

# ***Pathogen Indicator Bacteria Testing Quality Assurance Project Plan***

## **Monitoring Organizations**

*Southwest Conservation District, Save the Sound*

**Quality Assurance Project Plan Coordinating Organization**  
Save the Sound

## **Funded By**

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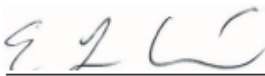
## A. Project Management

### A.1. APPROVAL PAGE



date: 9/8/2022

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date: 9/8/2022

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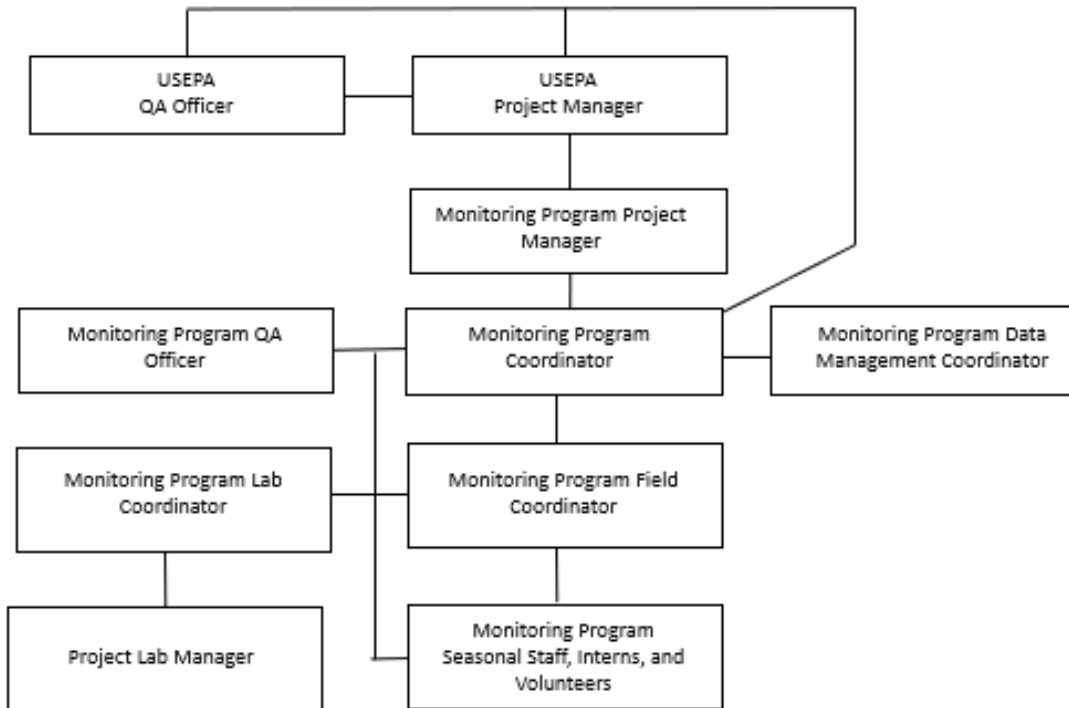
## A.4. PROJECT / TASK ORGANIZATION

**Table 1: Project Organization.**

Key project personnel and their corresponding responsibilities.

Name(s)	<i><b>Project Title</b></i> - Responsibility
Peter Linderoth	<i><b>Monitoring Program Project Manager</b></i> – Oversees all aspects of project that incorporate the monitoring program including: fiscal management, project objectives, data uses, program changes, etc. Develops the QAPP.
Elena Colón & Chris Sullivan	<i><b>Monitoring Program Coordinator</b></i> – Sampler recruitment and training. Produces monitoring report. Produces or oversees outreach efforts, in coordination with project manager.
Seasonal Staff	<i><b>Monitoring Program Field Coordinator</b></i> – Assist in training and quality assurance of monitoring groups for field work. Ensures field datasheets are properly filled out, samples and forms are transported to laboratories as needed, Standard Operations Procedures (SOPs) are being followed in entirety; and performs QA checks, including field audits, to make sure procedures are followed or corrected as needed (in collaboration QA office).
Elena Colón	<i><b>Monitoring Program Lab Coordinator</b></i> – Makes arrangements with any lab(s) used to perform analyses according to QAPP. Manages STS lab. Ensures correct procedures are used, holding times are met, and adequate documentation is provided.
Elena Colón	<i><b>Monitoring Program Data Management Coordinator</b></i> – Maintains the data systems for the program. Performs/oversees data entry and checks entries for accuracy against field and lab forms.
Peter Linderoth	<i><b>Monitoring Program Quality Assurance Officer</b></i> – Runs Quality Assurance (QA) program.
TBD	<i><b>USEPA Project Officer</b></i> – Oversees US EPA Cooperative Agreement compliance including processing recipient/subrecipient requests for QA/QC within EPA Regions
TBD	<i><b>USEPA Quality Assurance Officer</b></i> – Reviews, comments and approves QAPP.
SWCD Staff and Internships	<i><b>Project Laboratory Manager (SWCD)</b></i> – managing SWCD lab and working with samplers, maintaining supplies, and analyzing results
<b>Changes by year. Individual names are not listed.</b>	<i><b>Monitoring Program Seasonal Staff, Interns, and Volunteers</b></i> – Sample, perform field analyses, and assist in laboratory analyses and/or data entry.





**Figure 1: Organizational Chart.**  
 Lines between boxes indicate direct communication.

### **A.5. PROBLEM DEFINITION / BACKGROUND**

Sewage and polluted runoff into Long Island Sound and its watershed close beaches too frequently, contaminate tributaries and other waterbodies leading to designated use impairments, and are detrimental to fish and other wildlife through eutrophication and the introduction of toxic chemicals and materials. Beach closures have more than tripled in the Sound over the past two decades, according to the United States Environmental Protection Agency (USEPA) data. In 2012, the Sound suffered approximately 1,500 lost beach days when beaches, primarily in the western basin, were closed because of high levels of fecal indicator bacteria and associated pollution. Public education on these challenges is desperately needed. The general public is relatively unaware of the problems posed by leaking and aging sanitary sewer infrastructure that drains into Long Island Sound and how the resulting bacterial pollution is implicated in the number of beach closings and waterbody impairments in our

region. Furthermore, contamination from stormwater runoff, often laden with pathogens in urbanized areas, has detrimental impacts to the Sound and its tributaries.

The actions listed in this QAPP ultimately address the following questions:

- Where is fecal contamination entering Long Island Sound and its tributaries?
- What is the severity of that fecal contamination?

### **Organizational History and Mission**

The mission of Save the Sound is to protect and improve the land, air, and water of Connecticut and Long Island Sound. We use legal and scientific expertise and bring people together to achieve results that benefit our environment for current and future generations.

Our vision is of a Connecticut and Sound region where the vitality of nature will be protected for people and wildlife, now and for many decades to come. A region in which citizens from all walks of life can unite in transforming struggling habitats, polluted waters, endangered wildlife, and a threatened planet into resilient, healthy, vibrant, and inspiring places that sustain communities.

The mission of the Southwest Conservation District is to provide technical assistance, information, and education in natural resource conservation and management to agricultural cooperators, landowners, and the public across 43 municipalities in southwest Connecticut.

Improved soil conservation and water quality means healthier natural systems. These systems provide water for drinking, eating, bathing and various recreational activities. These same systems create healthy habitats for organisms, both inland and along the Long Island Sound. Improved habitats, protected natural resources, and native populations are also beneficial to human health.

### **Water Quality Monitoring History and Status**

The New York Office of Save the Sound initiated a pathogen-indicator bacteria and water quality monitoring program in 2013 and has since expanded the spatial and temporal scale of the water quality monitoring program overall. In early years of the monitoring effort, samples were collected by trained community scientists and processed at partnering laboratories in NY and CT. Save the Sound developed a laboratory setup in its Mamaroneck office in 2015 for processing samples. This effort was kickstarted by an equipment loan from the US EPA Region II. Save the Sound now owns its own equipment and has outfitted a base laboratory in Larchmont, NY.

Initially pathogen bacteria indicator monitoring conducted by Save the Sound was focused primarily in Westchester County, NY as it is in close proximity to Save the Sound's Westchester Office. Seasonal monitoring locations have expanded to include lower Fairfield County (CT),

Queens, NYC (NY), and Nassau County (NY). Additional regions are planned to be added through partnerships with additional laboratories and Monitoring Program Coordinators throughout the entire LIS watershed. Southwest Conservation District (SWCD) has joined this effort with plans of monitoring the waters in and around New Haven County (CT). Exploratory samples (e.g. response to pollution report and other investigative efforts) have expanded to the entire Sound and its coastal watershed.

### **Monitoring and Data Use Objectives**

The objective of the program is to collect water samples to analyze for pathogen indicator bacteria concentrations in Long Island Sound and its tributary waterways, including but not limited to upstream and downstream of potential sewage pollution sources, at sub-tributary confluences, direct discharges from outfalls and pipes, and at public access points.

Save the Sound will make the program data available online and use the data to educate the public and engage them in local and regional strategies to reduce sewage-related pollution and improve water quality. Data will be shared with municipal leaders in all communities in which sampling occurs. Data will also be shared with regulatory agencies. Additionally, the activities covered under this QAPP will provide quality-assured data that can support additional goals such as the assessment and restoration of coastal waterways and watersheds through the implementation of programs such as but not limited to:

- EPA's 305(b) water body health assessments and 303(d) listings
- TMDL development for impaired waters
- Clean Water Act Section 319 projects
- Connecticut Department of Energy and Environmental Protection and New York Department of Environmental Conservation Watershed Management Plans
- Long Island Sound Study's Comprehensive Conservation and Management Plan

### **A.6. PROJECT / TASK DESCRIPTION**

There are two types of monitoring stations covered under this quality assurance project plan.

- 1) *Seasonal pathogen indicator bacteria* stations can be sampled at any day of the week or time of day. Seasonal monitoring stations are scheduled for weekly monitoring starting as early as May and concluding as late as October. Locations monitored are grouped by USGS HUC 12 watersheds when considering which stations will be sampled on any given day. A typical season for this monitoring effort is approximately 12 weeks. If unforeseen circumstances make 12 weeks of sampling not possible, a minimum of 8 samples in the seasonal interval will be considered a complete season for a station. Freshwater stations will be sampled for *E. coli* and Total Coliforms and brackish water stations will be sampled

for Enterococci. *E. coli* will not be sampled in brackish waters under any circumstances. The project maintains flexibility to collect samples outside of this objective with the understanding a given indicator cannot substitute for another in geometric mean calculations nor will it count towards a complete season of minimum of 8 samples of the same indicator. The monitoring program manager, monitoring program coordinator and field coordinator have the authority to make determination of indicator(s) sampled when preparing for sampling efforts. The number and location of seasonal stations are provided in Section A.6.b.

- 2) *Exploratory pathogen indicator bacteria stations* can be sampled at any day of the week or time of day. Exploratory sampling is conducted all year. These locations are based on pollution reports, track down efforts, or investigative sampling from other external information. Priority is given to immediate pollution threats then to other instances that may require sampling such as sampling around a known bacteria hotspot or screening of outfalls in a target area. These are circumstantial and require flexibility. This flexibility is important as some water quality interests will be very localized and will drive additional exploratory station selection. Exploratory sampling procedures, regardless of purpose, will adhere to the requirements detailed in this document. Enterococci can be sampled at any exploratory location. *E. coli* and Total Coliforms may be sampled instead, or in addition to Enterococci. *E. coli* will not be sampled in brackish waters under any circumstances. The project maintains flexibility to collect samples outside of this objective. The monitoring program manager, monitoring program coordinator and field coordinator have the authority to make determination of indicator(s) sampled when preparing for sampling efforts. There is no minimum requirement for the frequency of sample collection at these locations.

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### Seasonal pathogen indicator bacteria –

- for each station
  - Date and time
  - GPS coordinates
  - 0.5 ft below the surface, approximate mid depth in shallow waters, or end of pipe; any one or combination of
    - Enterococci
    - *E. coli*
    - Fecal Coliforms
    - Total Coliforms
  - Once per field day collect a blank field sample
- Obtain from an online NOAA tide table and weather station approved by Monitoring Program Coordinator:
  - Time of high and low tide nearest time of sampling
  - Precipitation out 72 hours from sample collection

3) *Exploratory pathogen indicator bacteria* stations can be sampled at any day of the week or time of day. These locations are sampled based on pollution reports, suspicious discharges or status of ambient water, or follow up to seasonal monitoring to assist in tracking down potential sources of pathogen indicator bacteria contributing to high results recorded during seasonal monitoring.

### Exploratory pathogen indicator bacteria –

for each station

- Date and time
- GPS coordinates
- 0.5 ft below the surface, approximate mid depth, alternative depth documented on chain of custody, or end of pipe; any one or combination of
  - Enterococci
  - *E. coli*
  - Fecal Coliforms
  - Total Coliforms
- Obtain from an online NOAA tide table and weather station approved by Monitoring Program Coordinator:
  - Time of high and low tide nearest time of sampling
  - Precipitation out 72 hours from sample collection

## **Roles of Project Participants**

The Monitoring Program Coordinator, Monitoring Program Field Coordinator, and Monitoring Program Lab Coordinator or designees will provide guidance and advisement to individuals participating in the field monitoring and assisting in project laboratories. They will conduct trainings, field audits, station selection guidance, ongoing technical support, and lab coordination among other activities. The full set of participants and their respective roles can be referenced in Table 1 of this document. Figure 1 outlines the lines of communications between project participants.

All participants in the project are responsible for conducting field work and analyses following the requirements presented in the QAPP and associated SOPs. Annual trainings will be attended by all project participants. Field and lab personnel will complete all required datasheets and chain of custody forms. Any problems or deviance from this QAPP or SOPs will immediately be reported to the Monitoring Program Field Coordinator who will confer with the Monitoring Program Quality Assurance Officer on corrective course of action

## **How the proposed sampling plan supports the Monitoring Program objectives**

Data collected under this QAPP will be collected in a manner to allow the data to be used as listed in section A.5. This is a coordinated effort among groups monitoring Long Island Sound, its tributaries, and other waters in its watershed with the goal of releasing high quality data to the public and water quality managers. Data released by participants in this QAPP will have high comparability and quality assurance for end users to consider.

## **Overview of data handling processes**

Sampling event and field data will be collected on standardized field datasheets which will also have a section for chain of custody record keeping. This standardized field datasheet is in Appendix A of this document.

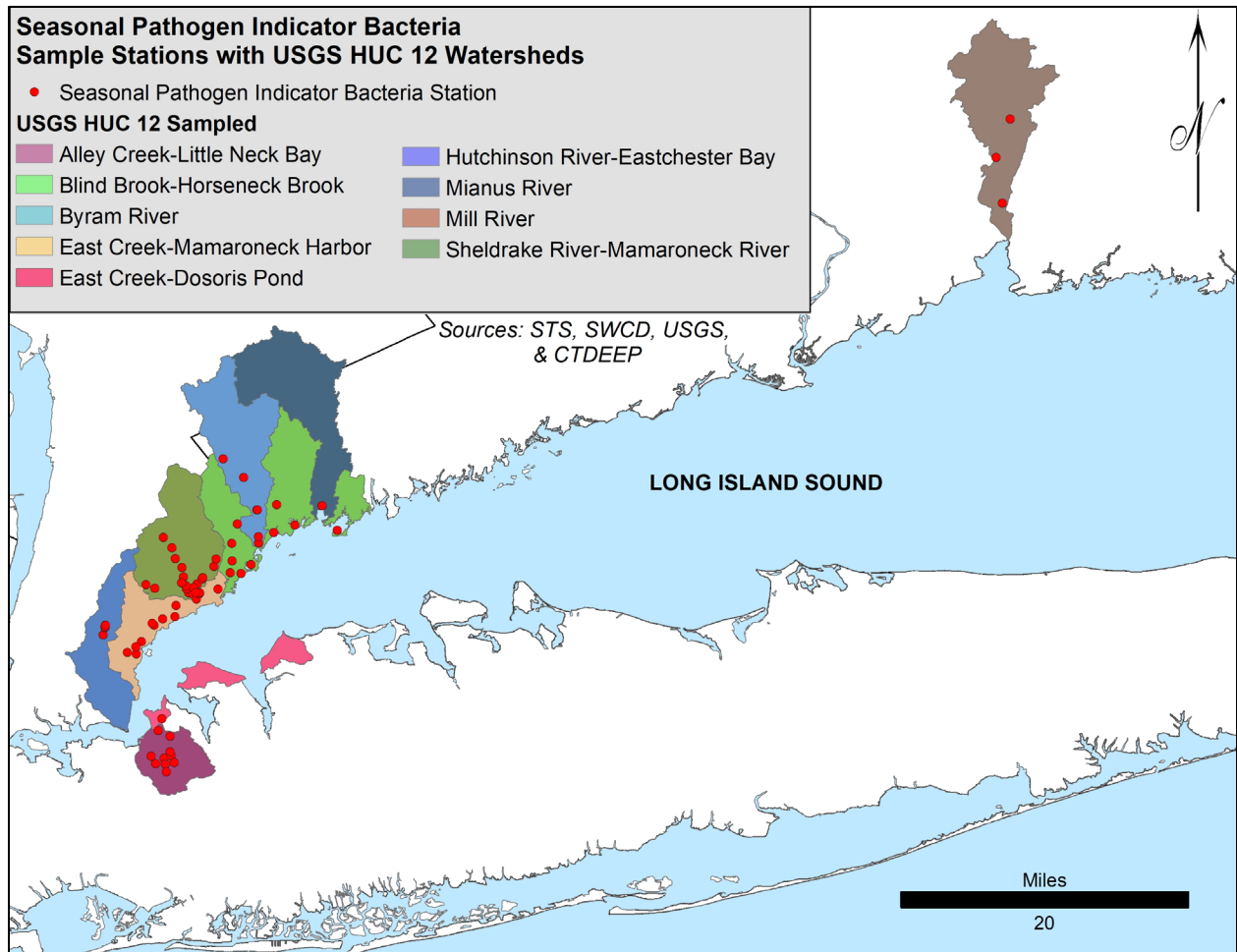
Laboratory data will be collected on a standardized laboratory datasheet. This standardized laboratory datasheet is in the Appendix A of this document.

### **A.6.a. Sampling Types Covered by this QAPP**

The type of sample information that can be collected under this QAPP includes:

- GPS location to record locations
- Enterococci
- *E. coli*
- Fecal Coliforms
- Total Coliforms

### A.6.b. Map of Study Area



**Figure 2: This map shows seasonal pathogen indicator bacteria station locations detailed in Table 2 and the HUC 12 Watersheds Sampled. Additional sample stations may be added to this project at which time the map will be updated.**

Station ID	Station Name	City	State	Water Type	Longitude	Latitude	USGS HUC 12 Watershed	Primary Sampling Group
BE-BHa	Byram Park	Greenwich	CT	Brackish	-73.64438	41.00466	Blind Brook-Horseneck Brook	Save the Sound
BE-LBd	Douglas Manor Beach	Queens, NYC	NY	Brackish	-73.75471	40.77774	Alley Creek-Little Neck Bay	Save the Sound
BE-MHa	Harbor Island Beach	Mamaroneck Village	NY	Brackish	-73.73023	40.94399	Sheldrake River-Mamaroneck River	Save the Sound
BE-MHd	Shore Acres Yacht Club	Mamaroneck Village	NY	Brackish	-73.72556	40.94266	Sheldrake River-Mamaroneck River	Save the Sound
BS-WCWa	Rye Playland Park	Rye	NY	Brackish	-73.67758	40.96355	Blind Brook-Horseneck Brook	Save the Sound
BS-WCWb	Beach Point Club	Mamaroneck Village	NY	Brackish	-73.72237	40.93763	Blind Brook-Horseneck Brook	Save the Sound
BS-WLISa	Glen Island Park	New Rochelle	NY	Brackish	-73.78280	40.88280	East Creek-Mamaroneck Harbor	Save the Sound
E-GCa	Greenwich Cove	Greenwich	CT	Brackish	-73.58047	41.00701	Blind Brook-Horseneck Brook	Save the Sound
E-GHa	Indian Harbor Yacht Club	Greenwich	CT	Brackish	-73.62315	41.01226	Blind Brook-Horseneck Brook	Save the Sound
E-LBa	Bayside Marina	Queens, NYC	NY	Brackish	-73.76801	40.77967	Alley Creek-Little Neck Bay	Save the Sound
E-LBb	Little Neck Bay at Cross Island Parkway & 35th Avenue	Queens, NYC	NY	Brackish	-73.76333	40.77242	Alley Creek-Little Neck Bay	Save the Sound
E-LBc	Parsons Beach at 233rd Street	Queens, NYC	NY	Brackish	-73.75391	40.77208	Alley Creek-Little Neck Bay	Save the Sound
E-LBe	Memorial Park	Queens, NYC	NY	Brackish	-73.74779	40.78046	Alley Creek-Little Neck Bay	Save the Sound
E-LBf	Little Neck Bay at Shore Drive & North Circle Drive	Great Neck Estates	NY	Brackish	-73.74889	40.78442	Alley Creek-Little Neck Bay	Save the Sound
E-LBg	Little Neck Bay at Martin Court	Kings Point	NY	Brackish	-73.76088	40.80577	Alley Creek-Little Neck Bay	Save the Sound
E-LHa	Larchmont Harbor at Park Avenue	Larchmont	NY	Brackish	-73.74401	40.92016	East Creek-Mamaroneck Harbor	Save the Sound
E-LHb	Flint Park	Larchmont	NY	Brackish	-73.74300	40.93150	East Creek-Mamaroneck Harbor	Save the Sound



E-MHb	Mamaroneck Harbor at Taylor Lane	Mamaroneck Village	NY	Brackish	-73.71940	40.94321	East Creek-Mamaroneck Harbor	Save the Sound
E-MHc	Mamaroneck Harbor East Basin	Mamaroneck Village	NY	Brackish	-73.73238	40.94805	Sheldrake River-Mamaroneck River	Save the Sound
E-MLa	Rye Marshlands Conservancy	Rye	NY	Brackish	-73.70050	40.94793	Blind Brook-Horseneck Brook	Save the Sound
E-NRHa	Neptune Boat Club	New Rochelle	NY	Brackish	-73.77782	40.89492	East Creek-Mamaroneck Harbor	Save the Sound
E-NRHc	Shore Park	Pelham Manor	NY	Brackish	-73.79194	40.88425	East Creek-Mamaroneck Harbor	Save the Sound
E-NRHd	Glen Island Approach	New Rochelle	NY	Brackish	-73.78338	40.88967	East Creek-Mamaroneck Harbor	Save the Sound
E-PLa	Playland Lake at Edith Read Natural Park	Rye	NY	Brackish	-73.66738	40.97255	Blind Brook-Horseneck Brook	Save the Sound
E-UMP	Udalls Mill Pond	Saddle Rock	NY	Brackish	-73.74891	40.79994	Alley Creek-Little Neck Bay	Save the Sound
E-VAMa	Van Amringe Millpond	Mamaroneck Village	NY	Brackish	-73.71908	40.94398	East Creek-Mamaroneck Harbor	Save the Sound
E-WLISb	Five Islands Park on Beach	New Rochelle	NY	Brackish	-73.76498	40.91151	East Creek-Mamaroneck Harbor	Save the Sound
E-WLISc	Five Islands Approach at Le Fevres Lane	New Rochelle	NY	Brackish	-73.76686	40.91334	East Creek-Mamaroneck Harbor	Save the Sound
R-AC-0.20	Alley Creek Outfall at Northern Boulevard	Queens, NYC	NY	Brackish	-73.75282	40.76417	Alley Creek-Little Neck Bay	Save the Sound
R-BB-0.6	Blind Brook at Disbrow Park	Rye	NY	Brackish	-73.68834	40.96424	Blind Brook-Horseneck Brook	Save the Sound
R-BB-2.9	Blind Brook at Rye Nature Center	Rye	NY	Brackish	-73.68646	40.97626	Blind Brook-Horseneck Brook	Save the Sound
R-BB-4.27	Blind Brook at Purchase Street	Rye	NY	Freshwater	-73.68660	40.99382	Blind Brook-Horseneck Brook	Save the Sound

R-BB-5.79	Blind Brook at North Ridge Street and Ridge Boulevard	Rye	NY	Freshwater	-73.68108	41.01338	Blind Brook-Horseneck Brook	Save the Sound
R-BR-0.55	Byram River at Columbus Park	Port Chester	NY	Brackish	-73.65970	40.99424	Byram River	Save the Sound
R-BR-1.01	Byram River at South Water Street	Greenwich	CT	Brackish	-73.65996	41.00060	Byram River	Save the Sound
R-BR-3.15	Byram River at Comley Avenue	Greenwich	CT	Freshwater	-73.66132	41.02735	Byram River	Save the Sound
R-BR-7.55	Byram River at Cliffdale Road	Greenwich	CT	Freshwater	-73.69552	41.07896	Byram River	Save the Sound
R-BREB-0.20	East Branch Byram River at Riversville Road	Greenwich	CT	Freshwater	-73.67475	41.06023	Byram River	Save the Sound
R-BSB-0.06	Beaver Swamp Brook at Boston Post Road	Mamaroneck Village	NY	Freshwater	-73.72143	40.95300	Sheldrake River-Mamaroneck River	Save the Sound
R-BSB-0.46	Beaver Swamp Brook at Rye Neck High School	Mamaroneck Village	NY	Freshwater	-73.71653	40.95801	Sheldrake River-Mamaroneck River	Save the Sound
R-BSB-1.67	Beaver Swamp Brook at Truxton Street	Harrison	NY	Freshwater	-73.70461	40.97060	Sheldrake River-Mamaroneck River	Save the Sound
R-BSB-2.2	Beaver Swamp Brook at Greenwood Union Cemetery	Harrison	NY	Freshwater	-73.70235	40.97795	Sheldrake River-Mamaroneck River	Save the Sound
R-BSBTa-0.02	Tributary to Beaver Swamp Brook	Harrison	NY	Freshwater	-73.71603	40.95939	Sheldrake River-Mamaroneck River	Save the Sound
R-GA-0.40	Gabblers Creek at Sandhill Road	Queens, NYC	NY	Freshwater	-73.74479	40.77329	Alley Creek-Little Neck Bay	Save the Sound
R-GC-0.25	Guion Creek at South Barry Avenue Bridge	Mamaroneck Village	NY	Brackish	-73.72522	40.94937	Sheldrake River-Mamaroneck River	Save the Sound
R-HNB-1.6	Horseneck Brook at Eagle Hill	Greenwich	CT	Freshwater	-73.64136	41.03262	Blind Brook-Horseneck Brook	Save the Sound

R-HUT-3.87	Glover Field	Mount Vernon	NY	Brackish	-73.81630	40.90178	Hutchinson River-Eastchester Bay	Save the Sound
R-HUT-4.40	Outfall at Farrell and Beechwood	Mount Vernon	NY	Freshwater	-73.81433	40.90939	Hutchinson River-Eastchester Bay	Save the Sound
R-HUT-4.42	Upstream of Farrell and Beechwood	Mount Vernon	NY	Freshwater	-73.81441	40.90968	Hutchinson River-Eastchester Bay	Save the Sound
R-HUT-4.55	Pelham Lake at Willson's Woods Park	Mount Vernon	NY	Freshwater	-73.81409	40.91163	Hutchinson River-Eastchester Bay	Save the Sound
R-MIR-1.40	Mianus River at Cos Cob Marina	Greenwich	CT	Brackish	-73.59599	41.03175	Mianus River	Save the Sound
R-MR-0.24	Mamaroneck River at Phillips Park Road	Mamaroneck Village	NY	Freshwater	-73.73272	40.95134	Sheldrake River-Mamaroneck River	Save the Sound
R-MR-0.61	Mamaroneck River at Station Park Road	Mamaroneck Village	NY	Freshwater	-73.73644	40.95488	Sheldrake River-Mamaroneck River	Save the Sound
R-MR-0.76	Mamaroneck River at North Barry Avenue Extended	Mamaroneck Village	NY	Freshwater	-73.73535	40.96017	Sheldrake River-Mamaroneck River	Save the Sound
R-MR-2.01	Mamaroneck River at Joint Water Works	Mamaroneck Village	NY	Freshwater	-73.73693	40.96933	Sheldrake River-Mamaroneck River	Save the Sound
R-MR-2.66	Mamaroneck River at Saxon Woods Park	Mamaroneck Town	NY	Freshwater	-73.74375	40.97833	Sheldrake River-Mamaroneck River	Save the Sound
R-MR-3.82	Mamaroneck River at Saxon Woods Road	White Plains	NY	Freshwater	-73.74696	40.98923	Sheldrake River-Mamaroneck River	Save the Sound
R-MR-5.12	Mamaroneck River at Reynal Road	White Plains	NY	Freshwater	-73.75562	40.99996	Sheldrake River-Mamaroneck River	Save the Sound
R-OC-0.22	Otter Creek at South Barry Avenue Bridge	Mamaroneck Village	NY	Brackish	-73.72324	40.94449	Sheldrake River-Mamaroneck River	Save the Sound

R-PC-0.01	Pemberwick Creek at Pemberwick Road	Greenwich	CT	Freshwater	-73.66107	41.02733	Byram River	Save the Sound
R-PR-0.1	Premium River at Pryer Manor Road	New Rochelle	NY	Brackish	-73.75622	40.91791	East Creek-Mamaroneck Harbor	Save the Sound
R-SHR-0.07	Sheldrake River at Columbus Park	Mamaroneck Village	NY	Freshwater	-73.73735	40.95427	Sheldrake River-Mamaroneck River	Save the Sound
R-SHR-2.28	Sheldrake River at Bonnie Briar Lane	Mamaroneck Town	NY	Freshwater	-73.76414	40.94871	Sheldrake River-Mamaroneck River	Save the Sound
R-SHR-2.91	Sheldrake Lake	New Rochelle	NY	Freshwater	-73.77341	40.95240	Sheldrake River-Mamaroneck River	Save the Sound
S-WLISd	Steppingstone Park	Kings Point	NY	Brackish	-73.75728	40.81753	East Creek-Dosoris Pond	Save the Sound
R-MR-2.79	Mill River at Whitney Water Center	Hamden	CT	Freshwaterwater	-72.910452	41.335996	Mill River	Southwest Conservation District
R-MR-7.39	Mill River at Mt Carmel Avenue	Hamden	CT	Freshwaterwater	-72.902758	41.42045	Mill River	Southwest Conservation District
R-SB-0.91	Shepherd Brook at Sanford Street	Hamden	CT	Freshwaterwater	-72.917126	41.382102	Mill River	Southwest Conservation District

**Table 2: Station identifying information, coordinates in WGS\_84, USGS HUC 12 watershed, city, state, water type, and primary sampling group, for seasonal pathogen indicator bacteria monitoring stations.**

**A.6.c. Annual Task Calendar**

The annual task calendar describes when certain activities will occur.

**Table 3: Annual Task Calendar**

These tasks are repeated annually.

Activity	J	F	M	A	M	J	J	A	S	O	N	D
Kickoff meeting with project team	x	x										
Develop draft changes/additions to QAPP if needed	x	x	x									
Finalize QAPP, responding to comments (if needed)			x	x	x							
Laboratory inventory, purchase, inspection, and testing	x	x	x	x	x	X	X	X	X	x	x	X
Seasonal pathogen indicator bacteria sampling					x	X	X	X	x	x		
Exploratory sampling	x	x	x	x	x	x	x	x	x	x	x	x
Project laboratory split sampling			x	x								
Field audits with monitoring programs							x					
Technical support to Monitoring Groups	X	X	X	x	x	x	x	x	x	x	x	x
Sampling events	X	X	X	X	X	X	X	X	X	X	X	X
Data entry	X	X	X	X	x	x	x	x	x	x	x	x
Seasonal Data review and validation of data entry										x	x	
Data uploads to program websites and EPA’s Water Quality Exchange (must follow data review)										x	x	x
Targeted meetings with stakeholders	X	X								X	X	X
Data to QAPP distribution list (seasonal and full set of annual exploratory samples)										X	x	

## A.7. DATA QUALITY OBJECTIVES

Taken together, precision, accuracy and bias, representativeness, comparability, completeness, and sensitivity comprise the major data quality indicators used to assess the quality of the program’s data. A summary of criteria are provided in Table 4.

### Definitions of these data quality indicator terms:

- **Precision** is the degree of agreement among repeated field measurements of the same indicator and gives information about the consistency of methods. It is typically defined as relative percent difference, or RPD.
- **Accuracy** is a measure of confidence that describes how close a measurement is to its “true” or expected value; it includes a combination of random error (precision) and systematic error (bias) components of both sampling and analytical operations.
- **Bias** is the systematic or persistent distortion of a measurement process that causes errors in one direction.

- **Representativeness** is the extent to which measurements actually represent the true environmental condition. Parameters, station selection (including location of sampling point within the water column), time, and frequency of sample collection can all play a role in determining how representative a sample is.
- **Comparability** is the extent to which data can be compared between sample locations or periods of time within a project, or between different sites.
- **Completeness** is the comparison between the amount of valid or usable data the program originally intended to collect versus how much was actually collected.
- **Sensitivity** is the capability of a method or instrument to discriminate between measurement responses representing different levels of the variable of interest.

**Table 4: Measurement Performance Criteria**

Data Quality Indicators	Measurement Performance Criteria	QC Sample and/or Activity Used to Assess Criteria
Precision – overall	RPD ≤ value indicated in Table 5	field duplicates
Precision – analytical	RPD ≤ value indicated in Table 5	analytical duplicates
Accuracy / Bias	QC sample within 95% confidence interval	certified reference material and laboratory duplicate when RPD>30%
Comparability	standard procedures followed	NA
Completeness	data from each station	66% of targeted seasonal pathogen indicator bacteria stations
Sensitivity	value ≥ MDL*	sample value check

\* MDL = method detection limit. This is a reporting limit based on the lowest standard accurately analyzed in the analysis.

**Precision** - Precision objectives are listed in Table 5. GPS coordinate precision is obtained by collecting one set of GPS coordinates in replicate per sampler per field day. Laboratory precision is obtained by a replicate lab analysis.

Exploratory monitoring will not be required to have field or lab replicates.

The frequency of lab replicate measurements for each parameter are described in Table 7.

Relative percent difference (RPD) of replicate samples is used as one index of precision; see Table 5. This is defined as the absolute difference between the replicates divided by the average of the replicates. The allowable RPDs for each parameter are provided in Table 7. A difference greater than the designated RPD requires further investigation of the laboratory methods. If the difference is large enough, it indicates failure (unless the average of the two

samples is less than 10 times the method detection limit), and results in potential disqualification or flagging of data from that station, unless there is a reasonable and supported explanation for the inconsistency. Replicate precision will be analyzed by calculating the RPD using the equation:

$$\text{RPD (\%)} = |x_1 - x_2| / ((x_1 + x_2)/2) * 100$$

where x1 is the original sample concentration and x2 is the replicate sample concentration.

The Microsoft Excel formula for calculating the RPD is:

$$= \text{ABS}(X1-X2) / ((X1+X2) / 2) * 100$$

where X1 is the original concentration and X2 is the replicate concentration. The RPD is calculated in the program's data entry template for replicate laboratory samples.

An RPD of over 30% will require a 95% confidence interval calculation to determine if data are disqualified. The 95% confidence interval verification will be calculated using IDEXX's Water MPN Generator.

**Accuracy and Bias** - Accuracy objectives are listed in Table 4. Procedures used to test or ensure accuracy are described in Table 11. While training and audits help to ensure measurement accuracy and precision, quantitative measures of accuracy for water quality monitoring are estimated using laboratory QC data which includes analysis of certified reference material and laboratory blanks using ASTM Type III or higher purified water.

The activities in this QAPP are intended to detect fecal contamination at specific locations and times. Sampling points are distributed to provide insight into possible sewage exposure by water users, to locate contamination hot spots, and to identify potential pollutant sources. The full subset of samples may not always describe general waterway conditions. Cumulative results could be biased higher as some sample stations are selected with potential fecal contamination sources considered. Safe and legal access to sampling stations is also a consideration

**Representativeness** – Seasonal pathogen indicator stations are selected purposefully for reasons listed in the Accuracy and Bias section above. End considerations of representativeness of the data set will be determined by end usages. Consideration will be given to adding stations to meet spatial water quality regulatory requirements or to enhance resolution of results.

Exploratory samples are often taken after a pollution report and are representative of the specific water sampled at the time of sampling.

**Comparability** - The comparability of the data collected can be assured by using known protocols and documenting methods, analysis, sampling sites and stations, times and dates,

sample storage and transfer, as well as laboratories and identification specialists; so that future surveys can produce comparable data by following similar procedures. Examples of project procedures are available in the collection of Standard Operating Procedures (SOPs) provided in Appendix A of this document.

**Completeness** –Minimum sample events for inclusion in seasonal monitoring are included in section A.6 of this document.

**Sensitivity** – Sensitivity objectives are listed in Table 5. Sensitivity is the lowest detection limit of the method or instrument for each of the measurement parameters of interest. For analytical methods, these are the method detection limits (MDLs).

**Table 5: Data Quality Objectives**

Parameter	Units	Accuracy	Precision (allowable RPD)	Approx. Expected Range	Sensitivity (Resolution or MDL)
Enterococci (Freshwater sample)	Most Probable Number/100 mL (MPN/100 mL)	Lab result of certified reference material within 95% confidence interval provided by IDEXX	30%	<1 MPN/100 mL - >24,196 MPN/100 mL	1 MPN/100 mL
Enterococci (Brackish sample)	Most Probable Number/100 mL (MPN/100 mL)	Lab result of certified reference material within 95% confidence interval provided by IDEXX	30%	<10 MPN/100 mL - >24,196 MPN/100 mL	1 MPN/100 mL
<i>E. coli</i> (Freshwater sample)	Most Probable Number/100 mL (MPN/100 mL)	Lab result of certified reference material within 95% confidence interval provided by IDEXX	30%	<1 MPN/100 mL - >24,196 MPN/100 mL	1 MPN/100 mL
Total Coliforms	Most Probable Number/100 mL (MPN/100 mL)	Lab result of certified reference material within 95% confidence interval provided by IDEXX	30%	<1 MPN/100 mL - >24,196 MPN/100 mL	1 MPN/100 mL
Fecal Coliforms	Most Probable Number/100 mL (MPN/100 mL)	Lab result of certified reference material within 95% confidence interval provided by IDEXX	30%	<1 MPN/100 mL - >24,196 MPN/100 mL	1 MPN/100 mL
GPS coordinates	decimal degrees (dec. deg.)	± 7.8 m <a href="http://www.gps.gov/systems/gps/performance/accuracy/">http://www.gps.gov/systems/gps/performance/accuracy/</a>	for reference point on land, within 11 m (=0.0001 dec. deg.)	NA	1.02 m



## **A.8. SPECIAL TRAINING / CERTIFICATION**

Pathogen indicator bacteria sampling trainings are hands-on events. They are designed for a wide range of experience in water quality monitoring; ranging from individuals with an extensive experience to individuals with little to no related experience. The trainings help to ensure all participants are on the same understanding of the project QAPP and SOPs. All participants in this project are provided the SOPs and QAPP. The objective of trainings is to have all participants, regardless of previous experience, following the project requirements in a unified manner.

The Monitoring Program Coordinator(s) or designated appointee shall ensure that all participants receive appropriate training by organizing and conducting training events. Designated appointees will only be staff from either SWCD or STS who have completed all pathogen indicator bacteria sampling training. The trainings are mandatory for new and veteran participants and have hands-on elements for sample collection, sample preservation, filling in datasheets, laboratory methods, and data management. All topics are covered in a guided step by step approach. Training will be assessed as described by checks in Section C.1 Assessment and Response Actions.

The Monitoring Program Coordinator(s) enter training into the project database and records the following information: subject matter (i.e. what type of monitoring and procedures are covered), training course title, date and agenda, name and qualification of trainers, and names of participants trained with associated monitoring group association. The trainings and technical support offered through the Monitoring Program Coordinator(s), Monitoring Program Laboratory Coordinator(s) and Monitoring Program Field Coordinator(s) are in place for the duration of the project. This will ensure new and veteran participants have a reliable source for prompt answers to their inquiries. Participants are prompted to email or call the listed personnel above with their questions. This support is mentioned throughout the project duration and emphasized at the trainings. Trainers remind trainees to call at any hint of a question or issue so it can be resolved.

The Monitoring Program Coordinator(s) will work closely with the Monitoring Project Manager and other signatories on this QAPP to confirm procedures are appropriate.

The Monitoring Project Manager was part of the three-person team leading UWS Tier I trainings around Long Island Sound in 2017 and continues to manage the trainings and project as it expands. He is the Director of Water Quality at Save the Sound and oversees, among other objectives, sampling water for pathogen indicator bacteria and environmental health indicators and stressors. He holds a Bachelor of Science Degree in Environmental Studies from University California Santa Barbara and a Master's of Science Degree in Environmental Science and Management from Sacred Heart University.

The Save the Sound Monitoring Program Coordinator is an integral member of the water quality team. She coordinates the pathogen indicator monitoring program, Unified Water Study, and manages the John and Daria Barry Foundation Water Quality Laboratory housed in Save the Sound’s Westchester Office. She holds a B.A. in Anthropology from Temple University. She worked as a staff scientist for The Patrick Center for Environmental Research at The Academy of Natural Sciences of Drexel University in Philadelphia, where she monitored sites across the Delaware River basin and worked with the Partnership for the Delaware Estuary in conducting freshwater mussel surveys.

The Southwest Conservation District Monitoring Program Coordinator holds a Bachelors in Environmental Science from Allegheny College and a Masters in Public Administration from the University of New Haven. He previously worked at CT DEEP in the Water Bureau for 11 .5 years where he collected water samples, was trained on bacteria sample processing and analysis by CT DPH and developed QAPP documents and monitoring plans as part of his workload. Prior to this he operated the largest volunteer water quality monitoring program in the State of Connecticut.

Project training shall take place as specified in Table 6.

**Table 6: Project-Specific Training**

Training: Type & Description	Trainer(s)	Training Date(s)	Trainees	Location of Training Records
Pathogen Indicator Bacteria Field Sampling <ul style="list-style-type: none"> <li>- General water quality parameter information</li> <li>- Chain of Custody record keeping</li> <li>- Pathogen indicator bacteria field collection, preservation, &amp; transport</li> <li>- GPS coordinate collection</li> </ul>	Monitoring Program Coordinator(s), Monitoring Program Field Coordinator(s), Project Manager	Annual; Spring before sampling season commences	All participants in the project conducting field sampling	Offices of the Monitoring Program Coordinator(s) and shared server location

Training: Type & Description	Trainer(s)	Training Date(s)	Trainees	Location of Training Records
Pathogen Indicator Bacteria Laboratory Analyses <ul style="list-style-type: none"> <li>- Laboratory methods</li> <li>- Data management and reporting</li> <li>- Demonstration of competence analyses</li> <li>- Data qualifications</li> </ul>	Monitoring Program Coordinator(s), Monitoring Program Laboratory Coordinator, Project Laboratory Manager(s), Project Manager	Annual; Spring before seasonal sampling season commences	All participants in the project conducting laboratory analyses	Offices of the Monitoring Program Coordinator(s) and shared server location

### A.9. DOCUMENTS AND RECORDS

**Field Datasheet and Chain of Custody** will be completed by participants before, during, and after pathogen indicator monitoring event. This form will have the address of the laboratory processing samples on it.

**Laboratory Datasheet** will be completed by participants prior to sample collecting, during processing, and for result entries. This form will have the address of the laboratory processing samples on it.

**Sample Labels** will be put on all field and lab sample containers. Labels on field sample containers will include the station name and space for writing the time of sample collection in the field. Lab containers will have the station ID and analyte. IDEXX trays will have station ID, date, and analyte on labels.

**Chain of Custody (COC) forms** are part of the field datasheet.

**Training records and field audit information** will be kept by the Monitoring Program Coordinator(s), and kept in a shared location.

The electronic project database shall be organized and protected from loss and damage through daily back-up of digital data on Save the Sound’s S-Drive. SWCD will also have a cloud-based database of stations and data to further enhance redundancy and security of information. This database will be used by Save the Sound to populate the S-Drive storage of data.

No scientific collecting permits or certificates of permission are required.

The specific forms to be used for this project are provided in the Appendices of this document.

## **B. Data Generation and Acquisition**

### **B.1. SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)**

#### *Seasonal pathogen indicator bacteria*

Seasonal pathogen indicator bacteria stations are assigned an ID consisting of the station type (e.g., “R” for river, “B” for recreational beach, “S” for shoreline, “E” for embayment, “L for lake”, “O” for outfall), waterway initials and approximate river mile when applicable (e.g., “R-MR-0.24”). Lower case letters are used for multiple sites in a waterbody other than a river (e.g., “E-MHb”) Each station is also assigned a name usually consisting of the waterway and a local landmark, street name, or other identifying feature (e.g., “Mamaroneck River at Joint Waterworks” and “Gabblers Creek at Sandhill Road”).

Waterway names are often identified using EPA, NYSDEC, and CTDEEP 305(b) assessment and 303(d) impairment documents. This mostly applies to sites along the Long Island Sound coastline to account for the differences in regulatory and local naming observed in some embayments and rivers.

Station selection is informed by reviewing the NYSDEC Priority Waterbodies List, CTDEEP Integrated Water Quality Report, the United States Environmental Protection Agency (EPA) 303(d) list, MS4 reports and audits, EPA LIS Beacon Database, sanitary sewage overflow reports, regional testing that has already been undertaken, Sewage Pollution Right to Know discharge reports and other sources as they become available. We also take into consideration input from community groups, state and local environmental agency staff, members of sports and outdoor associations, and other people with local knowledge. Ease of access, permanence of access, and volunteer safety are also considered during sample stations selection process.

The Monitoring Program Manager and Monitoring Program Coordinator(s) work together to select new seasonal stations based on information listed in the above paragraph. Other members of the project team may also join the discussion.

Sample stations will be grouped together according to United States Geological Survey Hydrologic Units identified with Hydrologic Unit Code (HUC) 12 classifications. Stations within a HUC 12 watershed boundary will be sampled together on any given weekly monitoring sampling event. In rare events, a station may need to be sampled on a different day of the week than others in its watershed. This occurrence will be noted with data from that day.

*Exploratory pathogen indicator bacteria*

Exploratory pathogen indicator bacteria are assigned an ID that consists of the year the samples are taken followed by a number. The number is assigned based on samples collected that year (e.g., EXP2022-01, EXP2022-02...). A name is also assigned in the same method used for seasonal pathogen indicator bacteria stations.

Sampling design components are described below:

**Sampling Safety.** Personal safety shall be a primary consideration in all activities, including selection of sampling stations, dates, and training programs. No sampling shall occur when personal safety is thought to be compromised. The Monitoring Program Coordinator of each participating group in this QAPP shall confer with their respective field teams before each sampling event to decide whether adverse weather or other conditions pose a threat to safety and will cancel/postpone sampling when necessary. Samplers shall wear life vests, or have them available for usage, on boats as required by United States Coast Guard, Connecticut, and New York regulations. Life vests will be worn when wading in waters under difficult conditions. Samplers shall wear proper clothing to protect against the elements and field hazards.

**Design Considerations.** A summary of general design approaches to the number of stations, depth of sampling, frequency of sampling, and time of day are included here:

**Table 7: Sampling Approaches.**

**Assessment Type: Seasonal Pathogen Indicator Bacteria Stations**

Indicators	Number of sample locations	Frequency, duration, special conditions	Quality Control
GPS: latitude & longitude in decimal degrees; NAD83 coordinate system or record system used	every station	Weekly from May - October	repeat reading of coordinates at one station per sampler and collect every time a station is sampled.  coordinates indicating a 25 m or greater discrepancy will be assessed and documented in final report.
Enterococci, <i>E. coli</i> , Total Coliforms, and/or Fecal Coliforms			once per sampling event conduct a field blank, once per sampling event conduct a laboratory duplicate

**Assessment Type: Exploratory Pathogen Indicator Bacteria Stations**

Indicators	Number of sample locations	Frequency, duration, special conditions	Quality Control
GPS: latitude & longitude in decimal degrees; NAD83 coordinate system or record system used	every station	As needed annually	repeat reading of coordinates at one station per sampler and collect every time a station is sampled.  coordinates indicating a 25 m or greater discrepancy will be assessed and documented in the final report.
Enterococci, <i>E. coli</i> , Total Coliforms, and/or Fecal Coliforms			QC of the initial sampling at exploratory stations is not conducted for ease of process and saving resources and time. If/when an exploratory station becomes a seasonal pathogen indicator bacteria station, all QC protocols and sampling will be added.

**B.2. SAMPLING METHODS**

Pre-coordination shall occur with the project labs to ensure that sample collection procedures meet lab needs. The project labs for this study are below:

*Pathogen Indicator Bacteria*

Save the Sound, John and Daria Barry Foundation Water Quality Laboratory, contact: Elena Colón & Peter Linderoth, [ecolon@savethesound.org](mailto:ecolon@savethesound.org); [plinderoth@savethesound.org](mailto:plinderoth@savethesound.org); 914-381-3140, 1385 Boston Post Rd, 2nd Fl, Larchmont, NY 10538

*Pathogen Indicator Bacteria*

Southwest Conservation District Laboratory, contact: Chris Sullivan, csullivan@conservect.org, 203-859-7014 51 Mill Pond Road, Hamden, CT 06514

A laboratory of equal or higher certification than Save the Sound or Southwest Conservation District can be considered if Save the Sound or Southwest Conservation District are unable to complete project analyses. This surrogate laboratory must adhere to analytical methods in Table 10.

To comply with this project’s guidelines, all sample collections shall follow detailed methods on how samples will be collected and preserved as stated in the standard operating procedures (SOPs) contained in Appendix A of this document. The lab has reviewed the SOPs and confirms they are appropriate for the select analyses. Results from any laboratory not listed in this QAPP will include the respective laboratory name and information that did the analysis listed.

A summary overview of sample collection methods is provided in Table 8.

Any field sample bottles collected for analysis will be stored in a cooler, in wet ice during the sampling trip. The cooler designated for these samples will not be used for the storage of any other materials.

All deviations from the Standard Operating Procedures of this QAPP will be documented and subsequently reviewed by the Monitoring Program Coordinator. Information will be available to all signatories at the completion of this project at which time acceptability of data will be determined.

**Table 8: Overview of Sample Collection Methods**  
**Pathogen Indicator Bacteria**

(NA = not applicable)

Parameter(s)	Container Type(s) and Preparation	Minimum Sample Quantity per Sample Station	Sample Preservation	Maximum Holding Time
GPS: latitude & longitude in decimal degrees; NAD83 coordinate system or record alternate system used	<i>in situ</i>	1	transfer to digital format, maintain back-up copies of digital data	NA
<i>E. coli</i> , Enterococci, Fecal Coliforms, Total Coliforms	<i>120 mL polyethylene bottles labeled with station id and time of sample collection</i>	Minimum 1 analyte per sample station	transfer to digital format, maintain back-up copies of digital data	8 hours

### B.3. SAMPLE HANDLING AND CUSTODY

Sample handling and labeling procedures shall comply with project Standard Operating Procedures (SOPs). Pathogen Indicator Bacteria samples will be transported in ice in a cooler at all times prior to analyses in the laboratory.

Sample labels will be associated with: station id and time of sample. These details will be written on the label. This information in addition to station name will also be filled in the field datasheet.

Chain of Custody shall be tracked as detailed in the SOPs. The project Chain of Custody forms are provided in Appendix A.

**The following steps shall be taken to avoid sample mislabeling:**

Labels will be prepared in advance and cross checked with the field datasheet before sampling events. Field samplers will complete the respective section of the field datasheet at each station prior to leaving. Laboratory personnel will check the field bottle label against the IDEXX quanti tray label to confirm they are in agreement.

### B.4. ANALYTICAL METHODS

To comply with the requirements of this project, all analytical methods used in the Monitoring Program, including methods used by laboratories performing analyses for the project, shall be based on standardized laboratory methods.

All analytical methods used for this project are provided in Appendix C

Table 10 provides an overview of the analytical methods utilized in this Monitoring Program. The SOP associated with these methods are included in Appendix C.

**Table 10: Overview of Analytical Methods.**

Parameter	Method #	Source of Method	MDL	Alternative Applications Special Provisions
Enterococci	SM 9230D-2013 (Enterolert)	SM	1 MPN/100 mL	
<i>E. coli</i>	Colilert-18	IDEXX	1 MPN/100 mL	
Total Coliforms	Colilert-18	IDEXX	1 MPN/100 mL	
Fecal Coliforms	Colilert-18	IDEXX	1 MPN/100 mL	



## B.5. QUALITY CONTROL

Quality control shall be discussed and defined prior to sampling (e.g., during training).

Details on quality control procedures are provided in Table 11.

**Table 11: Quality Control Measures**

Sample Type	Instrument/ Parameter	Accuracy Checks	Precision Checks	QC Samples (blanks and field duplicates)
GPS coordinates	GPS unit or Smart Phone app / GPS coordinates	Compare location to known location of sample station	Duplicate GPS readings at one station per sampler	1 / field day
Pathogen indicator bacteria - grab sample	Determination of pathogen indicator bacteria (Enterococci, <i>E. coli</i> , Total Coliforms and Fecal Coliforms)	Laboratory reagent blank, certified reference material analysis, laboratory fortified blanks, laboratory negative controls, field blank, field blank	Lab duplicate	Certified reference materials analysis as required for any certifications and fortified blanks per lot of IDEXX reagent  Laboratory reagent blank, laboratory sample duplicate, and field blank per sample event per laboratory

## B.6. INSTRUMENT / EQUIPMENT TESTING, INSPECTION AND MAINTENANCE

Maintenance of instruments and equipment shall occur as needed during the duration of the project. Equipment manuals will be kept in an accessible location at each project location for quick retrieval when needed.

The Monitoring Program Coordinator will ensure records of equipment inspection, maintenance, repair and replacement shall be kept in a logbook. Entries into the logbook are entered by the Laboratory Coordinator and Field Coordinator for their respective equipment and supplies lists. If the logbook is digital, appropriate backups of the computer files will be maintained by the Monitoring Program Coordinator and put on Save the Sound's S-Drive which is backed up daily.

Types of inspection include cleanness and operationality. Cleanness inspections are for cleanliness both in respect to visual contamination such as mud but also for disinfecting contamination that may not be visible. Operation inspections are undertaken to assess if a piece of equipment is operating as expected.

Disinfectant products used in this project can vary but will always have antiviral and antibacterial properties.

**Table 12: Instrument / Equipment Inspection and Testing Procedures**

Equipment Type	Inspection Frequency	Type Inspection	Maintenance, Corrective Action	Frequency
GPS unit or smartphone	before each sampling date	battery life	charge batteries	As needed during the season
Sample pole	After each sampling event	Check the basket at the end of the pole for solid connection, loosen and adjust length	Replace broken components or tighten when loose. Wipe after each use with isopropyl alcohol, rinse with freshwater and allow to dry prior to storage	As needed during season and prior to storage
Cooler	Before each sampling date After each sampling date	Cleanness	Empty cooler, rinse inside with water, clean inside and outside (if needed) with disinfectant after every use  Check the cooler has been cleaned after last use, clean with disinfectant if questionable	As required during season and prior to storage
IDEXX Sealer	After incident/spill and annually	Cleanness	Follow instruction manual for guidance on disassembling sealer for cleaning internal components. Wipe external components annually or after a spill with disinfectant	As needed and annually close to the beginning of the calendar year

Equipment Type	Inspection Frequency	Type Inspection	Maintenance, Corrective Action	Frequency
Incubator	After incident/spill and annually  Incubator temperature reading	Cleanness  Operational	Follow instruction manual for guidance on cleaning chamber  Compare incubator temperature display with internal thermometer. Contact incubator manufacturer for maintenance if difference is >0.2 C	As needed and annually close to the beginning of the calendar year  When recording internal thermometer temperature on lab datasheet
UV viewing cabinet	After incident/spill and annually	Cleanness	Clean with nonabrasive cloth and light disinfectant	As needed and annually close to the beginning of the calendar year
Incubator thermometer	Annually	Operational	Verify with a NIST-certified thermometer. Note difference and list $\pm$ correction on incubator thermometer	Annually
Infrared thermometer	Annually	Operational	Verify against NIST-certified thermometer. Note difference and list $\pm$ correction on infrared thermometer	Annually

## B.7. INSPECTION / ACCEPTANCE OF SUPPLIES AND CONSUMABLES

The procedures for inspection and acceptance of supplies and consumables listed in Table 14 shall be followed by the project participants. Supplies and consumables information will be kept in an accessible location at each project location for quick retrieval when needed.

**Table 14: Supplies Inspection and Acceptance Procedures**

Supplies	Inspection Frequency	Type of Inspection	Available Parts	Maintenance
Certified reference materials	Upon receipt, prior to storage, and check every time used	Visual inspection of quantity and expiration date	Spare certified materials	Storage according to manufacturer's recommendations
Quanti-Tray /2000	Before each sampling date	Visual inspection of quantity and expiration date	Spares	Storage according to manufacturer's recommendations
Dilution bottles	Before each sampling date	Visual inspection of quantity, volume checks, and expiration date	Spares	Storage according to manufacturer's recommendations
Pipettes	Before each sampling date	Visual inspection of quantity and expiration date	Spares	Storage according to manufacturer's recommendations
Field and lab sample sheets	Before each sampling date	Visual	Additional copies	N/A
Sample bottles	Before each sampling date	Integrity, cleanness and seal	Spares	Bottles single use and recycled
UV bulb in UV viewing cabinet	Every time used	Visual inspection confirming bulb is working	Spares	Replace bulb if needed
Incubator thermometer	Every time used	Visual inspection for any separation	Spares	Put thermometer in freezer overnight. Replace if needed

## B.8. NON-DIRECT MEASUREMENTS

To provide high-quality data to enhance the interpretation of data collected as part of this Monitoring Program, data may be acquired from qualified sources approved by the Monitoring Program Coordinator(s). NOAA tide gauges will be used for tide information. Specifically, sample stations will be mapped and the closest NOAA tide gauge in Long Island Sound will be utilized for tidal data and noted on data spreadsheet. Precipitation will be acquired from local weather stations that log reasonable (in respect to northeastern USA conditions) volumes. Precipitation data out of the expected annual volumes and the observed conditions will be flagged and discussed with Monitoring Program Coordinator(s) and shared with quality

assurance personnel for review and potential disqualification. External data sources are described in Table 15.

**Table 15: Non-Project Data Validity**

The following data will be used as part of the Monitoring Program. This is a secondary use of data.

Title or descriptive name of data document.	Source of data.	QAPP written? Y/N	Notes on quality of data.	Planned restrictions in use of the data due to questions about data quality.
Time of low and high tide	NOAA tide gauges recorded on data spreadsheet	N	NOAA has internal requirements for data suitability. High and low tide data are not generally available at the embayment. Data from NOAA tide gauges are acceptable; data are used in broad scale, to determine the potential impact of stage in tidal cycle on the day of sampling.	Data quality is acceptable. However, local tidal stage will differ from the nearest NOAA gauge even when corrected for difference in location. These data are rough predictors only.
Precipitation out to 72+ hours prior to the sample collection	Weather Underground data recorded on data spreadsheet	N	Data from nearby weather stations are acceptable; data are used in broad scale, to determine the potential impact of weather on the day of sampling.	Data quality is acceptable and an approximate for the exact location the sample is collected. Hourly data are required for day of sampling precipitation totals and weather stations may change at discretion of Monitoring Program Coordinator or designee

## B.9. DATA MANAGEMENT

Field samplers shall record data on field datasheets, review them, and turn over to respective Monitoring Program Field Coordinator or designated appointee. The latter can include any member of the project team.

Each person who handles or transports samples shall sign the custody form on the field datasheet upon receipt of the samples. Chain of custody forms will follow samples to the lab and back to Monitoring Program Lab Coordinator by mail or direct handoff after each sample run is completed.

Monitoring Program Field Coordinator(s) or designated appointees shall review sheets and confer with field teams on any needed corrective action. Designated appointee can be any member of the project team.

Once laboratory analyses are complete, the laboratory personnel shall deliver lab results to the Monitoring Program Lab Coordinator.

The Monitoring Program Lab Coordinator or other trained designee will enter raw field and lab data into the project computer system. The trained designee can be any member of the project team. Non-direct measurements of tide and precipitation data will be entered at this time.

Computer-entered data shall be compared with field and lab datasheets for accuracy. Review of non-direct measurements will be undertaken at this time as well.

Original datasheets will be stored by the Monitoring Program Coordinator following digital data entry.

Digital back-ups and copies of the non-digitized data will be made and stored in a separate location designated by the Monitoring Program Coordinator.

Documentation of data recording and handling, including all problems and corrective actions, shall be included in all preliminary and final reports.

Final Data will be entered into EPA’s Water Quality Exchange by the Monitoring Program Coordinator and/or Monitoring Program Lab Coordinator annually near the end of the calendar year.

Table 16 in this document accurately represents the procedures utilized by the project for data management, review, validation, and verification.

**Table 16: Data Management, Review, Validation, Verification Process Summary**

The term “Field sampler” refers to the person conducting the sampling in the field.

Activity	By whom	Corrective action, if needed
Conduct field audits of field samplers	Monitoring Program Field Coordinator, Monitoring Program Quality Assurance Officer, or appointed designee	Correct any discrepancies with this QAPP or SOPs
Check labels just prior to sampling, to ensure correct labeling of container.	Field sampler and Monitoring Program Field Coordinator or designated appointee	Correct label
At time of sampling, record data, sign field sheets.	Field sampler and all that control sample bottles	Remind project personnel of proper procedures; retrain if needed.
Fill out, sign chain of custody (CoC) forms for any samples going to lab.	Field sampler and all that control sample bottles	Remind project personnel of proper procedures; retrain if needed.
Upon receipt of field sheets, recheck for completeness	Monitoring Program Field Coordinator or designated appointee	Confer with field sampler(s) immediately or within 24 hours. Resample if needed or obtain information from responsible personnel

Activity	By whom	Corrective action, if needed
<p>Upon receipt of samples, field sheets and CoC forms, check to see that sheets and forms correspond to number of samples, condition of samples as stated on CoC forms. Sign CoC forms.            Copies of field sheets and CoC forms are made, given to the Monitoring Program Coordinator.</p>	<p>Monitoring Program Lab Coordinator or designated appointee</p>	<p>Contact field samplers as needed to locate missing samples, data records. In case of missing/spoiled samples or data records, authorize resampling as needed and feasible. If resampling is not feasible, flag all suspect data.</p>
<p>Upon completion of laboratory analyses, fill out lab sheets, including data on QC tests.</p>	<p>Monitoring Project Laboratory Coordinator, Monitoring Project Manager, or designated appointee</p>	<p>Re-analyze if possible. If not, confer with Monitoring Program Coordinator and Monitoring Program Quality Assurance Officer. Flag all suspect data using identifiers listed in project laboratory SOPs found in Appendix C.</p>
<p>Upon receipt of lab data, review for completeness and legibility.</p>	<p>Monitoring Program Lab Coordinator or Project Laboratory Manager</p>	<p>Confer with the Monitoring Program Coordinator, Monitoring Program Laboratory Coordinator, and Monitoring Program Quality Assurance Officer.</p>
<p>Upon completion of data entry, compare with field/lab sheets for accuracy.</p>	<p>Monitoring Program Lab Coordinator or designated appointee.            Data entry personnel may review their own work, but it cannot be on the same day as data entry whenever possible.</p>	<p>Re-enter or correct data.</p>
<p>Translate data into preliminary data reports: run statistical analyses and/or prepare graphical summaries of data. Check for agreement with QC objectives for completeness.</p>	<p>Monitoring Program Coordinator, Monitoring Program Laboratory Coordinator, and Project Laboratory Manager</p>	<p>Confer with the Monitoring Program Quality Assurance Officer and Monitoring Program Project Manager. Assign necessary identifier(s) or discard suspect data.</p>
<p>In-season (at least once) and end of season review of collected data sets (individual sample runs and season-total compilations); review for completeness and agreement with QC objectives and DQOs.</p>	<p>Monitoring Program Coordinator</p>	<p>Flag suspect data. Confer with the Monitoring Program Quality Assurance Officer.</p>

**Data Management Systems** – spreadsheets, databases, location of data records (paper and electronic), are described here:

All data will be entered from field and laboratory datasheets to an Excel spreadsheet for storage and retrieval by project personnel. Digital copies of all datasheets will be kept on file on the S-Drive server in Save the Sound office for at least 5 years with a plan to keep records for duration of the project and beyond. The S-Drive is backed up weekly.

## **C. Assessment and Oversight**

### **C.1. ASSESSMENT AND RESPONSE ACTIONS**

The Monitoring Program Coordinator(s), Monitoring Program Laboratory Coordinator, Project Laboratory Manager, Monitoring Program Field Coordinator and Quality Assurance Officer will identify and effectively address any issues that affect data quality, personal safety, and other important project components. The progress and quality of the monitoring program shall be assessed to ensure the objectives are being accomplished. The Monitoring Program Coordinator(s) or appointed designees will check at the end of every month to confirm the following:

- a. Monitoring is occurring as planned.
- b. Sufficient written commentary and supporting photographs, if listed on chain of custody, exist.
- c. Sufficient field members are available for all sampling events.
- d. Samplers are collected in accordance with project schedules.
- e. Datasheets and custody control sheets are being properly completed and signed.
- f. Retraining or other corrective action is implemented at the first hint of non-compliance with the QAPP or SOPs.
- g. Labs are adhering to the requirements of this QAPP in terms of work performed, accuracy, acceptable holding times, timely and understandable results and delivery process.
- h. Data management is being handled properly, i.e. data are entered on a timely basis, is properly backed up, is easily accessed, and raw data are properly stored in a safe place.
- i. Procedure for developing and reporting the results exists.
- j. All samplers and laboratory personnel have undertaken the training.

Project personnel engaging in field and lab data collection and analysis will be assessed on their ability to follow procedures during field audits overseen by the Monitoring Program Quality Assurance Officer and Monitoring Program Coordinator(s). The Monitoring Program Field Coordinator or designee will observe each field sampler undertaking field procedures once during the season and follow up actions, such as meetings or additional training, will be



scheduled after initial field audits. Field procedures can be reviewed from a set location that does not need to be a seasonal monitoring station.

The Monitoring Program Coordinator(s) shall confer with the Monitoring Program Quality Assurance Officer and Project Manager as necessary to discuss any problems that occur and what corrective actions are needed to maintain program integrity. In addition, the Monitoring Program Coordinator(s) and Project Manager shall meet at the end of the sampling season, to review the draft report and discuss all aspects of the program and identify necessary program modifications for future sampling activities. All problems discovered and program modifications made shall be documented in the final version of the project report. If modifications require changes in the Quality Assurance Project Plan, these changes shall be submitted to the QAPP signatories for review.

If data are found to be consistently outside the Data Quality Objectives as defined in section A.7. of this document the Monitoring Program Coordinator(s) shall review the program and correct problems as needed. Corrections will be reported to the Project Manager prior to action(s). Corrections may include retraining project personnel; rewriting sampling instructions; replacement of staff/samplers; alteration of sampling schedules, stations or methods; or other actions deemed necessary. This information will be logged and maintained by the Monitoring Program Quality Assurance Officer. It will also be included in the QAPP Final Report.

## **C.2. REPORTS TO MANAGEMENT**

Data that have passed the project quality assurance may be posted on websites, shared with the local media or at other venues (e.g. kiosks at recreation access sites), and submitted to the EPA Long Island Sound Study, Interstate Environmental Commission, New York State Department of Environmental Conservation, New York City Department of Environmental Protection, Connecticut Department of Energy and Environmental Protection. Data are uploaded to the Water Quality Exchange portal. Preliminary data that has not been through the full quality assurance procedures can be shared but will contain clear messaging that it is preliminary data that has not been through the full quality assurance process.

The Monitoring Program Coordinator(s) and Project Manager will write a final report. This will be sent to the distribution list on this QAPP. A final workbook of data from all stations will accompany the report. The final report will describe the program's goals, methods, quality control results, and recommendations.

All reports, preliminary or final, will include discussion of steps taken to assure data quality, findings on data quality, and decisions made on use, censorship, or flagging of questionable data. Any data that are censored in reports will be either referred to in this discussion, or presented but noted as censored.

In short, the final report will include:

- Raw data
- QC data
- Associated metadata
- Questionable data, flagged
- Identification of status as “preliminary” or “final” report

**Table 17: Report Mechanisms, Responsibilities, and Distribution**

Reporting Mechanism	Person Responsible for writing report	Distribution list
Final Monitoring Report	Monitoring Program Coordinator and Project Manager	Signatories of this QAPP
Final Monitoring Data	Monitoring Program Coordinator and Project Manager	QAPP distribution list

## D. Data Validation and Usability

### D.1. DATA REVIEW, VERIFICATION, AND VALIDATION

All project data, metadata, and quality control data shall be critically reviewed to look for problems that may compromise data usability.

Data collected before the 2022 season will be flagged as not being conducted under this QAPP when distributed. Save the Sound will be tasked with maintaining this QAPP in all aspects for the duration of the project.

The Monitoring Program Field Coordinator or designated appointee will review field data after each sampling run and take corrective actions as described in Table 16 of this document. At least once during the season, at the end of the season and if questions arise, the Monitoring Program Coordinator or designated appointee will share the data with the Monitoring Program Quality Assurance Officer to determine if the data appear to meet the objectives of the QAPP. Together, they will decide on any actions to take if problems are found.

## **D.2. VERIFICATION AND VALIDATION METHODS**

All project data and metadata are reviewed and approved as usable data, or as un-usable data.

Data verification and validation will occur as described in Table 16, and will include checks on:

- Completion of all fields on datasheets; missing datasheets
- Completeness of sampling runs (e.g. number of stations visited / samples taken vs. number proposed, were all parameters sampled / analyzed)
- Completeness of QC checks (e.g. number and type of QC checks performed vs. number or type proposed)
- Number of samples exceeding QC limits for accuracy and precision and how far limits were exceeded.

## **D.3. RECONCILIATION WITH USER REQUIREMENTS**

At the conclusion of the sampling season, after all in-season quality control checks, assessment actions, validation and verification checks and corrective actions have been taken, the resulting data set will be compared with the program's data quality objectives (DQOs) as defined in section A.7. This review will include, for each parameter, calculation of the following:

- Completeness goals: 66% of targeted seasonal pathogen indicator bacteria stations
- Percent of samples exceeding accuracy and precision limits.
- Average departure from accuracy and precision targets.

After reviewing these calculations, and taking into consideration such factors as clusters of unacceptable data the Monitoring Program Coordinator and Quality Assurance Officer will evaluate overall program attainment of DQOs and determine what limitations to place on the use of the data, or if a revision of the DQOs is allowable.

## **E. Appendices**

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# Pathogen Indicator Bacteria Field Sample Collection

## POINT OF CONTACT

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EMAIL: [plinderoth@savethesound.org](mailto:plinderoth@savethesound.org)  
PHONE: 914-381-3140

## OBJECTIVE

Collect field samples for subsequent laboratory analyses of *E. coli*, Enterococci, Total Coliforms, and Fecal Coliforms.

## OVERVIEW

Sterilized sample vessels are used to collect a grab sample from non-potable water sources, such as rivers, lakes or bays. For most samples, a 1:10 sample dilution is prepared by diluting 10 mL of water sample with 90 mL of deionized water to increase detection range. The dilution of the sample can be changed depending on expected results.

## SOURCES

U.S. EPA Reg 2 Laboratory. "Guidance Document for Citizen Science Pathogen Monitoring of Total Coliforms and *E. coli* Using IDEXX Colilert-18 with Quanti-Tray 2000." Revision 1.1. Feb 2015.

## MATERIALS AND EQUIPMENT

### Field Collection

- Sterilized 120 mL Sample Vessel
  - Containing sodium thiosulfate for neutralizing chlorine if present
  - Bottle without sodium thiosulfate can be used if noted
- Nitrile Gloves
- Cooler filled with Ice
- Sampling Extension Pole (optional)
- Permanent Marker
- Pen or Pencil
- GPS unit or smartphone
- Field Datasheet with Chain-of-Custody Section

## QUALITY CONTROL

### Field Blanks

#### Field Blank

A minimum of one field blank per sampling day per laboratory shall be prepared. To prepare a field blank, the sample collector will pour 99 mL of deionized water into a sterile sample vessel at the assigned sample field station. The field blank sample shall be stored and processed in the same manner as all other samples collected. The field blank will be checked for temperature when it returns to the laboratory.

## METHODS

### Sample Collection and Storage

#### Sample Collection

- a. Record the GPS Coordinates where sample is to be collected.
- b. Write the sample time on the sample vessel prior to putting the bottle in the water.
- c. Wear nitrile gloves on both hands prior to removing cap from sample bottle.
- d. Remove cap from sterile sample bottle (NEVER TOUCH INSIDE OF CAP OR BOTTLE).
- e. Submerge bottle 6 inches below surface of water, right side up under the surface.
- f. If not using a pole, keep cap on until sample depth is reached, remove cap, then allow water to fill bottle.
- g. If using a pole, remove cap prior to putting bottle in the water, lower bottle at end of pole at an angle to keep water from filling on the way down, then turn right side up at 6 inches below the surface to fill.
- h. If a separate depth is required, note on field datasheet process on depth measurement (e.g. sample pole has alternative depth markings on it for target sample depth).
- i. Bring bottle up out of water after full, pour out any excess water above the 100 mL line.
- j. Recap the bottle and seal it tightly.
- k. Immediately place the closed bottle in the cooler and cover it with ice.
- l. Deliver the sample to the lab as soon as possible.

#### Storage

Samples are to be stored at temperatures  $<10^{\circ}\text{C}$  and the field blank will be measured using an infrared thermometer upon arrival to the laboratory. If the commute time between the lab and time of collection is short, evidence of icing is sufficient to meet storage criteria.

## Holding Time

Samples should be returned to the laboratory within 6 hours of collection and placed in the incubator within 8 hours of collection.

## TROUBLESHOOTING / HINTS

- Collecting sediment in the sample should be avoided.
- Every attempt should be made to limit light exposure to the sample during field collection.

## DATA PROCESSING AND STORAGE

### Sample Data Handling

Field datasheet should be evaluated for completeness and any deviations to the SOP should be identified and noted.

Each sample collector is responsible for recording the field sample data (date, time, GPS coordinates, and field conditions) and providing the data to the lab via a chain-of-custody at the time the sample is transferred over.

Field datasheets, which also serve as chain-of-custody will be reviewed for completeness prior to transfer to the laboratory by field sampler. Chain-of-custody records will be kept by the project laboratory coordinator with respective laboratory data.

### Data Analysis

There are two types of results for most pathogen analyses when being compared to a Water Quality Standard (WQS): the “Single Maximum Concentration” and the “Geometric Mean”. The single maximum concentration is each individual result. Individual sample results are compared to the criteria that you have established for your project.

Also, the project can prescribe a longer-term depiction of bacterial contamination of multiple samples collected over more than one day. This is called a Geometric Mean. A minimum of 5 individual results are recommended to be used to calculate a Geometric Mean.

$$\text{Geometric Mean} = \text{nth root of } (X_1)(X_2)\dots(X_n)$$

The “nth” root is the number of total results used in your geometric mean calculation, times the multiple all of your test results together.

# GPS Coordinates

## POINT OF CONTACT

NAME: Peter Linderoth  
ADDRESS: 1385 Boston Post Rd, 2<sup>nd</sup> Floor, Larchmont, NY 10538  
EMAIL: [plinderoth@savethesound.org](mailto:plinderoth@savethesound.org)  
PHONE: 914-381-3140

## OBJECTIVE

Determine the GPS coordinates of the station in WGS 84.

## OVERVIEW

A GPS unit is used to obtain GPS coordinates for the station on each field day. A Smart Phone app is an acceptable alternative as backup. Google Maps is the preferred application for obtaining coordinates with a smartphone. GPS position of your actual location at each station for each sample date is required. A second reading is taken at one station per sampler per sample day to assess precision. If unforeseen circumstances make recording GPS points impossible and dead reckoning is used, your data may still be used as part of the project but will be flagged and reviewed in the quality assurance process.

## SOURCES

These procedures are based on the EPA Volunteer Estuary Monitoring Manual (EPA, 2007). The EPA Volunteer Estuary Monitoring Manual (EPA, 2007) provides a wealth of specific data for monitoring groups.

## MATERIALS AND EQUIPMENT

- GPS coordinate sampling device. Options include:
  - Handheld GPS unit.
  - Apps are available for your smart phone that allow you to easily log and return to GPS coordinates. The accuracy of the GPS on your phone is dependent on Wi-Fi signals and cellular signals.
  - Dead reckoning to get yourself to a station should only be used if your GPS options have failed. This will need to be included in field notes for the sampling event. Data will be reviewed in the quality assurance process.



## METHODS

### Preparation

- GPS device
  - Check batteries.
  - Confirm unit turns on and is able to record coordinates prior to station visit.

### Field Collection and Processing

1. Set GPS to WGS 84 if using a handheld with that functionality. If using a smartphone, Google Maps provides coordinates in WGS 84 datum.
2. Determine the GPS coordinates of the station by reading the display while standing still at sample station location.
3. Recording GPS coordinates in decimal degrees format is preferred (XX.XXXXX, XX.XXXXX)
  - a. If absolutely necessary, another standard format can be used such as degrees/minutes/seconds.
4. Record decimal degrees GPS coordinates out to 5 decimal points.
5. At least once per sampling day per field sampler (usually the last station of the day), take the reading again and record on field datasheet.

### Sample Storage

Not applicable.

### Laboratory Analysis

Not applicable.

## TROUBLESHOOTING / HINTS

- Always carry a copy of this SOP and the relevant parameter-specific SOPs.

## DATA PROCESSING AND STORAGE

GPS data are entered onto the field datasheet and relinquished to the laboratory upon delivery of samples.

## REFERENCES

EPA, 2007, Volunteer Estuary Monitoring, A Methods Manual, Second Edition. Orhrel Jr., R.L., Register, K.M. (Eds.). The Ocean Conservancy & EPA. 396 p. [https://www.epa.gov/sites/production/files/2015-09/documents/2007\\_04\\_09\\_estuaries\\_monitoruments\\_manual.pdf](https://www.epa.gov/sites/production/files/2015-09/documents/2007_04_09_estuaries_monitoruments_manual.pdf)



**Chain of Custody Record**  
 1385 Boston Post Road., 2<sup>nd</sup> Floor  
 Larchmont, NY 10538

**Date:**

Station ID	Station Name/Location & Field GPS Coordinates	Time Sampled

1a. Current Weather Conditions (circle):  
 Mostly Cloudy      Clear      Partly Cloudy      1b. Rain in Past 24 hours (circle)?    Yes    No  
                                  Foggy      Drizzle      Rain  
 Amount in inches if known:

2. Odor (Write Station ID below)	Musty	Sewage	Chlorine	Petroleum	Decay (dead organisms)	Sulfide (rotten eggs)

3. Animals (Write Sta. ID below)	Gulls	Geese	Ducks	Raccoons	Rats	Other

4. Tide at time of sampling (circle)?    Not Tidal      High      Ebb      Slack      Low      Flood

5. Other Observations (i.e. foam present, oil present, trash)? Please note on back of chain of custody and clearly label station ID with observation.

<b>Chain of Custody Record</b>					
_____	_____	_____	_____	_____	_____
Bottles relinquished from Lab by	Time	Bottles received by	Time	Bottles received by	Time
_____	_____	_____	_____	_____	_____
Bottles received by	Time	Bottles received by	Time	Bottles received by	Time
_____	_____	_____	_____	_____	_____



**Chain of Custody Record**  
 51 Mill Pond Road  
 Hamden, CT 06514

**Date:**

Station ID	Station Name/Location & Field GPS Coordinates	Time Sampled

1a. Current Weather Conditions (circle): Clear Partly Cloudy 1b. Rain in Past 24 hours (circle)? Yes No  
 Mostly Cloudy Foggy Drizzle Rain  
 Amount in inches if known:

2. Odor (Write Station ID below)	Musty	Sewage	Chlorine	Petroleum	Decay (dead organisms)	Sulfide (rotten eggs)

3. Animals (Write Sta. ID below)	Gulls	Geese	Ducks	Racoons	Rats	Other

4. Tide at time of sampling (circle)? Not Tidal High Ebb Slack Low Flood

5. Other Observations (i.e. foam present, oil present, trash)? Please note on back of chain of custody and clearly label station ID with observation.

**Chain of Custody Record**

_____	_____	_____	_____	_____	_____
Bottles relinquished from Lab by	Time	Bottles received by	Time	Bottles received by	Time
_____	_____	_____	_____	_____	_____
Bottles received by	Time	Bottles received by	Time	Bottles received by	Time



Save the Sound  
 Pathogen Indicator Bacteria Monitoring Program  
 Laboratory Data Sheet

Date:		Parameter (circle): Enterococcus    E. coli    Total coliforms    Fecal coliform							
Site ID	Site Name	Sample Time	DF	Yellow Small Well	Yellow Large Well	Fluorescent Small Well	Fluorescent Large Well	MPN/100 mL	Qualifier (U, J, or R)



Southwest Conservation District  
 Pathogen Indicator Bacteria Monitoring Program  
 Laboratory Data Sheet

Date:		Parameter (circle): Enterococcus    E. coli    Total coliforms    Fecal coliform							
Site ID	Site Name	Sample Time	DF	Yellow Small Well	Yellow Large Well	Fluorescent Small Well	Fluorescent Large Well	MPN/100 mL	Qualifier (U, J, or R)



Southwest Conservation District  
 Pathogen Indicator Bacteria Monitoring Program  
 Laboratory Data Sheet

<b>Date:</b>					
Incubator Manufacturer: Serial Number:		Temp (°C) at <u>start</u> of incubation?  Temp (°C) at <u>end</u> of incubation?			Thermometer#:
Reagent/Supply	Manufacturer	Cat#	Lot #	Expiration Date	Notes
	IDEXX				
Quanti-Tray 2000	IDEXX	WQT2K			
Sample Bottle	IDEXX	WVI20PET-200			
Sterile DI Water 90 ml	Hardy Diagnostics	DO90			
Sterile DI Water 99 ml	Hardy Diagnostics	DO99			
Test Set up by (Date/Time)		Date/Time Test Startup by	Date & Time Incubated	Results Read by (Date/Time)	

# Standard Test Method for Enterococci in Water Using Enterolert

## POINT OF CONTACT

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PHONE: 914-381-3140 ext. 201

## OBJECTIVE

Detect the presence of *Enterococcus* bacteria, such as *E. faecium*, *E. faecalis*, in non-potable water samples using IDEXX's Enterolert, a patented Defined Substrate Technology (DST).

## OVERVIEW

The presence of enterococci in water samples is determined using IDEXX's Enterolert. Enterolert utilizes a nutrient indicator that fluoresces when metabolized by enterococci. Combined with the Quanti-Tray/2000, enterococci can be enumerated by counting the number of wells fluorescing after a 24-hour incubation period at  $41\pm 0.5^{\circ}\text{C}$ . The detection limit for this method is 1 Most Probable Number (MPN)/100 mL.

Sterilized sample vessels are used to collect a grab sample from non-potable water sources, such as rivers, lakes or bays. For most samples, a 1:10 sample dilution is prepared by diluting 10 mL of water sample with 90 mL of deionized water to increase detection range.

## SOURCES

U.S. EPA Reg 2 Laboratory. "Guidance Document for Citizen Science Pathogen Monitoring of Enterococci Using IDEXX Enterolert with Quanti-Tray 2000." Revision 5. Feb 2015.

ASTM 6503-99. Standard Test Method for Enterococci in Water Using Enterolert. Reapproved 2009.

## MATERIALS AND EQUIPMENT

### Lab Analysis

- Incubator
- Enclosed Chamber Bottle Thermometer



- 6-Watt Fluorescent UV Lamp
- UV Viewing Cabinet
- Quanti-Tray Sealer Plus
- Quanti-Tray/2000 Rubber Insert
- IDEXX Quanti-Tray/2000
- Pipette Controller
- 10 mL Serological Pipet
- Infrared Thermometer
- IDEXX Enterolert
- Sterilized Dilution Bottles
- Deionized Water
- Nitrile Gloves
- Disinfecting Spray
- IDEXX WQC Enterococci

## QUALITY CONTROL

### Initial Demonstration of Capability (iDOC)

An Initial Demonstration of Capability (iDOC) should be completed by any person processing and analyzing samples for *Enterococcus* to demonstrate proficiency in performing the method properly. A laboratory volunteer or intern may prepare samples if they are under the direct supervision of someone who has a valid iDOC on file.

The iDOC is conducted by analyzing four quantitative positive control samples. Using the four results calculate the mean percent recovery. True values are provided by IDEXX. An acceptable percent recovery should be 50-200% of the true value. If the iDOC does not meet acceptance criteria the analyst shall proceed accordingly:

- i. Locate and correct source of the problem
- ii. Repeat the iDOC

In addition to establishing the initial performance capabilities, laboratory blanks and quality control samples will be used to continually check on performance. All quality control records will be maintained by the laboratory.

### Field and Laboratory Blanks

#### Field Blank

The field blank will be used to assess contamination from field conditions during sampling. There should be no fluorescing after the incubation period to meet acceptable quality assurance criteria.

A minimum of one field blank per sampling day shall be prepared. To prepare a field blank, the sample collector will pour 99 mL of deionized water into a sterile sample vessel at the assigned sample field site. The field blank sample shall be stored and processed the same manner all other samples collected.

#### Laboratory Reagent Blank (LRB)

The LRB will be used to assess the aseptic techniques of the analyst processing the samples. There should be no fluorescing after the incubation period to meet acceptable quality assurance criteria.

For every batch of samples processed, a laboratory reagent blank shall be prepared by the analyst. To prepare the LRB, a packet of Enterolert is mixed with 99 mL of deionized water. The LRB will be processed along with the batch of samples.

#### Laboratory Sample Duplicate

The laboratory sample duplicate assesses the precision of the analytical method and the relative percent difference (RPD) between the results. An acceptable RPD should be <30%. If an RPD is >30% test procedures, techniques and reagents should be reviewed prior to the next batch of samples to be processed.

For every batch of samples processed, a laboratory sample duplicate shall be prepared by the analyst. Two aliquots of a sample are taken from the same container under laboratory conditions and processed and analyzed independently.

#### Laboratory Fortified Blank (LFB)

A laboratory fortified blank serves as a positive control and is a mixture of reagent-water sample and a known concentration of *Enterococcus faecalis*. The percent recovery should be between 50-200% of the true value.

The LFB should be prepared for every new box (lot number batch) of Enterolert.

#### Laboratory Negative Controls

In addition to preparing the LFB, two negative controls should be prepared (*S. aureus* & *E. coli*) to document the effect of non-*Enterococcus* bacteria with the Enterolert reagent. The negative controls should not show any fluorescence.

## INTERFERENCES

The presence of *Bacillus* spp. (a non-target organism) can interfere with the testing of marine water samples. To eliminate interference, a 1:10 dilution is required with sterile deionized or distilled water. High concentrations of *Enterococcus* may require further dilution of a sample (1% or 0.1%) to obtain a quantifiable result.

## METHODS

### Sample Storage

#### Storage

Samples are to be stored in temperatures  $<10^{\circ}\text{C}$  and measured using an infrared thermometer upon arrival to the laboratory. If the commute time between the lab and time of collection is short, evidence of icing is sufficient.

#### Holding Time

Samples should be returned to the laboratory within 6 hours of collection and placed in the incubator within 8 hours of collection.

### Laboratory Analysis Procedure

#### Sample Preparation

- a. Turn on the Quanti-Tray Plus sealer to warm it up. This takes approximately 10 minutes.
- b. Confirm the temperature of your incubator is  $40.5^{\circ}\text{C} - 41.5^{\circ}\text{C}$  (read certified incubator thermometer in chamber, do not rely solely on digital display on the incubator unit).
- c. Wipe down your workspace with disinfectant spray.
- d. Wash your hands and put on disposable nitrile gloves.
- e. Label a sterile deionized water sample bottle that contains 90 mL (test sample) of sterile deionized water bottles that corresponds to each sample collected.
- f. Label a Quanti-Tray/2000 to match each location at which a sample was collected. If not using labels, write only on the back of the trays with a sharpie permanent marker to keep from puncturing tray. The information on the back of the trays (or label) should include station ID, site, sample site and time placed inside incubator. **It is recommended that water proof labels be prepared in advance to expedite sample processing.**
- g. Remove samples from cooler and arrange on bench in order of collection time (start with the earliest time). Work with one sample at a time.
- h. Arrange your test sample bottles and sterile deionized dilution bottles in the order that you will prepare dilutions and analyze the samples. When handling sample bottles and sterile deionized water bottles, never touch the top of the bottle or inside the lid with your fingers after the lid has been removed. Only the outside of lid and the sides and bottom of the bottle can be touched.
- i. Gently invert the bottles containing the test samples 25 times to suspend anything that may have settled to the bottom of the sample before making dilutions.
- j. Sample Dilutions: After mixing approximately 25 times, open the lid of the sample container.
  - i. Tear open or peel back the protective sleeve from the top of the pipette (do not touch the tip with your fingers or have the tip come in contact with the bench top).

- ii. Insert the pipet into the pipette controller, then place the tip into the water sample (this should be done immediately after inverting your water sample approximately 25 times to ensure even distribution of the potential bacteria in the sample).
  - iii. Extract exactly 10 ml of the water sample into the pipette with the bottom of meniscus at 10 ml mark.
  - iv. Place the pipette inside of the top of the opened deionized water bottle and discharge the sample water into the sterile 90 mL mixing bottle of deionized water.
  - v. Close the lid on the deionized water with 10 mL of sample and remix 25 times. This is your 10% sample dilution.
  - vi. Dispose of pipette into a dedicated waste container and repeat above steps until all samples have been completed (remember to analyze to samples in the order of collection (early to later). Pipettes must only be used once and then disposed.
  - vii. As a reminder, all test samples are prepared in 90 mL of sterile deionized water while the LRB will be prepared in a 100 mL sterile deionized water container (99 mL nominal).
- k. Add one packet of Enterolert reagent to each sample bottle including the control and positive control. Hold the snap packs facing away from your face and pop open the top.
  - l. Snap the bottle lids back on and gently shake or invert in a gentle arc until all the powder dissolves and the bubbles disappear. The Enterolert reagent has a typical light-yellow color. Your sample is now ready for placement into the Quanti-Tray.

#### Quanti-Tray/2000 Preparation

- a. After mixing sample by inverting the bottle approximately 25 times, pour the reagent/sample mixture into its corresponding Quanti-Tray while avoiding contact with the foil tab.
- b. Use one hand to hold a Quanti-Tray upright with the well side facing the palm. Squeeze the upper part of the Quanti-Tray so that it bends towards the palm in a U-Shape. Gently pull the foil tab to separate the foil from the tray. Avoid touching the inside of the foil or tray.
- c. After adding sample, tap the end of the Quanti-Tray with the small wells closest to your bench top to release any air bubbles. Allow foam to settle for a few seconds.
- d. Place the sample-filled tray into the rubber insert of the Quanti-Tray Sealer with the plastic well side facing down. Check that the Green Light is lit on the sealer indicating that the proper temperature of the sealer is adequate.
- e. Place the rubber sealer form with the tray on top the inlet hopper of the sealer machine with the large reservoir cell placed the farthest away from the machine and the small cells entering the machine first.
- f. Gently feed the tray and holder into the machine gently. The sealing machine will automatically grab the rubber form and tray and draw them through the sealer.
- g. Retrieve the sealed tray on the other side of the machine (don't pull on it).
- h. Check to see that all plastic wells on the tray are filled with the sample. A well is

considered full if it is at least ½ full. Up to 2 wells can even be completely empty and the tray can still be used as the results will still be statistically valid.

- i. If there is a problem sealing the tray or there are more than 2 empty cells you will have to discard tray and prepare a new sample/reagent mixture from the reserved sample and pour into a new tray.
- j. Set aside your sealed Quanti-Trays and place all together into the incubator at 41°C. It is best to minimize the number of times you open the incubator door so as not to disturb the temperature setting.
- k. The trays can be stacked but should be spread out as much as possible in the incubator.
- l. Note on your data sheet the time you placed the trays into the incubator. They will stay there for a MINIMUM of 24 hours but no longer than 28 hours.
- m. Wash hands with anti-bacterial soap and wipe down the lab bench with disinfectant spray.
- n. Turn off the Quanti-Tray Sealer.

#### Sample Analysis (24-28 Hours Later)

- a. Take the Quanti-Trays out of the incubator 24 hours after they were put in (but no later than 28 hours). Turn UV light on and darken the room if possible. Enterolert results are definitive at 24–28 hours. In addition, positives observed before 24 hours and negatives observed after 28 hours are also valid
- b. Shine UV light on each tray or place in UV light holder box available from IDEXX. You are looking for wells fluorescing blue. Wells that are not blue or do not fluoresce ARE NOT counted as a positive result. It may help to look at your blank if you are having difficulty determining what is a positive result in some of your test samples.
- c. If any wells in the Blank are positive, then there was a contamination of your lab procedure or reagents. Blanks are considered contaminated if results are greater than 10 MPN/mL if associated sample concentrations are prepared as 10% solutions.
- d. Count large and small positive wells that:
  - i. Fluoresce under a long-wave ultraviolet light as enterococci.
  - ii. A permanent marker may be used to place a slash over each positive well to be used as a confirmation of initial counts under the UV light source.
  - iii. Off-color fluorescence is not counted as positive results.
  - iv. The large overflow well at the top of the tray is counted as a large well.
  - v. The Quanti-Tray 2000 has 49 large wells (**including the one large well at the top of the tray**) and 48 small wells on the opposite side of the tray. Record 2 numbers for each tray on the data sheet: # of Large Wells Positive and # of Small Wells positive on the data sheet.
- e. Refer to the MPN table to obtain results. For a 1:10 dilution, multiply the MPN table result by 10 to obtain the final MPN/100 mL.
- f. Record the results on the data sheet and record the LRB result as well. Record your name and the time and date that the tray results were read and recorded on the data sheet.
- g. Dispose of used trays in biohazard bags/containers and dispose of in accordance with local requirements. Remember to wash hands before and after and change disposable gloves often to minimize chances of cross-contamination.

## TROUBLESHOOTING / HINTS

- Collecting sediment in the sample should be avoided.
- Every attempt should be made to limit light exposure to the sample during field collection.

## DATA PROCESSING AND STORAGE

### Sample Data Handling

Data sheet should be evaluated for completeness and any deviations to the SOP should be identified and noted. Qualifiers will be used as a means to rapidly indicate confidence and usability of a data. Qualifiers are typically letter designations which are attached to individual data points to relay some additional information regarding the usability of the data.

#### a. “U” Qualifier

This qualifier is attached to a result to indicate that the organism was not detected at the prescribed reporting limit of the method. For *Enterococcus*, using Enterolert, the reporting limit for a 10% test sample is: “10 MPN/100 mL”. Therefore, if no wells fluoresce, then your result is not zero, but “10U”. Sample dilution will affect your reporting limit. If you dilute your sample to 1%, then the reporting limit would correspond with a change from 10 to 100 MPN/100 mL.

#### b. “R” Qualifier

An “R” qualifier stands for “Rejected” data. This letter designation will let the data user know that something occurred which renders the data non-usable. What types of things may require data to be rejected?

- Missed holding times – Methods are developed with maximum holding times. If exceeded, then the confidence in the data is not very good.
- Blank contamination greater than the reporting limit – If you have *Enterococcus* growing in your blank, then reassess your procedures and improve your aseptic technique before your next set of samples.

There may be others scenarios which may affect the quality of the data. These should be identified in your project QAPP.

#### c. “J” Qualifier

The “J” qualifier designates an “estimated value”. Unlike the “R” designation, a data with a “J” designation is considered valid and usable data. A “J” designation may be used for the following scenarios:

- There is blank contamination, but it is below the laboratory reporting limit.
- The positive control was positive but the percent recovery measured during the test was either above or below the QC acceptance limits for percent recovery.
- Incubator temperature was 41.6°C, or 0.1°C above the specified maximum

- range. Major deviations of incubator temperature may require an “R” designation.
- iv. Samples not stored on ice and/or temperature above 10.0°C. Extreme variations in temperature may require the data be rejected as well.
  - v. The Relative Percent Difference between the test sample and duplicate was greater than 30%.
  - vi. Other SOP deficiencies or method anomalies.

Any result above the reporting limit, with no QC or test anomalies, will not have any qualifier associated with it, but it will be reported with a “>” sign in front of the highest value.

The laboratory manager will be the custodian of the finalized data files and final sample analysis results.

Each sample collector is responsible for recording the field sample data (date, time and field conditions) and providing the data to the lab via a chain-of-custody at the time the sample is transferred over.

## Data Analysis

There are two types of results for most pathogen analyses when being compared to a Water Quality Standard (WQS): the “Single Maximum Concentration” and the “Geometric Mean”. The single maximum concentration is each individual result obtained during your study. Individual sample results can be compared to the criteria that you have established for your project. The national WQS (Water Quality Standard) for *Enterococcus* for marine bathing beaches is 104 CFU/100 mL (61 for freshwater samples). This is typically used as the criterion for single maximum concentrations as a not to exceed point in time measurement for primary contact.

Also, the WQS prescribe a longer-term depiction of bacterial contamination over a 30 day or monthly period. This is called a Geometric Mean. A minimum of 5 individual results are recommended to be used to calculate a Geometric Mean. The National Water Quality Standard for a minimum of 5 samples over a 30-day period is 35 MPN/100 mL for *Enterococcus* in saltwater and 33 MPN/100 mL in freshwater (1986 Water Quality Standards).

$$\text{Geometric Mean} = \text{nth root of } (X_1)(X_2)\dots(X_n)$$

The “nth” root is the number of total results used in your geometric mean calculation, times the multiple all of your test results together.

## REFERENCES

U.S. EPA Reg 2 Laboratory. "Guidance Document for Citizen Science Pathogen Monitoring of Enterococci Using IDEXX Enterolert with Quanti-Tray 2000." Revision 5. Feb 2015.

ASTM 6503-99. Standard Test Method for Enterococci in Water Using Enterolert. Reapproved 2009.

IDEXX. "Enterolert Test Kit" Instruction Insert.



# Standard Test Method for Total Coliforms and *E. coli* in Water Using Colilert-18

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## OBJECTIVE

Detect the presence of total coliforms and *E. coli* in non-potable water samples using IDEXX's Colilert-18, a patented Defined Substrate Technology (DST).

## OVERVIEW

The presence of total coliforms and *E. coli* in water samples is determined using IDEXX's Colilert-18. Colilert-18 utilizes a nutrient indicator that fluoresces when metabolized by *E. coli*. Combined with the Quanti-Tray/2000, *E. coli* bacteria can be enumerated by counting the number of wells fluorescing after an 18-hour incubation period at 35±0.5°C. For total coliforms the number of wells yellow in color under normal lighting is counted after the incubation period. The detection limit for this method is 1 Most Probable Number (MPN)/100 mL.

Sterilized sample vessels are used to collect a grab sample from non-potable water sources, such as rivers, lakes or bays. For most samples, a 1:10 sample dilution is prepared by diluting 10 mL of water sample with 90 mL of deionized water to increase detection range.

## SOURCES

U.S. EPA Reg 2 Laboratory. "Guidance Document for Citizen Science Pathogen Monitoring of Total Coliforms and *E. coli* Using IDEXX Colilert-18 with Quanti-Tray 2000." Revision 1.1. Feb 2015.

## MATERIALS AND EQUIPMENT

### Field Collection

- Sterilized 120 mL Sample Vessel
  - Containing sodium thiosulfate for neutralizing chlorine
- Nitrile Gloves
- Cooler filled with ice
- Sampling Extension Pole (optional)

- Permanent Marker

## Lab Analysis

- Incubator
- Enclosed Chamber Bottle Thermometer
- 6-Watt Fluorescent UV Lamp
- UV Viewing Cabinet
- Quanti-Tray Sealer Plus
- Quanti-Tray/2000 Rubber Insert
- IDEXX Quanti-Tray/2000
- Pipette Controller
- 10 mL Serological Pipet
- Infrared Thermometer
- IDEXX Colilert-18
- Sterilized Dilution Bottles
- Deionized Water
- Nitrile Gloves
- Disinfecting Spray
- IDEXX-QC Coliform and *E. coli* Kit

## QUALITY CONTROL

### Initial Demonstration of Capability (iDOC)

An Initial Demonstration of Capability (iDOC) should be completed by any person processing and analyzing samples for *E. coli* and total coliforms to demonstrate proficiency in performing the method properly. A laboratory volunteer or intern may prepare samples if they are under the direct supervision of someone who has a valid iDOC on file.

The iDOC is conducted by analyzing four quantitative positive control samples. Using the four results calculate the mean percent recovery. True values are provided by IDEXX. An acceptable percent recovery should be 50-200% of the true value. If the iDOC does not meet acceptance criteria the analyst shall proceed accordingly:

- iii. Locate and correct source of the problem
- iv. Repeat the iDOC

In addition to establishing the initial performance capabilities, laboratory blanks and quality control samples will be used to continually check on performance. All quality control records will be maintained by the laboratory.

## Field and Laboratory Blanks

### Field Blank

The field blank will be used to assess contamination from field conditions during sampling. There should be no fluorescing under UV light nor any yellow wells after the incubation period to meet acceptable quality assurance criteria.

A minimum of one field blank per sampling day shall be prepared. To prepare a field blank, the sample collector will pour 99 mL of deionized water into a sterile sample vessel at the assigned sample field site. The field blank sample shall be stored and processed the same manner all other samples collected.

### Laboratory Reagent Blank (LRB)

The LRB will be used to assess the aseptic techniques of the analyst processing the samples. There should be no fluorescing under UV light nor any yellow wells after the incubation period to meet acceptable quality assurance criteria.

For every batch of samples processed, a laboratory reagent blank shall be prepared by the analyst. To prepare the LRB, a packet of Colilert-18 is mixed with 99 mL of deionized water. The LRB will be processed along with the batch of samples.

## Laboratory Sample Duplicate

The laboratory sample duplicate assesses the precision of the analytical method and the relative percent difference (RPD) between the results. An acceptable RPD should be <30%. If an RPD is >30% test procedures, techniques and reagents should be reviewed prior to the next batch of samples to be processed.

For every batch of samples processed, a laboratory sample duplicate shall be prepared by the analyst. Two aliquots of a sample are taken from the same container under laboratory conditions and processed and analyzed independently.

## Laboratory Fortified Blank (LFB)

A laboratory fortified blank serves as a positive control and is a mixture of reagent-water sample and a known concentration of *E. coli*. The percent recovery should be between 50-200% of the true value.

The LFB should be prepared for every new box (lot number batch) of Colilert-18.

## Negative Controls

In addition to preparing the LFB, two negative controls should be prepared (*E. aerogenes* & *P. aeruginosa*) to document the effect of non-*E. coli* bacteria with the Colilert-18 reagent. The

negative controls should not show any fluorescence.

## INTERFERENCES

In samples with excessive chlorine, a flash may be observed when adding Coli-18 reagent. If this is seen, consider the sample invalid and discontinue testing.

## METHODS

### Sample Storage

#### Storage

Samples are to be stored in temperatures  $<10^{\circ}\text{C}$  and measured using an infrared thermometer upon arrival to the laboratory. If the commute time between the lab and time of collection is short, evidence of icing is sufficient.

#### Holding Time

Samples should be returned to the laboratory within 6 hours of collection and placed in the incubator within 8 hours of collection.

### Laboratory Analysis Procedure

#### Sample Preparation

- m. Turn on the Quanti-Tray Plus sealer to warm it up. This takes approximately 10 minutes.
- n. Confirm the temperature of your incubator is  $34.5^{\circ}\text{C} - 35.5^{\circ}\text{C}$  (read certified incubator thermometer in chamber, do not rely solely on digital display on the incubator unit).
- o. Wipe down your workspace with disinfectant spray.
- p. Wash your hands and put on disposable nitrile gloves.
- q. Label a sterile deionized water sample bottle that contains 90 mL (test sample) of sterile deionized water bottles that corresponds to each sample collected.
- r. Label a Quanti-Tray/2000 to match each location at which a sample was collected. If not using labels, write only on the back of the trays with a sharpie permanent marker to keep from puncturing tray. The information on the back of the trays (or label) should include station ID, site, sample time and time placed inside incubator. **It is recommended that water proof labels be prepared in advance to expedite sample processing.**
- s. Remove samples from cooler and arrange on bench in order of collection time (start with the earliest time). Work with one sample at a time.
- t. Arrange your test sample bottles and sterile deionized dilution bottles in the order that you will prepare dilutions and analyze the samples. When handling sample bottles and sterile deionized water bottles, never touch the top of the bottle or inside the lid with your fingers after the lid has been removed. Only the outside of lid and the sides and bottom of the bottle can be touched.

- u. Gently invert the bottles containing the test samples 25 times to suspend anything that may have settled to the bottom of the sample before making dilutions.
- v. Sample Dilutions: After mixing approximately 25 times, open the lid of the sample container.
  - i. Tear open or peel back the protective sleeve from the top of the pipette (do not touch the tip with your fingers or have the tip come in contact with the bench top).
  - ii. Insert the pipet into the pipette controller, then place the tip into the water sample (this should be done immediately after inverting your water sample approximately 25 times to ensure even distribution of the potential bacteria in the sample).
  - iii. Extract exactly 10 ml of the water sample into the pipette with the bottom of meniscus at 10 ml mark.
  - iv. Place the pipette inside of the top of the opened deionized water bottle and discharge the sample water into the sterile 90 mL mixing bottle of deionized water.
  - v. Close the lid on the deionized water with 10 mL of sample and remix 25 times. This is your 10% sample dilution.
  - vi. Dispose of pipette into a dedicated waste container and repeat above steps until all samples have been completed (remember to analyze to samples in the order of collection (early to later). Pipettes must only be used once and then disposed.
  - vii. As a reminder, all test samples are prepared in 90 mL of sterile deionized water while the LRB will be prepared in a 100 mL sterile deionized water container (99 mL nominal).
- w. Add one packet of Colilert-18 reagent to each sample bottle. Hold the snap packs facing away from your face and pop open the top.
- x. Snap the bottle lids back on and gently shake or invert in a gentle arc until all the powder dissolves and the bubbles disappear. The Colilert-18 reagent has a typical light-yellow color. Your sample is now ready for placement into the Quanti-Tray.

#### Quanti-Tray/2000 Preparation

- o. After mixing sample by inverting the bottle approximately 25 times, pour the reagent/sample mixture into its corresponding Quanti-Tray while avoiding contact with the foil tab.
- p. Use one hand to hold a Quanti-Tray upright with the well side facing the palm. Squeeze the upper part of the Quanti-Tray so that it bends towards the palm in a U-Shape. Gently pull the foil tab to separate the foil from the tray. Avoid touching the inside of the foil or tray.
- q. After adding sample, tap the end of the Quanti-Tray with the small wells closest to your bench top to release any air bubbles. Allow foam to settle for a few seconds.
- r. Place the sample-filled tray into the rubber insert of the Quanti-Tray Sealer with the plastic well side facing down. Check that the Green Light is lit on the sealer indicating that the proper temperature of the sealer is adequate.

- s. Place the rubber sealer form with the tray on top the inlet hopper of the sealer machine with the large reservoir cell placed the farthest away from the machine and the small cells entering the machine first.
- t. Gently feed the tray and holder into the machine gently. The sealing machine will automatically grab the rubber form and tray and draw them through the sealer.
- u. Retrieve the sealed tray on the other side of the machine (don't pull on it).
- v. Check to see that all plastic wells on the tray are filled with the sample. A well is considered full if it is at least  $\frac{1}{2}$  full. Up to 2 wells can even be completely empty and the tray can still be used as the results will still be statistically valid.
- w. If there is a problem sealing the tray or there are more than 2 empty cells you will have to discard tray and prepare a new sample/reagent mixture from the reserved sample and pour into a new tray.
- x. Set aside your sealed Quanti-Trays and place all together into the incubator at 35.0°C. It is best to minimize the number of times you open the incubator door so as not to disturb the temperature setting.
- y. The trays can be stacked but should be spread out as much as possible in the incubator.
- z. Note on your data sheet the time you placed the trays into the incubator. They will stay there for a MINIMUM of 18 hours but no longer than 22 hours.
- aa. Wash hands with anti-bacterial soap and wipe down the lab bench with disinfectant spray.
- bb. Turn off the Quanti-Tray Sealer.

#### Sample Analysis (18-22 Hours Later)

- h. Take the Quanti-Trays out of the incubator 18 hours after they were put in (but no later than 22 hours). Turn UV light on and darken the room if possible. Colilert-18 results are definitive at 18-22 hours. In addition, positives observed before 18 hours and negatives observed after 22 hours are also valid
- i. Shine UV light on each tray or place in UV light holder box available from IDEXX. You are looking for wells fluorescing. Wells that do not fluoresce ARE NOT counted as a positive result for *E. coli*. It may help to look at your blank if you are having difficulty determining what is a positive result in some of your test samples.
- j. If any wells in the LRB are positive, then there was a contamination of your lab procedure or reagents. Blanks are considered contaminated if results are greater than 10 MPN/mL if associated sample concentrations are prepared as 10% solutions.
- k. Count large and small positive wells that:
  - vi. Fluoresce under a long-wave ultraviolet light as positive for *E.coli*.
  - vii. A permanent marker may be used to place a slash over each positive well to be used as a confirmation of initial counts under the UV light source.
  - viii. Off-color fluorescence is not counted as positive results.
  - ix. The large overflow well at the top of the tray is counted as a large well.
  - x. The Quanti-Tray 2000 has 49 large wells (**including the one large well at the top of the tray**) and 48 small wells on the opposite side of the tray. Record 2 numbers for each tray on the data sheet: # of Large Wells Positive and # of Small Wells positive on the data sheet.

- l. After counting fluorescing wells, remove tray from UV light and count the # of large wells with a yellow color as well as the # of small wells yellow in color. The yellow color indicates a positive for total coliforms. Record the two numbers for each tray on the data sheet: # of Large Wells Positive and # of Small Wells positive on the data sheet.
- m. Refer to the MPN table to obtain the separate results for total coliforms and *E. coli*. For a 1:10 dilution, multiply the MPN table result by 10 to obtain the final MPN/100 mL.
- n. Record the results on the data sheet and record the LRB result as well. Record your name and the time and date that the tray results were read and recorded on the data sheet.
- o. Dispose of used trays in biohazard bags/containers and dispose of in accordance with local requirements. Remember to wash hands before and after and change disposable gloves often to minimize chances of cross-contamination.

## TROUBLESHOOTING / HINTS

- Collecting sediment in the sample should be avoided.
- Every attempt should be made to limit light exposure to the sample during field collection.

## DATA PROCESSING AND STORAGE

### Sample Data Handling

Data sheet should be evaluated for completeness and any deviations to the SOP should be identified and noted. Qualifiers will be used as a means to rapidly indicate confidence and usability of a data. Qualifiers are typically letter designations which are attached to individual data points to relay some additional information regarding the usability of the data.

#### d. “U” Qualifier

This qualifier is attached to a result to indicate that the organism was not detected at the prescribed reporting limit of the method. For total coliforms and *E. coli*, using Colilert-18, the reporting limit for a 10% test sample is: “10 MPN/100 mL”. Therefore, if no wells fluoresce (*E. coli*) or turn yellow (total coliforms), then your result is not zero, but “10U”. Sample dilution will affect your reporting limit. If you dilute your sample to 1%, then the reporting limit would correspond with a change from 10 to 100 MPN/100 mL.

#### e. “R” Qualifier

An “R” qualifier stands for “Rejected” data. This letter designation will let the data user know that something occurred which renders the data non-usable. What types of things may require data to be rejected?

- iii. Missed holding times – Methods are developed with maximum holding times. If exceeded, then the confidence in the data is not very good.
- iv. Blank contamination greater than the reporting limit – If you have *E. coli* growing in your blank, then reassess your procedures and improve your aseptic technique before your next set of samples.

There may be others scenarios which may affect the quality of the data.

**f. “J” Qualifier**

The “J” qualifier designates an “estimated value”. Unlike the “R” designation, a data with a “J” designation is considered valid and usable data. A “J” designation may be used for the following scenarios:

- vii. There is blank contamination, but it is below the laboratory reporting limit.
- viii. The positive control was positive but the percent recovery measured during the test was either above or below the QC acceptance limits for percent recovery.
- ix. Incubator temperature was 35.6°C, or 0.1°C above the specified maximum range. Major deviations of incubator temperature may require an “R” designation.
- x. Samples not stored on ice and/or temperature above 10.0°C. Extreme variations in temperature may require the data be rejected as well.
- xi. The Relative Percent Difference between the test sample and duplicate was greater than 30%.
- xii. Other SOP deficiencies or method anomalies.

Any result above the reporting limit, with no QC or test anomalies, will not have any qualifier associated with it, but it will be reported with a “>” sign in front of the highest value.

The laboratory manager will be the custodian of the finalized data files and final sample analysis results.

Each sample collector is responsible for recording the field sample data (date, time and field conditions) and providing the data to the lab via a chain-of-custody at the time the sample is transferred over.

## Data Analysis

There are two types of results for most pathogen analyses when being compared to a Water Quality Standard (WQS): the “Single Maximum Concentration” and the “Geometric Mean”. The single maximum concentration is each individual result obtained during your study. Individual sample results can be compared to the criteria that you have established for your project. The national WQS (Water Quality Standard) for *E. coli* in recreational waters (freshwater) is 235 CFU/100 mL. This is typically used as the criterion for single maximum concentrations as a not to exceed point in time measurement for primary contact.

Also, the WQS prescribe a longer-term depiction of bacterial contamination over a 30 day or monthly period. This is called a Geometric Mean. A minimum of 5 individual results are recommended to be used to calculate a Geometric Mean. The National Water Quality Standard for a minimum of 5 samples over a 30-day period is 126 MPN/100 mL for *E. coli* in freshwater (1986 Water Quality Standards).

$$\text{Geometric Mean} = \text{nth root of } (X_1)(X_2)\dots(X_n)$$

The “nth” root is the number of total results used in your geometric mean calculation, times the



multiple all of your test results together.

## REFERENCES

U.S. EPA Reg 2 Laboratory. "Guidance Document for Citizen Science Pathogen Monitoring of Total Coliforms and *E. coli* Using IDEXX Colilert-18 with Quanti-Tray 2000." Revision 1.1. Feb 2015.

IDEXX. "Colilert-18 Test Kit" Instruction Insert.

# Standard Test Method for Total Coliforms and Fecal Coliform in Water Using Colilert-18

## POINT OF CONTACT

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## OBJECTIVE

Detect the presence of Fecal coliforms in water samples using IDEXX's Colilert-18, a patented Defined Substrate Technology (DST).

## OVERVIEW

The presence of Fecal coliforms in water samples is determined using IDEXX's Colilert-18. Colilert-18 utilizes a nutrient indicator that changes it from colorless to yellow when metabolized by the fecal coliforms. Combined with the Quanti-Tray/2000, Fecal coliform bacteria can be enumerated by counting the number of yellow-colored wells after an 18-hour incubation period at  $44.5 \pm 0.2^\circ\text{C}$ . The detection limit for this method is 1 Most Probable Number (MPN)/100 mL.

Sterilized sample vessels are used to collect a grab sample from the water source. For most samples, a 1:10 sample dilution is prepared by diluting 10 mL of water sample with 90 mL of deionized water to increase detection range.

## SOURCES

U.S. EPA Reg 2 Laboratory. "Guidance Document for Citizen Science Pathogen Monitoring of Total Coliforms and Fecal coliform Using IDEXX Colilert-18 with Quanti-Tray 2000." Revision 1.1. Feb 2015.

## MATERIALS AND EQUIPMENT

### Lab Analysis

- Incubator
- Enclosed Chamber Bottle Thermometer
- 6-Watt Fluorescent UV Lamp
- UV Viewing Cabinet
- Quanti-Tray Sealer Plus

- Quanti-Tray/2000 Rubber Insert
- IDEXX Quanti-Tray/2000
- Pipette Controller
- 10 mL Serological Pipet
- Infrared Thermometer
- IDEXX Colilert-18
- Sterilized Dilution Bottles
- Deionized Water
- Nitrile Gloves
- Disinfecting Spray
- IDEXX-QC Fecal coliform Kit

## QUALITY CONTROL

### Initial Demonstration of Capability (iDOC)

An Initial Demonstration of Capability (iDOC) should be completed by any person processing and analyzing samples for Fecal coliforms to demonstrate proficiency in performing the method properly. A laboratory volunteer or intern may prepare samples if they are under the direct supervision of someone who has a valid iDOC on file.

The iDOC is conducted by analyzing four quantitative positive control samples. Using the four results calculate the mean percent recovery. True values are provided by IDEXX. An acceptable percent recovery should be 50-200% of the true value. If the iDOC does not meet acceptance criteria the analyst shall proceed accordingly:

- v. Locate and correct source of the problem
- vi. Repeat the iDOC

In addition to establishing the initial performance capabilities, laboratory blanks and quality control samples will be used to continually check on performance. All quality control records will be maintained by the laboratory.

### Field and Laboratory Blanks

#### Field Blank

The field blank will be used to assess contamination from field conditions during sampling. There should be no fluorescing under UV light nor any yellow wells after the incubation period to meet acceptable quality assurance criteria.

A minimum of one field blank per sampling day shall be prepared. To prepare a field blank, the sample collector will pour 99 mL of deionized water into a sterile sample vessel at the assigned sample field site. The field blank sample shall be stored and processed the same

manner all other samples collected.

#### Laboratory Reagent Blank (LRB)

The LRB will be used to assess the aseptic techniques of the analyst processing the samples. There should be no fluorescing under UV light nor any yellow wells after the incubation period to meet acceptable quality assurance criteria.

For every batch of samples processed, a laboratory reagent blank shall be prepared by the analyst. To prepare the LRB, a packet of Colilert-18 is mixed with 99 mL of deionized water. The LRB will be processed along with the batch of samples.

#### Laboratory Sample Duplicate

The laboratory sample duplicate assesses the precision of the analytical method and the relative percent difference (RPD) between the results. An acceptable RPD should be <30%. If an RPD is >30% test procedures, techniques and reagents should be reviewed prior to the next batch of samples to be processed.

For every batch of samples processed, a laboratory sample duplicate shall be prepared by the analyst. Two aliquots of a sample are taken from the same container under laboratory conditions and processed and analyzed independently.

#### Laboratory Fortified Blank (LFB)

A laboratory fortified blank serves as a positive control and is a mixture of reagent-water sample and a known concentration of *E. coli*. The percent recovery should be between 50-200% of the true value.

The LFB should be prepared for every new box (lot number batch) of Colilert-18.

#### Negative Controls

In addition to preparing the LFB, a negative control should be prepared (*P. aeruginosa*) to document the effect of non-Fecal coliform bacteria with the Colilert-18 reagent. The negative control should not show any fluorescence or yellow coloring.

## INTERFERENCES

In samples with excessive chlorine, a flash may be observed when adding Coli-18 reagent. If this is seen, consider the sample invalid and discontinue testing.

## METHODS

### Sample Storage

#### Storage

Samples are to be stored in temperatures  $<10^{\circ}\text{C}$  and measured using an infrared thermometer upon arrival to the laboratory. If the commute time between the lab and time of collection is short, evidence of icing is sufficient.

#### Holding Time

Samples should be returned to the laboratory within 6 hours of collection and placed in the incubator within 8 hours of collection.

### Laboratory Analysis Procedure

#### Sample Preparation

- y. Turn on the Quanti-Tray Plus sealer to warm it up. This takes approximately 10 minutes.
- z. Confirm the temperature of your incubator is  $44.3^{\circ}\text{C} - 44.7^{\circ}\text{C}$  (read certified incubator thermometer in chamber, do not rely solely on digital display on the incubator unit).
  - aa. Wipe down your workspace with disinfectant spray.
  - bb. Wash your hands and put on disposable nitrile gloves.
  - cc. Label a sterile deionized water sample bottle that contains 90 mL (test sample) of sterile deionized water bottles that corresponds to each sample collected.
  - dd. Label a Quanti-Tray/2000 to match each location at which a sample was collected. If not using labels, write only on the back of the trays with a sharpie permanent marker to keep from puncturing tray. The information on the back of the trays (or label) should include station ID, site, sample site and time placed inside incubator. **It is recommended that water proof labels be prepared in advance to expedite sample processing.**
  - ee. Remove samples from cooler and arrange on bench in order of collection time (start with the earliest time). Work with one sample at a time.
  - ff. Arrange your test sample bottles and sterile deionized dilution bottles in the order that you will prepare dilutions and analyze the samples. When handling sample bottles and sterile deionized water bottles, never touch the top of the bottle or inside the lid with your fingers after the lid has been removed. Only the outside of lid and the sides and bottom of the bottle can be touched.
  - gg. Gently invert the bottles containing the test samples 25 times to suspend anything that may have settled to the bottom of the sample before making dilutions.
  - hh. Sample Dilutions: After mixing approximately 25 times, open the lid of the sample container.
    - i. Tear open or peel back the protective sleeve from the top of the pipette (do not touch the tip with your fingers or have the tip come in contact with the bench top).

- ii. Insert the pipet into the pipette controller, then place the tip into the water sample (this should be done immediately after inverting your water sample approximately 25 times to ensure even distribution of the potential bacteria in the sample).
  - iii. Extract exactly 10 ml of the water sample into the pipette with the bottom of meniscus at 10 ml mark.
  - iv. Place the pipette inside of the top of the opened deionized water bottle and discharge the sample water into the sterile 90 mL mixing bottle of deionized water.
  - v. Close the lid on the deionized water with 10 mL of sample and remix 25 times. This is your 10% sample dilution.
  - vi. Dispose of pipette into a dedicated waste container and repeat above steps until all samples have been completed (remember to analyze to samples in the order of collection (early to later). Pipettes must only be used once and then disposed.
  - vii. As a reminder, all test samples are prepared in 90 mL of sterile deionized water while the LRB will be prepared in a 100 mL sterile deionized water container (99 mL nominal).
- ii. Add one packet of Colilert-18 reagent to each sample bottle. Hold the snap packs facing away from your face and pop open the top.
  - jj. Snap the bottle lids back on and gently shake or invert in a gentle arc until all the powder dissolves and the bubbles disappear. The Colilert-18 reagent has a typical light-yellow color. Your sample is now ready for placement into the Quanti-Tray.

#### Quanti-Tray/2000 Preparation

- cc. After mixing sample by inverting the bottle approximately 25 times, pour the reagent/sample mixture into its corresponding Quanti-Tray while avoiding contact with the foil tab.
- dd. Use one hand to hold a Quanti-Tray upright with the well side facing the palm. Squeeze the upper part of the Quanti-Tray so that it bends towards the palm in a U-Shape. Gently pull the foil tab to separate the foil from the tray. Avoid touching the inside of the foil or tray.
- ee. After adding sample, tap the end of the Quanti-Tray with the small wells closest to your bench top to release any air bubbles. Allow foam to settle for a few seconds.
- ff. Place the sample-filled tray into the rubber insert of the Quanti-Tray Sealer with the plastic well side facing down. Check that the Green Light is lit on the sealer indicating that the proper temperature of the sealer is adequate.
- gg. Place the rubber sealer form with the tray on top the inlet hopper of the sealer machine with the large reservoir cell placed the farthest away from the machine and the small cells entering the machine first.
- hh. Gently feed the tray and holder into the machine gently. The sealing machine will automatically grab the rubber form and tray and draw them through the sealer.
- ii. Retrieve the sealed tray on the other side of the machine (don't pull on it).
- jj. Check to see that all plastic wells on the tray are filled with the sample. A well is

- considered full if it is at least ½ full. Up to 2 wells can even be completely empty and the tray can still be used as the results will still be statistically valid.
- kk. If there is a problem sealing the tray or there are more than 2 empty cells you will have to discard tray and prepare a new sample/reagent mixture from the reserved sample and pour into a new tray.
  - ll. Set aside your sealed Quanti-Trays and place all together into the incubator at 44.5°C. It is best to minimize the number of times you open the incubator door so as not to disturb the temperature setting.
  - mm. The trays can be stacked but should be spread out as much as possible in the incubator.
  - nn. Note on your data sheet the time you placed the trays into the incubator. They will stay there for a MINIMUM of 18 hours but no longer than 22 hours.
  - oo. Wash hands with anti-bacterial soap and wipe down the lab bench with disinfectant spray.
  - pp. Turn off the Quanti-Tray Sealer.

#### Sample Analysis (18-22 Hours Later)

- p. Take the Quanti-Trays out of the incubator 18 hours after they were put in (but no later than 22 hours). Turn UV light on and darken the room if possible. Colilert-18 results are definitive at 18-22 hours. In addition, positives observed before 18 hours and negatives observed after 22 hours are also valid
- q. Wells that are yellow in color are counted as a positive result for Fecal coliform. It may help to look at your blank if you are having difficulty determining what is a positive result in some of your test samples.
- r. If any wells in the LRB are positive, then there was a contamination of your lab procedure or reagents. Blanks are considered contaminated if results are greater than 10 MPN/mL if associated sample concentrations are prepared as 10% solutions.
- s. Count large and small positive wells that:
  - xi. Turned yellow in color as positive for Fecal coliforms.
  - xii. A permanent marker may be used to place a slash over each positive well to be used as a confirmation of initial counts.
  - xiii. The large overflow well at the top of the tray is counted as a large well.
  - xiv. The Quanti-Tray 2000 has 49 large wells (**including the one large well at the top of the tray**) and 48 small wells on the opposite side of the tray. Record two numbers for each tray on the data sheet: # of Large Wells Positive and # of Small Wells positive on the data sheet.
- t. Refer to the MPN table to obtain the results for Fecal coliform. For a 1:10 dilution, multiply the MPN table result by 10 to obtain the final MPN/100 mL.
- u. Record the results on the data sheet and record the LRB result as well. Record your name and the time and date that the tray results were read and recorded on the data sheet.
- v. Dispose of used trays in biohazard bags/containers and dispose of in accordance with local requirements. Remember to wash hands before and after and change disposable gloves often to minimize chances of cross-contamination.

## TROUBLESHOOTING / HINTS

- Collecting sediment in the sample should be avoided.
- Every attempt should be made to limit light exposure to the sample during field collection.

## DATA PROCESSING AND STORAGE

### Sample Data Handling

Data sheet should be evaluated for completeness and any deviations to the SOP should be identified and noted. Qualifiers will be used as a means to rapidly indicate confidence and usability of a data. Qualifiers are typically letter designations which are attached to individual data points to relay some additional information regarding the usability of the data.

#### g. “U” Qualifier

This qualifier is attached to a result to indicate that the organism was not detected at the prescribed reporting limit of the method. For Fecal coliform, using Colilert-18, the reporting limit for a 10% test sample is: “10 MPN/100 mL”. Therefore, if no wells turn yellow, then your result is not zero, but “10U”. Sample dilution will affect your reporting limit. If you dilute your sample to 1%, then the reporting limit would correspond with a change from 10 to 100 MPN/100 mL.

#### h. “R” Qualifier

An “R” qualifier stands for “Rejected” data. This letter designation will let the data user know that something occurred which renders the data non-usable. What types of things may require data to be rejected?

- v. Missed holding times – Methods are developed with maximum holding times. If exceeded, then the confidence in the data is not very good.
- vi. Blank contamination greater than the reporting limit – If you have Fecal coliforms growing in your blank, then reassess your procedures and improve your aseptic technique before your next set of samples.

There may be others scenarios which may affect the quality of the data.

#### i. “J” Qualifier

The “J” qualifier designates an “estimated value”. Unlike the “R” designation, a data with a “J” designation is considered valid and usable data. A “J” designation may be used for the following scenarios:

- xiii. There is blank contamination, but it is below the laboratory reporting limit.
- xiv. The positive control was positive but the percent recovery measured during the test was either above or below the QC acceptance limits for percent recovery.
- xv. Incubator temperature was 44.8°C, or 0.1°C above the specified maximum range. Major deviations of incubator temperature may require an “R” designation.
- xvi. Samples not stored on ice and/or temperature above 10.0°C. Extreme



- variations in temperature may require the data be rejected as well.
- xvii. The Relative Percent Difference between the test sample and duplicate was greater than 30%.
  - xviii. Other SOP deficiencies or method anomalies.

Any result above the reporting limit, with no QC or test anomalies, will not have any qualifier associated with it, but it will be reported with a ">" sign in front of the highest value.

The laboratory manager will be the custodian of the finalized data files and final sample analysis results.

Each sample collector is responsible for recording the field sample data (date, time and field conditions) and providing the data to the lab via a chain-of-custody at the time the sample is transferred over.

## Data Analysis

There are two types of results for most pathogen analyses when being compared to a Water Quality Standard (WQS): the "Single Maximum Concentration" and the "Geometric Mean". The single maximum concentration is each individual result obtained during your study. Individual sample results can be compared to the criteria that you have established for your project.

Also, the WQS prescribe a longer-term depiction of bacterial contamination over a 30 day or monthly period. This is called a Geometric Mean. A minimum of 5 individual results are recommended to be used to calculate a Geometric Mean.

$$\text{Geometric Mean} = \text{nth root of } (X_1)(X_2)\dots(X_n)$$

The "nth" root is the number of total results used in your geometric mean calculation, times the multiple all of your test results together.

## REFERENCES

U.S. EPA Reg 2 Laboratory. "Guidance Document for Citizen Science Pathogen Monitoring of Total Coliforms and Fecal coliform Using IDEXX Colilert-18 with Quanti-Tray 2000." Revision 1.1. Feb 2015.

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