

OTHER ____________

Nassau Co. DOH PHL 209 Main Street Hempstead, NY 11550	FORM NAME: □ QC □ Equip Maint □ Training □ Comp Doc □ Other	
LABORATORY SECTION □ Chemistry □ Environmental Microbiology □ Clinical Microbiology	Form. No.: Date:	Rev: 0 Created By: CONNIE IANNUCCI

Beach Monitoring Daily Sampling Log

COALITION TO SAVE HEMPSTEAD HARBOR

Elap ID #10339

NASSAU COUNTY DEPARTMENT OF HEALTH
DIVISION OF PLBLIC HEALTH LABORATORIES
209 MAIN STREET, HEMPSTEAD, NY 11550
RODGER P. SILLETTI Ph.D., D (ABMM), DIRECTOR TELEPHONE (516) 572-1202 FAX (516) 572-1206

COLLECTOR'S NAME DATE

ALL SAMPLES SUBMITTED IN STERILE POLYSTYRENE VESSELS CONTAINING SODIUM THIOSULFATE (UNLESS OTHERWISE SPECIFIED)

Field No.	Area No.	Point No.	Sample Type	Location	Time	Temperature		Wind	Weather	Wave Height	Laboratory Use Only			
						Air	Water				Lab Number	Fecal Coliforms	Enterococci	Comments
CSHH-1	10		5	BEACON ELEVEN										
CSHH-2	10		5	BELL BUOY 6										
CSHH-3	10		5	RED MARKER GLEN COVER CREEK										
CSHH-4	10		5	BAR BEACH SPIT										
CSHH-5	10		5	MOTT'S COVE										
CSHH-6	10		5	EAST OF INCEINERATOR										
CSHH-7	10		5	BRYANT LANDING										
CSHH-8	10		5	GLEN COVE STP										
CSHH-9				FIRST PIPE WEST OF STP OUTFALL										
CSHH-10				PIPE AT CORNER OF SEAWALL WEST OF STP OUTFALL										
CSHH-11				50 YARDS EAST OF STP OUTFALL										
CSHH-12				EAST OF STP OUTFALL BY BEND IN SEAWALL										
CSHH-13				60 FEET WEST OF MILL POND WEIR										

COMMENTS/REMARKS

*ESTIMATED COUNTES: ALL COUNTS ARE ABOVE UPPER ACCEPTANCE LIMIT (20-60), OR NO COUNTS WITHIN ACCEPTANCE LIMIT (20-60)

TNTC = "TOO NUMEROUS TO COUNT"

DATA ENTRY PROOFED

TEST	METHOD	CODE
Fecal Coliform/100 ml.	Membrane Filtration	SM-18-20 9222 D
Enterococci/100 ml	Membrane Filtration	EPA Method 1600

TEMP CONTROL: TIME RECEIVED: DATE ANALYZED:

DATE RECEIVED:

SAMPLE ACCEPTABLE: YES ☐ NO ☐ ANALYSIS SUCCESSFUL: YES ☐ NO ☐

LABORATORY ACCREDITATION NOTICE:

The results provided on this report have been produced in compliance with "NELAC" (National Environmental Laboratory Accreditation Conference) standards and relate only to the identified sample. Any deviations from the accepted "NELAC" collection requirements for non-potable samples are appropriately noted. This rpeort shall not be reproced except in full without the written approval of the laboratory. Current New York State laboratory certification status is maintained under ELAP ID #10339.

VERIFICATION REVIEW

Name: Title: Date:

Comments:

Nassau Co. DOH PHL 209 Main Street Hempstead, NY 11550	FORM NAME: □ QC □ Equip Maint □ Training □ Comp Doc □ Other	
LABORATORY SECTION □ Chemistry □ Environmental Microbiology □ Clinical Microbiology	Form. No.: Date:	Rev: 0 Created By: CONNIE IANNUCCI

Beach Monitoring Daily Sampling Log

COALITION TO SAVE HEMPSTEAD HARBOR

Elap ID #10339

NASSAU COUNTY DEPARTMENT OF HEALTH
DIVISION OF PLBLIC HEALTH LABORATORIES
209 MAIN STREET, HEMPSTEAD, NY 11550
RODGER P. SILLETTI Ph.D., D (ABMM), DIRECTOR TELEPHONE (516) 572-1202 FAX (516) 572-1206

COLLECTOR'S NAME DATE

ALL SAMPLES SUBMITTED IN STERILE POLYSTYRENE VESSELS CONTAINING SODIUM THIOSULFATE (UNLESS OTHERWISE SPECIFIED)

Field No.	Area No.	Point No.	Sample Type	Location	Time	Temperature		Wind	Weather	Wave Height	Laboratory Use Only			
						Air	Water				Lab Number	Fecal Coliforms	Enterococci	Comments
CSHH-13A	10		5	HEAD OF GLEN COVE CREEK-COUNTY PIPE										
CSHH-13B	10		5	DOG LEG @ HEAD OF GLEN COVE CREEK										
CSHH-14	10		5	NW CORNER OF POWER PLANT ~ 50 YARDS FROM CEMENT OUTFALL										
CSHH-14A	10		5	CEMENT OUTFALL ADJACENT TO POWER PLANT										
CSHH-15	10		5	NW CORNER OF TAPPEN POOL										
CSHH-15A	10		5	SCUDDER'S POND OUTFALL @ SEAWALL N. OF TAPPEN POOL										
CSHH-15B	10		5	SCUDDER'S POND WEIR										

COMMENTS/REMARKS

*ESTIMATED COUNTES: ALL COUNTS ARE ABOVE UPPER ACCEPTANCE LIMIT (20-60), OR NO COUNTS WITHIN ACCEPTANCE LIMIT (20-60)

TNTC = "TOO NUMEROUS TO COUNT"

DATA ENTRY PROOFED **24hr rain:** **48hr rain:**

TEST	METHOD	CODE
Fecal Coliform/100 ml.	Membrane Filtration	SM-18-20 9222 D
Enterococci/100 ml	Membrane Filtration	EPA Method 1600

TEMP CONTROL: TIME RECEIVED: DATE ANALYZED:

DATE RECEIVED:

SAMPLE ACCEPTABLE: YES ☐ NO ☐ ANALYSIS SUCCESSFUL: YES ☐ NO ☐

LABORATORY ACCREDITATION NOTICE:
The results provided on this report have been produced in compliance with "NELAC" (National Environmental Laboratory Accreditation Conference) standards and relate only to the identified sample. Any deviations from the accepted "NELAC" collection requirements for non-potable samples are appropriately noted. This rpeort shall not be reproced except in full without the written approval of the laboratory. Current New York State laboratory certification status is maintained under ELAP ID #10339.

VERIFICATION REVIEW
Name: Title: Date:
Comments:

CODES FOR DAILY SAMPLING LOG

TIME - use military time; 9:00 a.m.= 0900, 2:30 p.m.= 1430

TEMP - use two spaces and report in degrees centigrade to nearest whole number
(if 9 degrees, report as 09, if 23 degrees, report as 23)

WIND - use one or two places for direction and two places for speed:
south at 15 mph should be reported as S-15; northwest at 2 mph
should be entered as as NW-02. No wind or "calm" should be
be entered as N-00.

WEATHER - 1 = fair, 2 = partly cloudy, 3 = cloudy, 4 = rain, 5 = snow, 6 = fog

WAVE HEIGHT - should be entered to nearest half foot, using two digits:
CALM = 0.0; 2 1/2 FOOT WAVES = 2.5

SAMPLE TYPE - 3 = fresh water pond/stream/drain,
4 = sewage, 5 = beach, 6 = other

COMMENTS, REMARKS - use this area to record any unusal conditions or
observations.

Appendix G

Electronic Data Format

CSHH Water-Monitoring Program 2010

Date	Water Temp (°C)		Salinity (ppt)		DO (ppm)		pH (ppm)		Air Temp	Secchi(m)	Turbidity	Depth (m)	Time
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	(°C)		(NTUs)	(Bottom)	(AM)
CSHH #1 - Beacon 11													
11/3/10	11.29	12.83	26.13	27.25	8.47	8.33	8.0	8.0	4.0	2.3	1.17/1.20	5.7	8:15
10/28/10	16.5	16.48	26.61	27.01	6.57	6.64	7.8	7.8	19.9	2.0	1.16/1.16	3.5	8:19
10/20/10	14.75	15.76	25.43	26.46	7.75	7.65	7.9	8.0	10.6	2.2	0.84/1.13	5.0	8:20
10/13/10	17.43	17.45	25.63	25.74	7.45	7.44	7.8	7.9	11.1	3.0	0.59/0.28	3.8	8:15
10/7/10	18.12	18.86	25.91	26.67	7.32	6.47	7.9	7.8	13.9	2.0	1.55/1.83	3.7	8:00
9/29/10	20.77	20.87	26.53	26.94	4.48	4.74	7.8	7.7	20.1	1.3	2.03/2.87	3.5	8:35
9/22/10	19.91	20.33	25.83	26.58	7.83	7.47	8.0	8.0	21.0	1.5	1.29/196	3.4	7:50
9/15/10	21.03	21.03	26.19	26.19	7.81	7.74	8.0	7.9	17.5	0.9	2.34/2.90	4.3	8:07
9/8/10	23.05	23.02	26.17	26.54	4.26	3.48	7.6	7.5	24.3	1.0	3.02/2.81	3.9	8:20
9/2/10	25.36	23.80	26.37	26.98	6.59	3.83	7.8	7.5	28.6	1.0	2.29/4.66	4.8	8:45
8/26/10	21.67	21.98	25.70	27.10	4.76	3.35	7.6	7.4	21.9	1.3	1.97/2.73	3.3	8:05
8/18/10	23.87	23.19	26.63	27.22	6.34	3.65	7.6	7.4	23.1	1.0	1.67/2.04	5.0	8:00
8/11/10	24.69	23.24	26.28	26.82	3.7	2.24	7.4	7.4	24.7	0.8	3.08/3.16	2.7	8:01
8/4/10	22.57	21.91	26.56	26.85	4.16	2.60	7.1	7.4	26.4	1.3	2.03/1.79	4.2	8:20
7/28/10	24.78	22.69	25.36	26.26	4.01	2.73	7.7	7.5	27.4	1.0	2.84/2.84	2.9	8:40
7/21/10	26.39	23.25	25.42	26.29	8.01	2.80	7.9	7.5	29.0	0.8	2.79/2.56	5.1	8:14
7/15/10	23.78	23.09	25.11	25.45	2.76	2.71	7.4	7.4	24.1	1.5	1.87/1.48	3.2	8:26
7/7/10	23.07	19.5	25.75	26.23	8.27	5.67	7.8	7.5	29.2	1.5	1.91/1.85	5.0	8:32
6/30/10	22.70	22.50	24.88	25.20	7.54	7.91	8.0	8.0	19.7	1.0	2.77/2.87	3.0	8:43
6/23/10	21.98	16.81	24.72	25.84	9.93	5.21	8.1	7.7	26.1	1.0	1.89/1.79	5.0	8:30
6/16/10	17.90	18.10	No electronic meter.			7.5			21.1	1.0	2.3/1.85	4.0	9:00
6/9/10	19.67	16.46	23.32	24.81	DO probe malfur		8.4	8.0	19.2	1.0	2.65/2.34	5.5	8:40
6/2/10	18.03	17.16	23.45	23.90	4.93	6.09	7.9	7.9	21.2	1.0	3.88/3.54	4.3	8:55
5/26/10	16.00	13.83	23.67	24.26	8.94	8.16	7.9	7.8	20.9	1.75	0.63/0.65	5.1	8:50
5/20/10	13.36	12.21	22.69	23.95	9.23	8.84	7.7	6.7	15.3	1.5	2.36/2.31	5.3	9:25
CSHH #2 - Bell Marker 6													
11/3/10	11.92	12.66	26.83	27.43	10.52	10.14	8.0	8.0	9.6	2.8	1.05/1.34	9.1	10:40
10/28/10	16.40	16.20	27.35	27.75	7.99	6.40	8.0	7.8	19.0	3.0	0.56/0.32	8.0	8:45
10/20/10	15.20	16.40	25.69	26.99	10.92	8.38	8.1	7.9	14.5	1.7	1.08/0.77	10.0	11:40
10/13/10	17.87	17.87	26.43	26.44	7.87	7.83	8.0	8.0	11.5	2.0	0.69/1.47	6.7	8:44
10/7/10	No data colelcted at this station--platform detached from sonde.												
9/29/10	20.89	20.90	27.36	27.54	6.15	4.96	7.8	7.7	20.3	1.5	1.08/1.51	8.1	9:00
9/22/10	20.44	20.76	26.85	27.13	8.62	7.48	8.1	8.0	21.5	1.75	2.64/1.14	9.0	8:25
9/15/10	21.26	20.90	27.01	26.91	6.95	7.26	7.8	7.8	17.9	2.0	1.14/2.11	9.1	8:38
9/8/10	23.12	22.77	26.61	26.86	6.85	4.70	7.7	7.6	27.7	2.2	1.43/1.32	10.6	11:00
9/2/10	25.12	23.14	26.55	26.89	11.20	2.53	8.2	7.4	28.7	1.0	5.54/3.32	8.7	9:25
8/26/10	22.37	22.17	27.00	27.14	5.67	4.56	7.7	7.6	22.5	2.0	1.34/0.95	8.4	8:45
8/18/10	24.12	22.97	27.16	27.39	10.33	3.65	8.2	7.5	23.8	1.0	1.80/2.25	8.6	9:15
8/11/10	23.49	21.96	26.92	27.29	4.81	1.29	7.6	7.3	25.8	1.0	1.51/1.38	8.1	8:30
8/4/10	22.75	21.25	26.60	27.00	6.23	1.15	7.7	7.3	26.7	1.5	1.51/1.23	9.3	8:48
7/28/10	23.48	20.65	25.93	26.80	5.86	0.58	7.7	7.3	27.9	1.3	1.23/1.24	8.3	9:45
7/21/10	26.62	21.18	25.83	26.56	8.80	1.00	8.1	7.3	30.4	1.0	2.32/1.81	9.6	10:14
7/15/10	22.60	21.39	25.91	26.47	6.01	3.36	7.7	7.5	23.5	1.7	0.97/0.61	5.7	9:50
7/7/10	23.91	17.63	25.65	26.52	10.35	2.43	8.2	7.3	30.0	1.5	1.63/1.85	8.3	9:27
6/30/10	21.36	17.91	25.86	26.20	9.39	4.36	8.3	7.5	19.4	1.2	1.42/1.65	8.0	9:08
6/23/10	20.44	16.27	25.59	26.00	10.45	5.01	8.2	7.6	25.6	1.0	1.14/1.22	9.2	10:30
6/16/10	17.50	16.80	No electronic equipment.			6.00			20.1	1.6	2.03/1.38	7.0	9:25`
6/9/10	Trouble with electonic meters--DO probe malfunction. No sampling at this station.												
6/2/10	19.47	16.54	23.10	24.34	7.95	8.20	8.3	8.0	21.3	1.3	2.52/1.78	6.9	9:30
5/26/10	15.76	14.80	24.18	24.25	10.13	9.57	8.0	7.9	26.5	2.0	0.98/0.74	9.2	10:45
5/20/10	14.96	11.33	23.24	24.42	9.30	7.94	7.8	7.7	21.1	2.0	1.44/1.27	7.4	10:40

CSHH Water-Monitoring Program 2010

Date	Water Temp (°C)		Salinity (ppt)		DO (ppm)		pH (ppm)		Air Temp	Secchi(m)	Turbidity	Depth (m)	Time
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	(°C)		(NTUs)	(Bottom)	(AM)
CSHH #3 - Glen Cove Creek, Red Marker													
11/3/10	12.19	12.72	26.82	27.29	11.20	10.25	8.1	8.0	9.1	3.0	0.8/1.12	5.4	11:00
10/28/10	16.49	16.54	26.51	27.01	7.88	7.63	7.9	7.8	19.2	2.5	0.79/0.66	3.3	9:05
10/20/10	15.59	15.85	25.82	26.49	10.14	9.34	8.0	8.0	15.5	1.9	0.67/0.62	5.3	11:25
10/13/10	17.89	18.00	25.75	26.35	8.06	7.82	8.0	8.0	13.0	2.3	0.31/0.46	3.5	9:35
10/7/10	No data collected at this station--platform detached from sonde.												
9/29/10	20.77	20.98	26.66	27.47	6.27	4.46	7.8	7.7	20.5	1.75	0.85/1.06	3.3	9:28
9/22/10	20.33	20.37	26.43	26.70	8.09	7.56	8.0	8.0	22.4	1.75	2.06/2.37	4.3	8:40
9/15/10	21.00	20.89	26.74	26.81	8.23	7.80	7.9	7.9	17.9	1.0	1.91/2.12	3.8	9:00
9/8/10	23.18	22.98	26.54	26.63	6.31	5.03	7.7	7.6	28.0	1.5	2.35/2.13	5.6	11:15
9/2/10	25.90	23.95	25.99	26.77	11.91	4.94	8.2	7.7	29.0	1.0	3.30/3.02	3.9	9:55
8/26/10	21.65	21.91	25.63	26.27	5.17	4.28	7.6	7.5	22.8	2.0	1.16/1.43	3.6	9:10
8/18/10	24.22	23.33	26.73	27.29	8.61	5.23	8.0	7.6	24.3	1.0	1.91/2.23	4.8	9:34
8/11/10	23.96	23.69	26.76	26.89	6.17	4.77	7.7	7.7	26.8	0.8	1.44/1.73	3.2	8:55
8/4/10	22.86	22.36	26.27	26.74	5.94	4.18	7.6	7.5	27.3	1.0	2.03/1.68	4.4	9:14
7/28/10	24.99	22.00	25.53	26.65	7.76	2.10	8.0	7.4	30.0	1.0	1.89/2.20	3.9	10:05
7/21/10	26.83	22.73	25.29	26.38	9.72	2.79	8.2	7.4	30.3	1.1	2.25/2.26	4.8	10:30
7/15/10	22.61	22.08	25.25	25.81	5.91	3.72	7.6	7.5	24.4	2.0	0.75/0.89	3.0	9:12
7/7/10	23.97	19.58	25.60	26.31	10.12	7.31	8.2	7.7	32.5	1.5	1.54/--	4.7	9:55
6/30/10	21.82	21.77	25.68	25.78	9.61	9.94	8.3	8.2	19.9	1.5	1.73/2.21	3.0	9:40
6/23/10	21.67	16.74	25.01	25.83	11.15	4.69	8.3	7.6	26.5	0.9	2.08/2.28	5.0	11:05
6/16/10	18.00	18.00	No electronic equipment.			6.50			22.1	1.5	1.46/1.28	2.5	10:05
6/9/10	No data collected at this station--trouble with electronic meter--DO probe malfunction.												
6/2/10	19.64	17.58	23.79	24.14	8.70	9.25	8.4	8.0	22.2	1.0	2.14/2.04	3.2	10:00
5/26/10	16.56	14.50	23.80	24.24	9.53	9.37	8.0	7.9	28.9	1.8	0.31/1.17	5.6	11:05
5/20/10	13.89	12.27	23.37	24.11	8.94	8.44	7.8	7.8	20.5	2.4	1.08/1.34	3.4	11:05
CSHH #8 - Glen Cove Sewage Treatment Plant Outfall													
11/3/10	12.57	12.85	25.41	26.96	10.59	9.82	8.0	8.0	10.0	2.0	1.22/1.35	4.0	11:30
10/28/10	15.91	16.26	26.26	26.69	8.26	7.73	7.8	7.8	20.0	1.0	2.71/2.03	2.6	9:26
10/20/10	15.86	15.63	25.24	26.04	9.03	8.67	7.9	7.9	15.9	1.8	1.01/1.03	4.5	11:09
10/13/10	18.41	18.16	25.69	26.15	7.04	7.23	7.9	7.9	13.3	1.2	1.25/0.62	2.5	10:00
10/7/10	No data collected at this station--platform detached from sonde.												
9/29/10	21.22	21.12	26.24	26.70	3.61	3.45	7.6	7.7	20.9	1.5	0.64/1.44	2.7	9:55
9/22/10	20.32	20.99	22.78	26.48	7.26	6.17	7.9	7.8	21.5	0.75	1.21/1.53	3.3	9:00
9/15/10	21.32	21.60	22.97	26.67	7.8	7.17	7.8	7.8	17.6	1.0	2.86/2.34	2.7	9:30
9/8/10	22.88	23.18	21.95	26.00	6.47	5.37	7.7	7.6	28.9	1.0	3.43/3.71	4.4	11:35
9/2/10	25.82	24.34	23.89	26.49	15.62	6.17	8.4	7.8	30.6	0.75	3.84/4.66	3.1	10:40
8/26/10	22.28	22.33	24.99	26.62	3.70	2.05	7.4	7.3	23.1	1.5	1.53/2.01	3.0	8:40
8/18/10	24.50	23.41	26.13	26.93	11.03	4.07	8.1	7.5	25.0	0.75	2.54/2.49	3.5	10:00
8/11/10	24.58	23.8	26.12	26.85	7.05	4.8	7.8	7.6	27.8	0.80	1.93/2.41	2.6	9:17
8/4/10	23.94	23.87	22.4	26.31	6.42	4.18	7.6	7.5	28.5	1.00	3.37/2.49	2.9	10:00
7/28/10	25.68	24.39	22.02	25.96	9.73	4.37	8.1	7.6	30.5	0.75	2.95/2.95	3.0	10:35
7/21/10	26.36	23.58	23.28	26.28	10.9	2.46	8.2	7.4	31.3	0.80	2.59/3.45	3.9	10:50
7/15/10	22.65	22.46	25.75	25.88	4.27	3.16	7.5	7.4	24.4	1.10	1.14/1.93	1.8	9:42
7/7/10	24.67	20.68	23.39	26.15	10.83	6.88	8.2	7.8	33.4	1.00	2.45/2.73	3.5	10:30
6/30/10	23.39	22.18	23.78	25.45	12.92	8.21	8.4	8.0	19.7	0.80	2.70/2.70	2.3	10:02
6/23/10	21.81	17.75	25.45	25.79	15.22	5.57	8.3	7.6	29.4	0.70	2.43/2.92	4.9	11:33
6/16/10	18.80	18	No electronic equipment.			7.25			23.1	1.50	1.86	2.3	10:20
6/9/10	No data collected at this station--trouble with electronic meter--DO probe malfunction.												
6/2/10	20.04	18.5	17.93	23.95	10.72	11.51	8.2	8.1	23.4	0.50	3.18	2.5	10:25
5/26/10	16.95	15.59	21.02	23.86	9.39	9.49	7.7	7.9	29.8	0.90	2.62/3.18	4.5	11:30
5/20/10	13.62	13.21	22.9	23.48	8.59	8.24	7.7	7.7	22.4	1.50	1.51/1.19	2.3	11:26

CSHH Water-Monitoring Program 2010

Date	Water Temp (°C)		Salinity (ppt)		DO (ppm)		pH (ppm)		Air Temp	Secchi(m)	Turbidity	Depth (m)	Time
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	(°C)		(NTUs)	(Bottom)	(AM)
CSHH #13 - 60' West of the Mill Pond Weir													
11/3/10	13.88	13.53	19.41	27.02	9.86	8.27	8.0	7.8	10.8	1.50	2.97/2.55	2.2	11:50
10/28/10	15.80		25.80		5.64		7.5		22.3	0.4*	1.59	0.4	9:45
10/20/10	16.21	15.94	24.85	25.76	8.81	6.26	7.8	7.7	16.1	1.5	3.71/3.12	2.4	10:19
10/13/10	18.36	18.23	25.64	26.19	5.73	6.42	7.7	7.8	14.3	1.1	3.63/3.69	2.5	10:23
10/7/10	No data collected at this station--platform detached from sonde.												
9/29/10	20.80	21.18	24.21	26.11	4.48	2.49	7.6	7.5	21.6	1.2	2.33/3.11	0.9	10:15
9/22/10	19.88	21.18	19.72	26.27	6.24	3.88	7.6	7.5	22.5	1.75	2.29/3.52	2.9	9:20
9/15/10	21.01	21.36	23.29	25.79	4.93	4.98	7.5	7.5	19.4	1.0*	3.49/3.79	1.0	9:55
9/8/10	Ran out of time--no sampling at CSHH #13.												
9/2/10	23.69	25.26	19.30	25.84	11.64	9.04	8.1	7.9	31.8	1.25	3.36/4.96	1.8	10:55
8/26/10	21.96	22.53	25.00	26.66	4.25	0.50	7.4	7.3	23.9	1.25	2.42/4.65	2.9	10:02
8/18/10	23.86	24.44	25.97	26.81	11.25	8.25	8.0	7.9	25.5	0.75	5.64/3.17	2.0	10:35
8/11/10	24.11	24.14	24.88	26.60	7.43	3.91	7.8	7.5	28.1	0.80	6.56/3.36	2.0	9:24
8/4/10	23.32	23.33	25.52	26.05	3.18	2.50	7.4	7.3	29.7	0.90	6.38/4.95	1.6	10:25
7/28/10	22.88	25.02	15.60	25.26	11.01	5.38	8.1	7.6	31.2	1.00	5.68/4.36	1.9	10:55
7/21/10	26.02	25.85	24.91	25.63	8.27	6.46	7.9	7.8	31.6	1.00	3.50/3.62	1.5	11:05
7/15/10	Tide too low--couldn't hold position for sampling at this station.												
7/7/10	22.81	20.27	25.69	26.03	6.80	1.67	7.7	7.3	34.2	1.00	2.61/2.45	3.5	10:50
6/30/10	20.33	21.04	15.59	25.40	9.24	3.55	7.9	7.5	20.0	1.00	3.06/4.06	2.3	10:18
6/23/10	21.14	19.10	23.91	25.34	9.98	4.39	8.1	7.4	28.9	0.70	3.12/3.02	2.1	11:50
6/16/10	18.00	18.00	No electronic equipment.			4.00			18.5	1.00	3.77/4.45	2.0	10:45
6/9/10	No data collected at this station--trouble with electronic meter.												
6/2/10	18.82	17.40	14.14	23.80	9.81	4.54	8.1	7.4	24.9	0.75	3.40/3.85	2.4	10:43
5/27/09	Barges blocking head of Glen Cove Creek--no access to CSHH #13.												
5/20/10	Barges blocking head of Glen Cove Creek--no access to CSHH #13.												
										*bottom			
CSHH #14 - 50 yds from Powerhouse Drain													
11/3/10	11.32	12.28	26.28	26.86	9.22	8.43	8.0	8.0	3.6	2.3*	1.02/1.02	2.7	8:40
10/20/10	14.74	15.14	25.51	25.79	8.61	7.77	7.9	7.9	14.3	1.8	0.64/1.06	1.9	9:45
9/22/10	20.22	20.27	26.50	26.50	7.14	7.16	8.0	8.0	22.1	1.5	2.34/2.13	1.6	9:55
9/8/10	22.99	22.91	26.65	26.65	4.52	4.03	7.6	7.5	27.7	0.75	5.05/5.18	2.4	10:28
8/11/10	22.76	22.60	27.02	27.03	2.07	1.97	7.3	7.3	29.2	1.8	1.75/1.24	4.1	10:20
7/21/10	25.69	25.27	25.43	25.78	6.03	5.12	7.7	7.6	30.0	0.8	3.17/3.17	2.0	9:45
										*bottom			
CSHH #15 - 50 yds from Scudders Pond Outfall, North of Tappen Pool													
11/3/10	11.64	12.81	26.19	27.24	10.31	10.01	8.0	8.0	9.2	2.5*	1.06/0.98	2.5	10:20
10/20/10	15.18	15.72	25.84	26.33	8.94	7.8	8.0	7.9	15.1	2.2*	0.68/1.12	2.6	10:10
9/22/10	19.85	19.83	25.88	25.89	7.85	7.87	7.8	7.9	21.9	2.0	1.97/2.32	2.3	9:40
9/8/10	22.89	23.07	26.30	26.34	5.84	5.09	7.6	7.6	27.5	1.2	2.85/2.34	2.6	10:46
8/11/10	23.89	23.83	26.41	26.41	6.20	6.20	7.6	7.6	27.0	1.0	no sample	1.7	10:07
7/21/10	25.72	24.67	25.73	26.03	6.08	4.18	7.8	7.6	29.8	1.0	2.9/3.68	1.8	10:01
										*bottom			
CSHH #4 - Bar Beach Spit													
11/3/10	11.20	12.67	26.25	27.16	8.87	8.39	8.0	8.0	5.9	2.3*	1.25/2.94	2.9	8:55
10/20/10	15.09	15.71	25.68	26.27	7.89	7.75	7.9	7.9	14.0	2.0	1.33/1.02	2.6	9:46
9/22/10	20.21	20.28	26.55	26.60	7.66	7.60	8.0	8.0	23.4	1.5	2.29/1.72	5.4	10:05
9/8/10	23.05	22.98	26.34	26.42	4.48	4.13	7.5	7.5	24.8	1.0	3.44/3.83	6.7	9:00
8/11/10	23.82	23.53	26.74	26.81	5.60	4.49	7.6	7.5	29.5	1.0	2.70/2.80	5.3	10:35
7/21/10	25.70	23.20	25.58	26.15	5.74	2.18	7.7	7.3	30.4	0.8	2.75/3.17	7.2	9:35
6/9/10	Trouble with electronic meter--no sample collection at this station.												
										*bottom			

CSHH Water-Monitoring Program 2010

Date	Water Temp (°C)		Salinity (ppt)		DO (ppm)		pH (ppm)		Air Temp	Secchi(m)	Turbidity	Depth (m)	Time
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	(°C)		(NTUs)	(Bottom)	(AM)
CSHH #5 - Mott's Cove													
11/3/10	11.92	12.32	26.30	26.84	7.80	7.65	8.0	8.0	5.7	2.3	1.0/1.82	2.3	9:05
10/20/10	15.40	15.63	25.63	26.11	7.49	7.34	7.9	7.9	12.5	2.0*	1.36/1.36	1.9	9:31
9/22/10	19.99	20.03	25.68	25.79	7.68	7.83	8.0	8.0	22.7	1.25	3.37/3.63	1.6	10:15
9/8/10	22.98	23.06	25.62	26.04	4.18	3.93	7.5	7.5	25.6	1.0	3.56/3.89	1.5	9:24
8/11/10	24.64	24.17	26.38	26.77	3.65	3.24	7.4	7.4	30.7	1.0	2.50/2.84	1.8	10:53
7/21/10	26.65	25.17	24.83	25.59	7.30	5.16	7.8	7.5	29.2	0.5	5.98	1.8	9:21
										*bottom			
CSHH #6 - East of Former Incinerator Site													
11/3/10	10.96	11.64	25.37	26.22	10.35	9.75	8.0	8.0	6.9	1.8	1.95/1.82	2.8	9:30
10/20/10	15.07	15.18	25.39	25.56	7.88	7.32	7.9	7.9	12.0	1.8	2.19/1.89	2.3	9:12
9/22/10	20.19	20.14	25.78	26.02	8.01	7.95	8.0	8.0	23.7	1.75	2.72/3.06	2.4	10:30
9/8/10	23.16	23.09	25.94	25.98	4.31	4.01	7.5	7.5	26.7	1.2	4.67/4.28	2.3	9:45
8/11/10	24.72	24.79	26.56	26.57	4.97	4.39	7.5	7.4	27.6	0.5	3.52	2.4	11:04
7/21/10	27.04	25.66	25.16	25.65	6.00	4.76	7.5	7.5	29.7	0.8	4.33/3.78	1.3	9:05
CSHH #7 - West of Bryant Landing (formerly site of oil dock)													
11/3/10	10.57	11.06	23.92	25.40	9.58	9.81	7.9	7.9	7.3	1.0	3.05/3.31	2.1	9:50
10/20/10	14.30	14.94	24.64	25.21	7.62	7.12	7.9	7.9	10.7	1.3	2.7/3.0	2.0	8:58
9/22/10	20.26	20.12	25.49	25.62	7.44	7.50	8.0	8.0	24.2	1.25	4.67/4.30	1.8	10:40
9/8/10	23.22	23.09	25.56	25.73	4.69	3.94	7.6	7.5	28.0	1.0	5.18/4.59	2.0	10:00
8/11/10	26.00	25.70	25.50	26.16	6.68	6.11	7.6	7.5	29.1	0.5	6.38	2.2	11:15
7/21/20	26.65	26.98	24.80	25.17	4.89	4.82	7.5	7.5	29.0	0.5	4.56	1.5	8:45

Appendix H

Hach Nitrate and Nitrite and LaMotte Kit Manuals

DETERGENT TEST KIT

MODEL DS-1-DC • CODE 4507-01

QUANTITY	CONTENTS	CODE
2 x 30 mL	*DS Indicator Reagent	*4508-G
15 mL	DS Reference Solution	4513-E
50 g	pH Adjustment Powder	4509-H
1	Test Tube, Test Sample w/cap	0282
1	Test Tube, Reference Sample w/cap	0283
1	Test Tube, 1-8 mL, plastic, w/cap	0755
1	Pipet, glass	0347
1	Spoon, 0.25 g, plastic	0695

*WARNING: Reagents marked with an * are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents see MSDS CD or www.lamotte.com. To obtain a printed copy, contact LaMotte by e-mail, phone or fax.

To order individual reagents or test kit components, use the specified code number.

WARNING! This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision

PROCEDURE

STEP I - Determine if Detergent is Present

1. Use the calibrated test tube (0755) to measure 5 mL of the sample solution. Add to the screw cap tube marked Test Sample (0282).
2. Use the 0.25 g spoon (0695) to add one measure of pH Adjustment Powder (4509). Shake until dissolved.
3. Fill the pipet (0347) with *DS Indicator Reagent (4508) by squeezing the rubber bulb, then inserting pipet into reagent. Add this amount of *DS Indicator Reagent to the Test Sample tube. Cap and shake for one minute.
4. Allow the tube to stand until the two layers of the solution separate. The water layer will settle to the bottom and the reagent layer will rise to the top. Use chart below to determine if detergent is present.

Bottom Layer	Top Layer	Quick Reading
Colorless	Colored	No Detergent in sample
Some Color	Some Color	Some Detergent in sample
Colored	Colorless	High Detergent in sample

NOTE: If the amount of detergent in the sample is to be determined, save this Test Sample and proceed to Step 2.

STEP 2 - Determine the Amount of Detergent Present

1. Use the calibrated test tube (0755) to measure 5 mL of detergent-free water. Add to the screw cap tube marked Reference Sample (0283). (On field trips it may be necessary to carry a small supply of detergent free water.)
2. Use the 0.25 g spoon (0698) to add one measure of pH Adjustment Powder (4509). Shake until dissolved.
3. Fill the pipet (0347) with *DS Indicator Reagent (4508) by squeezing the rubber bulb, then inserting pipet into reagent. Add this amount of *DS Indicator Reagent to the Reference Sample tube.
4. Add one drop of DS Reference Solution (4513). Cap and shake for one minute.
5. Allow the tube to stand until the two layers of solution separate. The color produced in the bottom (water) layer is equivalent to 1 ppm of detergent.
6. Compare the color in the bottom layer of the Test Sample Tube from Part I to the color of the bottom of the Reference Sample Tube.

If Test Sample Color Is:	Test Sample Contains:
Lighter than Reference	Less than 1.0 ppm Detergent
Same as Reference	1.0 ppm Detergent
Darker than Reference	More than 1.0 ppm Detergent

7. Add one drop of DS Reference Solution (4513) to the Reference Sample Tube. Shake to mix. Compare the color as before. The color in the Reference Sample is now equal to 2.0 ppm. Continue this procedure, counting the number of drops of DS Reference Solution (4513) added, until the color of the bottom layer in each tube is the same. Each drop of the DS Reference Solution (4513) added to the Reference Sample Tube is equal to 1 ppm detergent in the sample.

   NOTE: If at any time the top layer of the Test Sample or Reference Sample becomes colorless, add more DS Indicator Reagent (4508). The amount of this reagent added is not important as long as there is some color in the top layer.

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12.09

Nitrogen, ammonia, colorimetry, salicylate-hypochlorite, automated-segmented flow

Parameters and Codes:
Nitrogen, ammonia, dissolved, I-2522-90 (mg/L as N): 00608
Nitrogen, ammonia, total-in-bottom-material, I-6522-90
(mg/L as N): 00611

1. Application

1.1 This method is used to analyze samples of surface, domestic, and industrial water, and brines containing from 0.01 to 1.5 mg/L of ammonia-nitrogen. Concentrations greater than 1.50 mg/L must be diluted. This modified method was implemented in the National Water Quality Laboratory in March 1988.

1.2 This method also is used to determine concentrations of ammonia-nitrogen in samples of bottom material containing at least 0.2 mg/kg NH_3-N. Prepared sample solutions containing more than 1.5 mg/L NH_3-N need to be diluted.

1.3 Sodium ion is a good replacement for ammonium ion in the slow-exchange positions of soil minerals (Jackson, 1958). Bottom material is treated with an acidified sodium chloride solution, and the resulting mixture is allowed to settle and then decanted to obtain a clear supernatant solution for analysis.

2. Summary of method

Ammonia reacts with salicylate and hypochlorite ions in the presence of ferricyanide ions to form the salicylic acid analog of indophenol blue (Reardon and others, 1966; Patton and Crouch, 1977; Harfmann and Crouch, 1989). The resulting color is directly proportional to the concentration of ammonia present.

3. Interferences

3.1 Sulfide interferes. Bromide and nitrite can interfere. Calcium and magnesium in highly alkaline waters (pH greater than 13.6) can exceed the ability of the tartrate to complex both ions.

3.2 The samples are easily contaminated by ammonia in the laboratory atmosphere; therefore, sample handling and analysis need to be performed where there is no possibility of ammonia contamination.

4. Apparatus

4.1 *Shaker,* wrist action.

4.2 *Alpkem rapid flow analyzer (RFA),* consisting of sampler, peristaltic pump, analytical cartridge, heating bath, colorimeter, data station, and printer.

With this equipment, the following operating conditions are satisfactory for the range from 0.01 to 1.50 mg/L of ammonia-nitrogen:

Flow cell	15 mm
Wavelength	660 nm
Sample time	24 seconds
Wash time	16 seconds
Sampling rate	90 per hour
Heating Bath (2mL)	37°C
Pecking	ON
Damp (RC)	*1* second

5. Reagents

5.1 Ammonia standard solution 1, 1.00 mL = 0.50 mg NH_3-N: Dissolve 1.9095 g NH_4Cl, dried overnight over sulfuric acid, in ammonia-free water and dilute to 1,000 mL. Refrigerate.

5.2 Ammonia standard solution II, 1.00 mL = 0.0015 mg NH_3-N: Dilute 3.0 mL ammonia standard solution I to 1,000 mL with ammonia-free water. Prepare fresh weekly and refrigerate.

5.2.1 Ammonia working solutions, bottom materials: Prepare an ammonia-free blank and 250 mL each of a series of ammonia working solutions by appropriate quantitative dilution of ammonia standard solution II or working solutions with acidified sodium chloride solution (paragraph 5.7), as follows:

Working solution No.	Solution added (mL)	Solution used	Ammonia concentration (mg/L)
1	250	Standard solution II	1.50
2	125	Standard solution II	.75
3	50	Standard solution II	.30
4	25	Standard solution II	.15
5	25	Working solution No. 2	.075
6	25	Working solution No. 4	.015

Ammonia working solutions, water: Prepare an ammonia-free blank and *250* mL each of a series of ammonia working solutions by dilution of ammonia standard solution II or working solutions with ammonia-free water as listed in the following table. If the samples to be analyzed are preserved, the ammonia working solutions need to contain an equivalent concentration of the same preservative.

Working solution No.	Solution added (mL)	Solution used	Ammonia concentration (mg/L)
1	250	Standard solution II	1.50
2	125	Standard solution II	.75
3	50	Standard solution II	.30
4	25	Standard solution II	.15
5	25	Working solution No. 2	.075
6	25	Working solution No. 4	.015

Prepare weekly and refrigerate.

5.3 *Buffer stock solution,* 71 g/L: Dissolve 134 g $Na_2HPO_4 \cdot 7H_2O$ in 800 mL of ammonia-free water. Add 100 mL *5M* NaOH, dilute to 1 L with ammonia-free water, and mix thoroughly.

5.4 *Buffer working solution:* Add, while stirring, 250 mL stock potassium sodium tartrate solution to 200. mL buffer stock solution. Slowly, while stirring, add 120 mL 5*M* NaOH. Dilute to 1 L with ammonia-free water, add 1 mL of Brij-35 solution, and mix thoroughly.

5.5 *Hydrochloric acid,* concentrated (sp gr 1.19).

5.6 Potassium sodium tartrate solution, 149 g/L: Dissolve 200 g $NaKC_4H_4O_6 \cdot 4H_2O$ in about 600 mL ammonia-free water. Dilute to 1 L.

5.7 Sodium chloride solution, 100 g/L: Dissolve 100 g NaCl in 800 mL ammonia-free water, mix thoroughly, adjust the pH to 2.5 using concentrated HCl (sp gr 1.19), and dilute to 1 L.

5.8 *Sodium hydroxide solution, 5M:* CAUTION: Add, while cooling and stirring, 200 g NaOH to about 800 mL ammonia-free water. Cool and dilute to 1 L.

5.9 Sodium hypochlorite solution: Dilute 50 mL sodium hypochlorite solution (a commercial bleach solution containing 5.25-percent available chlorine is satisfactory) to 500 mL with ammonia-free water. Prepare fresh daily.

5.10 *Sodium salicylate—sodium nitroferricyanide solution:* Dissolve 150 g sodium salicylate and 0.30 g sodium nitroferricyanide [$Na_2Fe(CN)_5NO \cdot 2H_2O$] in about 600 mL ammonia-free water. Filter through Whatman 41 filter paper or equivalent, and dilute to 1 L. Add 1.0 mL Brij-35 solution and store in a light-resistant container.

5.11 *Sulfuric acid,* concentrated (sp gr 1.84).

5.12 *Sulfuric acid, 2.5M:* Cautiously add 138 mL concentrated H_2SO_4 (sp gr 1.84) to about 700 mL ammonia-free water. Cool and dilute to 1 L with ammonia-free water.

6. Procedure

6.1 Proceed to paragraph 6.2 for water. For bottom materials, begin with paragraph 6.1.1.

6.1.1 Weigh, to the nearest milligram, about 5 g of sample prepared as directed in method P-0520, and transfer to a 250-mL Erlenmeyer flask.

6.1.2 Add 50 mL of the acidic sodium chloride solution (paragraph 5.7), shake on the wrist-action shaker for 30 minutes, and allow to settle.

6.1.3 Transfer the supenatant solution to a 200-mL volumetric flask, taking care not to disturb the residue in the bottom of the Erlenmeyer flask.

6.1.4 Wash the sediment in the Erlenmeyer flask with 20 mL acidic sodium chloride solution (paragraph 5.7), let settle, and transfer the clear wash solution to the volumetric flask. Adjust to volume with acidic sodium chloride solution (paragraph 5.7). Proceed to paragraph 6.2.

6.2 Set up manifold (fig. 4). If the laboratory air is contaminated with ammonia, the air needs to be passed through a scrubber containing 2.5*M* H_2SO_4 before it enters the air manifold tube.

6.3 Allow the colorimeter, recorder, and heating bath to warm for at least 10 minutes or until the temperature of the heating bath reaches 37°C.

6.4 After all reagents are on line (NOTE 1), adjust the sample output of the photometer to 5 V. Then switch the photometer to "absorbance" mode and use the reference detector "fine gain" control to adjust the baseline absorbance to about 0.2 V. See operation manuals for complete details (Alpkem Corp., 1986). The solution remaining in the wash reservoir from previous determinations might be contaminated; therefore, this reservoir needs to be emptied and rinsed, and then refilled with fresh solution before proceeding.

NOTE 1. Place each reagent line except salicylate into its respective container; allow at least 5 minutes for the introduction of these reagents, and then place the salicylate line into its reagent container. If a precipitate forms after the addition of the salicylate, the pH of the solution stream is too low; check for contaminated reagents and remake them, and start again using the aforementioned procedure.

6.5 Place the most concentrated working solution in two cups before analysis. As the peaks appear on the recorder, adjust the STD CAL control until the peak obtains 95 percent of full scale.

6.6 When the system is clear of all working solutions, determine a dwell time using the most concentrated working solution.

6.7 Place a complete set of working solutions and a blank in the first positions of the sample tray beginning with the most concentrated working solution (NOTES 2 and 3). Place individual working solutions of differing concentrations in approximately every tenth position on the tray following the accepted protocol. Fill the remainder of each tray with unknown samples.

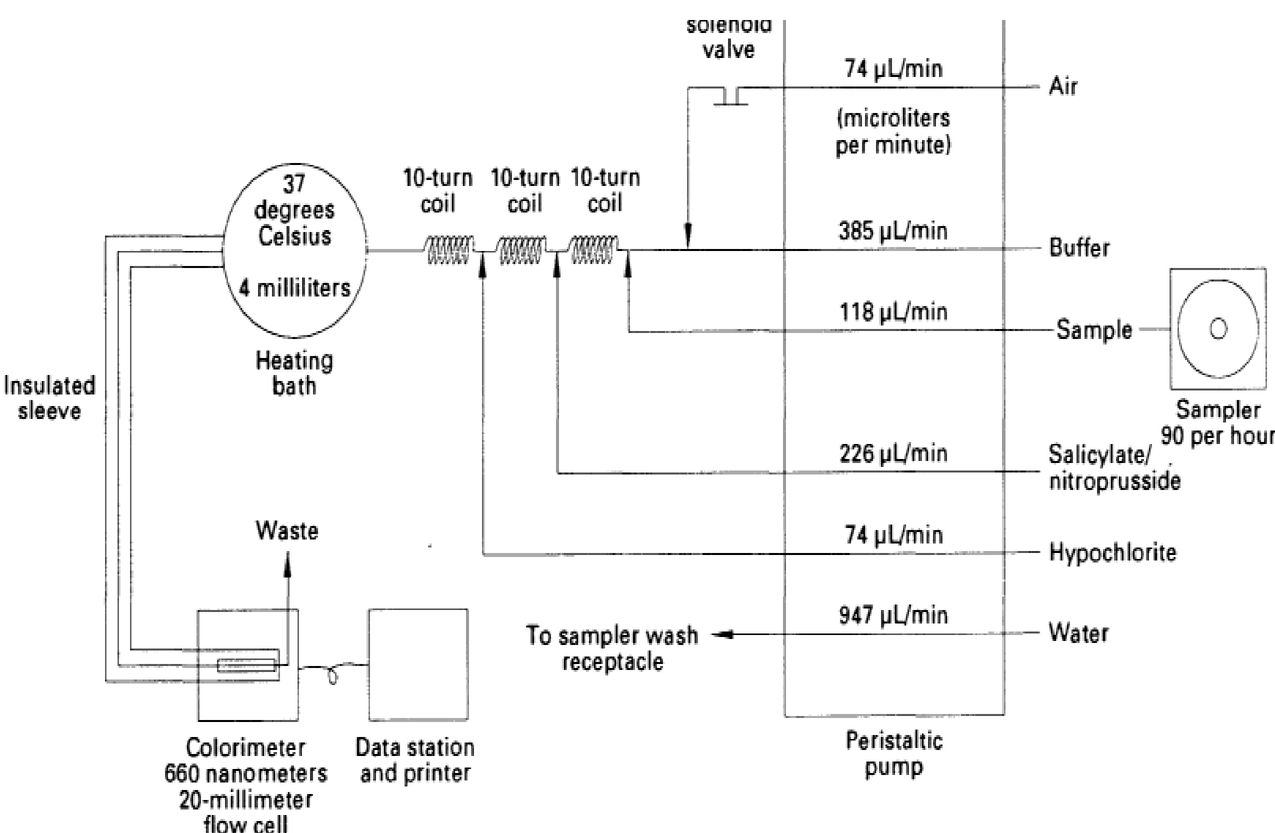

Figure 4.—Nitrogen, ammonia, salicylate-hypochlorite manifold.

NOTE 2. For analysis of bottom materials, use blank and working solutions as prepared in paragraph 5.2.1. For analysis of water, use blank and working solutions as prepared in paragraph 5.2.2.

NOTE 3. To avoid possible contamination of the sample cups, they need to remain sealed in their packages until just prior to use. Rinse each sample cup with sample prior to filling.

6.8 Begin analysis.

7. Calculations

7.1 Prepare an analytical curve by plotting the voltage of each working solution peak in relation to its respective ammonia-nitrogen concentration, or by using the RFA Softpak data reduction package. See operation manuals for complete details (Alpkem Corp., 1986).

7.2 Compute the concentration of dissolved ammonia-nitrogen in each sample by comparing its voltage to the analytical curve or by using the software. Any baseline drift needs to be accounted for when computing the voltage of a sample or standard peak; the RFA software automatically corrects for baseline drift.

7.3 Compute the concentration of ammonia-nitrogen in each sample of bottom material, as follows:

$$NH_3\text{-N (mg/kg)} = \frac{C_N \times 200 \text{ (NOTE 4)}}{\text{wt of sample, in g}}$$

where CN = NH_3-N concentration in sample, in milligrams per liter.
NOTE 4. The factor 200 is used in converting to milligrams per kilogram.

8. Report

8.1 Report concentrations of ammonia-nitrogen, dissolved (00608), as follows: less than 1.0 mg/L, two decimals; 1.0 mg/L and greater, two significant figures.

8.2 Report ammonia-nitrogen, total-in-bottom-material (00611), as follows: less than 10 mg/kg, one decimal; 10 mg/kg and greater, two significant figures.

9. Precision

Single operator precision for ammonia-nitrogen, as determined for natural-water samples, expressed as standard deviation and percentage relative standard deviation, is as follows:

Number of determinations	Mean (mg/L)	Standard deviation (mg/L)	Relative standard deviation (percent)
180	1.23	0.011	0.89
193	.17	.001	.59
147	.28	.004	1.4
252	.71	.005	.70
240	.54	.006	1.1
209	.06	.001	1.7
240	.12	.002	1.7

References

Alpkem Corp., 1986, Rapid flow analyzer operator’s manual, ALPKEM, methodology section.

Harfmann, R.G., and Crouch, S.R., 1989, Kinetic study of Berthelot reaction steps in the absence and presence of coupling reagents: Talanta, v. 36, p. 261-269.

Jackson, M.L., 1958, Soil chemical analysis: Englewood Cliffs, N.J., Prentice-Hall, p. 193.

Patton, C.J., and Crouch, S.R., 1977, Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia: Analytical Chemistry, v. 49, p. 464-469.

Reardon, J., Foreman, J.A., and Searcy, R.L., 1966, New reactants for the colorimetric determination of ammonia: Clinical Chimica Acta, v. 14, p. 403-405.

Dissolved Oxygen

Water Quality Test Kit

Instruction Manual • Code 7414/5860

LaMotte

INTRODUCTION

Aquatic animals need dissolved oxygen to live. Fish, invertebrates, plants, and aerobic bacteria all require oxygen for respiration. Oxygen dissolves readily into water from the atmosphere until the water is saturated. Once dissolved in the water, the oxygen diffuses very slowly and distribution depends on the movement of the aerated water. Oxygen is also produced by aquatic plants, algae, and phytoplankton as a by-product of photosynthesis.

The amount of oxygen required varies according to species and stage of life. Dissolved Oxygen levels below 3 ppm are stressful to most aquatic organisms. Dissolved Oxygen levels below 2 or 1 ppm will not support fish. Levels of 5 to 6 ppm are usually required for growth and activity.

This test kit uses the azide modification of the Winkler method for determining dissolved oxygen.

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KIT CONTENTS

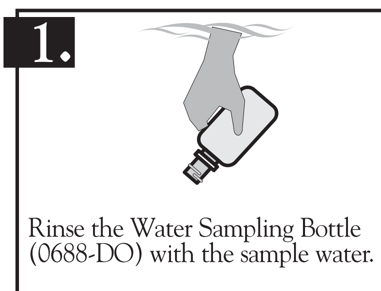
QUANTITY	CONTENTS	CODE
30 mL	*Manganous Sulfate Solution	*4167-G
30 mL	*Alkaline Potassium Iodide Azide	*7166-G
50 g	*Sulfamic Acid Powder (7414 Kit)	*6286-H
30 mL	*Sulfuric Acid, 1:1 (5860 Kit)	*6141WT-G
60 mL	*Sodium Thiosulfate, 0.025N	*4169-H
30 mL	Starch Indicator Solution	4170WT-G
1	Spoon, 1.0 g, plastic (7414 Kit)	0697
1	Direct Reading Titrator	0377
1	Test Tube, 5-10-12.9-15-20-25 mL, glass, w/cap	0608
1	Water Sampling Bottle, 60 mL, glass	0688-DO

***WARNING:** Reagents marked with a * are considered hazardous substances. To view or print a Material Safety Data Sheet (MSDS) for these reagents see MSDS CD or our website. To obtain a printed copy, contact us by e-mail, phone or fax..

To order individual reagents or test kit components, use the specified code numbers.

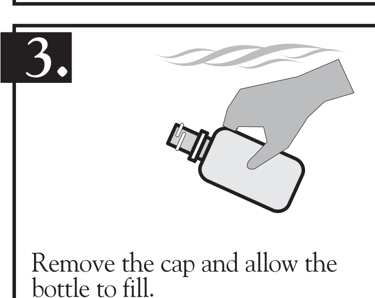
TEST PROCEDURE

PART 1 - COLLECTING THE WATER SAMPLE



Rinse the Water Sampling Bottle (0688-DO) with the sample water.

Tightly cap the bottle, and submerge it to the desired depth.



Remove the cap and allow the bottle to fill.

Tap the sides of the bottle to dislodge any air bubbles.

Replace the cap while the bottle is still submerged.

Retrieve the bottle and make sure that no air bubbles are trapped inside.

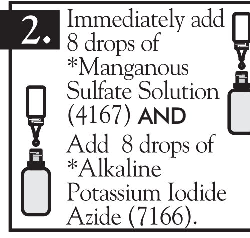
TEST PROCEDURE

PART 2 - ADDING THE REAGENTS

NOTE: Be careful not to introduce air into the sample while adding the reagents.

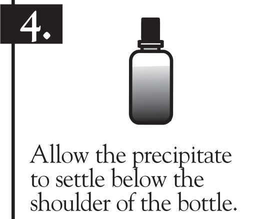
1. Remove the cap from the bottle.

2. Immediately add 8 drops of *Manganous Sulfate Solution (4167) **AND** Add 8 drops of *Alkaline Potassium Iodide Azide (7166).

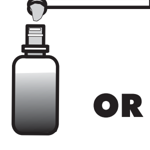


3. Cap the bottle and mix by inverting several times. A precipitate will form.

4. Allow the precipitate to settle below the shoulder of the bottle.



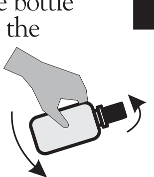
5. *For Kit Code 7414:* Immediately use the 1.0 g spoon (0697) to add one level measure of *Sulfamic Acid Powder (6286).



OR

For Kit Code 5860: Add 8 drops of *Sulfuric Acid, 1:1 (6141WT).

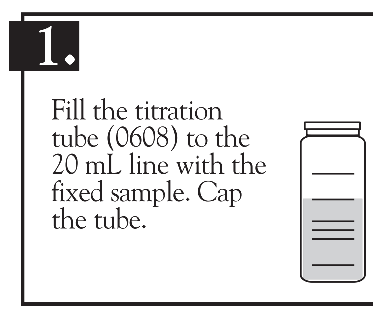
6. Cap and gently invert the bottle to mix the contents until the precipitate and the reagent have totally dissolved. The solution will be clear yellow to orange if the sample contains dissolved oxygen.



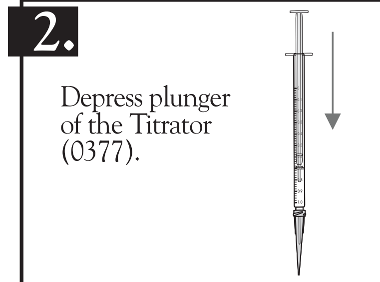
NOTE: At this point the sample has been "fixed" and contact between the sample and the atmosphere will not affect the test result. Samples may be held at this point and titrated later.

TEST PROCEDURE

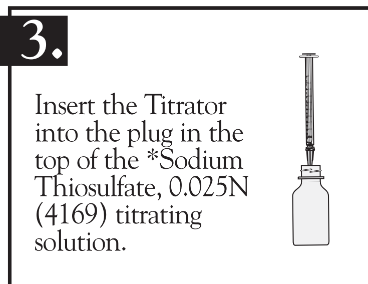
PART 3 - THE TITRATION



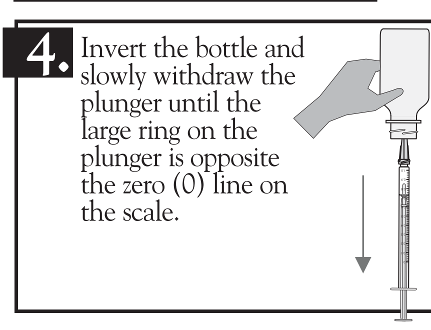
1. Fill the titration tube (0608) to the 20 mL line with the fixed sample. Cap the tube.



2. Depress plunger of the Titrator (0377).

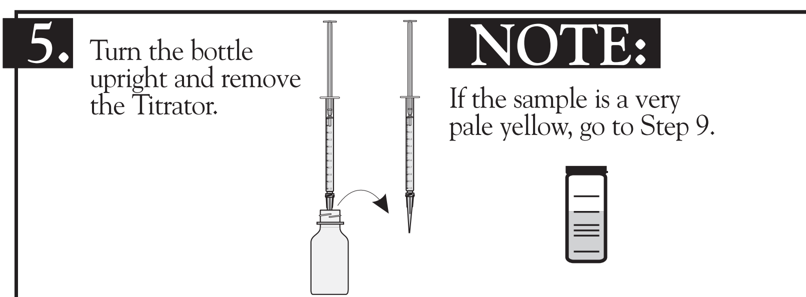


3. Insert the Titrator into the plug in the top of the *Sodium Thiosulfate, 0.025N (4169) titrating solution.



4. Invert the bottle and slowly withdraw the plunger until the large ring on the plunger is opposite the zero (0) line on the scale.

NOTE: If small air bubbles appear in the Titrator barrel, expel them by partially filling the barrel and pumping the titration solution back into the reagent container. Repeat until bubble disappears.

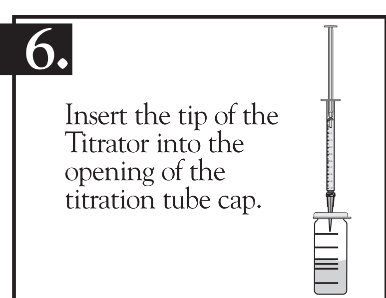


5. Turn the bottle upright and remove the Titrator.

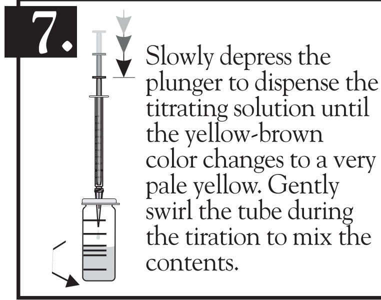
NOTE: If the sample is a very pale yellow, go to Step 9.

continued . . .

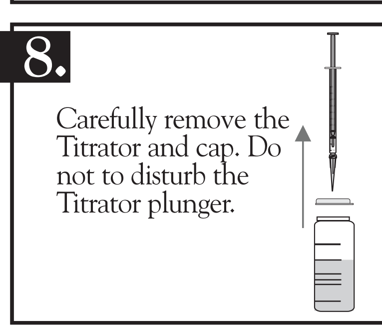
TEST PROCEDURE



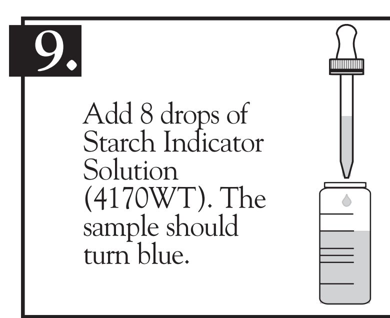
6. Insert the tip of the Titrator into the opening of the titration tube cap.



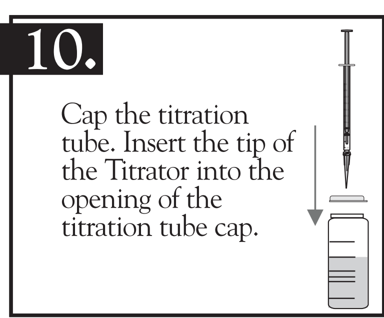
7. Slowly depress the plunger to dispense the titrating solution until the yellow-brown color changes to a very pale yellow. Gently swirl the tube during the tiration to mix the contents.



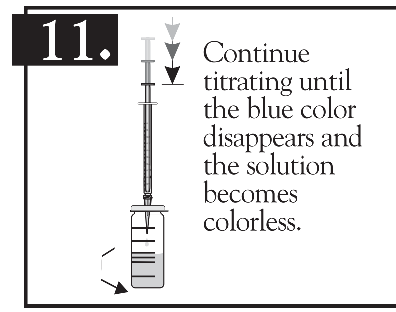
8. Carefully remove the Titrator and cap. Do not to disturb the Titrator plunger.



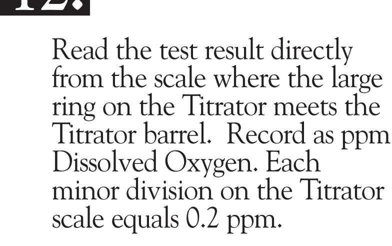
9. Add 8 drops of Starch Indicator Solution (4170WT). The sample should turn blue.



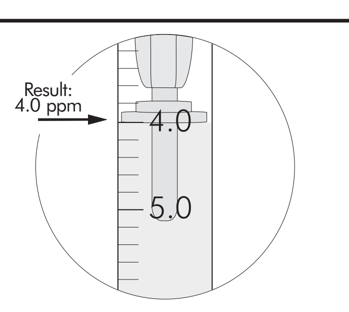
10. Cap the titration tube. Insert the tip of the Titrator into the opening of the titration tube cap.



11. Continue titrating until the blue color disappears and the solution becomes colorless.



12. Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Record as ppm Dissolved Oxygen. Each minor division on the Titrator scale equals 0.2 ppm.



TEST PROCEDURE

NOTE:

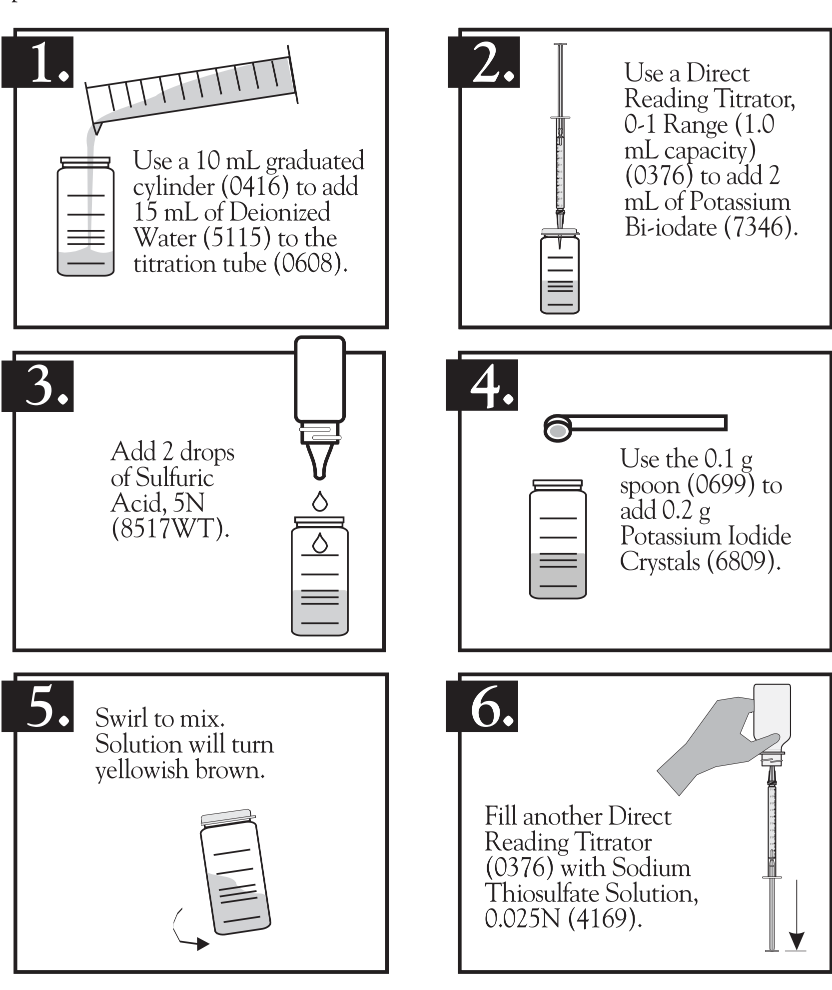
If the plunger ring reaches the bottom line on the scale (10 ppm) before the endpoint color change occurs, refill the Titrator and continue the titration. Include the value of the original amount of reagent dispensed (10 ppm) when recording the test result.

NOTE:

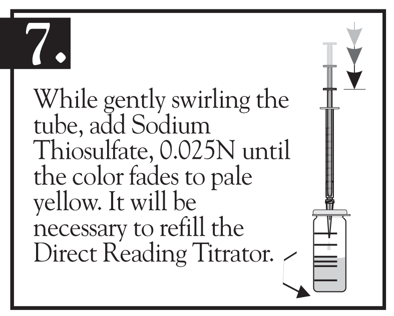
When testing is complete, discard titrating solution in Titrator. Rinse Titrator and titration tube thoroughly. DO NOT remove plunger or adapter tip.

EPA COMPLIANCE

To qualify as an EPA accepted test, and to achieve the greatest accuracy, the Sodium Thiosulfate Solution, 0.025N (4169) must be standardized daily. This procedure follows Standard Methods for the Examination of Water and Wastewater. Numbers in ( ) are for LaMotte products. These products are not included in this kit but can be ordered from LaMotte Company by using the specified code number.

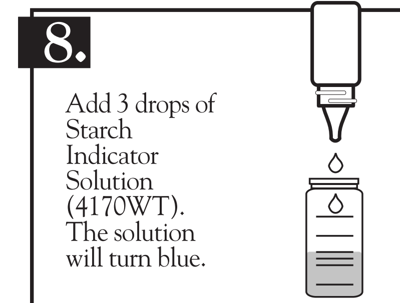


EPA COMPLIANCE



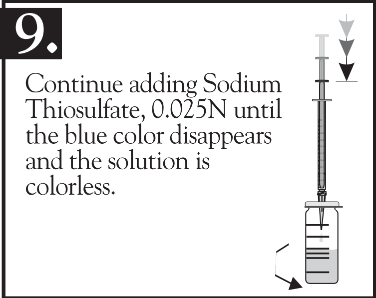
7.

While gently swirling the tube, add Sodium Thiosulfate, 0.025N until the color fades to pale yellow. It will be necessary to refill the Direct Reading Titrator.



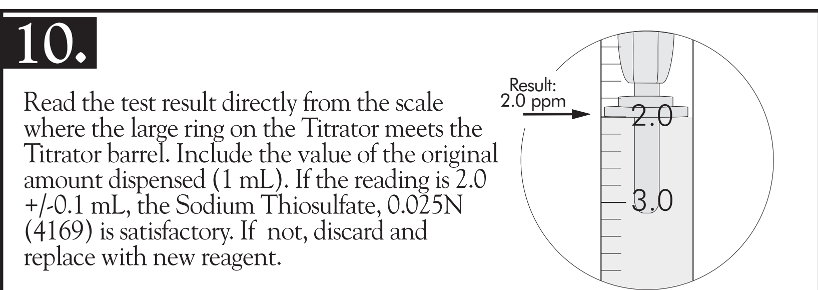
8.

Add 3 drops of Starch Indicator Solution (4170WT). The solution will turn blue.



9.

Continue adding Sodium Thiosulfate, 0.025N until the blue color disappears and the solution is colorless.



10.

Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Include the value of the original amount dispensed (1 mL). If the reading is 2.0 +/-0.1 mL, the Sodium Thiosulfate, 0.025N (4169) is satisfactory. If not, discard and replace with new reagent.

DISSOLVED OXYGEN FACT SHEET

Oxygen is critical to the survival of aquatic plants and animals, and a shortage of dissolved oxygen is not only a sign of pollution, it is harmful to fish. Some aquatic species are more sensitive to oxygen depletion than others, but some general guidelines to consider when analyzing test results are:

5–6 ppm	**Sufficient for most species**
<3 ppm	**Stressful to most aquatic species**
<2 ppm	**Fatal to most species**

Because of its importance to the fish's survival, aquaculturists, or "fish farmers," and aquarists use the dissolved oxygen test as a primary indicator of their system's ability to support healthy fish.

WHERE DOES THE OXYGEN COME FROM?

The oxygen found in water comes from many sources, but the largest source is oxygen absorbed from the atmosphere. Wave action and splashing allows more oxygen to be absorbed into the water. A second major source of oxygen is aquatic plants, including algae; during photosynthesis plants remove carbon dioxide from the water and replace it with oxygen.

Absorption

Oxygen is continuously moving between the water and surrounding air. The direction and speed of this movement is dependent upon the amount of contact between the air and water. A tumbling mountain stream or windswept, wave-covered lake, where more of the water's surface is exposed to the air, will absorb more oxygen from the atmosphere than a calm, smooth body of water. This is the idea behind aerators: by creating bubbles and waves the surface area is increased and more oxygen can enter the water.

Photosynthesis

In the leaves of plants, one of the most important chemical processes on Earth is constantly occurring: photosynthesis. During daylight, plants constantly take carbon dioxide from the air, and in the presence of water convert it to oxygen and carbohydrates, which are used to produce additional plant material. Since photosynthesis requires light, plants do not photosynthesize at night, so no oxygen is produced. Chemically, the photosynthesis reaction can be written as:

$$\text{Light} + nCO_2 + nH_2O \longrightarrow (C_2HO)n + nO_2$$

$$\text{Light} + \text{Carbon Dioxide} + \text{Water} \longrightarrow \text{Carbohydrate} + \text{Oxygen}$$

WHERE DOES THE OXYGEN GO?

Once in the water, oxygen is used by the aquatic life. Fish and other aquatic animals need oxygen to breathe or respire. Oxygen is also consumed by bacteria to decay, or decompose, dead plants and animals.

Respiration

All animals, whether on land or underwater, need oxygen to respire, grow and survive. Plants and animals respire throughout the night and day, consuming oxygen and producing carbon dioxide, which is then used by plants during photosynthesis.

Decomposition

All plant and animal waste eventually decomposes, whether it is from living animals or dead plants and animals. In the decomposition process, bacteria use oxygen to oxidize, or chemically alter, the material to break it down to its component parts. Some aquatic systems may undergo extreme amounts of oxidation, leaving no oxygen for the living organisms, which eventually leave or suffocate.

OTHER FACTORS

The oxygen level of a water system is not only dependent on production and consumption. Many other factors work together to determine the potential oxygen level, including:

- **Salt vs. fresh water** - Fresh water can hold more oxygen than salt water.
- **Temperature** - Cold water can hold more oxygen than warm water.
- **Atmospheric pressure (Altitude)** - The greater the atmospheric pressure the more oxygen the water will hold.

TESTING DISSOLVED OXYGEN

Dissolved oxygen is often tested using the Azide modification of the Winkler method. When testing dissolved oxygen it is critical not to introduce additional oxygen into the sample. Many people avoid this problem by filling the sample bottle all the way and allowing the water to overflow for one minute before capping.

The first step in a DO titration is the addition of Manganous Sulfate Solution (4167) and Alkaline Potassium Iodide Azide Solution (7166). These reagents react to form a white precipitate, or floc, of manganous hydroxide, $Mn(OH)_2$. Chemically, this reaction can be written as:

$$MnSO_4 + 2KOH \longrightarrow Mn(OH)_2 + K2SO_4$$

Manganous Sulfate + Potassium Hydroxide ⟶ Manganous Hydroxide + Potassium Sulfate

Immediately upon formation of the precipitate, the oxygen in the water oxidizes an equivalent amount of the manganous hydroxide to brown-colored manganic hydroxide. For every molecule of oxygen in the water, four molecules of manganous hydroxide are converted to manganic hydroxide. Chemically, this reaction can be written as:

$$4Mn(OH)_2 + O_2 + 2H_2O \longrightarrow 4Mn(OH)_3$$

Manganous Hydroxide + Oxygen + Water ⟶ Manganic Hydroxide

After the brown precipitate is formed, a strong acid, such as Sulfamic Acid Powder (6286) or Sulfuric Acid, 1:1 (6141) is added to the sample. The acid converts the manganic hydroxide to manganic sulfate. At this point the sample is considered "fixed" and concern for additional oxygen being introduced into the sample is reduced. Chemically, this reaction can be written as:

$$2Mn(OH)_3 + 3H_2SO_4 \longrightarrow Mn_2(SO_4)_3 + 6H_2O$$

Manganic Hydroxide + Sulfuric Acid ⟶ Manganic Sulfate + Water

Simultaneously, iodine from the potassium iodide in the Alkaline Potassium Iodide Azide Solution is oxidized by manganic sulfate, releasing free iodine into the water. Since the manganic sulfate for this reaction comes from the reaction between the manganous hydroxide and oxygen, the amount of iodine released is directly proportional to the amount of oxygen present in the original sample. The release of free iodine is indicated by the sample turning a yellow-brown color. Chemically, this reaction can be written as:

$$Mn_2(SO_4)_3 + 2KI \longrightarrow 2MnSO_4 + K_2SO_4 + I_2$$

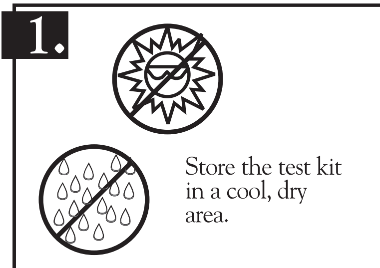
Manganic Sulfate + Potassium Iodide ⟶ Manganous Sulfate + Potassium Sulfate + Iodine

The final stage in the Winkler titration is the addition of sodium thiosulfate. The sodium thiosulfate reacts with the free iodine to produce sodium iodide. When all of the iodine has been converted the sample changes from yellow-brown to colorless. Often a starch indicator is added to enhance the final endpoint. Chemically, this reaction can be written as:

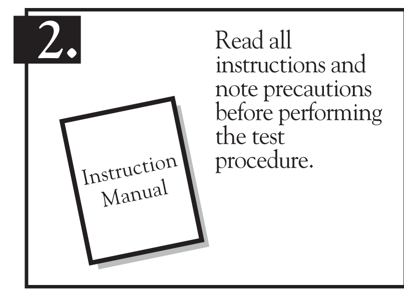
$$2Na_2S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI$$

Sodium Thiosulfate + Iodine ⟶ Sodium Tetrathionate + Sodium Iodide

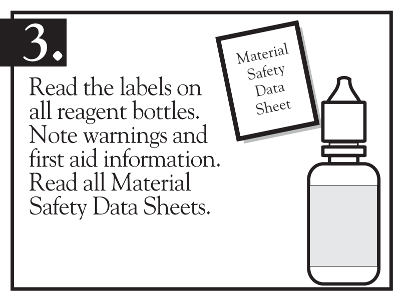
GENERAL SAFETY PRECAUTIONS



1. Store the test kit in a cool, dry area.



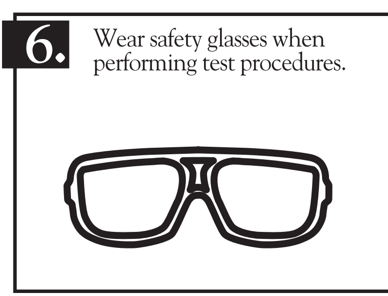
2. Read all instructions and note precautions before performing the test procedure.



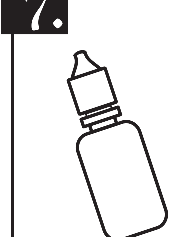
3. Read the labels on all reagent bottles. Note warnings and first aid information. Read all Material Safety Data Sheets.

4. Keep all equipment and reagent chemicals out of the reach of young children.

5. Avoid contact between reagent chemicals and skin, eyes, nose, and mouth.

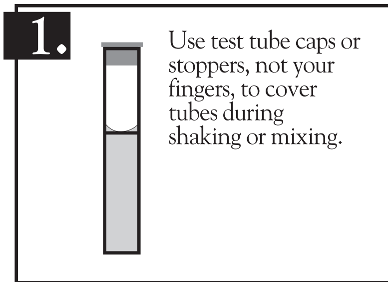


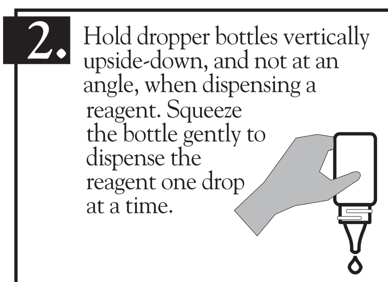
6. Wear safety glasses when performing test procedures.

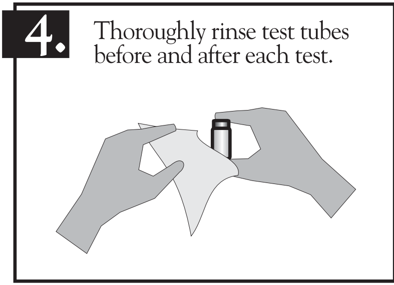


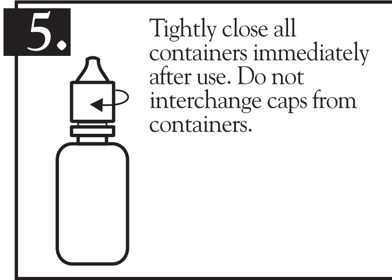
7. In the event of an accident or suspected poisoning, immediately call the Poison Center phone number in the front of your local telephone directory or call a physician. Additional information for all LaMotte reagents is available in the United States, Canada, Puerto Rico, and the US Virgin Islands from Chem-Tel by calling 1-800-255-3924. For other areas, call 813-248-0585 collect to contact Chem-Tel's International access number. Each reagent can be identified by the four digit number listed on the upper left corner of the reagent label, in the contents list and in the test procedures.

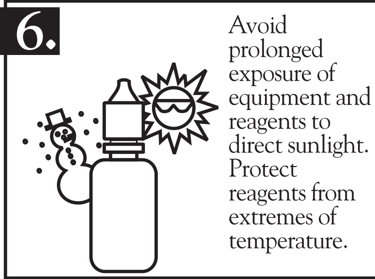
USE PROPER ANALYTICAL TECHNIQUES











MATERIAL SAFETY DATA SHEET

LaMOTTE COMPANY
PO BOX 329 - CHESTERTOWN - MARYLAND - 21620
TELEPHONE # FOR INFORMATION 410-778-3100
24 Hour Emergency Number (CHEM-TEL) 800-255-3924

1. PRODUCT IDENTIFICATION

Manganous Sulfate Solution *Code Nr.* **4167**

2. HAZARDOUS INGREDIENTS

NAME	*CAS #*	*TSCA #*	*%*	*PEL*	*TLV*
Manganese Sulfate monohydrate	10034-96-5	7785-87-7	36	5 mg/cubic m as Mn	C 5 mg/cubic m as Mn

3. NON-HAZARDOUS INGREDIENTS EXCEPT WATER (7732-18-5)

NAME	*CAS #*	*%*
Water to 100%		

4. PHYSICAL DATA

Appearance: Clear Pink Liquid
Solubility in Water: Soluble | *Odor:* None | *Boiling Point:* Unknown | *Melt. Point:* N/A
Vapor Pressure: <17 @ 20 deg C | *Vapor Density:* <1 (Air=1) | *pH:* 3

5. FIRE AND EXPLOSION DATA

Flash Point (method used) N/A | *Flammable Limit: LEL:* N/A | *UEL:* N/A
Extinguishing Media: Not a fire hazard
HMIS Hazard: Health - 1 *Flammability -* 0 *Reactivity -* 0 *Scale: 4 = Extreme, 3 = High, 2 = Moderate, 1 = Slight, 0 = Least*
Special Fire Fighting Procedures:
N/A
Unusual Fire & Explosion Hazard:
N/A

6. REACTIVITY DATA

Stability: ☒ *Stable* ☐ *Unstable*
Conditions to avoid: N/A
Incompatibility (Materials to avoid):
N/A
Hazardous Decomposition Products: N/A

7. HEALTH HAZARD DATA

Toxicity: Unknown
Primary Route of Entry: ☐ *Inhalation* ☒ *Ingestion* ☒ *Skin* ☐ *N/A*
Carcinogenicity: ☒ *None* ☐ *OSHA* ☐ *NTP* ☐ *IARC*
Other Health Related Comments:
Manganese investigated as a tumorigen, mutagen, reproductive effector.
Target Organs: N/A
Signs and symptoms of exposure:
May irritate eyes and skin. Harmful if swallowed.
Medical Condition Aggravated by Exposure: N/A

8. EMERGENCY FIRST AID PROCEDURES

Eye Contact: Immediately flush with water for 15 minutes. Consult a physician.
Ingestion: Induce vomiting immediately. Consult a physician.
Inhalation:
N/A
Skin Contact:
Flush thoroughly with water. Remove affected clothing and wash skin with soap and water. Consult physician.

9. SPILL AND DISPOSAL PROCEDURES

Spill and Leak:
Mop up carefully and hold for disposal.
Disposal:
Small quantity: Flush down drain with excess water. Large quantity: Containerize and dispose of as hazardous waste according to federal, state and local regulations.

10. PRECAUTIONARY MEASURES

In Handling: ☒ *Gloves* ☒ *Eye Protection* ☐ *N/A* ☒ *Other:* Lab Coat
Ventilation ☒ *Normal* ☐ *Mechanical* ☐ *Respiratory Protection*
Work/Hygienic Practices: Wash after handling.

11. SPECIAL PRECAUTIONS

N/A

DATE: 11/1/03 The above information is believed to be correct but does not claim to be all inclusive and should be used only as a guide.

† This is a toxic chemical subject to reporting requirements of section 313 of EPCRA and 40CFR372.

MATERIAL SAFETY DATA SHEET

LaMOTTE COMPANY
PO BOX 329 - CHESTERTOWN - MARYLAND - 21620
TELEPHONE # FOR INFORMATION 410-778-3100
24 Hour Emergency Number (CHEM-TEL) 800-255-3924

1. PRODUCT IDENTIFICATION

Sodium Thiosulfate, .025 N *Code Nr.* **4169**

2. HAZARDOUS INGREDIENTS

NAME	*CAS #*	*TSCA #*	*%*	*PEL*	*TLV*
Sodium Hydroxide	1310-73-2		<0.1	N/E	N/E

3. NON-HAZARDOUS INGREDIENTS EXCEPT WATER (7732-18-5)

NAME	*CAS #*	*%*
Sodium Thiosulfate, 5-hydrate	10102-17-7	<1
Water to 100%		

4. PHYSICAL DATA

Appearance: Clear Colorless Liquid
Solubility in Water: Soluble | *Odor:* None | *Boiling Point:* ca. 100 degC | *Melt. Point:* Unknown
Vapor Pressure: <17 @ 20 deg C | *Vapor Density:* <1 (Air=1) | *pH:* 12

5. FIRE AND EXPLOSION DATA

Flash Point (method used) N/A | *Flammable Limit: LEL:* N/A | *UEL:* N/A
Extinguishing Media: Not a fire hazard
HMIS Hazard: Health - 1 *Flammability -* 0 *Reactivity -* 0 *Scale: 4 = Extreme, 3 = High, 2 = Moderate, 1 = Slight, 0 = Least*
Special Fire Fighting Procedures:
N/A
Unusual Fire & Explosion Hazard:
N/A

6. REACTIVITY DATA

Stability: ☒ *Stable* ☐ *Unstable*
Conditions to avoid: Heat, light
Incompatibility (Materials to avoid):
N/A
Hazardous Decomposition Products: N/A

7. HEALTH HAZARD DATA

Toxicity: Non-toxic
Primary Route of Entry: ☐ *Inhalation* ☒ *Skin* ☒ *Ingestion* ☐ *N/A*
Carcinogenicity: ☒ *None* ☐ *NTP* ☐ *OSHA* ☐ *IARC*
Other Health Related Comments:
Target Organs: N/A
Signs and symptoms of exposure:
Large doses by mouth can cause GI irritation. May cause skin irritation.
Medical Condition Aggravated by Exposure: N/A

8. EMERGENCY FIRST AID PROCEDURES

Eye Contact: Flush with water for 15 minutes.
Ingestion: Drink plenty of water. Consult a physician.
Inhalation:
N/A
Skin Contact:
Flush with water. Wash with soap and water.

9. SPILL AND DISPOSAL PROCEDURES

Spill and Leak:
Neutralize with vinegar or other dilute acid and mop up.
Disposal:
Neutralize with dilute acid and wash down drain with excess water.

10. PRECAUTIONARY MEASURES

In Handling: ☒ *Gloves* ☒ *Eye Protection* ☐ *N/A* ☐ *Other:*
Ventilation ☒ *Normal* ☐ *Mechanical* ☐ *Respiratory Protection*
Work/Hygienic Practices: Avoid contact with eyes or skin.

11. SPECIAL PRECAUTIONS

Store away from heat and light.

DATE: 11/1/03 The above information is believed to be correct but does not claim to be all inclusive and should be used only as a guide.

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LaMOTTE COMPANY
PO BOX 329 - CHESTERTOWN - MARYLAND - 21620
TELEPHONE # FOR INFORMATION 410-778-3100
24 Hour Emergency Number (CHEM-TEL) 800-255-3924

1. PRODUCT IDENTIFICATION

Starch Indicator Solution *Code Nr.* **4170**

2. HAZARDOUS INGREDIENTS

NAME	*CAS #*	*TSCA #*	*%*	*PEL*	*TLV*
Salicylic Acid	69-72-7		0.13	N/E	N/E

3. NON-HAZARDOUS INGREDIENTS EXCEPT WATER (7732-18-5)

NAME	*CAS #*	*%*
Soluble Starch	9005-84-9	0.5
Water to 100%		

4. PHYSICAL DATA

Appearance: Colorless Liquid
Solubility in Water: Soluble | *Odor:* None | *Boiling Point:* ca. 100 degC | *Melt. Point:* Unknown
Vapor Pressure: <17 @ 20 deg C | *Vapor Density:* <1(Air=1) | *pH:* 3

5. FIRE AND EXPLOSION DATA

Flash Point (method used) N/A | *Flammable Limit: LEL:* N/A | *UEL:* N/A
Extinguishing Media: Not a fire hazard
HMIS Hazard: Health - 1 *Flammability -* 0 *Reactivity -* 0 *Scale: 4 = Extreme, 3 = High, 2 = Moderate, 1 = Slight, 0 = Least*
Special Fire Fighting Procedures:
N/A
Unusual Fire & Explosion Hazard:
N/A

6. REACTIVITY DATA

Stability: ☒ *Stable* ☐ *Unstable*
Conditions to avoid: Heat, light
Incompatibility (Materials to avoid):
N/A
Hazardous Decomposition Products: N/A

7. HEALTH HAZARD DATA

Toxicity: orl rat LD50: 891 mg/kg for salicylic acid solid
Primary Route of Entry: ☐ *Inhalation* ☐ *Skin* ☒ *Ingestion* ☐ *N/A*
Carcinogenicity: ☒ *None* ☐ *NTP* ☐ *OSHA* ☐ *IARC*
Other Health Related Comments:
Salicylic acid investigated as a possible mutagen.
Target Organs: N/A
Signs and symptoms of exposure:
May be harmful if swallowed.
Medical Condition Aggravated by Exposure: N/A

8. EMERGENCY FIRST AID PROCEDURES

Eye Contact: Flush with water.
Ingestion: Drink water or milk. Consult physician.
Inhalation:
N/A
Skin Contact:
Flush with water.

9. SPILL AND DISPOSAL PROCEDURES

Spill and Leak:
Mop up. Flush down drain.
Disposal:
Flush down drain with excess water. Dispose according to federal, state and local regulations.

10. PRECAUTIONARY MEASURES

In Handling: ☐ *Gloves* ☒ *Eye Protection* ☐ *N/A* ☐ *Other:*
Ventilation ☒ *Normal* ☐ *Mechanical* ☐ *Respiratory Protection*
Work/Hygienic Practices: N/A

11. SPECIAL PRECAUTIONS

Store at room temperature.

DATE: 11/1/03 The above information is believed to be correct but does not claim to be all inclusive and should be used only as a guide.

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LaMOTTE COMPANY
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TELEPHONE # FOR INFORMATION 410-778-3100
24 Hour Emergency Number (CHEM-TEL) 800-255-3924

1. PRODUCT IDENTIFICATION

Sulfuric Acid, 1:1 *Code Nr.* **6141**

2. HAZARDOUS INGREDIENTS

NAME	*CAS #*	*TSCA #*	*%*	*PEL*	*TLV*
† Sulfuric Acid	7664-93-9		64	1 mg/cubic m	1 mg/cubic m

3. NON-HAZARDOUS INGREDIENTS EXCEPT WATER (7732-18-5)

NAME	*CAS #*	*%*
Water to 100%		

4. PHYSICAL DATA

Appearance: Colorless Liquid
Solubility in Water: Soluble | *Odor:* None | *Boiling Point:* > 100 deg C | *Melt. Point:* N/A
Vapor Pressure: <1 @ 20 deg C | *Vapor Density:* >1 (Air=1) | *pH:* <1

5. FIRE AND EXPLOSION DATA

Flash Point (method used) N/A | *Flammable Limit: LEL:* N/A | *UEL:* N/A
Extinguishing Media: Dry chemical or CO_2, not water
HMIS Hazard: Health - 3 *Flammability -* 0 *Reactivity -* 2 *Scale: 4 = Extreme, 3 = High, 2 = Moderate, 1 = Slight, 0 = Least*
Special Fire Fighting Procedures:
Wear protective equipment and self-contained breathing apparatus.
Unusual Fire & Explosion Hazard:
A violent exothermic reaction occurs with water. Reacts with metals to form flammable, explosive hydrogen gas.

6. REACTIVITY DATA

Stability: ☒ *Stable* ☐ *Unstable*
Conditions to avoid: Moisture
Incompatibility (Materials to avoid):
Water, metals, organic or combustible materials, and strong bases.
Hazardous Decomposition Products: SOx, hydrogen gas

7. HEALTH HAZARD DATA

Toxicity: orl rat LD50: 2140 mg/kg for sulfuric acid
Primary Route of Entry: ☒ *Inhalation* ☒ *Ingestion* ☒ *Skin* ☐ *N/A*
Carcinogenicity: ☒ *None* ☐ *OSHA* ☐ *NTP* ☐ *IARC*
Other Health Related Comments:
Target Organs: Corrosive to all body parts, Skin,
Signs and symptoms of exposure:
Severe burns. Ingestion may be fatal. Inhalation can cause coughing, chest pains, damage to lungs.
Medical Condition Aggravated by Exposure: N/A

8. EMERGENCY FIRST AID PROCEDURES

Eye Contact: Immediately flush with water for 15 minutes. Call a doctor immediately.
Ingestion: Do not induce vomiting. Rinse mouth, drink plenty of water. Call a doctor immediately.
Inhalation:
Remove to fresh air. Give artificial respiration if not breathing. If breathing is difficult, give oxygen.
Skin Contact:
Immediately flush with water for 15 minutes while removing affected clothing. Consult physician.

9. SPILL AND DISPOSAL PROCEDURES

Spill and Leak:
Wear gloves & eye protection. Cover spill with sodium bicarbonate or soda ash/calcium hydroxide mixture. Mix and carefully add water to form slurry, avoiding heat, spattering, and fumes. Scoop up and flush to drain with excess water.
Disposal:
Add very slowly with stirring to a large volume of soda ash & calcium hydroxide. Pour neutralized solution down drain with excess water. Dispose according to federal, state and local regulations.

10. PRECAUTIONARY MEASURES

In Handling: ☒ *Gloves* ☒ *Eye Protection* ☐ *N/A* ☒ *Other:* Lab Coat
Ventilation ☐ *Normal* ☒ *Mechanical* ☐ *Respiratory Protection*
Work/Hygienic Practices: Avoid contact with skin and clothing and inhalation of vapor.

11. SPECIAL PRECAUTIONS

Store away from incompatible items (bases, metal powders, combustible materials).

DATE: 11/1/03 The above information is believed to be correct but does not claim to be all inclusive and should be used only as a guide.

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MATERIAL SAFETY DATA SHEET

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TELEPHONE # FOR INFORMATION 410-778-3100
24 Hour Emergency Number (CHEM-TEL) 800-255-3924

1. PRODUCT IDENTIFICATION

Sulfamic Acid Powder *Code Nr.* **6286**

2. HAZARDOUS INGREDIENTS

NAME	*CAS #*	*TSCA #*	*%*	*PEL*	*TLV*
Sulfamic Acid	5329-14-6		100	N/E	N/E

3. NON-HAZARDOUS INGREDIENTS EXCEPT WATER (7732-18-5)

NAME	*CAS #*	*%*
N/A		

4. PHYSICAL DATA

Appearance: White Crystals
Solubility in Water: Soluble | *Odor:* None | *Boiling Point:* N/A | *Melt. Point:* 200 deg C
Vapor Pressure: N/A | *Vapor Density:* N/A | *pH:* 1 (0.1g in 10mL water)

5. FIRE AND EXPLOSION DATA

Flash Point (method used) N/A | *Flammable Limit: LEL:* N/A | *UEL:* N/A
Extinguishing Media: Dry chemical, CO2, water spray, or alcohol-resistant foam
HMIS Hazard: Health - 2 *Flammability -* 1 *Reactivity -* 1 *Scale: 4 = Extreme, 3 = High, 2 = Moderate, 1 = Slight, 0 = Least*
Special Fire Fighting Procedures:
Firefighters wear self-contained breathing apparatus
Unusual Fire & Explosion Hazard:
Emits toxic fumes under fire conditions.

6. REACTIVITY DATA

Stability: ☒ *Stable* ☐ *Unstable*
Conditions to avoid: Moisture, heat. Temperatures above 190 deg C.
Incompatibility (Materials to avoid):
Strong acids, bases, oxidizers, chlorine and bleaches.
Hazardous Decomposition Products: SOx, NOx, ammonia

7. HEALTH HAZARD DATA

Toxicity: orl rat LD50: 3160 mg/kg
Primary Route of Entry: ☒ *Inhalation* ☒ *Ingestion* ☒ *Skin* ☐ *N/A* | *Carcinogenicity:* ☒ *None* ☐ *OSHA* ☐ *NTP* ☐ *IARC*
Other Health Related Comments:
Target Organs: N/A
Signs and symptoms of exposure:
Irritating and corrosive to eyes, nose, skin and respiratory tract.
Medical Condition Aggravated by Exposure: N/A

8. EMERGENCY FIRST AID PROCEDURES

Eye Contact: Immediately flush with water for 15 minutes. Contact a physician.
Ingestion: Rinse mouth. Give water with milk of magnesia. Get medical attention.
Inhalation:
Remove to fresh air. If breathing is difficult, give oxygen. Consult physician.
Skin Contact:
Flush skin thoroughly with water while removing affected clothing. Wash skin with soap and water. Consult a physician.

9. SPILL AND DISPOSAL PROCEDURES

Spill and Leak:
Sweep up, dissolve in water. Neutralize with sodium carbonate and wash down drain with excess water.
Disposal:
Dissolve in water. Neutralize with sodium carbonate and flush to drain with excess water. Dispose according to federal, state and local regulations.

10. PRECAUTIONARY MEASURES

In Handling: ☒ *Gloves* ☒ *Eye Protection* ☐ *N/A* ☒ *Other:* Lab Coat
Ventilation ☐ *Normal* ☒ *Mechanical* ☐ *Respiratory Protection*
Work/Hygienic Practices: Avoid breathing powder and contact with eyes, skin and clothing.

11. SPECIAL PRECAUTIONS

Store away from moisture, heat. Use with adequate ventilation.

DATE: 11/1/03 The above information is believed to be correct but does not claim to be all inclusive and should be used only as a guide.

† This is a toxic chemical subject to reporting requirements of section 313 of EPCRA and 40CFR372.

MATERIAL SAFETY DATA SHEET

LaMOTTE COMPANY
PO BOX 329 - CHESTERTOWN - MARYLAND - 21620
TELEPHONE # FOR INFORMATION 410-778-3100
24 Hour Emergency Number (CHEM-TEL) 800-255-3924

1. PRODUCT IDENTIFICATION

Alkaline Potassium Iodide Azide *Code Nr.* **7166**

2. HAZARDOUS INGREDIENTS

NAME	*CAS #*	*TSCA #*	*%*	*PEL*	*TLV*
Potassium Hydroxide	1310-58-3		60 - 70	C 2 mg/cubic m	C 2 mg/cubic m
† Sodium Azide	26628-22-8		<1	C 0.1 ppm (skin) as HN3	C 0.3 mg/cubic m as NaN3
Potassium Iodide	7681-11-0		14	N/E	N/E

3. NON-HAZARDOUS INGREDIENTS EXCEPT WATER (7732-18-5)

NAME	*CAS #*	*%*
Water to 100%		

4. PHYSICAL DATA

Appearance: Clear Colorless Liquid
Solubility in Water: Soluble *Odor:* None *Boiling Point:* Unknown *Melt. Point:* N/A
Vapor Pressure: Unknown *Vapor Density:* Unknown *pH:* 14

5. FIRE AND EXPLOSION DATA

Flash Point (method used) N/A *Flammable Limit: LEL:* N/A *UEL:* N/A
Extinguishing Media: Not a fire hazard
HMIS Hazard: Health - 3 *Flammability -* 0 *Reactivity -* 2 *Scale: 4 = Extreme, 3 = High, 2 = Moderate, 1 = Slight, 0 = Least*
Special Fire Fighting Procedures:
Wear self contained breathing apparatus and protective clothing to prevent inhalation and contact with eyes.
Unusual Fire & Explosion Hazard:
Violent exothermic reaction occurs with water.May produce enough heat to ignite combustibles. Can react with metals to produce hydrogen, forming explosive mix with air.

6. REACTIVITY DATA

Stability: ☒ *Stable* ☐ *Unstable*
Conditions to avoid: Heat
Incompatibility (Materials to avoid):
Strong acids, metals
Hazardous Decomposition Products: Hydrogen gas

7. HEALTH HAZARD DATA

Toxicity: orl rat LD50: 365 mg/kg for potassium hydroxide; 27 mg/kg for sodium azide solid
Primary Route of Entry: ☐ *Inhalation* ☒ *Skin* ☐ *Ingestion* ☐ *N/A*
Carcinogenicity: ☒ *None* ☐ *NTP* ☐ *OSHA* ☐ *IARC*
Other Health Related Comments:

Target Organs: Corrosive to all body parts, Eyes, Skin,
Signs and symptoms of exposure:
Severe burns, may be fatal if swallowed

Medical Condition Aggravated by Exposure: N/A

8. EMERGENCY FIRST AID PROCEDURES

Eye Contact: Immediately flush with water for 15 minutes. Get medical attention immediately.
Ingestion: Do not induce vomiting. Rinse out mouth, drink plenty of water and call a doctor immediately.
Inhalation:
Remove to fresh air.
Skin Contact:
Immediately flush with water while removing affected clothing and rinse skin thoroughly for 15 minutes. Consult physician.

9. SPILL AND DISPOSAL PROCEDURES

Spill and Leak:
Neutralize by carefully and slowly adding dilute hydrochloric acid (conc. 6M or less) to pH 7. Collect waste liquid. Dispose of as follows:

Disposal:
Small amt.<25 mL -Flush neutralized waste to drain with water. Large amt.-Sodium azide can react with metal--such as copper pipes--to form shock or friction sensitive metal azides (explosive). Dispose of larger amts. as hazardous waste, according to federal, state and local regulations.

10. PRECAUTIONARY MEASURES

In Handling: ☒ *Gloves* ☒ *Eye Protection* ☐ *N/A* ☒ *Other:* Lab Coat
Ventilation ☒ *Normal* ☐ *Mechanical* ☐ *Respiratory Protection*
Work/Hygienic Practices: Avoid contact with skin and clothing. Use Neoprene gloves, goggles, face shield, protective clothing

11. SPECIAL PRECAUTIONS

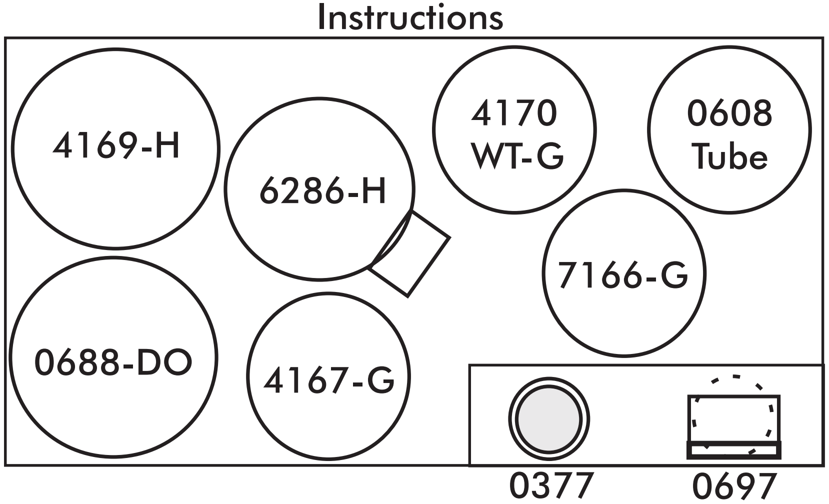
Store away from incompatible items (acids, metals).

DATE: 11/1/03 The above information is believed to be correct but does not claim to be all inclusive and should be used only as a guide.

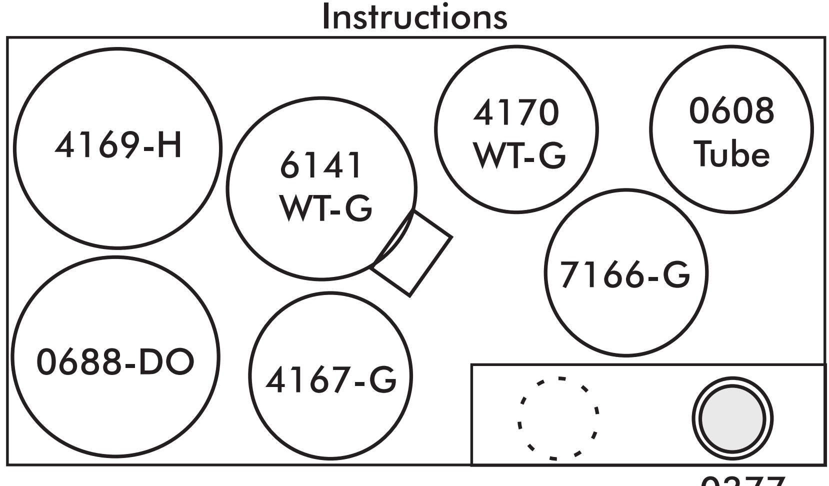
† This is a toxic chemical subject to reporting requirements of section 313 of EPCRA and 40CFR372.

KIT DIAGRAMS

DISSOLVED OXYGEN KIT • CODE 7414



DISSOLVED OXYGEN KIT • CODE 5860



SHORT FORM INSTRUCTIONS

Read all instructions before performing test. Use this guide as a quick reference.

1. Fill Water Sampling Bottle (0688-DO).
2. Add 8 drops of *Manganous Sulfate Solution (4167).
3. Add 8 drops of *Alkaline Potassium Iodide Azide (7166).
4. Cap and mix.
5. Allow precipitate to settle.
6. Use the 1.0 g spoon to add *Sulfamic Acid Powder (6286) or add 8 drops of Sulfuric Acid, 1:1 (6141WT).
7. Cap and mix until reagent and precipitate dissolve.
8. Fill test tube (0608) to the 20 mL line.
9. Fill Titrator with *Sodium Thiosulfate, 0.025N (4169).
10. Titrate until sample color is pale yellow. DO NOT DISTURB TITRATOR.
11. Add 8 drops of Starch Indicator (4170WT).
12. Continue titration until blue color just disappears and solution is colorless.
13. Read result in ppm Dissolved Oxygen.

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67414-MN • 11/04

AMMONIA-NITROGEN TEST KIT

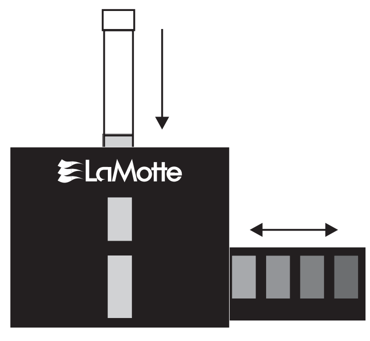
SALICYLATE METHOD • CODE 3304

QUANTITY	CONTENTS	CODE
60 mL	*Salicylate Ammonia #1	*3978LWT-H
30 mL	*Salicylate Ammonia #2	*3979WT-G
30 mL	Salicylate Ammonia #3	3982WT-G
2	Test Tubes, plastic, w/caps	0106
1	Octa-Slide Viewer	1100
1	Ammonia-Nitrogen Octa-Slide Bar, 0 - 2 ppm	3441

***WARNING:** Reagents marked with an * are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents see MSDS CD or our web site. To obtain a printed copy, contact us by e-mail, phone or fax.

To order individual reagents or test kit components, use the specified code number.

USE OF THE OCTA-SLIDE VIEWER



The Octa-Slide Viewer should be held so non-direct light enters through the back of the viewer. With sample tube inserted at top, slide the Octa-Slide bar through the viewer and match with color standard.

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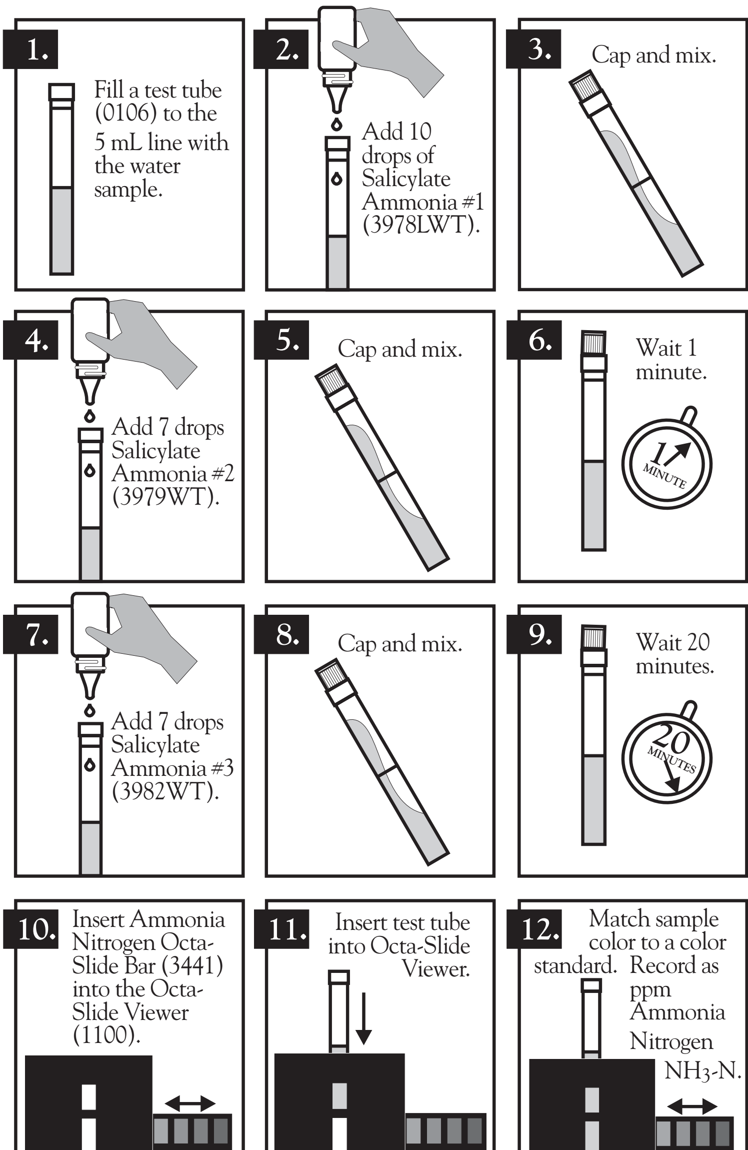
05.05

PROCEDURE

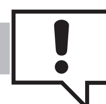


Nitrate

Method 8192 — Cadmium Reduction Method

Powder Pillows — LR (0.01 to 0.50 mg/L NO_3^-–N)

Scope and Application: For water, wastewater, and seawater



Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- A deposit of unoxidized metal will remain after the NitraVer® 6 dissolves. The deposit will not affect results.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Rinse the sample cell and mixing cylinder immediately after use to remove all cadmium particles.
- Properly dispose of the used sample. Prepared samples contain cadmium and must be disposed of according to Federal, State, and local hazardous waste regulations. For information on pollution prevention and waste management, see *Section 5* on page *79*.
- Shaking time and technique influence color development. Analyze a standard solution several times and adjust the shaking time to obtain the correct result. Use this time for analyzing samples

Powder Pillows — Method 8192

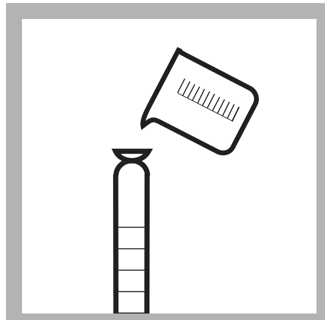
1. Touch

Hach Programs.

Select program

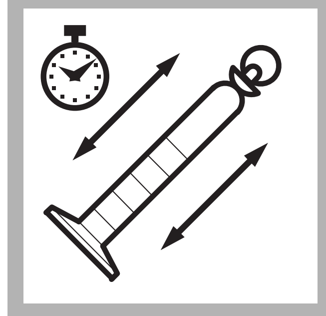
351 N, Nitrate LR.

Touch **Start**.



2. Fill a 25-mL graduated mixing cylinder with 15 mL of sample.

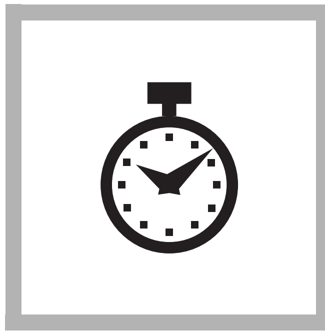
3. Add the contents of one NitraVer 6 Reagent Powder Pillow to the cylinder. Stopper.



4. Touch the timer icon.

Touch **OK**.

Shake the cylinder vigorously for three minutes.



5. When the timer beeps, touch the timer icon again.

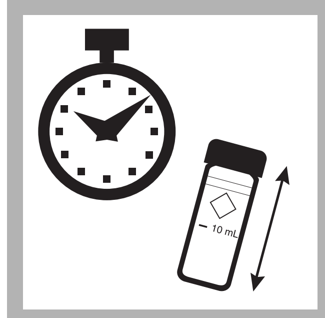
Touch **OK**.

A 2-minute reaction period will begin.

6. When the timer beeps, carefully pour 10 mL of the sample into a clean, round sample cell. Do not transfer any cadmium particles to the sample cell.

7. Add the contents of one NitriVer3 Nitrite Reagent Powder Pillow to the sample cell (this is the prepared sample).

Cap the sample cell.

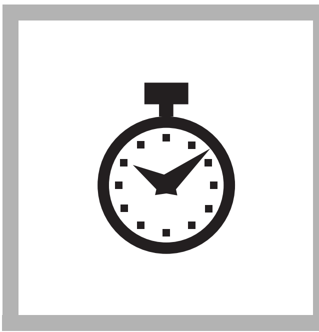


8. Touch the timer icon.

Touch **OK**.

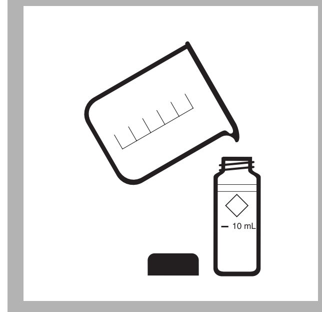
Shake the sample cell gently for 30 seconds.

A pink color will develop if nitrate is present.



9. Touch the timer icon.

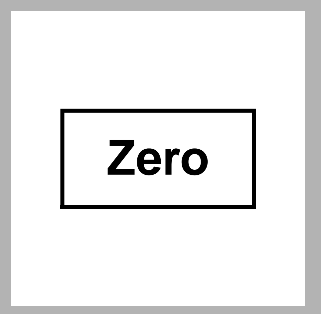
A 15-minute reaction period will begin.



10. When the timer beeps, fill a second sample cell with 10 mL of original sample (this is the blank).

Cap the sample cell.

11. Place the blank into the cell holder.



12. Touch **Zero**.

The display will show:

0.00 mg/L NO_3^-–N

13. Place the prepared sample into the cell holder.

Results will appear in mg/L NO_3^-–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium	100 mg/L
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration.
Ferric iron	All levels
Nitrite	All levels: This method measures both the nitrate and nitrite in the sample. If nitrite is present, the nitrite nitrogen test (Program #371) should be done on the sample. Pretreat the nitrate nitrogen sample with the following pretreatment. Then subtract the amount of nitrite found from the results of the LR nitrate nitrogen test. **1.** Add 30-g/L Bromine Water (Cat. No. 2211-20) dropwise to the sample in *step 3* until a yellow color remains. Mix after each drop. **2.** Add one drop of 30-g/L Phenol Solution (Cat. No. 2112-20) to destroy the color. **3.** Proceed with the LR Nitrate procedure.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage and Preservation

More reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 48 hours at 4 °C. To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid (Cat. No. 979-49) per liter and store at 4 °C.

Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-53). Do not use mercury compounds as preservatives. Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Nitrate Nitrogen Voluette® Ampule Standard, 12.0-mg/L NO_3^-–N (Cat. No. 14333-10).
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 15 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *46* for more information.

Standard Solution Method

1. To test accuracy, use a 0.20-mg/L NO_3^-–N standard in place of the sample and perform the procedure as described. Prepare this standard by diluting 2.00 mL of a 10-mg/L Nitrate Nitrogen Standard Solution to 100.0 mL with deionized water.
2. To adjust the calibration curve using the reading obtained with the 0.20-mg/L nitrate nitrogen standard, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *49* for more information.

Method Performance

Precision
Standard: 0.2 mg/L NO_3^-–N

Program	95% Confidence Limits of Distribution
351	0.18–0.23 mg/L NO_3^-–N

See *Section 3.4.3 Precision* on page *53* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	∆Abs	∆Concentration
Entire range	0.010	0.004 mg/L NO_3^-–N

See *Section 3.4.5 Sensitivity* on page *54* for more information.

Summary of Method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with chromotropic acid to form a pink-colored product. Test results are measured at 507 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Low Range Nitrate Reagent Set (100 tests)			24298-00
Includes:			
NitraVer® 6 Nitrate Reagent Powder Pillows	1 pillow	100/pkg	21072-49
NitriVer® 3 Nitrite Reagent Powder Pillows	1 pillow	100/pkg	21071-69

Required Apparatus

Description	Quantity Required per test	Unit	Cat. No.
Cylinder, graduated, mixing, 25-mL	1	each	20886-40
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Description	Quantity Required per test	Unit	Cat. No.
Nitrate Nitrogen Standard Solution, 10.0-mg/L NO_3^-–N		500 mL	307-49
Nitrate Nitrogen Standard Solution, Voluette® Ampule, 12-mg/L NO_3^-–N		16/pkg	14333-10
Water, deionized		4 liters	272-56

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✔Method 8507 — Diazotization Method

Powder Pillows or AccuVac® Ampuls — LR (0.002 to 0.300 mg/L NO_2^-–N)

Scope and Application: For water, wastewater, and seawater; USEPA approved for wastewater analysis*

* Federal Register, 44(85), 25505 (May 1, 1979)

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows — Method 8507

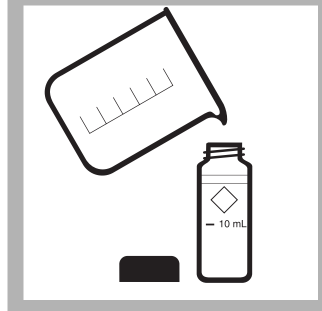
1. Touch

Hach Programs.

Select program

371 N, Nitrite LR.

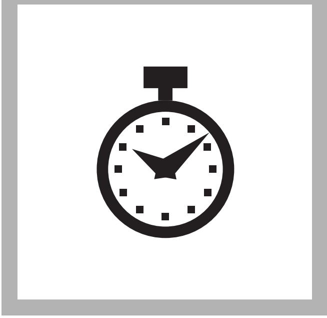
Touch **Start**.



2. Fill a round sample cell with 10 mL of sample.

3. Add the contents of one NitriVer 3 Nitrite Reagent Powder Pillow (the prepared sample).

Cap and shake to dissolve. A pink color will develop if nitrite is present.



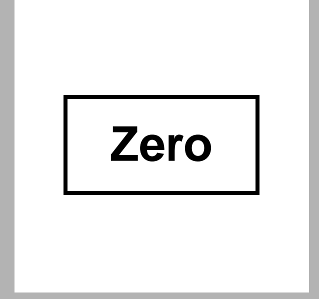
4. Touch the timer icon.

Touch **OK**.

A 20-minute reaction period will begin.

5. When the timer beeps, fill a second sample cell with 10 mL of sample (this is the blank).

6. Wipe the blank and place it into the cell holder.



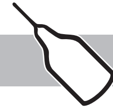
7. Touch **Zero**.

The display will show:

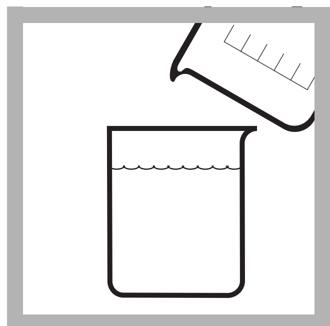
0.000 mg/L NO_2^-–N

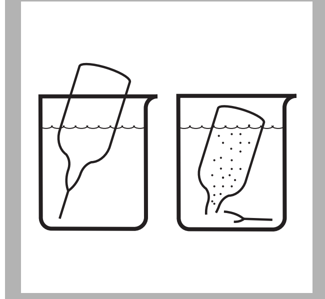
8. Wipe the prepared sample and place it into the cell holder.

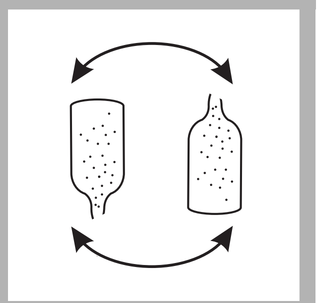
Results will appear in mg/L NO_2^-–N.



AccuVac® Ampul — Method 8507







1. Touch

Hach Programs.

Select program

375 N, Nitrite LR AV.

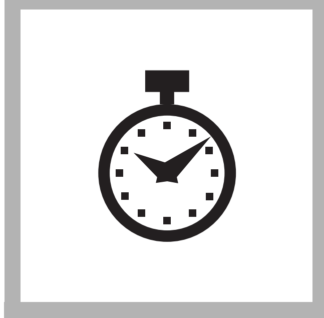
Touch **Start**.

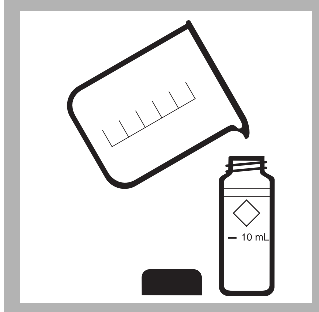
2. Pour at least 40 mL of sample into a 50-mL beaker.

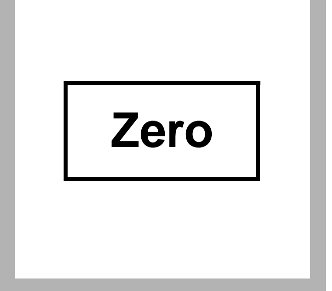
3. Fill a NitriVer 3 Nitrite AccuVac® Ampul with sample. Keep the tip immersed while the ampule fills.

4. Invert the ampule several times to mix.

A pink color will develop if nitrite is present.







5. Touch the timer icon.

Touch **OK**.

A 20-minute reaction period will begin.

6. When the timer beeps, fill a sample cell with at least 10 mL of sample (this is the blank).

7. Wipe the blank and place it into the cell holder.

8. Touch **Zero**.

The display will show:

0.000 mg/L NO_2^-–N

9. Wipe the AccuVac Ampul and place it into the cell holder. Read the results.

Results will appear in mg/L NO_2^-–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Antiminous ions	Interfere by causing precipitation
Auric ions	Interfere by causing precipitation
Bismuth ions	Interfere by causing precipitation
Chloroplatinate ions	Interfere by causing precipitation
Cupric ions	Cause low results
Ferric ions	Interfere by causing precipitation
Ferrous ions	Cause low results
Lead ions	Interfere by causing precipitation
Mercurous ions	Interfere by causing precipitation
Metavanadate ions	Interfere by causing precipitation
Nitrate	Very high levels of nitrate (>100 mg/L nitrate as N) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.
Silver ions	Interfere by causing precipitation
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Store at 4 °C (30 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. Do not use acid preservatives.

Accuracy Check

Standard Solution Method

Preparing nitrite standards is difficult. A standard should be prepared by a trained chemist. Hach recommends using the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, Method 4500—NO_2-B (p. 4–86 of 18th edition). Prepare a 0.150-mg/L standard.

Method Performance

Precision

Standard: 0.150 mg/L NO_2^-–N

Program	95% Confidence Limits of Distribution
371	0.146–0.154 mg/L NO_2^-–N
375	0.140–0.160 mg/L NO_2^-–N

See *Section 3.4.3 Precision* on page *53* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	∆Abs	∆Concentration
371	Entire range	0.010	0.002 mg/L NO_2^-–N
375	Entire range	0.010	0.002 mg/L NO_2^-–N

See *Section 3.4.5 Sensitivity* on page *54* for more information.

Summary of Method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present. Test results are measured at 507 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
NitriVer® 3 Nitrite Reagent Powder Pillows	1 pillow	100/pkg	21071-69
or			
NitriVer® 3 Nitrite Reagent AccuVac® Ampul	1 ampul	25/pkg	25120-25

Required Apparatus

Description	Quantity Required Per Test	Unit	Cat. No.
Beaker, 50-mL	1	each	500-41H
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Description	Quantity Required Per Test	Unit	Cat. No.
Sodium Nitrite, ACS		454 g	2452-01
Water, deionized		4 liters	272-56

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Appendix I

Bacterial Analysis Methods

5. VAN DER KOOIJ, D., W.A.M. HIJNEN & J.C. KRUITHOF. 1989. The effects of ozonation, biological filtration and distribution on the concentration of easily assimilable organic carbon (AOC) in drinking water. *Ozone Sci. Eng.* 11:297.
6. KAPLAN, L.A. & T.L. BOTT. 1990. Nutrients for bacterial growth in drinking water. Bioassay evaluation. EPA Project Summary, EPA-600/S2-89-030: 1-7. U.S. Environmental Protection Agency, Washington, D.C.

9. Bibliography

KING, E.O., M.K. WARD & D.E. RANEY. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. *J. Lab. Clin. Med.* 44:301.
MASON, J. & D.P. KELLY. 1988. Thiosulfate oxidation by obligately heterotrophic bacteria. *Microbial Ecol.* 15:123.

9221 MULTIPLE-TUBE FERMENTATION TECHNIQUE FOR MEMBERS OF THE COLIFORM GROUP*

9221 A. Introduction

The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. The historical definition of this group has been based on the method used for detection (lactose fermentation) rather than on the tenets of systematic bacteriology. Accordingly, when the fermentation technique is used, this group is defined as all facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 h at 35°C.

The standard test for the coliform group may be carried out either by the multiple-tube fermentation technique or presence-absence procedure (through the presumptive-confirmed phases or completed test) described herein, by the membrane filter (MF) technique (Section 9222) or by the enzymatic substrate coliform test (Section 9223). Each technique is applicable within the limitations specified and with due consideration of the purpose of the examination. Production of valid results requires strict adherence to quality control procedures. Quality control guidelines are outlined in Section 9020.

When multiple tubes are used in the fermentation technique, results of the examination of replicate tubes and dilutions are reported in terms of the Most Probable Number (MPN) of organisms present. This number, based on certain probability formulas, is an estimate of the mean density of coliforms in the sample. Coliform density, together with other information obtained by engineering or sanitary surveys, provides the best assessment of water treatment effectiveness and the sanitary quality of source water.

The precision of each test depends on the number of tubes used. The most satisfactory information will be obtained when the largest sample inoculum examined shows gas in some or all of the tubes and the smallest sample inoculum shows no gas in all or a majority of the tubes. Bacterial density can be estimated by the formula given or from the table using the number of positive tubes in the multiple dilutions (9221C.2). The number of sample portions selected will be governed by the desired precision of the result. MPN tables are based on the assumption of a Poisson distribution (random dispersion). However, if the sample is not adequately shaken before the portions are removed or if clumping of bacterial cells occurs, the MPN value will be an underestimate of the actual bacterial density.

1. Water of Drinking Water Quality

When drinking water is analyzed to determine if the quality meets the standards of the U.S. Environmental Protection Agency (EPA), use the fermentation technique with 10 replicate tubes each containing 10 mL, 5 replicate tubes each containing 20 mL, or a single bottle containing a 100-mL sample portion. When examining drinking water by the fermentation technique, process all tubes or bottles demonstrating growth with or without a positive acid or gas reaction to the confirmed phase (9221B.2). Apply the completed test (9221B.3) to not less than 10% of all coliform-positive samples per quarter. Obtain at least one positive sample per quarter. A positive EC broth (9221E) or a positive EC MUG broth (9221F) test result is considered an alternative to the positive completed test phase.

For the routine examination of public water supplies the object of the total coliform test is to determine the efficiency of treatment plant operation and the integrity of the distribution system. It is also used as a screen for the presence of fecal contamination. A high proportion of coliform occurrences in a distribution system may be attributed not to treatment failure at the plant or the well source, but to bacterial regrowth in the mains. Because it is difficult to distinguish between coliform regrowth and new contamination, assume all coliform occurrences to be new contamination unless otherwise demonstrated.

2. Water of Other than Drinking Water Quality

In the examination of nonpotable waters inoculate a series of tubes with appropriate decimal dilutions of the water (multiples and submultiples of 10 mL), based on the probable coliform density. Use the presumptive-confirmed phase of the multiple-tube procedure. Use the more labor-intensive completed test (9221B.3) as a quality control measure on at least 10% of coliform-positive nonpotable water samples on a seasonal basis. The object of the examination of nonpotable water generally is to estimate the density of bacterial contamination, determine a source of pollution, enforce water quality standards, or trace the survival of micro-

* Approved by Standard Methods Committee, 1994.

organisms. The multiple-tube fermentation technique may be used to obtain statistically valid MPN estimates of coliform density. Examine a sufficient number of samples to yield representative results for the sampling station. Generally, the geometric mean or median value of the results of a number of samples will yield a value in which the effect of sample-to-sample variation is minimized.

3. Other Samples

The multiple-tube fermentation technique is applicable to the analysis of salt or brackish waters as well as muds, sediments, and sludges. Follow the precautions given above on portion sizes and numbers of tubes per dilution.

To prepare solid or semisolid samples weigh the sample and add diluent to make a 10^{-1} dilution. For example, place 50 g sample in sterile blender jar, add 450 mL sterile phosphate buffer or 0.1% peptone dilution water, and blend for 1 to 2 min at low speed (8000 rpm). Prepare the appropriate decimal dilutions of the homogenized slurry as quickly as possible to minimize settling.

9221 B. Standard Total Coliform Fermentation Technique

1. Presumptive Phase

Use lauryl tryptose broth in the presumptive portion of the multiple-tube test. If the medium has been refrigerated after sterilization, incubate overnight at room temperature (20°C) before use. Discard tubes showing growth and/or bubbles.

a. Reagents and culture medium:

1) *Lauryl tryptose broth:*

Tryptose	20.0	g
Lactose	5.0	g
Dipotassium hydrogen phosphate, K_2HPO_4	2.75	g
Potassium dihydrogen phosphate, KH_2PO_4	2.75	g
Sodium chloride, NaCl	5.0	g
Sodium lauryl sulfate	0.1	g
Reagent-grade water	1	L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. pH should be 6.8 ± 0.2 after sterilization. Before sterilization, dispense sufficient medium, in fermentation tubes with an inverted vial, to cover inverted vial at least one-half to two-thirds after sterilization. Alternatively, omit inverted vial and add 0.01 g/L bromcresol purple to presumptive medium to determine acid production, the indicator of a positive result in this part of the coliform test. Close tubes with metal or heat-resistant plastic caps.

Make lauryl tryptose broth of such strength that adding 100-mL, 20-mL, or 10-mL portions of sample to medium will not reduce ingredient concentrations below those of the standard medium. Prepare in accordance with Table 9221:I.

b. Procedure:

1) Arrange fermentation tubes in rows of five or ten tubes each in a test tube rack. The number of rows and the sample volumes selected depend upon the quality and character of the water to be examined. For potable water use five 20-mL portions, ten 10-mL portions, or a single bottle of 100 mL portion; for nonpotable water use five tubes per dilution (of 10, 1, 0.1 mL, etc.).

In making dilutions and measuring diluted sample volumes, follow the precautions given in Section 9215B.2. Use Figure 9215:1 as a guide to preparing dilutions. Shake sample and dilutions vigorously about 25 times. Inoculate each tube in a set of five with replicate sample volumes (in increasing decimal dilutions, if decimal quantities of the sample are used). Mix test portions in the medium by gentle agitation.

2) Incubate inoculated tubes or bottles at 35 ± 0.5C. After 24 ± 2 h swirl each tube or bottle gently and examine it for growth, gas, and acidic reaction (shades of yellow color) and, if no gas or acidic reaction is evident, reincubate and reexamine at the end of 48 ± 3 h. Record presence or absence of growth, gas, and acid production. If the inner vial is omitted, growth with acidity signifies a positive presumptive reaction.

c. Interpretation: Production of an acidic reaction or gas in the tubes or bottles within 48 ± 3 h constitutes a positive presumptive reaction. Submit tubes with a positive presumptive reaction to the confirmed phase (9221B.2).

9221:I. Preparation of Lauryl Tryptose Broth

Inoculum *mL*	Amount of Medium in Tube *mL*	Volume of Medium + Inoculum *mL*	Dehydrated Lauryl Tryptose Broth Required *g/L*
1	10 or more	11 or more	35.6
10	10	20	71.2
10	20	30	53.4
20	10	30	106.8
100	50	150	106.8
100	35	135	137.1
100	20	120	213.6

The absence of acidic reaction or gas formation at the end of 48 ± 3 h of incubation constitutes a negative test. Submit drinking water samples demonstrating growth without a positive gas or acid reaction to the confirmed phase (9221B.2). An arbitrary 48-h limit for observation doubtless excludes occasional members of the coliform group that grow very slowly (see Section 9212).

2. Confirmed Phase

a. Culture medium: Use brilliant green lactose bile broth fermentation tubes for the confirmed phase.

Brilliant green lactose bile broth:

Peptone	10.0	g
Lactose	10.0	g
Oxgall	20.0	g
Brilliant green	0.0133	g
Reagent-grade water	1	L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. pH should be 7.2 ± 0.2 after sterilization. Before sterilization, dispense, in fermentation tubes with an inverted vial, sufficient medium to cover inverted vial at least one-half to two-thirds after sterilization. Close tubes with metal or heat-resistant plastic caps.

b. Procedure: Submit all presumptive tubes or bottles showing growth, any amount of gas, or acidic reaction within 24 ± 2 h of incubation to the confirmed phase. If active fermentation or acidic reaction appears in the presumptive tube earlier than 24 ± 2 h, transfer to the confirmatory medium; preferably examine tubes at 18 ± 1 h. If additional presumptive tubes or bottles show active fermentation or acidic reaction at the end of a 48 ± 3- h incubation period, submit these to the confirmed phase.

Gently shake or rotate presumptive tubes or bottles showing gas or acidic growth to resuspend the organisms. With a sterile loop 3.0 to 3.5 mm in diameter, transfer one or more loopfuls of culture to a fermentation tube containing brilliant green lactose bile broth or insert a sterile wooden applicator at least 2.5 cm into the culture, promptly remove, and plunge applicator to bottom of fermentation tube containing brilliant green lactose bile broth. Remove and discard applicator. Repeat for all other positive presumptive tubes.

Incubate the inoculated brilliant green lactose bile broth tube at 35 ± 0.5°C. Formation of gas in any amount in the inverted vial of the brilliant green lactose bile broth fermentation tube at any time (e.g., 6 ± 1 h, 24 ± 2 h) within 48 ± 3 h constitutes a positive confirmed phase. Calculate the MPN value from the number of positive brilliant green lactose bile tubes as described in Section 9221C.

c. Alternative procedure: Use this alternative only for polluted water or wastewater known to produce positive results consistently.

If all presumptive tubes are positive in two or more consecutive dilutions within 24 h, submit to the confirmed phase only the tubes of the highest dilution (smallest sample inoculum) in which all tubes are positive and any positive tubes in still higher dilutions. Submit to the confirmed phase all tubes in which gas or acidic growth is produced only after 48 h.

3. Completed Phase

To establish the presence of coliform bacteria and to provide quality control data, use the completed test on at least 10% of positive confirmed tubes (see Figure 9221:1). Simultaneous inoculation into brilliant green lactose bile broth for total coliforms and EC broth for fecal coliforms (see Section 9221E below) or EC-MUG broth for *Escherichia coli* may be used. Consider positive EC and EC-MUG broths elevated temperature (44.5°C) results as a positive completed test response. Parallel positive brilliant green lactose bile broth cultures with negative EC or EC-MUG broth cultures indicate the presence of nonfecal coliforms.

a. Culture media and reagents:

1) *LES Endo agar:* See Section 9222B. Use 100- × 15-mm petri plates.

2) *MacConkey agar:*

Peptone	17	g
Proteose peptone	3	g
Lactose	10	g
Bile salts	1.5	g
Sodium chloride, NaCl	5	g
Agar	13.5	g
Neutral red	0.03	g
Crystal violet	0.001	g
Reagent-grade water	1	L

Add ingredients to water, mix thoroughly, and heat to boiling to dissolve. Sterilize by autoclaving for 15 min at 121°C. Temper agar after sterilization and pour into petri plates (100 × 15 mm). pH should be 7.1 ± 0.2 after sterilization.

3) *Nutrient agar:*

Peptone	5.0	g
Beef extract	3.0	g
Agar	15.0	g
Reagent-grade water	1	L

Add ingredients to water, mix thoroughly, and heat to dissolve. pH should be 6.8 ± 0.2 after sterilization. Before sterilization, dispense in screw-capped tubes. After sterilization, immediately place tubes in an inclined position so that the agar will solidify with a sloped surface. Tighten screw caps after cooling and store in a protected, cool storage area.

4) Gram-stain reagents:

a) Ammonium oxalate-crystal violet (Hucker's): Dissolve 2 g crystal violet (90% dye content) in 20 mL 95% ethyl alcohol; dissolve 0.8 g $(NH_4)_2C_2O_4 \cdot H_2O$ in 80 mL reagent-grade water; mix the two solutions and age for 24 h before use; filter through paper into a staining bottle.

b) Lugol's solution, Gram's modification: Grind 1 g iodine crystals and 2 g KI in a mortar. Add reagent-grade water, a few milliliters at a time, and grind thoroughly after each addition until solution is complete. Rinse solution into an amber glass bottle with the remaining water (using a total of 300 mL).

c) Counterstain: Dissolve 2.5 g safranin dye in 100 mL 95% ethyl alcohol. Add 10 mL to 100 mL reagent-grade water.

d) Acetone alcohol: Mix equal volumes of ethyl alcohol (95%) with acetone.

b. Procedure:

1) Using aseptic technique, streak one LES Endo agar (Section 9222B.2) or MacConkey agar plate from each tube of brilliant green lactose bile broth showing gas, as soon as possible after the observation of gas. Streak plates in a manner to insure presence of some discrete colonies separated by at least 0.5 cm. Observe the following precautions when streaking plates to obtain a high

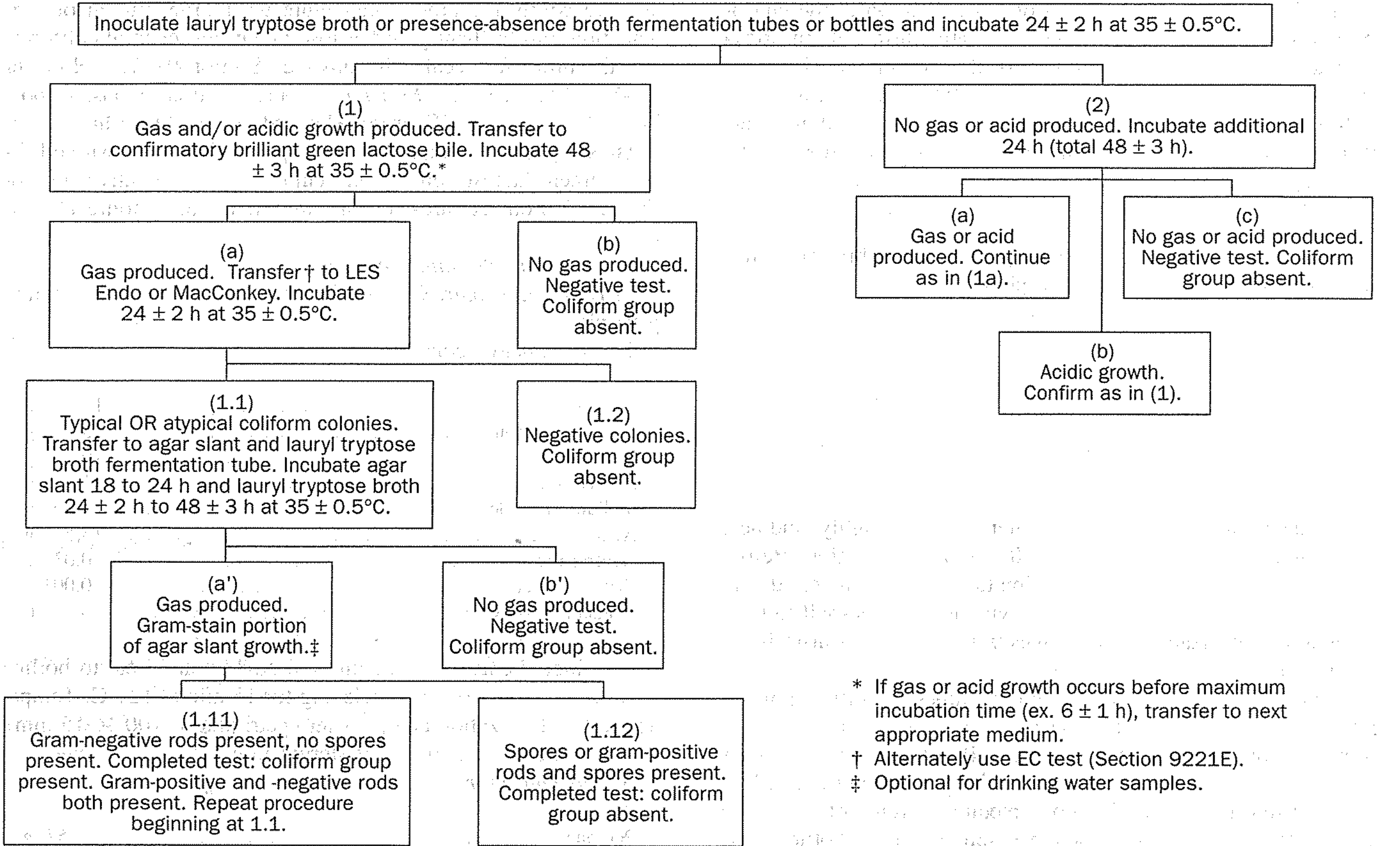

Figure 9221:1. Schematic outline of presumptive, confirmed, and completed phases for total coliform detection.

proportion of successful isolations if coliform organisms are present: (*a*) Use a sterile 3-mm-diam loop or an inoculating needle slightly curved at the tip; (*b*) tap and incline the fermentation tube to avoid picking up any membrane or scum on the needle; (*c*) insert end of loop or needle into the liquid in the tube to a depth of approximately 0.5 cm; and (*d*) streak plate for isolation with curved section of the needle in contact with the agar to avoid a scratched or torn surface. Flame loop between second and third quadrants to improve colony isolation.

Incubate plates (inverted) at 35 ± 0.5°C for 24 ± 2 h.

2) The colonies developing on LES Endo agar are defined as *typical* (pink to dark red with a green metallic surface sheen) or *atypical* (pink, red, white, or colorless colonies without sheen) after 24 h incubation. Typical lactose-fermenting colonies developing on MacConkey agar are red and may be surrounded by an opaque zone of precipitated bile. From each plate pick one or more typical, well-isolated coliform colonies or, if no typical colonies are present, pick two or more colonies considered most likely to consist of organisms of the coliform group, and transfer growth from each isolate to a single-strength lauryl tryptose broth fermentation tube and onto a nutrient agar slant. (The latter is unnecessary for drinking water samples.)

If needed, use a colony magnifying device to provide optimum magnification when colonies are picked from the LES Endo or MacConkey agar plates. When transferring colonies, choose well-isolated ones and barely touch the surface of the colony with a flame-sterilized, air-cooled transfer needle to minimize the danger of transferring a mixed culture.

Incubate secondary broth tubes (lauryl tryptose broth with inverted fermentation vials inserted) at 35 ± 0.5°C for 24 ± 2 h; if gas is not produced within 24 ± 2 h reincubate and examine again at 48 ± 3 h. Microscopically examine Gram-stained preparations from those 24-h nutrient agar slant cultures corresponding to the secondary tubes that show gas.

3) Gram-stain technique—The Gram stain may be omitted from the completed test for potable water samples only because the occurrences of gram-positive bacteria and spore-forming organisms surviving this selective screening procedure are infrequent in drinking water.

Various modifications of the Gram stain technique exist. Use the following modification by Hucker for staining smears of pure culture; include a gram-positive and a gram-negative culture as controls.

Prepare separate light emulsions of the test bacterial growth and positive and negative control cultures on the same slide using drops of distilled water on the slide. Air-dry and fix by passing slide through a flame and stain for 1 min with ammonium oxalate-crystal violet solution. Rinse slide in tap water and drain off excess; apply Lugol's solution for 1 min.

Rinse stained slide in tap water. Decolorize for approximately 15 to 30 s with acetone alcohol by holding slide between the fingers and letting acetone alcohol flow across the stained smear

until the solvent flows colorlessly from the slide. Do not over-decolorize. Counterstain with safranin for 15 s, rinse with tap water, blot dry with absorbent paper or air dry, and examine microscopically. Gram-positive organisms are blue; gram-negative organisms are red. Results are acceptable only when controls have given proper reactions.

c. Interpretation: Formation of gas in the secondary tube of lauryl tryptose broth within 48 ± 3 h and demonstration of gram-negative, nonspore-forming, rod-shaped bacteria from the agar culture constitute a positive result for the completed test, demonstrating the presence of a member of the coliform group.

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9221 C. Estimation of Bacterial Density

1. Precision of Fermentation Tube Test

Unless a large number of sample portions is examined, the precision of the fermentation tube test is rather low. For example, if only 1 mL is examined in a sample containing 1 coliform organism/mL, about 37% of 1-mL tubes may be expected to yield negative results because of random distribution of the bacteria in the sample. When five tubes, each with 1 mL sample, are used under these conditions, a completely negative result may be expected less than 1% of the time.

Consequently, exercise great caution when interpreting the sanitary significance of coliform results obtained from the use of a few tubes with each sample dilution, especially when the number of samples from a given sampling point is limited.

2. Computing and Recording of MPN

To calculate coliform density, compute in terms of the Most Probable Number (MPN). The MPN values, for a variety of planting series and results, are given in Tables 9221:II, III, and IV. Included in these tables are the 95% confidence limits for each MPN value determined. If the sample volumes used are those found in the tables, report the value corresponding to the number of positive and negative results in the series as the MPN/100 mL or report as total or fecal coliform presence or absence.

The sample volumes indicated in Tables 9221:II and III relate more specifically to finished waters. Table 9221:IV illustrates MPN values for combinations of positive and negative results when five 10-mL, five 1.0-mL, and five 0.1-mL volumes of samples are tested. When the series of decimal dilutions is different from that in the table, select the MPN value from Table 9221:IV for the combination of positive tubes and calculate according to the following formula:

$$\text{MPN value (from table)} \times \frac{10}{\text{largest volume tested in dilution series used for MPN determination}} = \text{MPN/100 mL}$$

When more than three dilutions are used in a decimal series of dilutions, use the results from only three of these in computing the MPN. To select the three dilutions to be used in determining the MPN index, choose the highest dilution that gives positive results in all five portions tested (no lower dilution giving any negative results) and the two next succeeding higher dilutions. Use the results at these three volumes in computing the MPN index. In the examples given below, the significant dilution results are shown in boldface. The number in the numerator represents positive tubes; that in the denominator, the total tubes planted; the combination of positives simply represents the total number of positive tubes per dilution:

Example	1 mL	0.1 mL	0.01 mL	0.001 mL	Combination of positives	MPN Index /100 mL
a	5/5	**5/5**	**2/5**	**0/5**	5-2-0	5000
b	**5/5**	**4/5**	**2/5**	0/5	5-4-2	2200
c	**0/5**	**1/5**	**0/5**	0/5	0-1-0	20

In *c*, select the first three dilutions so as to include the positive result in the middle dilution.

TABLE 9221:II. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 20-ML PORTIONS ARE USED

No. of Tubes Giving Positive Reaction Out of 5 of 20 mL Each	MPN Index/ 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0	<1.1	0	3.0
1	1.1	0.05	6.3
2	2.6	0.3	9.6
3	4.6	0.8	14.7
4	8.0	1.7	26.4
5	>8.0	4.0	Infinite

TABLE 9221.III. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN TEN 10-ML PORTIONS ARE USED

No. of Tubes Giving Positive Reaction Out of 10 of 10 mL Each	MPN Index/ 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0	<1.1	0	3.0
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12.0	4.3	27.1
8	16.1	5.9	36.8
9	23.0	8.1	59.5
10	>23.0	13.5	Infinite

TABLE 9221.IV. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 ML, 1.0 ML, 0.1 ML)

Combination of Positives	MPN Index/ 100 mL	95% Confidence Limits	
		Lower	Upper
0-0-0	<2	—	—
0-0-1	2	1.0	10
0-1-0	2	1.0	10
0-2-0	4	1.0	13
1-0-0	2	1.0	11
1-0-1	4	1.0	15
1-1-0	4	1.0	15
1-1-1	6	2.0	18
1-2-0	6	2.0	18
2-0-0	4	1.0	17
2-0-1	7	2.0	20
2-1-0	7	2.0	21
2-1-1	9	3.0	24
2-2-0	9	3.0	25
2-3-0	12	5.0	29
3-0-0	8	3.0	24
3-0-1	11	4.0	29
3-1-0	11	4.0	29
3-1-1	14	6.0	35
3-2-0	14	6.0	35
3-2-1	17	7.0	40
4-0-0	13	5.0	38
4-0-1	17	7.0	45
4-1-0	17	7.0	46
4-1-1	21	9.0	55
4-1-2	26	12	63
4-2-0	22	9.0	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9.0	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360
5-3-3	170	80	410
5-4-0	130	50	390
5-4-1	170	70	480
5-4-2	220	100	580
5-4-3	280	120	690
5-4-4	350	160	820
5-5-0	240	100	940
5-5-1	300	100	1300
5-5-2	500	200	2000
5-5-3	900	300	2900
5-5-4	1600	600	5300
5-5-5	≥1600	—	—

When a case such as that shown below in line *d* arises, where a positive occurs in a dilution higher than the three chosen according to the rule, incorporate it in the result for the highest chosen dilution, as in *e*:

Example	1 mL	0.1 mL	0.01 mL	0.001 mL	Combination of positives	MPN Index /100 mL
d	5/5	3/5	1/5	1/5	5-3-2	1400
e	5/5	3/5	2/5	0/5	5-3-2	1400

When it is desired to summarize with a single MPN value the results from a series of samples, use the geometric mean or the median.

Table 9221:IV shows the most likely positive tube combinations. If unlikely combinations occur with a frequency greater than 1% it is an indication that the technique is faulty or that the statistical assumptions underlying the MPN estimate are not being fulfilled. The MPN for combinations not appearing in the table, or for other combinations of tubes or dilutions, may be estimated by Thomas' simple formula:

$$\text{MPN/100 mL} = \frac{\text{no. of positive tubes} \times 100}{\sqrt{\left(\begin{matrix}\text{mL sample in}\\ \text{negative tubes}\end{matrix} \times \begin{matrix}\text{mL sample in}\\ \text{all tubes}\end{matrix}\right)}}$$

While the MPN tables and calculations are described for use in the coliform test, they are equally applicable to determining the MPN of any other organisms provided that suitable test media are available.

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9221 D. Presence-Absence (P-A) Coliform Test

The presence-absence (P-A) test for the coliform group is a simple modification of the multiple-tube procedure. Simplification, by use of one large test portion (100 mL) in a single culture bottle to obtain qualitative information on the presence or absence of coliforms, is justified on the theory that no coliforms should be present in 100 mL of a drinking water sample. The P-A test also provides the optional opportunity for further screening of the culture to isolate other indicators (fecal coliform, *Aeromonas, Staphylococcus, Pseudomonas,* fecal streptococcus, and *Clostridium*) on the same qualitative basis. Additional advantages include the possibility of examining a larger number of samples per unit of time. Comparative studies with the membrane filter procedure indicate that the P-A test may maximize coliform detection in samples containing many organisms that could overgrow coliform colonies and cause problems in detection.

The P-A test is intended for use on routine samples collected from distribution systems or water treatment plants. When sample locations produce a positive P-A result for coliforms, it may be advisable to determine coliform densities in repeat samples. Quantitative information may indicate the magnitude of a contaminating event.

1. Presumptive Phase

a. Culture media:

1) *P-A broth:* This medium is commercially available in dehydrated and in sterile concentrated form.

Beef extract	3.0	g
Peptone	5.0	g
Lactose	7.46	g
Tryptose	9.83	g
Dipotassium hydrogen phosphate, K_2HPO_4	1.35	g
Potassium dihydrogen phosphate, KH_2PO_4	1.35	g
Sodium chloride, NaCl	2.46	g
Sodium lauryl sulfate	0.05	g
Bromcresol purple	0.0085	g
Reagent-grade water	1	L

Make this formulation triple (3×) strength when examining 100-mL samples. Dissolve the P-A broth medium in water without heating, using a stirring device. Dispense 50 mL prepared medium into a screw-cap 250-mL milk dilution bottle. A fermentation tube insert is not necessary. Autoclave for 12 min at 121°C

with the total time in the autoclave limited to 30 min or less. pH should be 6.8 ± 0.2 after sterilization. When the PA medium is sterilized by filtration a 6× strength medium may be used. Aseptically dispense 20 mL of the 6× medium into a sterile 250-mL dilution bottle or equivalent container.

2) *Lauryl tryptose broth:* See Section 9221B.1.

b. Procedure: Shake sample vigorously for 5 s (approximately 25 times) and inoculate 100 mL into a P-A culture bottle. Mix thoroughly by inverting bottle once or twice to achieve even distribution of the triple-strength medium throughout the sample. Incubate at 35 ± 0.5°C and inspect after 24 and 48 h for acid reactions.

c. Interpretation: A distinct yellow color forms in the medium when acid conditions exist following lactose fermentation. If gas also is being produced, gently shaking the bottle will result in a foaming reaction. Any amount of gas and/or acid constitutes a positive presumptive test requiring confirmation.

2. Confirmed Phase

The confirmed phase is outlined in Figure 9221:1.

a. Culture medium: Use brilliant green lactose bile fermentation tubes (see 9221B.2).

b. Procedure: Transfer all cultures that show acid reaction or acid and gas reaction to brilliant green lactose bile (BGLB) broth for incubation at 35 ± 0.5°C (see Section 9221B.2).

c. Interpretation: Gas production in the BGLB broth culture within 48 ± 3 h confirms the presence of coliform bacteria. Report result as presence-absence test positive or negative for total coliforms in 100 mL of sample.

3. Completed Phase

The completed phase is outlined in Section 9221B.3 and Figure 9221:1.

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9221 E. Fecal Coliform Procedure

Elevated-temperature tests for distinguishing organisms of the total coliform group that also belong to the fecal coliform group are described herein. Modifications in technical procedures, standardization of methods, and detailed studies of the fecal coliform group have established the value of this procedure. The test can be performed by one of the multiple-tube procedures described here or by membrane filter methods as described in Section 9222. The procedure using A-1 broth is a single-step method.

The fecal coliform test (using EC medium) is applicable to investigations of drinking water, stream pollution, raw water sources, wastewater treatment systems, bathing waters, seawaters, and general water-quality monitoring. Prior enrichment in presumptive media is required for optimum recovery of fecal coliforms when using EC medium. The test using A-1 medium is applicable to source water, seawater, and treated wastewater.

1. Fecal Coliform Test (EC Medium)

The fecal coliform test is used to distinguish those total coliform organisms that are fecal coliforms. Use EC medium or, for a more rapid test of the quality of shellfish waters, treated wastewaters, or source waters, use A-1 medium in a direct test.

a. EC medium:

Tryptose or trypticase	20.0 g
Lactose	5.0 g
Bile salts mixture or bile salts No. 3	1.5 g
Dipotassium hydrogen phosphate, K_2HPO_4	4.0 g
Potassium dihydrogen phosphate, KH_2PO_4	1.5 g
Sodium chloride, NaCl	5.0 g
Reagent-grade water	1 L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. pH should be 6.9 ± 0.2 after sterilization. Before sterilization, dispense in fermentation tubes, each with an inverted vial, sufficient medium to cover the inverted vial at least partially after sterilization. Close tubes with metal or heat-resistant plastic caps.

b. Procedure: Submit all presumptive fermentation tubes or bottles showing any amount of gas, growth, or acidity within 48 h of incubation to the fecal coliform test.

1) Gently shake or rotate presumptive fermentation tubes or bottles showing gas, growth, or acidity. Using a sterile 3- or 3.5-mm-diam loop or sterile wooden applicator stick, transfer growth from each presumptive fermentation tube or bottle to EC broth (see Section 9221B.2).

2) Incubate inoculated EC broth tubes in a water bath at 44.5 ± 0.2°C for 24 ± 2 h.

Place all EC tubes in water bath within 30 min after inoculation. Maintain a sufficient water depth in water bath incubator to immerse tubes to upper level of the medium.

c. Interpretation: Gas production with growth in an EC broth culture within 24 ± 2 h or less is considered a positive fecal coliform reaction. Failure to produce gas (with little or no growth) constitutes a negative reaction. If multiple tubes are used, calculate MPN from the number of positive EC broth tubes as described in Section 9221C. When using only one tube for subculturing from a single presumptive bottle, report as presence or absence of fecal coliforms.

2. Fecal Coliform Direct Test (A-1 Medium)

a. A-1 broth: This medium may be used for the direct isolation of fecal coliforms from water. Prior enrichment in a presumptive medium is not required.

Lactose	5.0 g
Tryptone	20.0 g
Sodium chloride, NaCl	5.0 g
Salicin	0.5 g
Polyethylene glycol *p*-isooctylphenyl ether*	1.0 mL
Reagent-grade water	1 L

Heat to dissolve solid ingredients, add polyethylene glycol *p*-isooctylphenyl ether, and adjust to pH 6.9 ± 0.1. Before sterilization dispense in fermentation tubes with an inverted vial sufficient medium to cover the inverted vial at least partially after sterilization. Close with metal or heat-resistant plastic caps. Sterilize by autoclaving at 121°C for 10 min. Store in dark at room temperature for not longer than 7 d. Ignore formation of precipitate.

* Triton X-100, Rohm and Haas Co., or equivalent.

Make A-1 broth of such strength that adding 10-mL sample portions to medium will not reduce ingredient concentrations below those of the standard medium. For 10-mL samples prepare double-strength medium.

b. Procedure: Inoculate tubes of A-1 broth as directed in Section 9221B.1*b*1). Incubate for 3 h at 35 ± 0.5°C. Transfer tubes to a water bath at 44.5 ± 0.2°C and incubate for an additional 21 ± 2 h.

c. Interpretation: Gas production in any A-1 broth culture within 24 h or less is a positive reaction indicating the presence of fecal coliforms. Calculate MPN from the number of positive A-1 broth tubes as described in Section 9221C.

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9221 F. *Escherichia coli* Procedure (PROPOSED)

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* are defined as coliform bacteria that possess the enzyme β-glucuronidase 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 h 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 tube procedure as described here or by the membrane filter method as described in Section 9222. The chromogenic substrate procedure (Section 9223) can be used for direct detection of *E. coli*.

Tests for *E. coli* (using EC-MUG medium) are applicable for the analysis of drinking water, surface and ground water, 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.

1. *Escherichia coli* Test (EC-MUG medium)

Use EC-MUG medium for the confirmation of *E. coli*.

a. EC-MUG medium:

Tryptose or trypticase	20.0 g
Lactose	5.0 g
Bile salts mixture or bile salts No. 3	1.5 g
Dipotassium hydrogen phosphate, K_2HPO_4	4.0 g

Potassium dihydrogen phosphate, KH_2PO_4	1.5	g
Sodium chloride, NaCl	5.0	g
4-methylumbelliferyl-β-D-glucuronide (MUG)	0.05	g
Reagent-grade water	1	L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. pH should be 6.9 ± 0.2 after sterilization. Before sterilization, dispense in tubes that do not fluoresce under long-wavelength (366 nm) ultraviolet (UV) light. An inverted tube is not necessary. Close tubes with metal or heat-resistant plastic caps.

b. Procedure: Submit all presumptive fermentation tubes or bottles showing growth, gas, or acidity within 48 ± 3 h of incubation to the *E. coli* test.

1) Gently shake or rotate presumptive fermentation tubes or bottles showing growth, gas, or acidity. Using a sterile 3- or 3.5-mm-diam metal loop or sterile wooden applicator stick, transfer growth from presumptive fermentation tube or bottle to EC-MUG broth.

2) Incubate inoculated EC-MUG tubes in a water bath or incubator maintained at 44.5 ± 0.2°C for 24 ± 2 h. Place all EC-MUG tubes in water bath within 30 min after inoculation. Maintain a sufficient water depth in the water-bath incubator to immerse tubes to upper level of medium.

c. Interpretation: Examine all tubes exhibiting growth for fluorescence using a long-wavelength UV lamp (preferably 6 W). The presence of bright blue fluorescence is considered a positive response for *E. coli*. A positive control consisting of a known *E. coli* (MUG-positive) culture, a negative control consisting of a thermotolerant *Klebsiella pneumoniae* (MUG-negative) culture, and an uninoculated medium control may be necessary to interpret the results and to avoid confusion of weak auto-fluorescence of the medium as a positive response. If multiple tubes are used, calculate MPN from the number of positive EC-MUG broth tubes as described in Section 9221C. When using only one tube or subculturing from a single presumptive bottle, report as presence or absence of *E. coli*.

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9222 MEMBRANE FILTER TECHNIQUE FOR MEMBERS OF THE COLIFORM GROUP*

9222 A. Introduction

The membrane filter (MF) technique is highly reproducible, can be used to test relatively large sample volumes, and usually yields numerical results more rapidly than the multiple-tube fermentation procedure. The MF technique is extremely useful in monitoring drinking water and a variety of natural waters. However, the MF technique has limitations, particularly when testing waters with high turbidity or large numbers of noncoliform (background) bacteria. When the MF technique has not been used previously, it is desirable to conduct parallel tests with the method the laboratory is using currently to demonstrate applicability and comparability.

1. Definition

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 (golden) sheen within 24 h at 35°C on an Endo-type medium containing lactose. Some members of the total coliform group may produce dark red, mucoid, or nucleated colonies without a metallic sheen. When verified these are classified as atypical coliform colonies. When purified cultures of coliform bacteria are tested, they produce negative cytochrome oxidase and positive β-galactosidase test reactions.† Generally, pink (non-mucoid), blue, white, or colorless colonies lacking sheen are considered noncoliforms by this technique.

2. Applications

Turbidity caused by the presence of algae, particulates, or other interfering material 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 examination of saline waters, but not 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. For the detection of stressed total coliforms in treated drinking water and chlorinated secondary or tertiary wastewater effluents, use a method designed for stressed organism recovery (see Section 9212B.1). A modified MF technique for fecal coliforms (Section 9212) in chlorinated wastewater may be used if parallel testing over a 3-month period with the multiple-tube fermentation technique shows comparability for each site-specific type of sample.

* Approved by Standard Methods Committee, 1997.

† ONPG is a substrate for the β-galactosidase test.