QUALITY ASSURANCE PROJECT PLAN

Little Narragansett Bay and Pawcatuck River Bacteria Sampling Plan

Rhode Island Department of Environmental Management

May 14, 2007

PROJECT MANAGER

Heidi E. Travers — 15 May 2007 (Date)

Heidi E Travers
RIDEM, Office of Water Resources
235 Promenade Street — Providence, RI 02908
Tel: 401.222.4700 ext. 7613 — heidi.travers@dem.ri.gov

QUALITY ASSURANCE OFFICER

Elizabeth Scott — 5/16/07 (Date)

Elizabeth Scott
RIDEM, Office of Water Resources
235 Promenade Street — Providence, RI 02908
Tel: 401.222.4700 ext. 7300 — elizabeth.scott@dem.ri.gov

EPA PROJECT OFFICER

5/23/07 (Date)

Steve Winnett
EPA New England Region 1, Office of Ecosystem Protection
1 Congress Street — Boston, MA 02114
Tel: 617.918.1687 — winnett.steven@epa.gov

EPA QA MANAGER

05-18-07 (Date)

Steve DiMattei, Quality Assurance Chemist
EPA New England Region 1, Office of Environmental Measurement and Evaluation
11 Technology Drive — North Chelmsford, MA 01863
Tel: 617.918.8369 — dimattei.steve@epa.gov

2.0 Table of Contents

List of Tables

List of Figures

3.0 Distribution List

Table 3.1 Distribution List.

QAPP Recipient	Organization	Telephone Number	Address
Heidi Travers	RIDEM	401.222.4700 ext. 7613	235 Promenade Street Providence, RI 02908 heidi.travers@dem.ri.gov
Elizabeth Scott	RIDEM	401.222.4700 ext. 7300	235 Promenade Street Providence, RI 02908 elizabeth.scott@dem.ri.gov
Steve DiMattei	EPA New England Region 1	617.918.8369	11 Technology Drive North Chelmsford, MA 01863 dimattei.steve@epa.gov
Steve Winnett	EPA New England Region 1	617.918.1687	1 Congress Street Boston, MA 02114 winnett.steven@epa.gov
Joe Migliore	RIDEM	401.222.4700 ext. 7258	235 Promenade Street Providence, RI 02908 joseph.migliore@dem.ri.gov
Kerry Patterson	HEALTH Laboratory	401.222.5588	50 Orms Street Providence, RI 02904 kerryp@doh.state.us

4.0 Project Organization

4.1 Project Organizational Chart

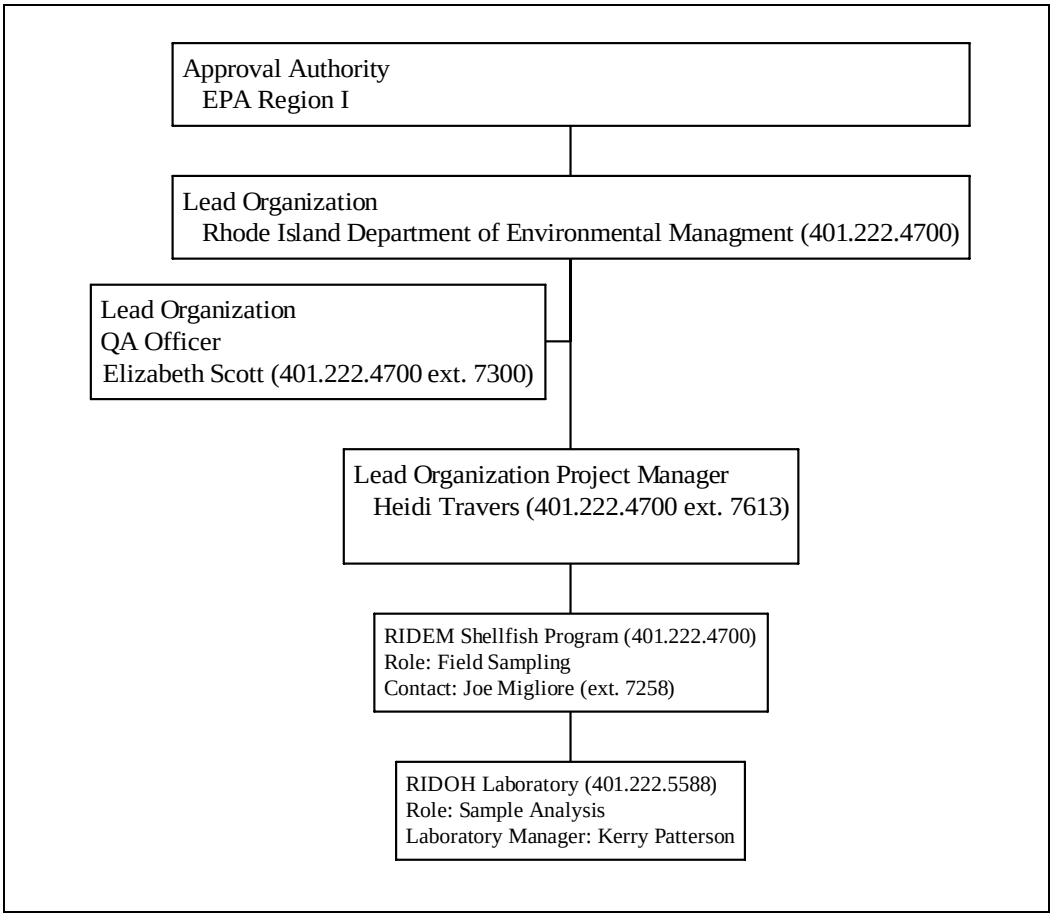


Figure 4.1 Project Organizational Chart.

4.2 Communication Pathways

The sampling plan details sample collection in the dry and wet weather in the estuarine waters of the Pawcatuck River and Little Narragansett Bay. Bacteria sources will also be sampled during wet weather. Sampling teams will be comprised of staff from the Rhode Island Department of Environmental Management (RIDEM). The Rhode Island Department of Health Laboratory (HEALTH) will analyze all samples for fecal coliform. Selected source samples will also be analyzed by HEALTH for male-specific bacteriophage.

The Project Manager will contact HEALTH to arrange for sample bottles prior to sampling. Enough sample bottles to begin a wet weather survey will be kept at RIDEM. When a potential storm is forecast, the Project Manager will alert the HEALTH to arrange for the pickup of additional bottles. The Project Manager will coordinate the arrangements with HEALTH.

Changes to the sampling plan may occur during the course of surveys. All changes made in the field by the field samplers will be documented in the field notes. The Project Manager will

discuss these changes with the field samplers within three days after sampling. It may become necessary to add and drop stations prior to sampling. The QA Officer and the Project Manager will make this decision jointly. All changes to the QA Plan will be reported in each event's Status Report and the Final Report.

4.3 Training

Each sampler will be given a copy of the Quality Assurance Plan that outlines the station locations and sampling protocol before sampling begins. Samplers will also be given a tour of their sampling locations before sampling begins. The Project Manager will detail the protocol for each station during the sampling station tour and distribute maps with station locations. All estuarine stations are well documented. Monitoring will be conducted by DEM staff who are familiar with bacteria sampling techniques and knowledgeable with the area.

The Total Maximum Daily Load (TMDL) Program keeps a list of all individuals trained. The Project Manager will document any additional training that occurs.

5.0 Problem Definition/Background

RIDEM is currently conducting a comprehensive water quality characterization of Little Narragansett Bay and the estuarine waters of the Pawcatuck River. During this characterization, RIDEM will organize all existing information and gather any additional information needed to develop a fecal coliform total maximum daily load report (TMDL) for the study area. Currently, the direct harvesting of shellfish is prohibited at all times in Little Narragansett Bay due to pollution closures. Water quality in the Pawcatuck River is not sufficient to allow shellfish harvesting in the adjacent waters of Little Narragansett Bay. The goal of this sampling program is to document instream water quality conditions in both wet and dry weather and to characterize the largest inputs into the bay before, during, and immediately after a storm event.

TMDLs are required under Section 303(d) of the Clean Water Act and USEPA's Water Quality Planning and Management Regulations (40 CFR Part 130). The goal of the TMDL study is to identify the existing pollution sources from nonpoint and point sources and to establish the impact that these loadings have on instream or inbay fecal coliform concentrations. At the completion of the study, the necessary TMDL reductions needed to achieve water quality standards will be established.

5.1 Study Area

The entire Pawcatuck River, including its freshwater reaches, is thirty-one miles long, flowing from its origin at the outlet of Worden's Pond in South Kingston, Rhode Island, to the inlet of Little Narragansett Bay in Westerly, Rhode Island. The Pawcatuck River basin drains an area approximately 302.4 square miles. Over eighty percent of the watershed is located in Rhode Island (Wild and Nimiroski, 2004).

The estuarine portion of the Pawcatuck River is considered to begin at the Route 1 highway bridge, at the junction of Main Street and Broad Street (RIDEM, 2006c). It forms the boundary between Westerly, Rhode Island and Stonington, Connecticut. It runs five miles, starting south of the Route 1 Bridge in Westerly and ending at Pawcatuck Point, where it flows into Little Narragansett Bay. This TMDL study focuses on the estuarine Pawcatuck River and Little Narragansett Bay. It will be referred to as the Pawcatuck River in this document. The freshwater reaches of the Pawcatuck River will be referred to as the freshwater Pawcatuck River. The study area is shown in Figure 5.1.

While two sub-watersheds drain into Little Narragansett Bay, only the Pawcatuck River sub-watershed will be sampled during this study. The second sub-watershed, the Wequetequock is located entirely in Connecticut and the portions of the estuary directly adjacent to Little Narragansett Bay meet water quality standards, indicating that problems in Little Narragansett Bay originate in the Pawcatuck River.

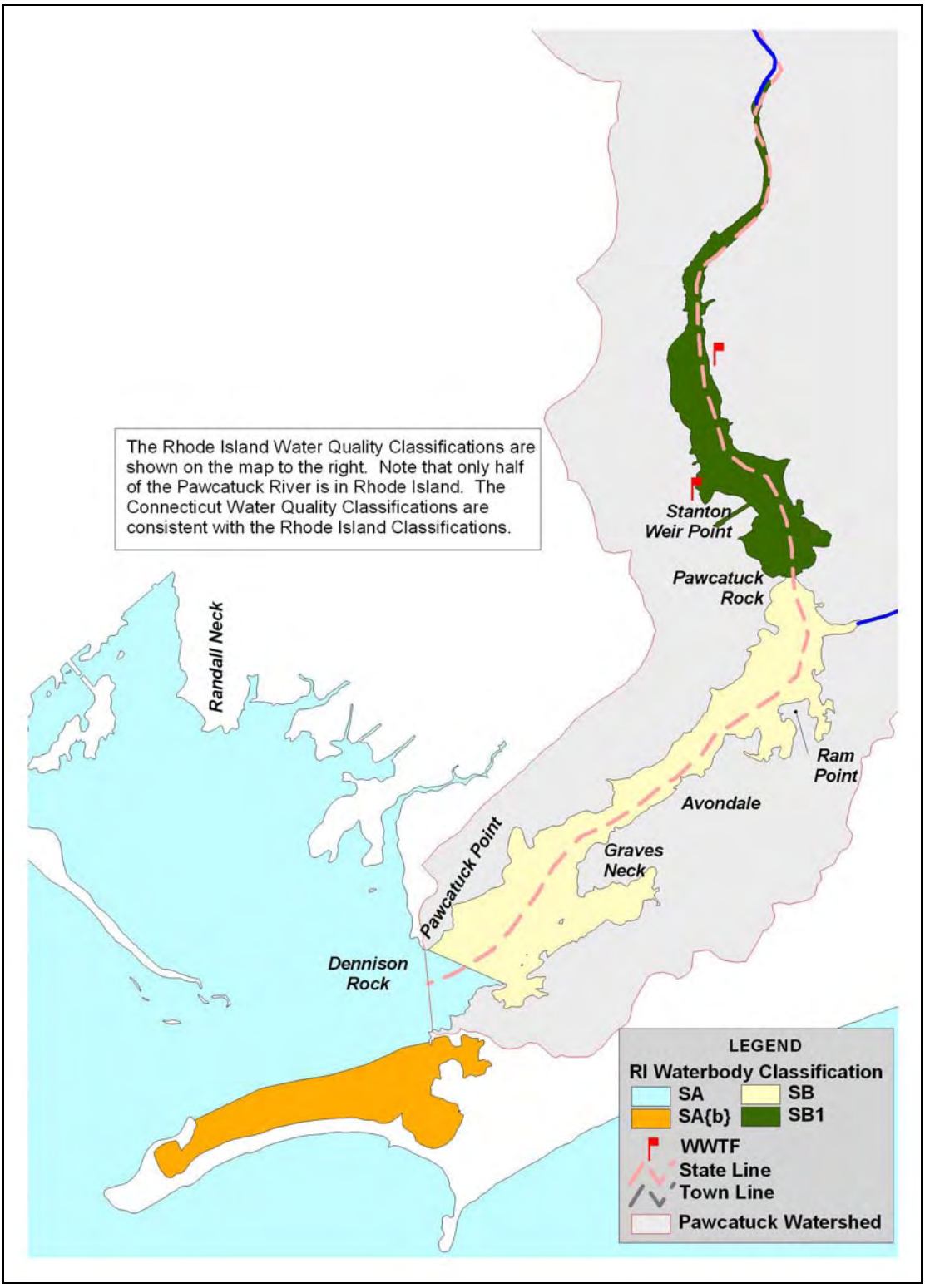


Figure 5.1 Little Narragansett Bay and the Pawcatuck River.

Table 5.1 Applicable Waterbodies in the Study Area (RIDEM, 2006a).

Waterbody Segment	Waterbody ID	RI Water Quality Classification	Description
Estuarine Pawcatuck River	RI0008038E-01A	SB1	Route 1 highway bridge to Pawcatuck Rock
	RI0008038E-01B	SB	Pawcatuck Rock to Pawcatuck Point
Little Narragansett Bay	RI0008038E-02A	SA	Little Narragansett Bay
	RI0008038E-02B	SA{b}	Watch Hill Cove and area north of Napatree

5.2 Water Quality History

A description of the Rhode Island shellfish closures in Little Narragansett Bay can be pieced together by analyzing the historic Rhode Island shellfish maps. Maps between 1946 and the present were analyzed. Since some years are missing, the exact closure dates may not be correct. In general, prior to 1966, harvesting shellfish was prohibited in the areas of Little Narragansett Bay now classified as Class SA{b} waters, but permitted in the remaining Rhode Island portions of Little Narragansett Bay. Harvesting shellfish was prohibited in all Rhode Island waters of Little Narragansett Bay between 1966 and 1981. Between 1981 and 1990, harvesting shellfish from the Class SA portions of Little Narragansett Bay was prohibited while seasonal closures existed in the Class SA{b} areas. The boundaries between the seasonal closure areas and the permanent closure areas appear to have changed between these times. Harvesting shellfish has been prohibited in all of the Rhode Island portions of Little Narragansett Bay since 1991 (RIDEM, 2002).

Connecticut has prohibited the direct harvesting of shellfish from the tidal Pawcatuck River and Little Narragansett Bay since 1948. In 1989, the Connecticut Shellfish Program opened Little Narragansett Bay and the lower tidal Pawcatuck River downstream of Pawcatuck Rock for the restricted relay of shellfish (depuration required in state certified waters) because of improved water quality and the results of dye studies conducted from the Westerly and Pawcatuck wastewater treatment facilities (Dillingham *et al.*, 1993).

During the summer of 2006, RIDEM, Connecticut Department of Environmental Protection (CTDEP), Connecticut Division of Agriculture / Bureau of Aquaculture (CT DA/BA), Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA) participated in a multi-agency study of the tidal Pawcatuck River and Little Narragansett Bay. The goal of the study was to characterize bacteria sources to the estuarine Pawcatuck River and to gauge their impact on the tidal Pawcatuck River and Little Narragansett Bay. Based on the results of this study, a monitoring plan has been developed to collect data needed to complete the TMDL study (RIDEM, 2006b).

A list of some applicable studies appears in Table 5.2. Section 14.0 discusses data acquisition requirements.

Table 5.2 Applicable Studies Conducted in the Pawcatuck River Watershed.

Primary Organization	Title	Date of Report	Approximate Date of Study
RIDEM	Summer 2006 Sampling Results**:** Pawcatuck River and Little Narragansett Bay Study	2006	Summer 2006
RIDEM	Shellfish Surface Water Monitoring Program	Ongoing	Ongoing
CT DA/BA	Shellfish Surface Water Monitoring Program	Ongoing	Ongoing
USGS			
Watershed Watch (University of Rhode Island)	Monitoring Data and Results	Ongoing	Ongoing

6.0 Project Description and Schedule

The requirements of the TMDL process help determine the scope of the Little Narragansett Bay and Pawcatuck River monitoring study. The goals of the sampling are to characterize water quality in Little Narragansett Bay and the estuarine Pawcatuck River in both wet and dry weather, confirm the water quality of the freshwater Pawcatuck River as it enters the estuarine river, and to sample a subset of pollution sources that were determined to have the greatest potential to impact receiving water quality in the study area.

Beginning in the spring of 2007, RIDEM will conduct a total of six dry and wet weather surveys of the Pawcatuck River and Little Narragansett Bay. Each of the four-planned dry weather surveys will consist of one sampling round, while each of the two-planned wet weather surveys will consist of multiple instream and pollution source sampling rounds as discussed below.

6.1 Tasks

The following tasks outline the steps needed to accomplish the objectives of the sampling program.

Task 1 Wet Weather Screening

The purpose of this wet weather screening to determine how long the study area is impacted by wet weather events. This information will used to determine how long the Pawcatuck River and Little Narragansett Bay needs to be sampled following a wet weather event. The limited wet weather data available for the Pawcatuck River shows that bacteria concentrations are highest at stations closest to the freshwater Pawcatuck River. Unfortunately, there is not enough information from the freshwater Pawcatuck River to determine the extent of wet weather impact (magnitude, timing, and length). The information collected from the freshwater Pawcatuck River will also be used target more-intensive wet weather source sampling from at this location.

Early in the sampling season (April or May), a short study will take place. Fecal coliform samples will be taken at three locations, one in the freshwater Pawcatuck River and two in the saltwater Pawcatuck River for several days following a significant rain event (at least one inch) that impacts the entire watershed area. Sampling will continue until bacteria concentrations reach pre-storm or dry weather concentrations.

These stations will be sampled by land. Samples will be sent to the HEALTH Laboratory. They will be analyzed using the MPN method.

Table 6.1 Analytical Services Table for Wet Weather Screening.

Medium/ Matrix	Analytical Parameter	Analytical Method/ SOP	No. of Sampling Locations[1]	No. of Field Duplicates	Total No. of Samples to Lab.	Data Package Turnaround	Laboratory Name
Surface Water	Fecal Coliform	MPN	15	5	20	10 Days	HEALTH

[1]Samples taken at separate times at the same location count as a separate sampling location/station.

Task 2 Instream Sampling

Instream sampling will take place in both dry and wet weather. At least four dry weather surveys will take place. Pre-storm samples taken prior to a wet weather survey will also be considered dry weather samples for TMDL analysis. Following a wet weather event of at least a half an inch of rain, the instream stations will be sampled four times. The timing of these samples is dependent of the results of Task 1 and on other environmental conditions. All shellfish program stations in the impaired waters will be sampled, except one station that is not accessible due to a sandbar. In addition, two stations that are located in waters that are not impaired will not be sampled. Stations that have been added include the freshwater Pawcatuck River and four saltwater stations. These stations have been added to investigate elevations in bacteria concentrations that became evident following the summer 2006 joint shoreline survey.

Instream stations will be sampled by boat. Samples will be sent to the HEALTH Laboratory. They will be analyzed using the MPN method.

Table 6.2 Analytical Services Table for Dry Weather Instream Sampling.

Medium/ Matrix	Analytical Parameter	Analytical Method/ SOP	No. of Sampling Locations[1]	No. of Field Duplicates	Total No. of Samples to Lab.	Data Package Turnaround	Laboratory Name
Surface Water	Fecal Coliform	MPN	76	8	84	10 Days	HEALTH

[1]Samples taken at separate times at the same location count as a separate sampling location/station.

Table 6.3 Analytical Services Table for Wet Weather Instream Sampling.

Medium/ Matrix	Analytical Parameter	Analytical Method/ SOP	No. of Sampling Locations[1]	No. of Field Duplicates	Total No. of Samples to Lab.	Data Package Turnaround	Laboratory Name
Surface Water	Fecal Coliform	MPN	190	20	210	10 Days	HEALTH

[1]Samples taken at separate times at the same location count as a separate sampling location/station. Total number of samples is two wet weather surveys.

Task 3 Source Sampling

Actual and potential pollution sources to the Pawcatuck River were identified during the joint 2006 shoreline survey. Thirty-six actual and potential pollution sources were identified along the Rhode Island shoreline and twenty actual and potential pollution sources were identified along the Connecticut Shoreline.

A subset of these sources was selected from the 2006 shoreline survey to be sampled during this study. Sources were chosen based on flow, size of outfall, fecal coliform concentration, and existing land use. The intent of sampling a subset of sources is to identify the major sources of fecal coliform and to assess the bacterial loading associated with different land uses.

All source stations will be sampled at two, six, and twelve hours after the beginning of the storm. Sources will only be sampled when they are flowing. Samples will be analyzed for fecal coliform, using the MPN method. Samples collected from areas with sewer lines will also be

analyzed for male-specific bacteriophage using the HEALTH methodology. The latter analysis serves as a screening tool to evaluate the presence of human sewage.

Table 6.3 Analytical Services Table for Wet Weather Source Sampling.

Medium/ Matrix	Analytical Parameter	Analytical Method/ SOP	No. of Sampling Locations[1]	No. of Field Duplicates	Total No. of Samples to Lab.	Data Package Turnaround	Laboratory Name
Surface Water	Fecal Coliform	MPN	72	8	80	10 Days	HEALTH
Surface Water	Male-Specific Bacteriophage	Attachment B	72	8	80	10 Days	HEALTH

[1]Samples taken at separate times at the same location count as a separate sampling location/station. Total number of samples is two wet weather surveys.

Task 4 Weather Criteria

Establishing criteria to determine when the area is experiencing dry versus wet weather is critical to the success of the monitoring study and the interpretation of the data. During this study, the area will be considered to be experiencing dry weather conditions when the area has received no rainfall within the last three days. In addition, it must be at least seven days since the area has received greater than 0.5 inches of rain.

One of the primary objectives when sampling the area during and directly following wet weather is to isolate the effect of a discrete wet weather event on instream and source conditions. During wet weather sampling, the instream impact of wet weather sources. The following rainfall criteria are proposed for this field program.

- Minimum rainfall of 0.5 inches in a 24-hr period
- Minimum duration of 5 hours
- Minimum antecedent dry period (ADP) of at least 3 days
- Minimum of three post-storm dry days
- Storm should cover a significant portion of the watershed based on model predictions.

All rainfall information will be measured at the National Weather Service's Westerly location, which is located within the watershed.

The Project Manager and the Quality Assurance Officer may need to adjust the dry and wet weather criteria based on actual weather conditions. Deviations from the plan will be noted in the sampling reports.

The Project Manager will keep track of atmospheric conditions and the development of potential storms. All potential samplers will be informed when conditions are favorable for a significant precipitation event to occur. Numerous numerical weather prediction models exist that display output on the Internet and forecast events 3 to 5 days in advance. The Project Manager is familiar with interpreting model output and will access them via the Internet to help target potential wet weather events. The following websites provide access to model output and discussion.

http://www.erh.noaa.gov/box/maps.shtml http://weather.unisys.com/ngm/
http://asp1.sbs.ohio-state.edu/ http://www.erh.noaa.gov/er/box/gfe/gridded.html

6.2 Project Schedule

Table 6.3 Project Schedule.

Task	Deliverable	2006			2007											
		O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Review Existing Data[1]	Monitoring Plan															
QAPP Preparation	QAPP Document															
Site Preparation	NA															
Sample Collection	NA															
Laboratory Analysis	Laboratory Report															
Final Data Report	Final Data Report															

[1]Section 14.0 of this report documents the existing data used to establish sampling stations and any data limitations.

7.0 Project Quality Objectives and Measurement Performance Criteria

Collecting high quality data is one of the most important goals of this project. Specific data quality objectives include method detection limits, precision, accuracy, representativeness, comparability, and completeness. All the data quality objectives will be met if the data collected are sufficient to complete the TMDL.

7.1 Measurement Performance Criteria

Representativeness

The selected stations and sampling frequency were chosen for their representativeness of conditions in the study area. The sampling frequency was chosen to complement the existing data set and to ensure that TMDL reductions are set using a data set that is representative of both wet and dry weather conditions. Monitoring stations were chosen based on pre-existing stations. Stations were added where additional information is needed. The extent to which the measurements represent actual environmental conditions will be somewhat restricted by the time of year the samples are taken and the overall weather conditions of that year (i.e. wet versus dry year).

Comparability

To maximize the quality of the data collected, and to collect data that is comparable with other studies, accepted sampling procedures will be used during this study. All samples collected will be sent to laboratories that use Standard Methods.

Sensitivity

Analytical methods were selected such that detection limits will not limit the usefulness of the data set.

Completeness

If the data collected is sufficient to complete the TMDL report, then the data is considered to be complete. Measurement performance criteria help determine the completeness of a data set. The measurement performance criteria for this project are documented in Tables 7.1 and 7.2 below.

Table 7.1 Fecal Coliform (MPN) Measurement Performance Criteria.

Sampling SOP	S-1
Medium/Matrix	Surface Water
Analytical Parameter	Fecal Coliform
Concentration Level	<1

Data Quality Indicator	**Analytical Method SOP Reference Laboratory**	**Measurement Performance Criteria**	**QC Sample and/or Activity Used to Assess Measurement Performance**	**QC Sample Assesses Error for Sampling (S), Analytical (A), or both (S/A)**
Precision	MPN Standard Method 9221A, B, E HEALTH	Within 95% Confidence Interval	Field Duplicates / Split	S/A
Accuracy / Bias Contamination	MPN Standard Method 9221A, B, E HEALTH	Positive Growth (>2)	Method Blank	A
Accuracy / Bias Contamination	MPN Standard Method 9221A, B, E HEALTH	No Growth	Reagent Blank	A
Data - Completeness	MPN Standard Method 9221A, B, E HEALTH		Anticipate 100%	A
Accuracy	MPN Standard Method 9221A, B, E HEALTH	Within 95% Confidence Interval	Field Duplicates / Split	S/A

Table 7.2 Male-Specific Bacteriophage Measurement Performance Criteria.

Sampling SOP	S-1
Medium/Matrix	Surface Water
Analytical Parameter	Male-Specific Bacteriophage
Concentration Level	<1

Data Quality Indicator	Analytical Method SOP Reference Laboratory	Measurement Performance Criteria	QC Sample and/or Activity Used to Assess Measurement Performance	QC Sample Assesses Error for Sampling (S), Analytical (A), or both (S/A)
Precision	MPN L-2 HEALTH	Within 95% Confidence Interval	Field Duplicates / Split	S/A
Accuracy / Bias Contamination	MPN L-2 HEALTH	Positive Growth (>2)	Method Blank	A
Accuracy / Bias Contamination	MPN L-2 HEALTH	No Growth	Reagent Blank	A
Data - Completeness	MPN L-2 HEALTH		Anticipate 100%	A
Accuracy	MPN L-2 HEALTH	Within 95% Confidence Interval	Field Duplicates / Split	S/A

8.0 Sampling Process Design

8.1 Sampling Design Rationale

Task 1 Wet Weather Screening

Task 1 outlined in Section 6.1 describes the process for deciding sampling stations. Stations were chosen based on the areas of the Pawcatuck River, which were assumed to be the most impacted by wet weather events. In addition, these stations were chosen based on their accessibility.

HEALTH Laboratory will use the MPN method to analyze all samples. Table 8.1 contains information about sampling and analytic methods for all the tasks.

Table 8.1 Sampling and Analysis Method/SOP Requirements.

Lab	Medium Matrix	Depth	Analytic Parameter	SOP		Container					Holding Time[1]
				Sampling	Analytical	No.	Size	Type	Requirements	Temp.	
HEALTH	Surface Water	6-12 inches	Fecal Coliform	S-1	L-1[2]	1	125 mL	Polyethylene	Ice	4°C	8 Hours
HEALTH	Surface Water	6-12 inches	Male-Specific Bacteriophage	S-1	L-2	1	500 mL	Polyethylene	Ice	4°C	8 Hours

[1]Samples may be held for up to twenty-four hours before being analyzed. Most samples will be delivered to the laboratory within eight hours.
[2]Standard Method 9221 A, B, E

Task 2 Instream Monitoring

Task 2 in Section 6.1 describes the process for deciding sampling sites within the Pawcatuck River and Little Narragansett Bay. All accessible Rhode Island Shellfish Program stations in impaired waters are sampled. Four additional stations were added to investigate elevations in bacteria concentrations. The freshwater Pawcatuck River will also be sampled. Figure A.2 and Tables A.3 and A.4 in Appendix A describe the exact location and monitoring protocol for each station. Dry and wet weather surveys are planned. A dry weather survey consists of one daily sample, while a wet weather survey consists of daily samples taken over a number of days. If possible, pre-storm samples will be taken. It is planned that four sampling rounds will occur following a rain event. The timing of these samples is dependent on the results of Task 1.

HEALTH Laboratory will use the MPN method to analyze all samples. Table 8.1 contains information about sampling and analysis methods.

Task 3 Source Sampling

Task 3 outlined in Section 6.1 describes the process for deciding sampling stations. Stations were chosen based on available information concerning flow, size of outfall, fecal coliform concentration, and existing land use. Figure A.3 and Tables A.5 and A.6 in Appendix A describe the exact location and monitoring protocol for each station. In general, sampling the fecal coliform sources will be intensive over the first six hours of the storm with a follow-up sample taken twelve hours after the storm begins.

HEALTH Laboratory will use the MPN method to analyze all samples. In addition, source samples taken in areas with sewage collections systems will be analyzed for male-specific bacteriophage. Table 8.1 contains information about sampling and analysis methods.

9.0 Sampling Procedures and Requirements

9.1 Sampling Procedures

Standard operating procedures for field sampling are located in Attachment A of this report.

Table 9.1 Project Sampling SOP Reference Table.

Reference Number /Title	Originating Organization	Equipment Identification	Modified for Work Project	Comments
Field Sampling SOP 1 (S-1) Fecal Coliform Sampling	RIDEM	Not Applicable	No	

9.2 Equipment Cleaning

The laboratory that completes the sample analysis will provide sterile bottles for bacteriological sampling.

9.3 Field Equipment Calibration and Maintenance

The Project Manager will ensure that all field equipment is operating properly. The only equipment needed for sampling is a 16-foot Jon Boat, typically maintained and operated by RIDEM Shellfish Program staff. Other equipment would typically include associated safety and maintenance gear, and sampling equipment.

10.0 Sample Handling, Tracking, and Custody Requirements

10.1 Field Notes

All sampling teams will be provided with either a field notebook or log sheet. A minimum entry includes the date, samplers name, station location, sample name and run (wet weather only), sample collection times, laboratory number, and any other information (tide, wind direction and speed, any source observations, etc.) that the team members may decide is significant.

At the completion of sampling, all log sheets and notebooks will be turned over to the Project Manager.

10.2 Sample Tracking

The proper identification of the sample is important. Before it is filled, the sample bottle must be labeled with the sample station id, sampler's initials, the date, and the time sampled. It is not necessary to write *GA12* on the sample bottles. (*GA12* indicates where in Rhode Island the sample was taken. All samples in this study are taken from *GA12*.) Additionally, during wet weather sampling, a number corresponding to the sample run should be inserted. For example, a sampler collecting the pre-storm sample at 0700 hrs at station GA17-3 would label the bottle "GA17-3-0700P". Replicate samples would have an "R" after the sample id. When taking the sample, the sampler should fill in the Sample ID on both the notebook and the sample bottle label.

Figure 10.2 depicts the Sample Bottle Label. The STATION, DATE, TIME, AND INITIALS fields should be filled in prior to sampling. The bottle should be labeled with permanent marker prior to taking the sample since it is difficult to write on wet sample bottles. Each sample bottle will also be given a laboratory number. This number must be written in the field notebook, on the sample bottle, and the chain of custody form. Note that samples taken in the vicinity of the wastewater treatment facility outfalls need to be collected in a THIO or thiosulfate bottle.

RI Department of Health Laboratory ( ) Legal
Sample #_____ Date/Time_____
Collection point_____
Collector_____ Program_____
Preservatives added:_____ THIO Sterilized on:
(X) By Lab

Figure 10.2 HEALTH Sample Bottle Label (The station, date, time, and initial fields should be labeled on the sample bottle).

Figure B.1 in Appendix B shows the chain of custody form for the HEALTH Laboratory. Before the samples are handed over to the laboratory, all fields must be filled in, especially the sample ID, laboratory id, and the time of sample collection field. The laboratory and RIDEM are given a copy of the completed chain of custody form. A 12 should be filled in after the initials GA and the last four columns of the chain of custody form should be marked in the following way:

Sample #	Collection Point:	# of tubes	dil.	thru	fecal col.
Laboratory #	GA 12 /STA.	*3*	*10*	*10^{-2}*	*3*

10.3 Sample Handling

All samples will be placed in a cooler with ice immediately after the sample is taken. The sample will be delivered to the laboratory within six hours. As long as some ice is still present in the cooler when it arrives at the lab, it will be assumed that the sample arrived to the lab at the correct temperature (4^{o} C).

A designee of the Project Manger will deliver the samples to the laboratory. Table 10.1 documents the sample handling system.

Table 10.1 Sample Handling System.

	Responsible Party	Samples
Sample Collection	RIDEM and designees	Source and Marine / Estuarine
Sample Delivery	RIDEM	Source and Marine / Estuarine
Sample Analysis	HEALTH Laboratory	Source and Marine / Estuarine
Sample Archival	None	Not Applicable
Sample Disposal	HEALTH Laboratory	Source and Marine / Estuarine

11.0 Field Analytical Method Requirements

During sampling, no field analyses will take place.

12.0 Fixed Laboratory Analytical Method Requirements

All samples taken from both marine and source waters will be taken to HEALTH Laboratory in Providence, Rhode Island. Estuarine samples will be analyzed for fecal coliform bacteria using the MPN method. Source samples will be analyzed for both fecal coliform bacteria using the MPN method and for male-specific bacteriophage using standard operating procedures detailed in Attachments B.1 and B.2. Table 12.1 provides the fixed laboratory analytical method / SOP reference table.

Table 12.1 Fixed Laboratory Analytical Method / SOP Reference Table.

Reference Number	Fixed Laboratory Performing Analysis	Title	Definitive or Screening Data	Analytical Parameter	Instrument	Modified for Work Project
L-1	HEALTH	MPN Method for Detection of Fecal Coliform	Definitive	Fecal Coliform	NA	N
L-2	HEALTH	Procedure for Determining Male-Specific Bacteriophage in Seawater, Wastewater and Shellfish Samples	Definitive	Male-Specific Bacteriophage	NA	N

13.0 Quality Control Requirements

Table 13.1 Field Sampling QC: Fecal Coliform and Male-Specific Bacteriophage.

Sampling SOP	S-1
Medium/ Matrix	Surface Water
Analytical Parameter	Fecal Coliform and Male-Specific Bacteriophage
Concentration Level	<1
Analytical Method/ SOP Reference	S-1

QC	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Person Responsible for Corrective Action	Data Quality Indicator	Measurement Performance Criteria
Field Duplicates[1]	Minimum 1 per 20 samples	L-1 Fecal Coliform L-2 Male-Specific Bacteriophage	Discuss any problems in the field with sampler.	Project Manager	Precision	Within 95% Confidence Interval

[1]Note QC procedures are the same for both fecal coliform and Male-Specific Bacteriophage.

Table 13.2 Fixed Laboratory Analytical QC: Fecal Coliform and Male-Specific Bacteriophage.

Sampling SOP	S-1
Medium/ Matrix	Surface Water
Analytical Parameter	Fecal Coliform and Male-Specific Bacteriophage
Concentration Level	<1
Analytical Method/ SOP Reference	Standard Method 9213D

QC	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Person Responsible for Corrective Action	Data Quality Indicator	Measurement Performance Criteria
Method Blank[1]	1 Per Batch	L-1 Fecal Coliform L-2 Male-Specific Bacteriophage	Reprepare Batch	Kerry Patterson	Bias - Contamination	Positive Growth (>2)
Reagent Blank[1]	1 Per Batch		Reprepare Batch	Kerry Patterson	Bias - Contamination	No Growth
Laboratory Duplicate[1]	1 per 10 samples		Reanalyze	Kerry Patterson	Precision - Lab	Within 95% Confidence Interval

[1]Note QC procedures are the same for both fecal coliform and Male-Specific Bacteriophage.

14.0 Data Acquisition Requirements

Shellfish Program sampling data from the Pawcatuck River and Little Narragansett Bay is limited since the area was permanently closed to shellfish harvesting in 1991. Over the last four years, the Shellfish Program has sampled the area more frequently in anticipation of a TMDL study. Shellfish Stations within Little Narragansett Bay and the Pawcatuck River have been sampled between four and eleven times since 2002. The stations in Little Narragansett Bay have been sampled fewer times because wind sometimes makes it difficult to reach these stations.

A joint shoreline survey was conducted along the Pawcatuck River in 2006. There is a very limited shoreline in Little Narragansett Bay. It was not surveyed because it is a sandy beach. Connecticut and Rhode Island data indicate that the elevated bacteria concentrations in Little Narragansett Bay can be traced to the Pawcatuck River. Fifty-six actual and potential bacteria sources were identified along the along Rhode Island and Connecticut shorelines with samples collected at sixteen of those sources. Follow-up samples were collected at five locations during the study, including four locations in Rhode Island. After the study, additional investigation was recommended for these five locations, along with two additional locations and a farm in Connecticut. One limitation to this data collection and source analysis investigations is that they were not conducted during wet weather conditions. No comprehensive wet weather dataset exists. It has become apparent that a few potential sources were missed during the 2006 Shoreline Survey. For example, Mastuxet Brook discharges to the Pawcatuck River downstream of Pawcatuck Rock. This tributary could not be seen from the water and was not identified and/or sampled. This tributary is currently being sampled by URI for DEM.

RIDEM will use rainfall information from the National Weather Service station in Westerly, located at the Westerly Airport. The airport is located within the watershed. Table 14.1 summarizes non-direct measurements used in setting up the Greenwich Bay wet weather study.

Table 14.1 Non-Direct Measurements Criteria and Limitations.

Non-Direct Measurement (Secondary Data)	Data Source	Data Generator	How Data Will Be Used	Limitations on Data Use
Rainfall	http://www.erh.noaa.gov/box/dailystns.shtml	National Weather Service	Quantify amount of rainfall received in watershed.	None
Bacteriological Monitoring	Shellfish Surface Water Monitoring Program	RIDEM	Evaluate bacteriological condition of growing area waters.	Limited Little Narragansett Bay Data.
	Shellfish Surface Water Monitoring Program	CT DA/BA	Evaluate bacteriological condition of growing area waters.	Data analyzed using different analytic techniques. Limited data from the Upper Pawcatuck River and the Rhode Island section of Little Narragansett Bay.
	Summer 2006 Sampling Results: Pawcatuck River and Little Narragansett Bay Study	CT DA/BA CT DEP EPA FDA RIDEM[1]	Rank bacteria sources and estimate their impact on estuarine water quality. Identify dry weather instream conditions that need further investigation.	No comprehensive source monitoring. Limited data from Little Narragansett Bay.

[1]Lead Organization.

15.0 Documentation, Records, and Data Management

All samplers will be given either a field notebook or log sheets. The monitoring plan given out when the each sampler collects his/her equipment includes specific information on what needs to be recorded on these sheets. All log sheets will be given to field leader at the conclusion of sampling. Initials on these sheets identify the sampler. The Project Manager will review the sheets within three days to identify any possible errors or omissions. The Project Manager will contact any sampler whose sheet shows any discrepancies. In addition, the Project Manager will try to contact all samplers to identify any problems or additional feedback that would make future sampling easier.

The Project Manager will designate a person to collect samples from the samplers during the storm. Each sampler will be responsible for filling out the chain of custody sheets (Figures B.1). When the samples are picked up from the samplers, the Project Manager or designee will check the chain of custody sheets. The samples and chain of custody sheets are also checked at the laboratory. A copy of the chain of custody form will be given to RIDEM when the samples are dropped off at the laboratory. After analysis is complete, sample results from the laboratory will be mailed to RIDEM.

After each sampling report, a brief Status Report will be written to document any changes to the Monitoring Plan. All information collected throughout the project will be summarized in the Final Data Report. Information included in the Final Data Report is described in Section 17.0. Table 15.1 lists records that will be generated throughout this project.

The Project Manager is responsible for the storage of all project files. RIDEM has a central filing system at its Providence Office where all original documents will be kept.

Table 15.1 Project Documentation and Records.

Sample Collection Records	Field Analysis Records	Fixed Laboratory Records	Data Assessment Records
Field Notes / Log Sheets	Field Notes / Log Sheets	Chain of Custody Records	Status Reports
Chain of Custody Records		Tabulated Data Summary Forms: draft and final	Final Data Report
Monitoring Plan			

16.0 Assessments and Response Actions

The Project Manager or designee will be responsible for each of the project tasks and their associated quality assurance and quality control procedures. The Project Manger will provide consistency between sampling events and sampling teams. Continual reports to the QA Officer about the status of sampling, quality assurance, and quality control will highlight any problems that are encountered during sampling. If needed, the QA Officer and Project Manager will halt sampling until problems are remedied.

Table 16.1 Project Assessment Table.

Assessment Type	Frequency	Internal or External	Person Responsible for Performing Assessment and Implementing Corrective Actions	Person Responsible for Monitoring the Effectiveness of the Corrective Action
Field Sampling Technical Systems Audit	Start of Sampling	I	Heidi Travers RIDEM	Elizabeth Scott RIDEM
HEALTH Laboratory Technical Systems Audit	Prior to Sample Receipt	E	Kerry Patterson HEALTH	Heidi Travers RIDEM

17.0 QA Management Reports

Table 17.1 lists the QA Management Reports that will be generated throughout this study.

As needed during this project, the Project Manager and the QA Officer will meet to discuss any issues related to sampling. These meetings will be verbal status reports. Problems encountered in the field will be discussed and any appropriate actions determined and implemented. Any changes and/or problems will be included in the final report.

After each sampling event, the Project Manager will generate a Status Report. This Status Report will be the written record of any changes to the QA Plan. If a station was not sampled, it will be documented here. Issues discussed during the Verbal Status Report can also be included.

At the completion of the study, the Project Manager will write a final report summarizing the three sampling events. Information in this final report will include the following information:

- Brief description of each sampling event
- Data tables of all data collected during the sampling event (including rainfall)
- Required Attachments
  - Status Reports

The Final Report may also include the Field Notes or sampling logs, chain of custody forms, and the laboratory data sheets provided by the labs. If this information is not included in the Final Report, it must be filed in the TMDL project files with the Final Report.

At the completion of the Final Report, the Quality Assurance Officer will complete the QA Review Form in Appendix C.

Table 17.1 QA Management Reports.

Type of Report	Frequency	Person(s) Responsible for Report Preparation	Report Recipient
Verbal Status Report	As needed	Heidi Travers RIDEM	Elizabeth Scott RIDEM
Written Status Report	After each wet weather survey	Heidi Travers RIDEM	Elizabeth Scott RIDEM
Final Report	Completion of sampling	Heidi Travers RIDEM	Elizabeth Scott RIDEM
QA Data Review Form	Completion of Final Report	Elizabeth Scott RIDEM	

18.0 Verification and Validation Requirements

Both the Project Manager and the QA Officer will review all data collected during this study to determine if the data meets QAPP Objectives. Decisions to qualify or reject data will be made by the Project Manager and QA Officer. Information from Tables 7.1, 7.2, 13.1, and 13.2 may be used during this process. All data collected will be included in the Final Report. To ensure correct interpretation of the data, all problems encountered in the field will be included in an Appendix to the report and discussed in the general text of the report. Problems will also be documented in each survey's written Status Report. To assist in data interpretation, statistical information on sampling events, including sampling size, sample mean, and sample variance, will be reported, where applicable. A discussion on duplicate precision and accuracy criteria and results will also be discussed in the Final Report.

19.0 Verification and Validation Procedures

All data collected during the wet weather studies will be included in the appendix of the report. Once the data has been collected, it will be entered into Microsoft Excel files. The Project Manager will proofread the data entry for errors. Errors will be corrected. Outliers and inconsistencies will be flagged for further review with the QA Officer. The decision to discard data will be made by the Project manager and QA Officer. Problems will be discussed in the Final Report. Table 19.1 discusses the data verification process.

Table 19.1 Data Verification Process.

Verification Task	Description	I/E	Responsible for Verification
Field Notes	Field notes will be collected at the end of each day and reviewed. Any required corrective actions will be addressed with the field samplers prior to further sampling. After the field notes have been entered into Excel, the data will be proofread for any data entry errors. Copies of the field notes will be maintained in the project file.	I	Heidi Travers RIDEM
Chain of Custody Forms	Chain of custody forms will be reviewed when samples are collected for delivery to the laboratory in the field and at the laboratory. The forms will be maintained in the project file.	I/E	Heidi Travers RIDEM Kerry Patterson HEALTH
Laboratory Data	All laboratory data packages will be verified internally by the laboratory performing the work for completeness prior to submittal. The data packages will also be reviewed by the sampling organization.	I/E	Heidi Travers RIDEM Kerry Patterson HEALTH

Data validation will utilize the measurement performance criteria documented in Tables 7.1, 7.2, 13.1, and 13.2 of this report.

20.0 Data Usability/Reconciliation with Project Quality Objectives

As soon as possible after each sampling event, calculations and determinations for precision, completeness, and accuracy will be made and corrective action implemented if needed. If data quality indicators meet those measurement performance criteria documented throughout this QA Plan, the project will be considered a success. If there are data that do not meet the measurement performance criteria established in this QA Plan, the data may be discarded and sampled again or the data may be used with stipulations written about its accuracy in the Final Report. The cause of the error will be evaluated. If the cause is equipment failure, calibration/maintenance techniques will be reassessed and improved. If the problem is sampling team error, retraining will occur. Any limitations with the data will be documented in the Status Reports and the Final Report.

References

Dillingham, Timothy P., Rush Abrams, Alan Desbonnet, and Jeffrey M. Willis. 1993. *The Pawcatuck River Estuary and Little Narragansett Bay: An Interstate Management Plan,* Rhode Island Coastal Resources Management Council, The Westerly RI, Connecticut Department of Environmental Protection, Stonington, CT.

Wild, Emily C. and Mark T. Nimiroski. 2004. *Estimated Water Use and Availability in the Pawcatuck Basin, Southern Rhode Island and Southeastern Connecticut*, Scientific Investigations Report 2004-5020, United Stated Geological Survey, Denver, CO.

RIDEM. 2002. *Historical Rhode Island Shellfish Closure Maps, 2001 Report,* Rhode Island Department of Environmental Management, Office of Water Resources, Providence, RI.

RIDEM. 2006a. *State of Rhode Island 2006 303(d) List: List of Impaired Waters, Final November 2006,* Rhode Island Department of Environmental Management, Office of Water Resources, Providence, RI.

RIDEM. 2006b. *Summer 2006 Sampling Results: Pawcatuck River and Little Narragansett Bay Study,* Draft Report Prepared by Rhode Island Department of Environmental Management, Office of Water Resources, Providence, RI.

RIDEM. 2006c. *Water Quality Regulations,* Rhode Island Department of Environmental Management, Office of Water Resources, Providence, RI.

Appendix A Sampling Station Information

Task 1 Pre-Wet Weather Screening

Table A.1 Monitoring Protocol and Rationale for Pre-Wet Weather Screening.

Station ID	WQ Class	Monitoring Protocol[1]					Rationale
		PS	12	24	48	72	
PR01	B	√	√	√	√	√	Determine impact (quantity and timing) of the freshwater Pawcatuck River during wet weather.
PR4	SB1	√	√	√	√	√	Determine wet weather impact (quantity and timing).
12-2B	SB1	√	√	√	√	√	Determine wet weather impact (quantity and timing).

[1]Monitoring protocol is dependent on the storm event being measured. Depending on factors such as storm intensity, storm duration, and daylight hours, the protocol may be varied to ensure that a representative set of samples is collected. Samples may be taken within six hours of the start of the storm. The remaining samples would be adjusted as deemed appropriate by the Project Manager and the QA Officer.

Table A.2 Pre-Wet Weather Screening Station Locations.

Station ID	Description
PR01	Freshwater Pawcatuck River
PR4	Upstream State Boat Ramp
12-2B	Westerly Yacht Club Dock

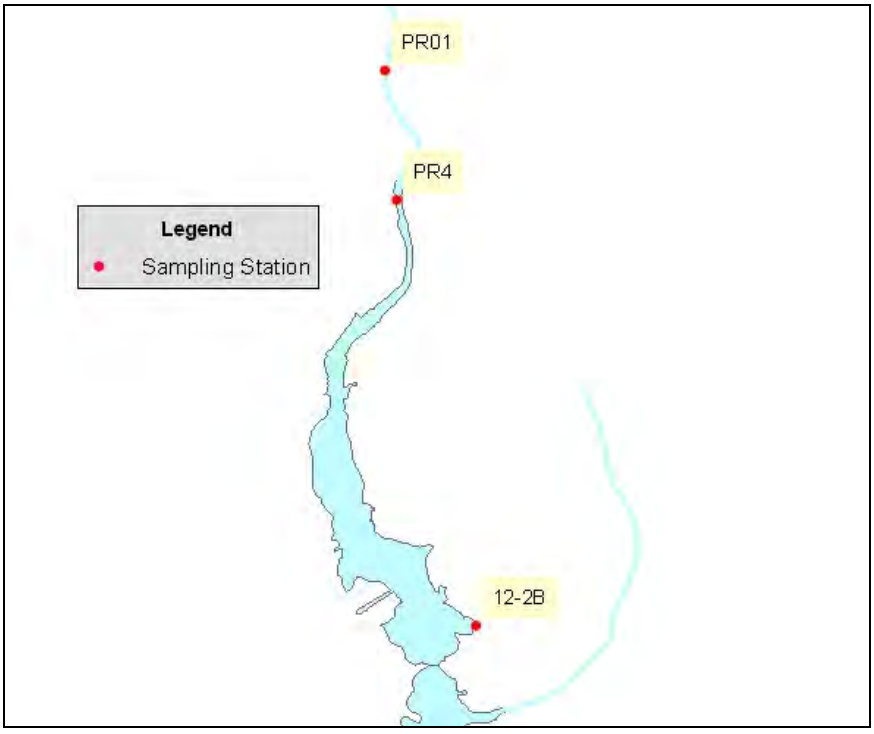


Figure A.1 Pre-Wet Weather Screening Station Locations.

Task 2 Instream Sampling

Table A.3 Monitoring Protocol and Rationale for Instream Sampling.

Station ID	WQ Class	Monitoring Protocol[1]					Rationale
		DW[2]	24	48	72	120	
PR01	B	√	√	√	√	√	Freshwater Pawcatuck River contribution.
PR4	SB1	√	√	√	√	√	Investigate potential sources between existing stations.
12-1	SB1	√	√	√	√	√	Pawcatuck River.
12-17	SB1	√[3]	√[3]	√[3]	√[3]	√[3]	Pawcatuck River.
17A	SB1	√	√	√	√	√	Investigate potential sources between existing stations.
12-2	SB1	√	√	√	√	√	Pawcatuck River.
19.6	SB1	√[3]	√[3]	√[3]	√[3]	√[3]	Pawcatuck River.
17B	SB1	√	√	√	√	√	Investigate potential sources between existing stations.
12-3	SB1	√	√	√	√	√	Pawcatuck River.
12-4	SB	√	√	√	√	√	Pawcatuck River.
12-5	SB	√	√	√	√	√	Pawcatuck River.
12-6	SB	√	√	√	√	√	Pawcatuck River.
12-7	SB	√	√	√	√	√	Pawcatuck River.
12-8	SB	√	√	√	√	√	Pawcatuck River.
12-9	SA	√	√	√	√	√	Little Narragansett Bay.
12-10	SA	√	√	√	√	√	Little Narragansett Bay.
12-15	SA{b}	√	√	√	√	√	Watch Hill Cove.
12-16	SA{b}	√	√	√	√	√	Watch Hill Cove.
12-11	SA	√	√	√	√	√	Little Narragansett Bay.

[1]While no more than four samples will be taken following a wet weather event; the timing of those samples depends on the results from the wet weather screening study and other environmental conditions. The first instream post-storm samples may be taken within hours of the end of the storm with the timing of remaining samples adjusted accordingly as deemed appropriate by the Project Manager and the QA Officer.

[2]Dry weather sampling may occur as a stand-alone sampling run or as a pre-storm sample taken prior to a wet weather event.

[3]Thiosulfate Bottle required at wastewater treatment facility outfall.

Table A.4 Instream Sampling Station Locations.

Station ID	Description
PR01	Freshwater Pawcatuck River
PR4	Upstream State Boat Ramp
12-1	Mid-channel opposite red brick building marked "1890 CB Cottrell + Sons", just north of rip-rap wall
12-17	From Westerly WWTF plume at outfall
17A	Duck Channel West of Major Island
12-2	At Nun Buoy #26
19.6	At Nun Buoy #20, close to Pawcatuck WWTF submerged outfall
17B	South of Gavitt Point, Near Shore
12-3	At Nun Buoy #20
12-4	At Nun Buoy #12
12-5	At Nun Buoy #8
12-6	At Can Buoy #7
12-7	At Nun Buoy #4
12-8	At Flashing Buoy #23, mouth of the Pawcatuck River
12-9	The intersection of a line from Nun Buoy #2 at Dennison Rock to Flashing Buoy #9, and a line from the southern tip of Barn Island to the southern tip of Sandy Pt.
12-10	At Nun Buoy #24 at Dennison Rock
12-14	Midway across the breached entrance to "The Kitchen" at Napatree Pt.
12-15	At the Flashing Buoy at the entrance channel to Watch Hill Cove.
12-16	In Watch Hill Cove, just off dock opposite Watch Hill Cove.
12-11	Midway between the southern extremity of Sandy Pt. and northeast extremely of Napatree Pt.
12-12	The intersection of a line from the western edge of Sandy Pt. to the western edge of Napatree Pt., and a line from the northeast end of the Bartlett Reef breakwater to Nun Buoy #2.
12-13	The intersection of a line from the northeast end of the Bartlett Reef breakwater to Bell Buoy #6 at Napatree Pt. Ledge, and a line from the light at the southeast end of the breakwater to the western edge of Napatree Pt; closest station to the Block Island Sound.

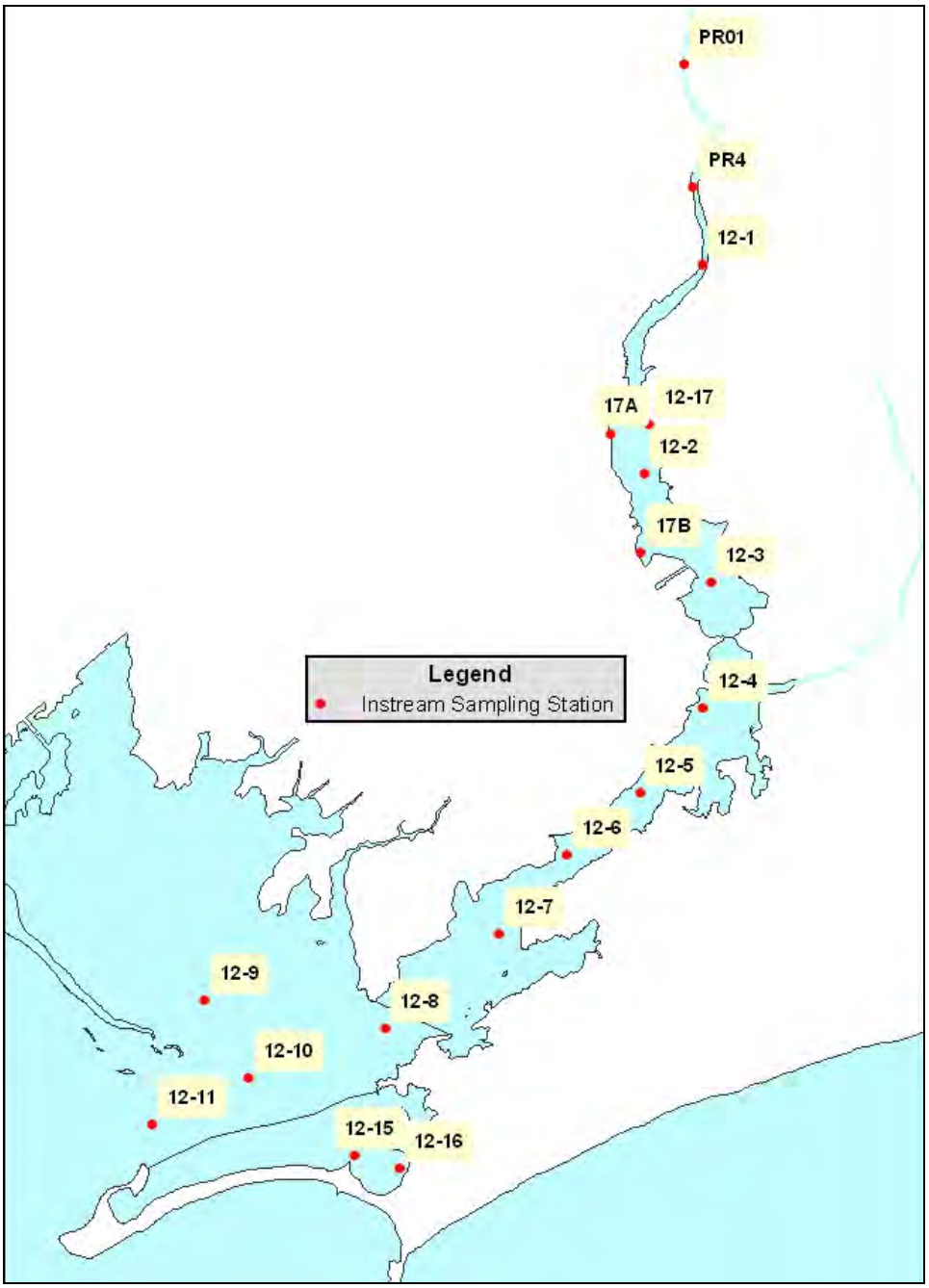


Figure A.2 Instream Station Locations.

Task 3 Source Sampling

Table A.3 Monitoring Protocol and Rationale for Source Sampling.

Station ID[1]	No. Storms[2]	Monitoring Protocol				Phage	Rationale
		PS	2	6	12		
PR01	Both	√	√	√	√	Y	Freshwater Pawcatuck River.
RI103	One	√	√	√	√	Y	High dry weather concentration. Access Problems.
RI122	One	√	√	√	√	Y	Target Area. Possible large drainage network.
RI121	One	√	√	√	√	Y	Target Area. Possible large drainage network.
RI119	One	√	√	√	√	Y	Potentially drains downtown. Still need to determine if it can be accessed.
RI101 RI102	Both	√	√	√	√	Y	High dry weather concentration. Sample one.
RI114	One	√	√	√	√	Y	Target Area. Possible large drainage network.
RI100	One	√	√	√	√	Y	High dry weather concentration. Access Problems.
MAS1	Both	√	√	√	√	Y	Mastuxet Brook. Largest tributary stream.
MAS2	Both	√	√	√	√	Y	Mastuxet Brook. Largest tributary stream.
RI701	Both	√	√	√	√	N	Watch Hill Cove. Still need to determine access.
RI700	Both	√	√	√	√	N	Watch Hill Cove. Still need to determine access.
CT106	One	√	√	√	√		Target Area. Possible large drainage network.
CT109	Both	√	√	√	√		Large storm drain. Winter flow. Priority Area. Extensive storm drain network.
CT112	One						Target Area.
CT403	Both	√	√	√	√		Priority Area. Submerged.

[1]Stations are still being selected. Priority has been given to those accessible storm drains in the target area.
[2]Approximately ten storm drains will be sampled per storm.

Table A.6 Source Sampling Station Locations.

Source ID	Description	Location	Latitude	Longitude
PR01	Freshwater Pawcatuck River			
RI103	30" Box Outfall	Under Main Street Bridge.	41.37765	71.83135
RI122	~24" Stone Sq, Submerged		41.37685	71.83195
RI121	~18" C	State Boat Ramp.	41.375283	71.832567
RI119	24" C		41.37325	71.8319
RI101	36" C Pipe (South-Right)		41.372883	71.8316
RI102	36" C Pipe (North-Left)		41.372883	71.8316
RI114	Box Shaped. 18" CMP		41.371833	71.831633
RI100	48" C Pipe	Margin Street.	41.369967	71.8318
MAS1	Mastuxet Brook	Between Airport Rd & Babcock Rd	82.369967	142.8318
MAS2	Mastuxet Brook	Before Outlet to Pawcatuck River		
RI701	12" CI	Watch Hill Cove	41.3119	71.857
RI700	12" CMP	Watch Hill Cove	41.3102	71.8585
CT106	56" C and rock slab culvert			
CT109	3' by 5' C Storm Drain		41.368193	71.836238
CT110	12" Black Plastic Pipe		41.365761	71.838596
CT112	24" C		41.364453	71.838529
CT403	3' - 4' CMP Mary Hall Road		41.352183	71.83765

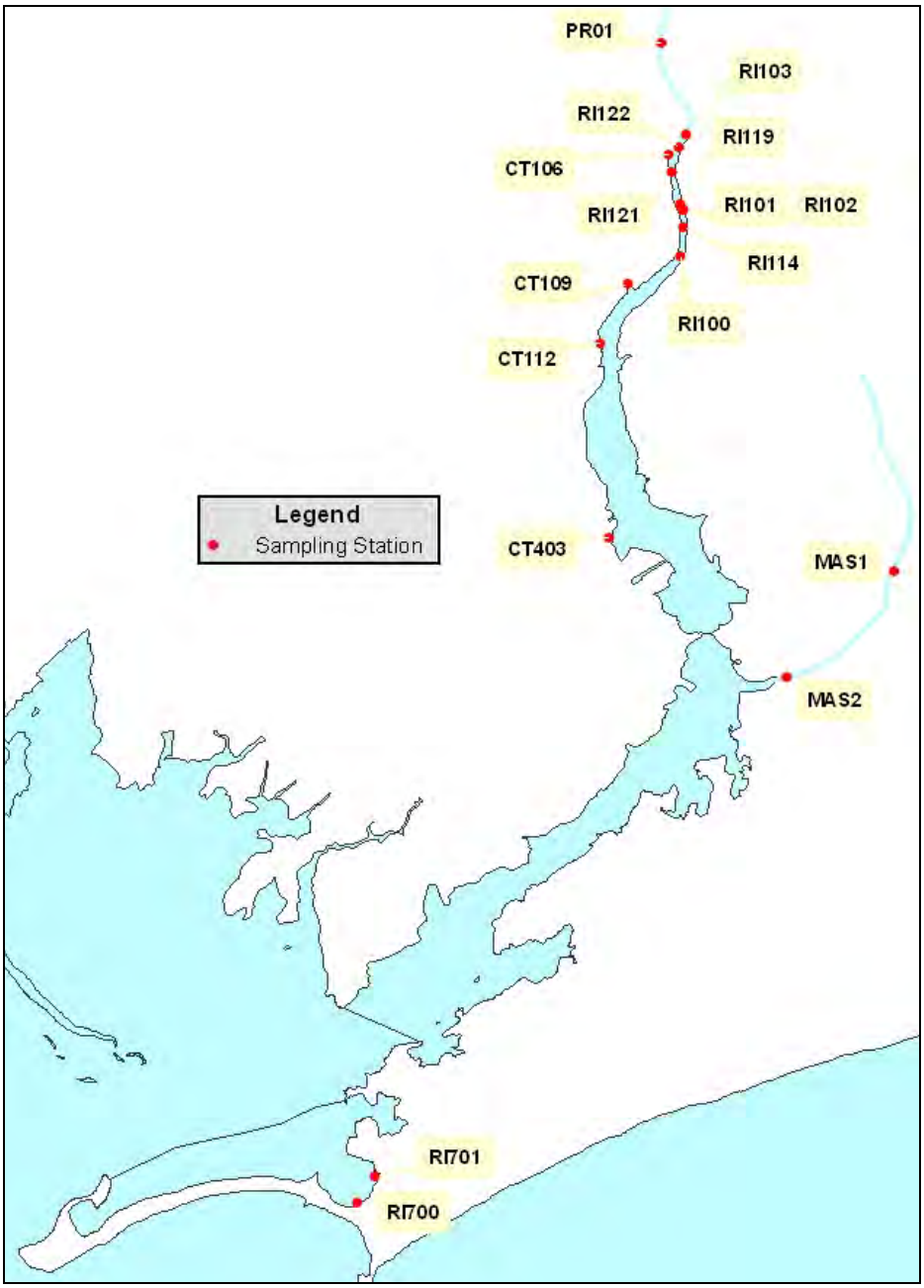
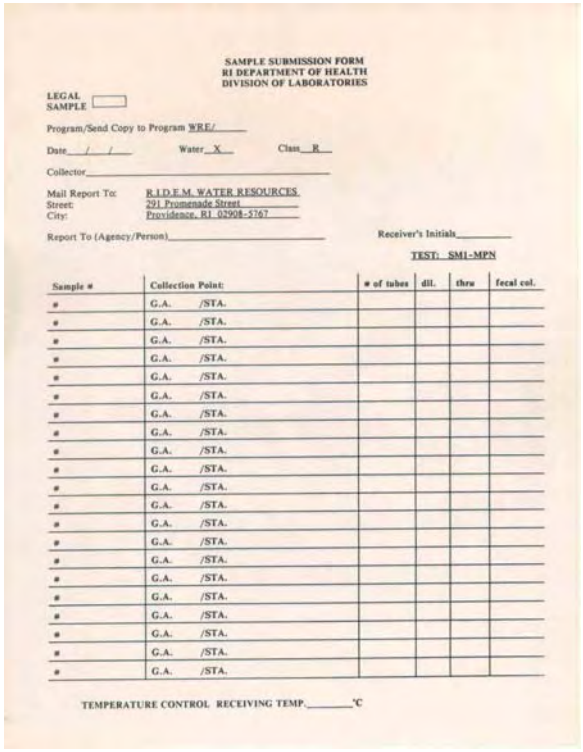


Figure A.3 Source Sampling Station Locations.

Appendix B Chain of Custody Form



SAMPLE SUBMISSION FORM
RI DEPARTMENT OF HEALTH
DIVISION OF LABORATORIES

LEGAL SAMPLE ☐

Program/Send Copy to Program WRE/_____

Date__/__/__ Water_X_ Class_B_

Collector_____

Mail Report To: R.I.D.E.M. WATER RESOURCES
Street: 291 Promenade Street
City: Providence, RI 02908-5767

Report To (Agency/Person)_____ Receiver's Initials_____

TEST: SM1-MPN

Sample #	Collection Point:	# of tubes	dil.	thru	fecal col.
#	G.A. /STA.				
#	G.A. /STA.				
#	G.A. /STA.				
#	G.A. /STA.				
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TEMPERATURE CONTROL RECEIVING TEMP._____°C

Figure B.1 Department of Health Chain of Custody Form.

Appendix C QA Review Form

QA REVIEW FORM

Reviewer _____(Project Quality Assurance Officer)

Date of Review _____

Title of Data Report _____

Date of Report _____

Author _____

Monitoring Period _____

Parameters _____

Approved QAPP	☐ Yes	☐ No	☐ Other	_____
Acceptable Laboratory	☐ Yes	☐ No	☐ Other	_____
Data Verification and Validation Procedures Followed	☐ Yes	☐ No	☐ Other	_____
Final Report Contains:				
Status Reports	☐ Yes	☐ No	☐ Other	_____
a. Information from Field Notes	☐ Yes	☐ No	☐ Other	_____
b. Written Record of Deviations from QAPP	☐ Yes	☐ No	☐ Other	_____
Final Data Report	☐ Yes	☐ No	☐ Other	_____
a. Measurement Performance Criteria and Quality Control Objectives	☐ Yes	☐ No	☐ Other	_____
b. Description of Each Sampling Event	☐ Yes	☐ No	☐ Other	_____
c. Data Tables	☐ Yes	☐ No	☐ Other	_____
d. Status Reports (as Attachment)	☐ Yes	☐ No	☐ Other	_____
Document Storage	☐ Yes	☐ No	☐ Other	_____
a. Chain of Custody Forms	☐ Yes	☐ No	☐ Other	_____
b. Laboratory Results	☐ Yes	☐ No	☐ Other	_____
c. Field Notes	☐ Yes	☐ No	☐ Other	_____

General Comments _____

Suggestions / Request for Additional Information _____

_____ _____

Signed **Date**
Quality Assurance Officer

Attachment A Field Sampling Standard Operating Procedures (SOP)

Field Sampling SOP 1 (S-1): Fecal Coliform Sampling

1. The laboratory-provided autoclaved sample bottles will be distributed to each team.

2. The following information is on the sample bottle label. The fields that should be filled in prior to sampling are in bold below. Label the bottle before taking the sample. It is difficult to write on wet sample bottles. The Station field should be composed of the Station ID followed by the hour when the sample was taken. A sample taken at hour 4 at Station HB07 would become HB07-4.

   Sample Bottle Label

Station:		
Depth:	Temp:	**Date:**
Initials	Salin.	**Time:**
Sample Type:		
Remarks:		

   The fields in **bold**, the station, date, time, and initial fields should be labeled on the sample bottle.

3. If you are using a sample stick, place the bottle in the stick.

4. When you are ready to take the sample, take the cap off the sample bottle. Hold the cap in one hand and the bottle (in the sample stick) in your other hand. Do not touch the inside of the bottle or cap. Do not put the cap on the ground.

5. Avoid contaminating the samples by not allowing the sample water to come in contact with anything before it is placed in the bottle. Be careful not to bring the rim or cap of the sample bottle into contact with anything. If possible, samples will be taken with a sample stick to avoid causing upstream disturbance prior to and during sampling.

6. Holding the bottle upside down, push the bottle through the water to mid-depth or as far as you can reach. Turn the bottle forward and scoop it forward and up and out of the water. Do this in one sweeping motion. Make sure you sample forward and away from you so that there is no chance that you will contaminate the sample with bacteria from your arm.

7. Pour off water to the neck of the bottle. Water should reach to within an inch to an inch and half of the top of the sample bottle. This provides space for mixing.

8. Cap the bottle tightly. Place the bottle upright in a cooler with ice to maintain a temperature of 4°C.

9. Be sure to record the time that the sample was taken in the sample log.

10. All bacteria samples (including field duplicates) should be taken using the steps discussed in Step 1 through Step 9.

Attachment B Fixed Laboratory Methods and Standard Operating Procedures

Fixed Laboratory Method 1 (L-1) MPN Method for Detection of Fecal Coliform Bacteria

HEALTH Laboratory

1. **Title:** Multiple Tube Fermentation Technique for the Members of the Total Coliform and Fecal Coliform Group

2. **References:** Standard Methods for the Examination of Water and Wastewater, 19th Edition. Section 9221 A, B, E pp.9-44-9-47, 9-51

3. **Scope and Principle of the Analysis:**
   3.1. The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. The historical definition of this group has been based on the method of detection, which is lactose fermentation, rather than on the tenets of systematic bacteriology. When the fermentation technique is used the coliform group is defined as all facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48h at 35°C. The fecal coliform test is applied to determine which total coliforms are also fecal coliforms
   3.2. When the Multiple Tube Fermentation Technique is used the results of the examination of replicate tubes and dilutions are reported in terms of Most Probable Number of organisms present. This number, based statistical probability formulas, is an estimation of mean bacterial density in the sample. This technique is used to determine the estimated bacterial density of non-potable samples and that the quality of potable water meets U.S E.P.A standards.
   3.3. Water of drinking water quality, non-potable water and brackish or salt water as well as mud, sludge and sediment samples can be analyzed by this technique
   3.4. This Standard Operating Procedure varies slightly depending of the matrix. The following procedure can be applied when testing non-potable water and brackish or salt water as well as mud, sludge and sediment samples.

4. **Interferences:** Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts. All reagents and apparatus must be routinely demonstrated to be free from significant interferences under the conditions of the analysis by:
   4.1. Sample bottles are autoclaved prior to sample collection. The bottles are checked for sterility before they are allowed out in the field.
   4.2. Glassware must by scrupulously cleaned and instructions for this cleaning should be described and performed as in Section 6.
   4.3. Only reagent-grade water that meets EPA/FDA criteria is used to prepare media and reagents. See table below for microbiological suitable water specifications.

Test for the Following Parameters	Limits	Frequency
Conductivity	>0.5 Megohms (resistivity) or <2 microsemiens	Monthly
Metals: Pb, Cd, Cr, Cu, Ni, Zn	Not Greater Than 0.5 mg/L per Contaminant	Annually
Total Chlorine Residue	<0.1ppm	Monthly
Heterotrophic Plate Count (HPC)	<500 CFU/mL	Monthly
pH	6.0-7.5	As Used
Bacteriological Quality of Rinse Water	Ratio 0.8-3.0	Annually

   4.4. Inadequate mixing of the sample will result in an underestimation of the actual bacterial density.
   4.5. Analysts need to wash hands thoroughly and wipe down the bench with disinfectant before analysis.
   4.6. Valid test results require strict adherence to all Quality control procedures.

5. **Safety Issues:**
   5.1. All media is purchased as a dehydrated powder. Analyst should wear gloves and a mask to avoid breathing the dust when weighing and mixing.

5.2. MSDS's for all media components/reagents used in the laboratory are located on a shelf at the end of aisle 5 in a black three ring binder.

6. **Apparatus and Equipment:**

6.1. Sample Bottles: Sample collection bottles should be wide-mouthed plastic (or non-corrosive glass-used only for mail-ins) and have a minimum capacity of 125mL and are able to withstand repeated autoclaving. Sample collecting containers- (sodium thiosulfate added prior to sterilization if needed), sterilize by autoclave for 30 min at 121°C. Sterility, detergent residue and the effectiveness of sodium thiosulfate are checked for each batch of bottles that are autoclaved, results are recorded.

6.2. Washing of sample bottles is performed in the washroom on the third floor. The washing machine is programmed to deliver a complete wash with suitable detergent for microbiological analysis followed by three fresh water rinses and a final rinse with distilled water. Sample containers returned to the lab from the washroom are checked for residue using 0.04% bromothymol blue (records are maintained for each batch of glassware).

6.3. Instrumentation

6.3.1. Incubator capable of holding the temperature at 35.0±0.5°C

6.3.2. Water bath capable of holding the temperature at 44.5±0.2°C.

6.3.3. pH meter- Accuracy and graduation scale to ±0.1 units.

6.3.4. Autoclave– Operation at 121±2°C

6.3.5. Hot air oven- Operation at 170±10°C

6.3.6. Balance-capable of detecting 0.1g with a150.0g load.

6.4. Other Equipment

6.4.1. Inoculating wire loops made of aluminum or stainless steel, size at least 3mm, which have been sterilized by dry heat at 170°C for two hours.

7. **Reagents, Solutions:**

7.1. REAGENT–GRADE/MICROBIOLOGICAL SUITABLE WATER

7.1.1. Reagent–Generated through a Barnstead/Thermolyne NaNOpure DIamond TOC Life Science (UV/UF with TOC) ULTRAPURE WATER SYSTEM located in the washroom on the third floor. This system provides water that meets the standards put forth by the E.P.A. (Regulations are stated in section 4.4). This water is used for all phosphate buffered water dilution water and media.

7.2. STOCK PHOSPHATE BUFFER SOLUTION

7.2.1. Prepare by dissolving 34.0g potassium dihydrogen phosphate (KH_2PO_4), in 500mL reagent grade water, adjust to pH 7.2±0.5 with sodium hydroxide (NaOH), and dilute to 1L with reagent grade water. Dispense in 100mL volumes, autoclave for 15 min. at 121^0C. Discard any bottle that has precipitate or is cloudy. Check for sterility by Standard Plate Count Method and record results. Label and store in refrigerator. Record in stock phosphate section of Q.C. RECORDS book.

7.3. STOCK MAGNESIUM CHLORIDE

7.3.1. Prepare by dissolving 81.4g $MgCl_2 \cdot 6H_2O$ in reagent- grade water and make up to a 1L volume with reagent-grade water. Dispense in 100mL volumes, autoclave for 15 min. at 121^0C. Discard any bottle that has precipitate or is cloudy. Check for sterility by Standard Plate Count Method and record results. Label and store in refrigerator. Record in magnesium chloride section of Q.C. RECORDS book.

7.4. BUFFERED WATER (*RINSE/DILUTION WATER)*

7.4.1. Prepare by adding 1.25mL stock phosphate buffer solution and 5.0mL magnesium chloride solution per liter of distilled water. Buffered water is dispensed into bottles fitted with nozzles and sterilized by autoclaving for 30 min. Q.C. 20mL from each bottle is added to 100mL of lactose broth and incubated for 48h as a sterility check. Any bottle exhibiting growth is discarded. Record in rinse water section of Q.C. RECORDS book.

7.5. 10% SODIUM THIOSULFATE $Na_2S_2O_3$

7.5.1. To prepare mix 15.8 grams of $Na_2S_2O_3$ with 200mL reagent water. Filter sterilize solution using the membrane filter apparatus. Label and store in refrigerator. This solution is added to sample collection containers in the amount sufficient to provide an approximate concentration of 100mg/L in the sample. This concentration is obtained by adding 0.5mL to a 4oz. bottle or 0.8mL. to the 8oz. prior to sterilization. The reagent acts as a chlorine neutralizer. Medium is made in clean, borosilicate glass, pre-sterilized, screw capped flasks, according to the manufacturer's directions.

Q.C. Perform a heterotrophic plate count on the filter-sterilized reagent, there should be <1CFU/plate. If >1CFU/plate is detected, discard reagent and prepare it again. Record in sodium thiosulfate section of Q.C. RECORDS book.

7.6. DISINFECTANT

7.6.1. Amphyll- Prepared in laboratory by measuring 200mL F/H® Disinfectant-Virucide Sanitizer-Cleaner for Hospital and Institutional Use and adding it to 18L of distilled water. This solution is prepared and stored in a carboy.

8. **Media**:

8.1. LAURYL TRYPTOSE BROTH (LTB)*

8.1.1. Prepared according to manufacturer.

8.1.1.1. The table below shows, grams needed to prepare single strength and double strength Lauryl Tryptose Broth.

Liters	***1X***	***2X***
2	71.2gm	142.4gm
3	106.8gm	213.6gm
4	142.4gm	284.8gm
5	178.0gm	356.0gm
6	213.6gm	427.2gm

8.2. BRILLIANT GREEN LACTOSE BILE BROTH (BGLBB)*

8.2.1. Preparation: according to manufacturer. Record quantity made, lot #, pH, etc. in media logbook.

8.3. EC BROTH*

8.3.1. Preparation: according to manufacturer. Record quantity made, lot #, pH, etc. in media logbook.

8.4. All tube media/broths are dispensed in 10mL amounts into fermentation tubes [size – 16x125 (EC, BGLBB, A-1 and LTB 1X) or 20x150 (A-1 and LTB 2X)] equipped with inverted inserts (unless otherwise directed).

8.4.1. Volume is checked at the start of the filling process and after autoclaving, volume should be 10±2.5%. Perform weekly and record in the Media Volume section of the Q.A. RECORDS book.

8.4.1.1. Using a couple of representative tubes, without a durham tube insert, dispense media as usual. Then measure volume in a 10 ml graduated cylinder (calibrated with a class A annually).

8.5. Media is autoclaved as directed by manufacturer.

8.5.1. Do not allow media to remain in autoclave for more than 30min.

8.6. All media has the pH measured before (if needed) and after autoclaving. If needed, the pH is adjusted (<0.5 units) prior to autoclaving, by adding either sodium hydroxide or hydrochloric acid.

8.7. All media is purchased as a dehydrated powder. Lot #'s are recorded in the new lot #/ media section of Q.A. RECORDS book

8.7.1. Bottles are dated upon receipt and when opened and are labeled with a laboratory expiration date which is 6 months from the opening date.

8.7.2. Powdered media is kept in a cool dry place.

8.7.3. Discard any powdered media if it appears caked, discolored.

8.8. Prepared media in tubes is dated when prepared and /or laboratory expiration date (tubes with loose fitting caps at room temperature are good for 7.days. Tubes with screw caps at room temperature are good for 1 month. Tubes with loose fitting caps refrigerated at 0-4.0C are good for 2 weeks, and tubes with screw caps refrigerated at 0-4.0C are good for 3 months. Prepared media is stored in a cool dry, dark place or in the refrigerator

8.8.1. A couple of representative tubes from every batch prepared are marked with a "sharpie" immediately after autoclaving at the volume height. Media should be discarded if evaporation loss is >1ml at the time of use.

8.8.2. Refrigerated media is allowed to come to room temperature. Remove from refrigerator 24h prior to use.

8.8.3. Prepared media is discarded if any growth or any air bubbles are present.

8.9. LEVINE'S EOSIN METHYLENE BLUE (EMB)

8.9.1. Purchased plate media used to grow positive tube cultures for gram staining confirmation.

9. **Sample Collection, Preservation and Storage:**

9.1. Sample collection bottles are wide-mouthed plastic or non-corrosive glass and a minimum capacity of 125mL that are able to withstand repeated autoclaving.

9.1.1. After clean samples bottles are returned from the washroom and have dried sufficiently they are capped and ready to be sterilized by autoclaving for 30 min at 121^0C. Sterility and the effectiveness of sodium thiosulfate, if added, are checked for each batch. Pouring 50mL of a non-selective medium (tryptic soy broth or Lauryl tryptose (1X) broth) into the bottle followed by incubation for 24-48H and observed for growth checks sterility of sample bottles. Results are recorded in the sample bottle section of Q.C. RECORDS book. The effectiveness of sodium thiosulfate is conducted by adding a solution of bleach and water (1ml bleach/500mL water) to the bottle then adding a drop of Orthotoludine. This reaction should not produce a color change if the sodium thiosulfate is working correctly.

9.1.2. Sodium thiosulfate (pipette 0.5mL to a 4oz. bottle or 0.8mL. to the 8oz.) is added prior to sterilization. Samples containers with sodium thiosulfate added are used for chlorinated test sites.

9.1.3. Sample bottles are then labeled and stamped with the date of sterilization.

9.1.4. Samples are collected, then held at 0-10.0ºC during a maximum transport time of 6 hours.

9.1.5. Once at the laboratory samples are held under refrigeration until analysis (within **2 hours** of receipt).

9.1.6. The requirements stated above can be unrealistic, so if needed analysis can be performed on samples when less than 24 hours have elapsed from collection to receipt in the laboratory. An observation of the collection time and date located on the Form A is a prerequisite to acceptance of the sample by the staff in Central Receiving. Laboratory personnel review the collection date and time when samples arrive in the laboratory for analysis.

9.2. There is a 24-hour maximum hold time on water samples. An observation of the collection time and date located on the Form A is a prerequisite to acceptance of the sample by the staff in Central Receiving. Laboratory personnel review the collection date and time when samples arrive in the laboratory for analysis.

10. **Quality Control:**

10.1. Known samples, accompany samples throughout incubation processes to insure that the proper temperatures are maintained. Daily quality control for media: Inoculate 4 tubes each of BGLBB, EC, and LTB 1X and 2X with *E.coli, Enterobacter aerogenes and H.alveii*, last tube is uninoculated for sterility. Incubate BGLBB, 1X and 2X tubes at 35^{+}–0.5°C for 24h., and EC tubes at 44.5±0.2°C for 24 h.

10.2. Results are recorded every day and should be;

10.2.1. BGLBB, 1X, 2X - growth and gas for *E.coli* and *Ent.aerog* growth and no gas for *H.alveii* no growth or gas in uninoculated tube.

10.2.2. EC - growth and gas for *E.coli* only - no gas or growth for *Ent.aerog., H.alveii,* or uninoculated tubes.

10.2.3. All new media of LTB, EC, BGLBB are checked as above when made.

10.3. Sterility of pipettes is checked by pippeting sterile water into a tube containing LTB for each new lot#.

10.3.1. Records are maintained in Purchased equipment sterilized by manufacturer section of Q.C. Book

11. **Sample Preparation:**

11.1. Analysis performed within 24h of collection.

11.2. Keep under refrigeration until time of analysis (within 2h of receipt).

12. **Procedure:**

12.1. **Presumptive Phase**

12.1.1. Work areas are wiped down with germicide prior to testing.

12.1.2. All tubes are checked for the presence of gas prior to inoculation, if any are found they are discarded.

12.1.3. Appropriate amounts of sample are determined. If sample is drinking water the standard volume is 10mL in 10 tubes. For other waters use multiples and sub-multiples of 10 to inoculate tubes. Volume of sample and medium should result in single strength medium

12.1.4. Tubes containing lauryl tryptose broth (LTB), 1X and 2X, broth are set up in racks, such that there are 12 tubes per sample.

12.1.4.1. **EXAMPLE:** Standard 3 tube, $10\text{-}10^{-2}$ dilution

12.1.4.1.1. First Row - Start with 2x(LTB), arrange in rack 3 tubes deep. Add 10 ml of sample in each of the three tubes.

12.1.4.1.2. Second Row- 1x(LTB), arrange in rack 3 tubes deep. Add 1ml of sample in each of the three tubes.
12.1.4.1.3. Third row-2x(LTB), arrange in rack 3 tubes deep. Add 10 ml of a 1:100 dilution of original sample in each of the three tubes.
12.1.4.1.4. Fourth Row-1x(LTB), arrange in rack 3 tubes deep. Add 1 ml of a 1:100 dilution of original sample in each of the three tubes
12.1.4.1.5. A 1:100 dilution is made by adding 1ml of original sample to 99ml dilution blank (phosphate buffered water).
12.1.4.2. Wastewater is usually analyzed using a 3 tube, 10-10 $^{-5}$ dilution.
12.1.4.2.1. This setup is accomplished by setting up rack as above with a fifth, sixth, and seventh row added.
12.1.4.2.2. Inoculate the same as above, making two more decimal dilutions (1ml into 99ml dilution blank (phosphate buffered water).
12.1.5. Samples and dilutions are shaken vigorously (25 times through a 1ft arc/7s) before any sample is withdrawn.
12.1.6. Dispense appropriate volumes of sample and dilutions using sterile pipettes and a pipetting aid (mouth pipetting is not permitted) into Lauryl Tryptose Broth (1x and 2x) as a presumptive test.
12.1.7. Tubes are then incubated 35±0.5°C. After 24h±2h tubes are gently swirled and observed for growth with or without gas (bubbles in the inverted insert). Record presence or absence of growth, gas, and acid production. Negative tubes are reincubated for another 24h. All positive and negative tubes are recorded. All presumptive positive tubes after 24 h or 48h are subjected to the confirmation and completed phase.

12.2. **Confirmation Phase and Fecal Coliform Test**
12.2.1. Brilliant Green Lactose Bile Broth is used for the confirmed test.
12.2.1.1. This test is performed not performed for shellfish harvesting areas.
12.2.2. EC medium is used for the fecal coliform test.
12.2.3. Using a sterile loop 3.0-3.5mm in diameter, transfer one or more loopfuls from each positive presumptive tube and inoculate a corresponding BGLBB tube and EC tube.
12.2.3.1. Incubate BGLBB tubes at 35±0.5°C. Tubes are checked at 24±2h and 48±3h for gas production.
12.2.3.1.1. Any amount of gas formation in the inverted vile after 48±h constitutes a positive test
12.2.3.2. Incubate EC tubes in water bath at 44.5±0.2°C. Tubes are read at 24±2h for gas production.
12.2.3.2.1. EC must be incubated within 20min. of inoculation.
12.2.3.2.2. Water level in bath must immerse upper level of medium.
12.2.3.2.3. Gas production with growth within 24±2h constitutes a positive fecal coliform reaction.
12.2.4. All positive and negative tubes are recorded.

12.3. **Completed Phase**
12.3.1. At least 10% of positive confirmed tubes are subject to the completed test phase. Positive BGLB tube is streaked onto Levine EMB agar plates for colony isolation and incubated at 35±0.5°C for 24h(Coliform morphology-Typical- is pink-dark red with a metallic green surface, Atypical- is pink, red, white, or colorless without a green sheen). Growth from coliform colonies is transferred to LTB medium and a nutrient agar slant (the latter can be omitted for drinking water samples). LTB tubes are incubated at 35±0.5°C for 24h-48h. If gas produced in LTB then cultures are considered coliforms. Optional for drinking water samples, agar slant is incubated for 18h-24h, then gram stain a portion of agar slant. Observe microscopically for coliforms (gram-negative rods, no spores present).

13. **Laboratory Action:**
13.1. The sequence of positive and negative tubes for the presumptive, confirmed, fecal tests and a calculated MPN value are entered into the Aspen system. The system electronically sends results to the appropriate offices.

14. **Expression of Results:**
14.1. Final results are based on the confirmatory test for total coliforms and fecal coliforms.
14.2. If multiple tubes are used, an MPN can be calculated. If only one tube is used for subculturing from a single presumptive bottle, report as present or absent for total and fecal coliforms.
14.3. Coliform density values can be found on the MPN index and 95% Confidence Limits Tables.
14.3.1. There are a variety of these tables available depending on the planting series used.

15. **Reporting**
    15.1. Prior to entering results electronically, worksheets must be reviewed.
        15.1.1. Daily data worksheets are accepted or rejected on the basis of correctness and completeness by a laboratorian. Date and initials are required.
            15.1.1.1. Any worksheets that are rejected are brought to the supervisor's attention for a corrective plan of action.
            15.1.1.2. Acceptable work sheets are ready to be entered into the Aspen system.
    15.2. Entering results into the Aspen system.
        15.2.1. An adhesive label designates each sample with a six-digit number that is applied to bottle in the field or upon receipt in the Central Receiving area.
        15.2.2. Electronic entering of data requires a new number be assigned called an Aspen #.
            15.2.2.1. This is achieved in Central Receiving where specimens are initially entered into Aspen.
    15.3. Retrieving the Aspen number from the Aspen workstation.
        15.3.1. Log into the system with the appropriate user designation and password.
        15.3.2. Click on the first tab under the Active Sample column-**Log in/Edit.**
            15.3.2.1. Scroll through the bottom left corner of the field marked "Record". When you encounter a sample that matches the six digit # found on the adhesive label applied by the collector in the "Lab ID" field record the Sample I.D # (Aspen #) in the appropriate spot the worksheet.
            15.3.2.2. Each sample now has two unique numbers assigned to it. (An adhesive label with six digit number that is applied in the field or upon receipt in the Central Receiving office by the collector and the Aspen #).
        15.3.3. Assigning a Worklist #;
            15.3.3.1. Click on the enter sample results tab (2nd under the Login/Edit column).
            15.3.3.2. Click on "Create New Worksheet" tab.
            15.3.3.3. Click on "By Test Group" tab.
            15.3.3.4. Move cursor to the "Select Test Group ID" field.
            15.3.3.5. Using the drop down menu scroll to the analysis test code (SM01).
            15.3.3.6. Hit "Filter Test Group".
            15.3.3.7. All samples that are pending with the test code of the previous step will be listed.
            15.3.3.8. Your cursor will automatically be in the "Select For Data Entry" field. Click on each sample that you want to group on one work list.
                15.3.3.8.1. Daily work lists should contain all samples for each day for each test code.
            15.3.3.9. Hit "Enter Results".
                15.3.3.9.1. A new screen will appear showing the Worksheet #. This number is recorded in the workbook.
    15.4. Entering results;
            15.4.1.1. Click on the enter sample results tab (2nd under the Login/Edit column).
            15.4.1.2. Click on "Review Existing Worksheet" tab.
                15.4.1.2.1. Scroll to the worksheet number you want, highlight it and press continue.
        15.4.2. All results are entered in the "Alpha Results" field.
                15.4.2.1.1. It is necessary to hit enter several times after the last result is entered.
            15.4.2.2. Next hit the "Mark Status" tab.
            15.4.2.3. Select the "Mark Tests Analyzed". Hit "O.K".
            15.4.2.4. Type in date of analysis, when prompted.
            15.4.2.5. Type in initials of analyst (person who actually performed test), when prompted.
            15.4.2.6. Always use the "Back" button to get out of an Aspen screen. When the first screen is reached, hit the "Stop" button to shut down Aspen.
            15.4.2.7. The workbook is initialed and dated in the space marked Entered.
    15.5. Reviewing Aspen entry;
        15.5.1. A person other than the person who did the original entering reviews results in a timely fashion.
        15.5.2. To review, hit the "Routine Reports" button in the Login/Edit column on Aspens first screen.
        15.5.3. Select "Worksheets".
        15.5.4. Select the work list # you want to review.
        15.5.5. Review entered results and accepted workbook results for accuracy.
        15.5.6. Attention should be made to assigned numbers to see that they correlate.

15.5.7. If a mistake is encountered print the page containing the mistake.

15.6. Changing an incorrect result.

15.6.1. In the first screen of Aspen hit enter results button.

15.6.2. Select the appropriate work list.

15.6.3. Scroll to the field where the mistake is found.

15.6.4. Make the needed changes and hit enter twice.

15.6.5. Print page with corrected results.

15.6.6. Fill out a corrective action report form and affix the incorrect and the corrected Aspen pages.

15.7. Once the Aspen results are reviewed and corrected if needed the worklist can be marked ready to report.

15.7.1. Begin by following section 15.4 – 15.4.2.2.

15.7.2. After hitting the "mark status" button, highlight the " mark tests approved" bullet.

15.7.3. Type in date approved (today's date), when prompted.

15.7.3.1. Type in initials of person who approved worklist (person reviewing results on Aspen), when prompted.

15.7.4. The workbook is initialed and dated in the space marked Release/Approve.

15.8. Always use the "Back" button to get out of an Aspen screen. When the first screen is reached, hit the "Stop" button to shut down Aspen.

Fixed Laboratory Method 2 (L-2) Standard Operating Procedures (SOP) for Determination of Male-Specific Bacteriophage

Procedure for Determining Male-specific Bacteriophage in Seawater and Wastewater

The protocol is based on the work of Dr. William Burkhardt, US FDA, Dauphin Island, Alabama. He taught the procedure to the Northeast Laboratory Evaluation Officers and Managers (NELEOMs) at the Male-specific Bacteriophage (MSB) Workshop in Gloucester, Massachusetts, on March 9-12, 2004.

Coliphage are bacterial viruses (bacteriophages) that infect and replicate in *Escherichia coli*. They are often found in high concentrations in municipal wastewater and to a lesser degree in human and animal feces. Coliphages are potentially important microorganisms for monitoring the microbial quality of waters because traditional bacterial monitoring does not accurately indicate the presence of non-bacterial organisms such as human pathogenic viruses; human virus detection is beyond the capabilities of most water laboratories; and coliphage detection is relatively inexpensive, easy to perform, and provides overnight results.

The male-specific coliphages infect male bacterial cells via the F-pilus. F-factor is the fertility factor in certain strains of *E. coli*. It is a plasmid that, when present, codes for pilus formation. The pilus allows the transfer of nucleic acid from one bacterium to another. Male-specific coliphages (F+) are RNA or DNA viruses and are unable to replicate in the environment. Although coliphages are not pathogenic for humans, other microorganisms that are human pathogens may be present in waters impacted by untreated or inadequately treated domestic wastes. Use good laboratory practices when working with potentially harmful samples.

The host bacteria used is *E. coli* F_{amp} for male-specific coliphage.

What are male-specific bacteriophages?

Lytic viruses of bacteria – (killing of host bacteria).
E. coli and *S. typhimurium*
(production of E. coli pili)

Requires a piliated host cell for adsorption, they do not attach to cell surface (somatic).

Requires host cell in log-phase of growth-cells do not produce pili at < 30°C.

Optimal growth temperature: 35 - 37°C.

Plaque size is generally self-limiting.

Norwalk-like viruses are presently referred to as Noroviruses. Gastroenteritis symptoms are indicative of viral infection. Norovirus infection causes vomiting, diarrhea, cramps and weakness within an 18-30 h onset with an illnesses lasting for up to 72 h. In addition, Noroviruses are highly contagious and spreads in a confined population (i.e., a cruise ship). Viruses like cold reduced sunshine environments. Bioaccumulation within shellfish in the U.S. occurs from October through January.

Fecal coliforms have no correlation to the bacteriophage levels during outbreak periods normally observed from November through January.

Apparatus and Materials

Equipment for collection and transport of samples

Sterile bottles with screw caps, 100 mL or 125 mL treated w/ sodium thiosulfate and untreated
4 mil plastic bags
Ice chest
Ice
Latex gloves
Temperature Control Blank
Disinfectant

Equipment for the laboratory

Centrifuge, sterile 50 mL conical tubes, 9000 x *g* performance capability, 4°C
Water Bath, 44-52°C
Laboratory Balance, 0.00 g
Incubator, 35-37°C
Balance
Stir Plate
Rotator
Vortex
Blender
Autoclave, 121°C
Cold Room, 4°C
Freezer, -20°C
Light Box (optional)
Thermometers, range 0 – 121°C
Timer

Erlenmeyer Flasks, 1 L and 2 L
Graduate Cylinders, 1000 and 500 mL
Stir Bar
Funnel
Shovel
Weigh Boats
Deionized or Distilled Water
Inoculating Loops (3 mm in diameter or 10 L volume)
Sterilization Filtration Equipment - 0.22 or 0.45-µm pore size
SyringesBsterile disposable, 5-, 10- or 20 mL
Potable Water Source
Scrub Brushes, sterile
Bone Snips, sterile
Knives, sterile
Blender Jars, sterile
Baskets, drying
KimWipes
Decontamination Bag
Disinfectant

Pipets-Pipettors, 500 µL, 1000 µL, 5 mL, 10 mL and 25 mL
Pipet Bulb
Centrifuge Tubes, sterile disposable 15 & 50 mL
Petri Dishes, sterile disposable 100 x 15 mm
Test Tubes 16 x 150 mm, s/s loose fitting caps or screw caps
Test Tube Rack--size to accommodate tubes
Freezer Vials- sterile 5 mL screw cap
Foil
Parafilm

Permanent Marker

Reagents
Reagent Water
Glycerol- sterile
Ethanol - 70% or laboratory disinfectant
Calcium Chloride- 1M

Antibiotic stocks
*****Antibiotics must always be added to medium after the medium has been autoclave and cooled.
Streptomycin/Ampicillin (50 µg/mL)
ampicillin sodium salt (Sigma A9518)
streptomycin sulfate (Sigma S6501)

MediaB
Bottom Agar
DS Soft Agar
Growth Broth

StockB Bacterial Host
E.coli $F_{amp}^{\sim}$ *E. coli* HS(pFamp)RR
- originated by Victor Cabelli, University of Rhode Island, Kingston, RI, USA, frozen stock ATCC #700609.

Bacteriophage-
MS2, ATCC # 15597

Media Composition
***E. coli* $\mathbf{F_{amp}}$**

Bottom Agar

Tryptone	10.0 g
Dextrose	1.0 g
NaCl	5.0 g
Agar	15.0 g
DI Water	990 ml

- With gentle mixing, add all the components to 990 mL of dH_2O in a 2000 mL flask. Dissolve and sterilize.
- Sterilize at 121ºC ± 2ºC for 15 minutes.
- Allow the agar to equilibrate in the water bath set at 44 - 46ºC.
- Aseptically, add 10 mL of Streptomycin sulfate/Ampicillin solution to the flask. (50 µg/mL final).
- Temper to 50ºC in the water bath.
- Aseptically, pipet 15 mL aliquots into sterile 100 x 15 mm petri dishes and allow the agar to harden.
- Store the dishes inverted at 4ºC overnight and warm to room temperature for 1 h before use.
- Plates are good for one month at 4ºC.
    - **Streptomycin sulfate/Ampicillin Solution** - Dissolve 0.5g of streptomycin sulfate and 0.5g of ampicillin in 100 mL of dH_20. Filter with the 0.22 µm filter. Store in 10 mL aliquots in sterile containers at 4ºC. Allow to come to room temperature.

DS Soft Agar

Tryptone	10.0 g
Dextrose	1.0 g
NaCl	5.0 g
1M $CaCl_2$	0.5 mL
Agar	7.0 g

DI Water 500 ml

[*$CaCl_2$ Anhydrous FW 111.0, Dihydrate FW 147]

- With gentle mixing, add all the components to 500 mL of dH_2O in a 1000 mL flask.
- Bring flask to a boil.
- Dispense in 2.5 mL aliquots into 16 x 100 mL tubes and freeze (-20ºC) for up to one month. Place a foil top on the tubes and sealed them in a zip-lock bag.
- Sterilize prior to use at 121ºC ± 2ºC for 15 minutes; temper to 50 - 52ºC for no longer than 2 h.
  - 1M $CaCl_2$ - Add 1.11g of $CaCl_2$ to 10 mL on dH_2O. Sterilize by autoclaving at 121ºC for 15 minutes. Tubes are good for one month at 4ºC. Use at room temperature. **[Divalent cations such as Ca+ are used to maintain phage stability and to facilitate efficient phage absorption to host.]**

Growth Broth

Tryptone	10.0 g
Dextrose	1.0 g
NaCl	5.0 g

DI Water 1000 ml

- With gentle mixing, add all the components to 1000 mL of dH_2O in a 2000 mL flask. Dissolve and dispense into 100 mL NALGENE containers. Fill the containers to 75 mL.
- Sterilize at 121ºC ± 2ºC for 15 minutes.
- Bottles are good for one month at 4ºC.

Propagation of E. coli Famp

- Bottom Agar streak plate - Transfer preferable < 1 week old.
- 10 mL of Growth Broth medium tempered to 35 - 37ºC - **vortex to aerate prior to inoculation.**
- Using a sterile 10 µL loop collect material from several colonies and transfer to the broth medium in a sterile 15 mL conical tube.

Gently shake to mix, and incubate at 35 - 37°C for 4 - 6 hours (turbidity $.10^7$cells/mL; O.D @ 540 nm = 0.4). Once turbidity is observed, you can start using the culture (log-phased growth).

Control Plates

- For a *negative* control plate add 2.5 mL of Growth Broth to the 2.5 mL DS Soft Agar tube (adding 0.2 mL of *E. coli* F_{amp} is optional, and recommended).
- For a *positive* control plate add 0.2 mL of a 4 h culture of host *E. coli* F_{amp} bacterium (approximately 30-80 PFU/mL). Make serial dilutions of your MS2 coliphage culture using 9 and 9.9 mL Growth Broth blanks. The tube should contain 2.5 mL of DS Soft Agar + 0.2 mL of host + 1 mL of MS2 dilution + 1.5 mL of Growth Broth.

Remove the tube from the water bath. Gently mix the tubes in your palm. Pour each tube onto a Bottom Agar plate containing Streptomycin/Ampicillin (50 µg/ml final). Invert and place in an incubator at 35 - 37°C for 18 - 24 h. Examine for plaques and record the results.

MSB Density Determinations in Low Contaminated Water Concentration Technique

Used for **shellfish Growing Area water** samples. For each water sample prepare (2) Bottom Agar plates and (2) 2.5 Soft Agar tubes for each plate if processing only a 50 mL portion. If processing the entire 100 mL portion prepare (4) Bottom Agar plates and (4) 2.5 Soft Agar tubes. Use a 4 to 6 h *E. coli* F_{amp}. Always run a positive control because during the summer months you may not find viruses. Also, run a negative control plate where plaque is not seen. Add (2) Bottom Agar plates and (2) DS Soft Agar tubes for the controls.

1. Weigh 100 mL of sea water in a sterile container. [You may need to put **45 mL** each into two centrifuge bottle.]

2. Allow water to warm to RT (20 - 30 min).

3. Add 1 g tryptone and 1 g beef extract to the 100 mL water sample, shake to dissolve. Bubbles will appear in the bottle. [Use 0.5 g of tryptone and beef extract if using 45 mL portions in the 50 mL centrifuge bottles.]

4. Add 10 mL of *E. coli* F_{amp} culture to 100 mL portion or 5 mL to each 45 mL portion - Do not shake. Clean any overflow on the centrifuge bottle with alcohol.

5. Incubate at 35 - 37ºC for 50 min - rotate at 100 rpm. With the shaker inside the incubator, lay the bottles down on the shaker platform and set the speed to a *very gentle* setting.

6. Centrifuged for 15 min. @ 9,000 x *g*; 4 C.

7. Aspirate or decant supernatant - Do Not Disturb Pellet! Decant into a waste container for biohazard sterilization.

8. Use 5 mL of Growth Broth to re-suspend pellet. Run the tryptone up and down over the pellet or vortexing may be needed to breakup the pellet. You do not want to see any clumps!

9. **Split the 5 mL** Growth Broth into two DS Soft agar tubes combine 2.5 mL aliquot of supernatant and 2.5 mL DS Soft agar (tempered to 52ºC). Gently mix contents by rolling the tube between your palms.

10. Each DS Soft agar tube is overlaid onto one Bottom Agar plate containing Streptomycin/Ampicillin (50 µg/ml final). Drag the mixture into a clear area and don=t over swirl the plates.

11. Plates are inverted and incubated for 18 - 24 h @ 35 - 37ºC. After incubation, count the circular zones of clearing (typically 1 to 10 mm in diameter) in the lawn of host bacteria in double agar plates. Report results in PFU/100 mL.

Calculations for Shellfish Growing Area Waters:

< If only a **50 mL portion** is used you must *multiply the sum of your results by 2* to account for 100 mL sample.

(Plate 1 + Plate 2) x 2 = PFU/100 mL

50 mL portion of Water - 12, 14 ≡ 12 + 14 = 26 x 2 = 52 PFU/100 mL

< If the entire **100 mL portion** is used the result is *the sum of all four plates*.

(Plate 1 + Plate 2 + Plate 3 + Plate 4) = PFU/100 mL

50 mL portion of Water - 6, 10 ≡ 6 + 10 + 8 + 7 = 31 PFU/100 mL
50 mL portion of Water - 8, 7

MSB Density Determinations in Highly Contaminated Water
∃ 100 PFU/100 mL

FDA recommends the following

For a **raw sewage** sample process 2.5 mL from (10^{-1}) and (10^{-2}) dilution. Pour 4 plates for each dilution. Run 2 control plates.

For a **treated sewage** sample process 2.5 mL for the "direct" technique, 2.5 mL from (10^{-1}) and 2.5 mL (10^{-2}) dilutions from the "concentration" technique. Pour 4 plates for each dilution. Run 2 control plates.

Tryptone Dilution Tubes—
Aseptically, dispense 9 mL aliquots of sterile Growth Broth into sterile 16 x 150 mm test tubes that have been sterilized at 121EC for 15 min.

To carry out a serial "ten-fold" dilution you would do the following:

From a 100 mL sewage water sample –

- Take 1 mL of the water sample and mix it with 9 mL of Growth Broth. The dilution becomes (1:10). Mix or vortex. Use a micropipette with new sterile tip to carry out a second ten-fold dilution.
- Take 1 mL of the (1:10) tube and mix it with a new 9 mL tube of Growth Broth. The dilution becomes (1:100). Repeat until you have reached your desired concentration.

1. Allow the water sample to warm to RT (20 - 30 min).
2. Prepare your serial dilution with the water sample.
3. *When running up to 3 plates per dilution*B Combine **2.5 mL aliquot of sample**, 0.2 ml of *E.coli* F_{amp} to 2.5 ml DS Soft agar (tempered to 52EC). Gently mix contents by rolling the tube between your palms. [Uses 7.5 mL of each dilution tube.]

   *When running up to 4 plates per dilution*B Combine **1.5 mL aliquot of sample**, 1.0 mL of Growth Broth, 0.2 ml of *E.coli* F_{amp} to 2.5 ml DS Soft agar (tempered to 52EC). Gently mix contents by rolling the tube between your palms. [Uses 6.0 mL of each dilution tube.]

4. Overlay onto a Bottom Agar plate containing Streptomycin/Ampicillin (50µg/ml final). Drag the mixture into a clear area and don=t over swirl the plates.

5. Plates are inverted and incubated for 18 - 24 h @ 35- 37ºC. After incubation, count the circular zones of clearing (typically 1 to 10 mm in diameter) in the lawn of host bacteria in double agar plates. Report results in PFU/100 mL

Computations for Sewage samples using four plates per dilution:

$$\textbf{MSB/100 mL} = \frac{\textbf{Avg of plate counts}}{\textbf{mL analyzed/plate}} \times \textbf{dilution factor} \times \frac{100}{100} = \textbf{PFU/100 mL}$$

10^{0} = TNTC, TNTC, TNTC, TNTC
10^{-1} = 164, 190, 170, 175 = Average 174.75 pfu/plate
10^{-2} = 16, 16

174.75) 1.5 mL aliquot = $116.5 \times \frac{1000}{100}$* = 116,500 ≡ 120,000 PFU/100 mL

[* 1000 ≡ 100 mL (water sample) x 10 (from 10^{-1})]

Storage of E. coli F_{amp}

- No Selective Pressure - Addition of glycerol (10% final) into a broth culture.*
  Storage: Freeze at - 80 C Indefinite. (*Most desirable method.)*
  [Pressure is reapplied when the culture is streaked back onto the Bottom Agar containing the two antibiotics.]

- Bottom Agar Streak Plate
  Storage: Refrigerator at 4 C for 2-3 weeks.

- Tryptic Soy Agar Slant w/ Mineral Oil overlay
  Storage: Room temperature in the dark for 2-5 years +.

- Selective Pressure - Tryptic soy Agar Slant w/Streptomycin and Ampicillin. Stab deep, let grow for 6 h, and then overlay with sterile mineral oil. (*Least desirable method*.)

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Glycerol Solution, 10% - Add 9 mL of distilled water to 1 mL of undiluted glycerol. Autoclave resulting 10% glycerol solution at 121EC for 15 min and use at room temperature.

- Add 1/5th volume of 10% glycerol solution. Let stand for 30 min. Dispense into 1 mL aliquots in 2-mL cryovials and store at -70 to -80EC is best, but -20EC is acceptable.

Source of Bacterial Host Strains

E. coli HS(pFamp)R, ATCC #700891- Bacterial host for male-specific coliphages.

Sample Collection and Storage

- Sterile sample containers of plastic or borosilicate glass are used to collect water samples. 4 mil plastic bags are used to collect shellfish shellstock samples. If the water sample is from a shellfish growing area follow your water and shellfish sample collection SOPs.

- If the sample is from a sewage treatment plate, gloves should be used when the sample is collect. If the sample was chlorinated use a sodium thiosulfate treated sample container. If the sample was unchlorinated use an untreated sample bottle.

- Record all pertinent information of the collection form.

- Maximum holding times for samples-
- raw and treated sewage samples up to 24 h,
- shellfish up to 24 h, and
- shellfish growing area water up to 30 h.

Quality Assurance

- Run positive and negative control plates with each sample.

- Check media sterility.

- Media log book should be maintained (volume, weights of each components, lot numbers, etc.).

- Develop an intra- and inter-laboratory performance program.

- Circular zones of clearing (typically 1 to 10 mm in diameter) in lawn of host bacteria after 18- 24 h of incubation are plaques. Count the number of plaques on each plate. (Use of a light box to evaluate results is recommended.) Plaque forming unit (PFU).

- Desired range of zero to 300 PFU per plate. If the count exceeds the upper range or if the plaques are not discrete, results should be recorded as *too numerous to count* (TNTC).

Calibration and Standardization

- Check temperatures in the water baths, refrigerators, freezers, and incubator twice a day (at least 4 h apart) to ensure operation within the stated limits of the method and record the measurements in a log book.

- Check thermometers at least annually against an NIST-certified thermometer.

- Calibrate the balance annually using ASTM-certified Class 1 or 2 or NIST Class S reference weights.

- Laboratories must adhere to all applicable quality control requirements set forth in the most recent version of FDA=s *Shellfish Laboratory Evaluation Checklist*.

Safety

- Samples, reference materials, and equipment known or suspected to have bacteriophage attached or contained must be sterilized prior to disposal.

Useful Terms

h - hour
°C - degrees Celsius
µL - microliter
g - gram
L - liter
M - molar
mL - milliliter
rpm - revolutions per minute
x - Atimes@
Ave. - average
Na2S2O3 - Sodium thiosulfate
MSB - male-specific bacteriophage
NIST - National Institute of Standards and Technology

PFU - number of plaque forming units
RT - room temperature
TNTC - too numerous to count

Coliphage - Viruses that infect *E. coli*.

Enrichment - Enrichment is meant as the increase in number of bacteriophage through the addition to the growth medium of host bacteria allowing coliphage replication.

Host Bacteria - Are those bacteria that allow the bacteriophage to penetrate and replicate with them, ultimately lysing, resulting in the release of the progeny bacteriophage. Host bacteria are essential for virus replication. The host used in this method is *E.coli* F_{amp} (*E.coli*HS(pFamp)R).

Lysis zone - In this method, typically a circular zone of clearing indication a sample is positive for coliphages.

Male-specific coliphage - Viruses (bacteriophages) that infect coliform bacteria only via the F-pilus.

Plaque - Circular zones of clearing (typically 1 to 10 mm in diameter) in lawn of host bacteria in double agar plates after incubation.

References

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Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure, USEPA, EPA 821-R-01-030, April 2001.