Citizen Science QAPP Template #1
Title and Approval Page

Citizen Science Pathogen Monitoring
Effective Date of Plan: May 1, 2014

HEP Project Manager: 5/2/14
Signature/Date
Gabriela Munoz/NY-NJ HEP

NEIWPCC Project Manager: 5/5/14
Signature/Date
Susy King/NEIWPCC

NEIWPCC QA Manager: 5/5/14
Signature/Date
Susan Sullivan/NEIWPCC

EPA Project Officer: 5/2/14
Signature/Date
Robert Nyman/EPA

EPA QA Officer: 05/01/14
Signature/Date
Carol Lynes/EPA

NJDEP QA Officer: 06/06/14
Signature/Date
Debra Waller/ NJDEP

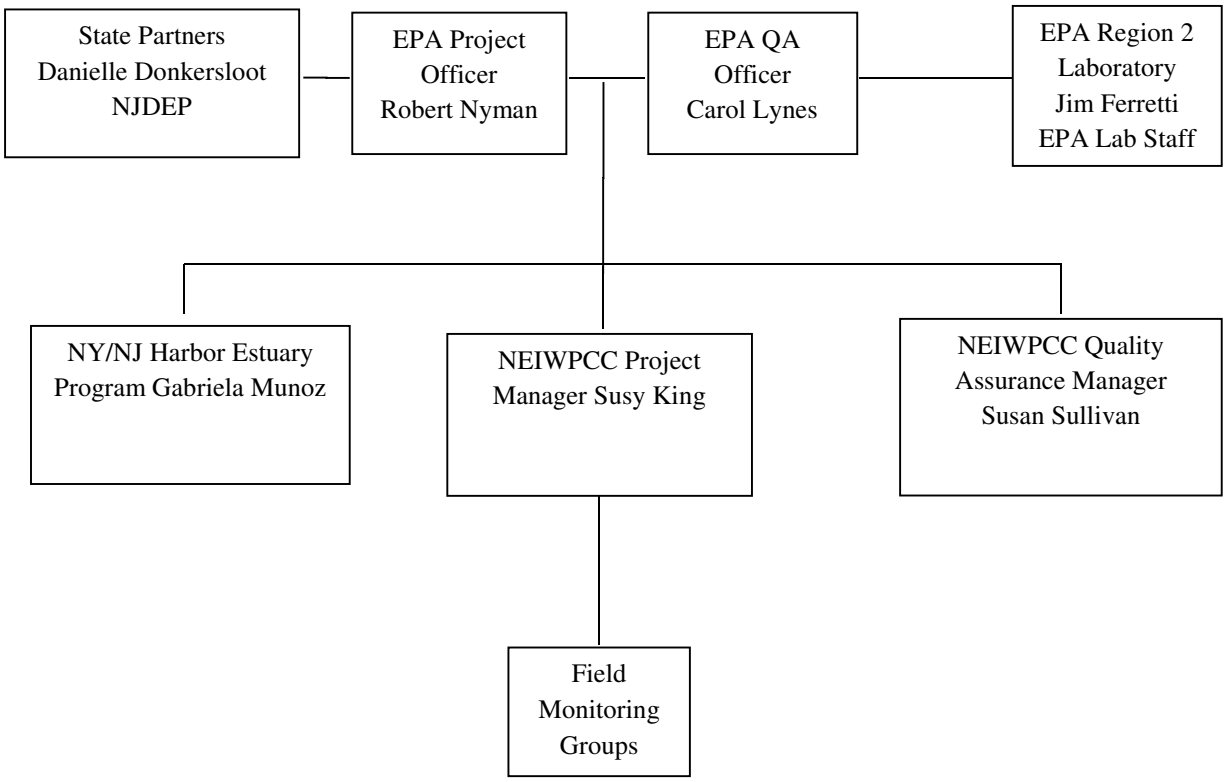
NJDEP Project officer 06/06/14 *
Signature / Date
Danielle Donkersloot/NJDEP

* Tier D approval for temperature, salinity, conductivity, DO, pH.
Tier C approval for IDEXX Enterolert for saline waters
Tier D approval for IDEXX Enterolert for freshwater
All conditions of QAPP must be met for Department data use consideration - DD

Table of Contents

Citizen Science QAPP Template #2A
Project Organization Chart

The organization chart shows the lines of communication and reporting for the project, similar to a chain of command. Fill in the names of the individuals and their titles (where applicable). If the project does not have all of the personnel in the chart, put N/A in the box where this applies. If necessary add more boxes to accurately reflect the communication and reporting structure of the project.



NOTE: Organizational information for the NEIWPCC Field Monitoring Groups is provided in the contractor specific addenda.

Citizen Science QAPP Template #2B
Project Distribution List

The distribution list ensures everyone involved with the project receives a copy of the QAPP and is aware/clear about the work being conducted. It also provides the contact information for those involved with the project. For this table, input the names and contact information for all individuals who will need to get a copy of the QAPP.

Name/Title	Contact Information
Gabriela Munoz NY-NJ HEP	Email: gabriela@harborestuary Phone: 212-483-7667
Susy King NEIWPCC Project Manager	Email: sking@neiwpcc.org Phone: 978-349-2506
Susan Sullivan NEIWPCC Quality Assurance Manager	Email: ssullivan@neiwpcc.org Phone: 978-323-7929 x503
Robert Nyman EPA Project Officer	Email: nyman.robert@epa.gov Phone: 212-637-3809
Carol Lynes EPA Quality Assurance Officer	Email: lynes.carol@epa.gov Phone: 732-321-6760
Jim Ferretti EPA Laboratory	Email: ferretti.jim@epa.gov Phone:732-321-6728
Erwin Smieszek EPA Environmental Engineer	Email: smieszek.erwin@epa.gov Phone: 732-321-6718
Stan Stephansen EPA Data Management	Email: stephansen.stanley@epa.gov Phone: 212-637-3776
Danielle Donkersloot NJDEP	Email: danielle.donkersloot@dep.state.nj.us Phone: 609-633-9241
Alene Onion NYSDEC	Email: amonion@gw.dec.state.ny.us Phone: 518-402-8166

Note: NEIWPCC Contractor contact information is provided in the individual contractor addenda.

Citizen Science QAPP Template #3
Project/Task Organization

Fill in the name, title, organization affiliation and responsibilities sections of the table below. For the responsibilities section, state what work/task each individual will be doing throughout the project. The responsibilities section provides an outline of the work that will be done for the project. Project specific details will be addressed in later sections of the QAPP. **NOTE**: The names and titles should be consistent in Templates #1, #2A, #2B, and #3.

Name	Title	Organizational Affiliation	Responsibilities (specific to this project)
Gabriela Munoz	Project Manager	NY-NJ HEP	Coordinates with contractors and partners
Susy King	NEIWPCC Project Manager	NEIWPCC	Manages 106 Grant (I98297411) and contractors, will approve addendums submitted by contractors
Susan Sullivan	NEIWPCC Quality Assurance Manager	NEIWPCC	Review of QAPP to ensure compliance with NEIWPCC QMP
Robert Nyman	EPA Project Officer	EPA	Provides grant oversight and will be the recipient for reports from NEIWPCC, will verify addendums submitted comply with requirements before final approval of addendums by NEIWPCC
Carol Lynes	EPA Quality Assurance Officer	EPA	Review the QAPP
Jim Ferretti	EPA Team Leader, Sanitary Chemistry and Biology Team	EPA Laboratory	Provide training for the IDEXX Enterolert analyses, coordinate/reviews contractor Enterolert results
Erwin Smieszek	EPA Environmental/ Chemical Engineer	EPA	Provide training for field equipment and sampling perform field assessments
Stan Stephansen	EPA Data Management	EPA	Will provide data management training and assist with uploading data into STORET
Danielle Donkersloot	NJDEP Contact	NJDEP	Coordinates activities between NJDEP and EPA
Debra Waller	NJDEP-Office of Quality Assurance	NJDEP	Assist with field and lab training

Citizen Science QAPP Template #4
Problem Definition and Project Objectives

Problem Definition

Clearly state the problem and environmental questions being addressed by the project.

This project provides an opportunity for community groups to collaborate with NEIWPCC, HEP, USEPA Region 2, NJDEP, and NYSDEC to gather water quality data in tributaries to the NY--NJ Harbor Estuary, with a focus on pathogen indicators. These data will be publicly available, enabling communities to gain knowledge about the health of their local streams. The skills gained by the community groups carrying out the monitoring will empower them to pursue a variety of citizen science projects, producing useful and valid environmental data. It is hoped that this project will constitute a model for future citizen science collaborative projects.

Project Objectives (linking data results with possible actions)

Describe how the project objectives will answer the problem presented in the problem definition provided above. Include the tasks that will be completed to provide or collect information to address the problem.

This project will enable citizen scientists to generate high quality data and gain knowledge of water quality monitoring and data collection. The project will engage four contractors, each of which will monitor one or more tributaries to the NY-NJ Harbor Estuary.
The goals of the project are:

- Goal 1: Raise the visibility level of Citizen Science in the NY--NJ Harbor Estuary
- Goal 2: Improve the data quality of citizen monitoring efforts by training citizen scientists
- Goal 3: Generate high quality data suitable for use by the states' environmental departments, at their discretion
- Goal 4: Make data publicly available for a wide range of users, from the general public to regulatory agencies
- Goal 5: Foster stewardship of shared waterways by engaging local residents directly in environmental data collection, analysis and management.

Data Users

State who will use the data and what decisions or conclusions will be made based on the data. Include any action levels or standards to which the data will be compared.

The primary data users for this project are the public/communities, the Harbor Estuary Program and the USEPA Region 2. Secondary potential users of the data would be the NEIWPCC, NJDEP and NYSDEC. The state environmental departments can obtain valuable datasets that could serve to assess and close data gaps, allow for more focused and targeted monitoring, and guide restoration efforts among other potential uses.

Citizen Science QAPP Template #5
Background and History

Background/History

In this section, state why this work needs to be done, identifying the reasons for conducting the work and/or the lack of information relating to the project.

Designated as an Estuary of National Significance under the Clean Water Act, the New York-New Jersey Harbor Estuary is a complex ecological system in the midst of a major urban center. The NY-NJ HEP was authorized in 1987 by the U.S. Environmental Protection Agency and is one of 28 National Estuary Programs in the country. The Program is an ongoing effort to protect, conserve, and restore the estuary. Participants in the Program include representatives from local, state and federal environmental agencies; scientists; citizens; businesses; and environmentalists, among others.

NEIWPCC is a not-for-profit interstate organization, established by Congress in 1947 to serve and assist its member states individually and collectively by providing coordination, research, public education, training, and leadership in the management and protection of water quality in the New England states and New York. NEIWPCC strives to coordinate activities and forums that encourage cooperation among the states, educate the public about key water quality issues, support research projects, train environmental professionals, and provide overall leadership in the management and protection of water quality. NEIWPCC is a partner of the HEP and received a grant to assist the program in conducting citizen science projects to monitor for pathogen indicators in tributaries to the NY-NJ Harbor Estuary.

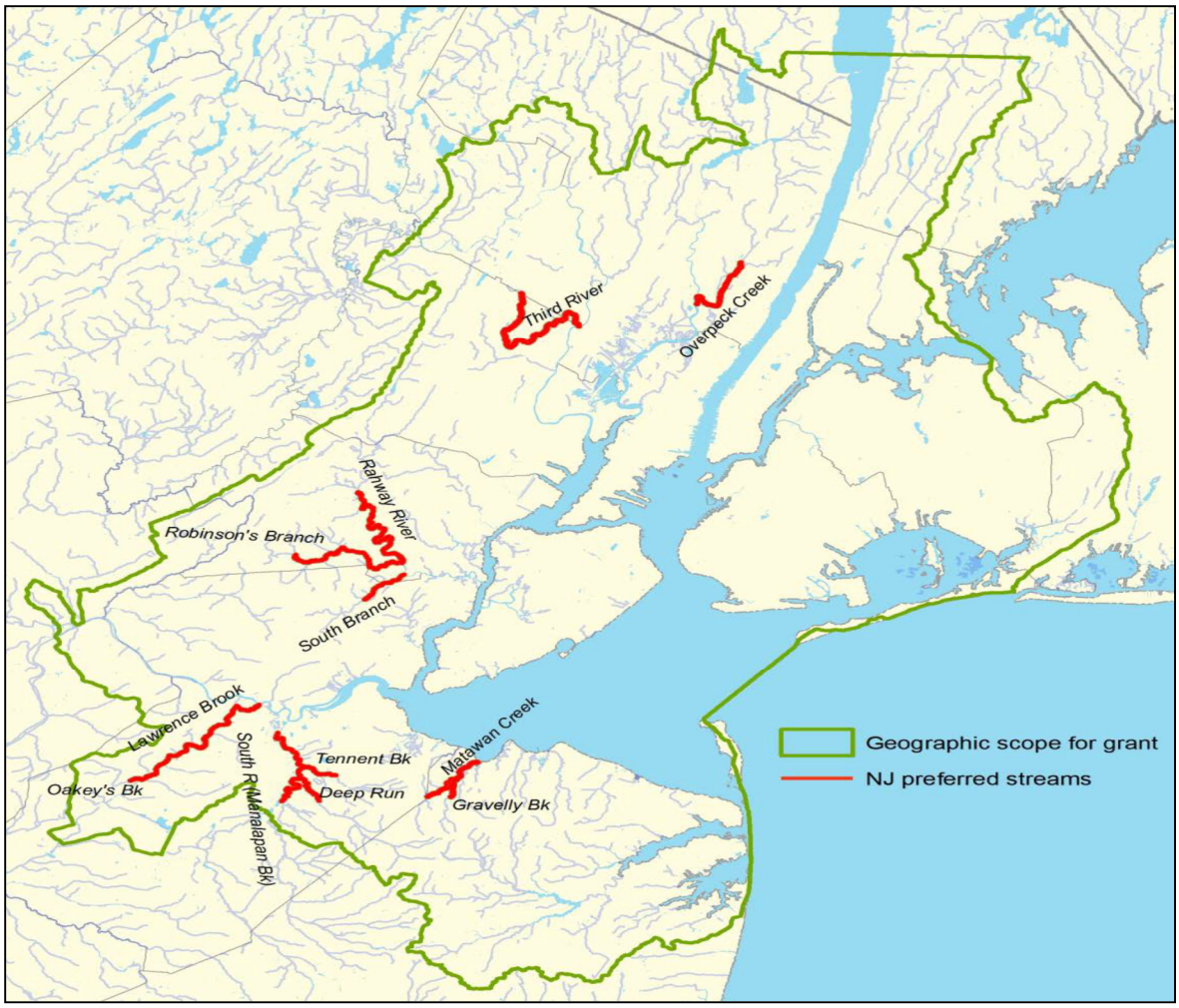
Citizen Science is a fast-growing field in which scientific investigations are conducted by volunteers. Individuals and community groups have long collected data to better understand their local environment and address issues of concern to them. As a results of advancing technology and increased empowerment, citizen science projects have increased greatly over the past decade. These projects have been successful in expanding scientific knowledge, raising people's awareness of their environment, and leveraging change. This project is intended to empower and engage citizen scientists with the tools they will need to produce credible water quality data from water bodies that have an impact in their communities. It will also serve as an opportunity for EPA Region 2 to evaluate the practicality of establishing a citizen science equipment loan program within the Region. A regional monitoring equipment loan program will provide citizen scientists, non-profit and community organizations with reliable tools and methodologies for environmental data collection, while enabling them to improve the health and sustainability of the communities in which they live.

Citizen Science QAPP Template #6
Project Location

Project Location

Provide a description of the site and sampling locations and how they were chosen. Provide the rationale for selecting sample locations and what is going to be sampled. Provide a map showing the location and any other relevant information for the project. Tie this information back to the goals and objectives of the project.

The project will be conducted in tributaries to the NY-NJ Harbor Estuary, within HEP's core area highlighted in the figure below. Pathogen indicators and water quality parameters in surface water will be collected as part of this project. Each contractor will provide specific proposed geographic locations including GPS coordinates in Appendices 5 through 8.



Citizen Science QAPP Template #7
Project Schedule

In the table below, list all major project activities that will be performed during the course of the project. Provide estimates of the timeframe expected for the activities to be conducted and/or completed.

Activities	Organization/Group responsible for activity completion	Timeframe work will be done
Approval of QAPP and Supplemental Addendums	EPA Quality Assurance Officer/NEIWPCC QA Manager	May 2014
Field Instrument and Sample Collection Training	EPA/NJDEP	May 14, 2014
Laboratory and Analytical Training	DESA Laboratory/NJDEP	May 14 and 15, 2014
Data Management Training	EPA	May 15, 2014
Sample Collection	Contractor	June-August 2014
Sample Analysis	Contractor w/EPA DESA Lab assistance	June –August 2014
Data Management	Contractor w/EPA assistance	June – September 2014
Data Evaluation	Contractor w/EPA DESA Lab assistance	September 2014
Submission of Final Report(s)	Contractor	December 15, 2014

Citizen Science QAPP Template #8
Existing Data

For many projects it may be necessary to use data that someone else has already collected, (i.e. existing data). Just because data was collected by a reliable source, such as a peer reviewed journal article, doesn't mean it was collected in a way that your project could use. It is important to perform a check on the data to see how the data was collected and if it is acceptable for the objectives of your project. You must complete this template if your project will be using existing data.

Identify all existing data that will be used for the project, and their originating sources. Specify how the existing data will be used, and the limitations on their use.

- In the **Existing Data** section state what existing data you will use.
- In the **Data Source** section state where that data will come from.
- In the **How Data Will Be Used** section state the need for this data and/or what purpose it will be used for.
- In the **Acceptance Criteria** section state what the requirements are for the data in order for them to be used in the project. For example, if you are looking for temperature data for a water body collected in July, then temperature data collected in June would not be acceptable for the project. Data collected with a certain instrument or by a certain method are also instances where the collected data may not be acceptable for the project.

Existing Data	Data Source	How Data Will Be Used	Acceptance Criteria
N/A	N/A	N/A	N/A

No existing data will be used for this project.

Citizen Science QAPP Template #9
Quality Objectives

Use this template to develop the data quality objectives (DQOs) that define the type, quantity and quality of data needed to answer specific environmental questions, and support proper environmental decisions. The examples provided below are neither inclusive nor appropriate for all projects. Fill in all information appropriate for the project. Complete this template for field, existing data and laboratory activities, if your project includes these components.

Precision is defined as the ability of a measurement to consistently be reproduced. Repeated measurements are usually used to determine precision. In the case of repeated measurements, one would see how close those measurements agree. If repeat measurements will be taken state how close those measurements need to agree by.

Precision:

Field – A duplicate YSI profile will be taken at one sampling location during each sampling event. For each sampling event, the duplicate YSI profile will be taken at a different sampling location. For example, the week 1 sampling event duplicate YSI profile will be taken at sampling location A and the week 2 sampling event duplicate YSI profile will be taken at sampling location D. The temperature readings must agree within ±0.1°C, the salinity readings within ±1.0 0/00 (part per thousand), pH readings within ±0.2 s/u, conductivity readings within ±500 µS/cm and the DO readings within ±0.5 mg/L. GPS units are accurate to within ± 15 meters.

Laboratory – A laboratory fortified blank sample will be performed with each batch (every 20 samples) and evaluated against a certified recovery value.

Bias is defined as any influence in the project that might sway or skew the data in a particular direction. Taking samples from one location where a problem is known to exist, instead of taking samples evenly distributed over a wide area, is one example of how data can be biased. State any biases that could potentially exist and how they will be addressed in the project.

Bias:

Field – This project sampling design is a judgmental design and considered a biased sampling approach. The sampling locations for the project have been selected due to known influences or previous data suggesting influences to a particular area of the waterbody. There will also be a seasonal influence on the data since sampling will occur during June through August. The seasonal variations in temperature and rainfall are known to influence enterococcus numbers.

Representativeness is how well the collected data depicts the true system. Describe how the collected data will accurately represent the population, place, time and/or situation of interest.

Representativeness:
Field- All sampling locations are known to be within tributaries of the NY-NJ Harbor Estuary where pathogen indicator levels are known or suspected to be elevated.

Comparability: is defined as the extent to which data from one data set can be compared directly to another data set. The data sets should have enough common ground, equivalence or similarity to permit a meaningful analysis. State if the data is intended to be compared to other data sets and how this will be achieved.

Comparability:
Field- The same make and model of GPS and YSI units will be used by each team during the training and for field sampling for the duration of the project. Standard units will be applied when recording data from the GPS and YSI (see table in Sensitivity section). GPS data will be recorded in DD.MMSSSS format. The field samplers will also use standard water sample collection methods which are described in Template #10A.

Laboratory – If possible, reagents and materials that will be used for the Enterolert analyses will be from the same lot and vendor for all groups. Also, the Enterolert Method is a standardized method.

Completeness is the amount of data that must be collected in order to achieve the goals and objectives stated for the project. State how much data will need to be collected in order for the project to be considered successful. This can be stated as a total number of samples or a percentage of data collected.

Completeness:
Field and Laboratory - The goal is to collect 100% of the samples; however, 90% would be acceptable for the purposes of the project. If weather or other issues impede a sampling event, the event will be rescheduled.

Sensitivity is essentially the lowest detection limit of a method, instrument or process for each of the measurement parameters of interest. State the sensitivity needed for the instruments, methods or processes used for the project in order to obtain meaningful data.

Sensitivity:
Field- See table below for YSI and GPS sensitivity criteria.

Laboratory - The detection limit for undiluted samples will be 0 Colony Forming Unit per 100 mLs of sample (CFU/100 mL) and 10 CFU/100 mLs for 10% diluted test samples.

Instrument	Range	Sensitivity
YSI 556 MPS*		
Temperature	-5 to 45ºC ± 0.15ºC	0.1ºC
Salinity	0 to 70 0/00 ± 0.1 0/00	0.01 0/00
Conductivity	0 to 200 mS/cm ±0.001 mS/cm	0.001 to 0.1 mS/cm
DO	0 to 50 mg/L ± 0.2 mg/L	0.01 mg/L
pH	0 to 14 units ± 0.2 units	0.01 units
Garmin Montana 650T GPS		± 15 meters

***Note: YSI 556 MPS Range and Sensitivity provided in the above table are manufacturer specified ranges.**

Citizen Science QAPP Template #10A
Data Collection Methods

Sampling Design

For this section, describe and justify the data collection activities. Include location specific information, such as GPS coordinates or landmarks, for the data collection locations. Provide information about the frequency of sampling and the collection of quality control samples. Include information about your plans for sample identification and transportation.

Sampling Schedule

Sampling will occur June through August 2014. There will be 5 sampling events in a 4 week period/per month. Sampling will occur once per week except for one week during the month when sampling will occur twice in one week. Sampling will occur on the same day each week for each group however the 5th sampling event may be scheduled as the contractor deems appropriate with approval of the EPA Region 2 Lab. One sample will be collected per sampling location. The sampling day of the week will be determined in advance by each group and must be approved by EPA Region 2. No weekend days are allowed for sampling and laboratory use. All groups must sample on Monday, Tuesday, Wednesday, or Thursday except any group using the Rocking the Boat Laboratory in the Bronx. Sampling days are restricted to Monday, Tuesday, or Wednesday of each week.

Sampling Locations

The number of sampling locations will be determined by the contractor and submitted as an attachment or addendum to this QAPP. It is the contractor's responsibility to select the number of locations that will allow the samples to be transported to the laboratory for analysis within the established holding times. Holding time will start after the collection of the first sample. GPS coordinates will be included for each sampling location. Handheld GPS units will be provided by EPA and used to find/identify the sampling locations. GPS coordinate readings will be taken each time the sampling team arrives at the sampling location.

Sampling Methodology

Appropriate Personal Protective Equipment (PPE) must be worn when sampling. It is the contractor's responsibility to determine what safety measures and PPE are adequate overall and for each sampling location, as established in the contractor's safety plan. The contractors will have their safety plans on file and available upon request or if audited. When the sampling teams arrive on station they will fill out the data sheets, label the sample bottles and then measure the water quality parameters. Water quality parameters will be collected using the YSI 556 Multi Parameter Sonde (MPS). Temperature, salinity/conductivity, DO, and pH parameters will be measured at each station. In general, sample away from the stream bank in the main current to avoid sampling in stagnant water. Water quality measurements will be taken, ideally between 0.5 to 1 meter below the surface of the water with the sample collector positioned downstream of

the YSI to avoid the dispersion of sediments, etc into the water column. A duplicate set of readings will be collected once per sampling event. The duplicate readings shall be taken at a different sampling location for each sampling event.

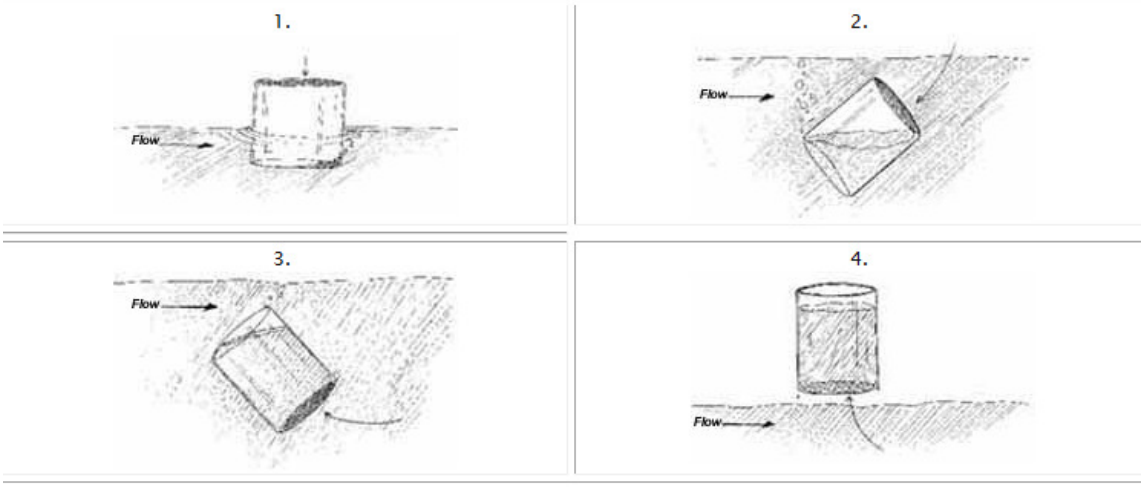
Water samples will then be collected using one of the following methodologies. The method used will be dependent on what the water depth and field/site conditions are when the sampling teams arrive at the station. Samples will be collected into sterilized 120 ml HDPE plastic bottles. **Care must be taken not to touch the cap or the inside of the bottle to avoid contamination. Care must also be taken not to disturb the waterbody substrate.**

Method A:

1. Label the bottle with the information provided in the Sample Labeling, Custody and Transport Section below.
2. Don the appropriate personal protective equipment. A new pair of gloves must be used at each sampling location.
3. *Wading:* (See Figure 5.2 below) Wade in until you reach the appropriate depth for sampling (i.e., at minimum knee depth). Try to disturb as little bottom sediment as possible. In any case, be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream. Collect the water sample on your upstream side, in front of you. With the collector's arms extended to the front, hold the container (cap still on) near its base and downward at a 45-degree angle under the water. Remove the cap while under water and fill the container in one slow sweeping motion in the upstream direction. The mouth of the container should be kept ahead of the collector's hand and the container recapped while it is still submerged.
4. *Boat:* Carefully reach over the side and collect the water sample on the upstream side of the boat. With the collector's arms extended to the front, hold the container (cap still on) near its base and downward at a 45-degree angle under the water. Remove the cap while under water and fill the container in one slow sweeping motion in the upstream direction. The mouth of the container should be kept ahead of the collector's hand and the container recapped while it is still submerged.
5. *Extension pole*: You may also tape your bottle to an extension pole to sample from a bridge or in other situations where the minimum sampling depth cannot be reached. In this case, while wearing a new pair of gloves, secure a capped sampling bottle to the pole, remove the cap just before sampling, and avoid touching the inside of the bottle or the cap. Plunge the pole and sampling bottle (opening upward) into the water. Replace the cap immediately after sampling, remove the bottle from the pole.
6. Note that all samples from a given location should be taken in the same manner throughout the sampling season and noted in the addendum (i.e., wading, from a boat, or using an extension pole).
7. If possible, leave a 1-inch air space or fill to the shoulder of the bottle. Do not fill the bottle completely to the top so that the sample can be shaken just before analysis.
8. Fill in the bottle number and/or site number on the appropriate field data sheet. This is important because it tells the lab coordinator which bottle goes with which site.
9. Seal the samples in a plastic bag and place samples in the cooler on ice for transport to the respective lab.

Figure 5.2

Getting into position to take a water sample
Volunteers should sample in the mail current, facing upstream.



(EPA Volunteer Stream Monitoring: A Methods Manual, Chapter 5 Water Quality Conditions)

Method B:
For water samples to be collected using a weighted sample bottle:

1. Place a sterile 1 liter sample bottle in the center of the weighted apparatus. A new, sterile 1 liter bottle must be used at each station to collect the sample.
2. Remove the cap.
3. Lower the weighted sample bottle via the nylon rope attached to the weight into the water to a minimum depth of 1 foot below the surface and allow it to fill.
4. Retrieve the sample.
5. Pour the sample from the 1 liter bottle into the 120 mL bottle until the water level reaches the shoulder of the 120 mL bottle. Cap the 120 mL bottle. The remaining sample in the 1 liter bottle may be discarded.
6. Fill in the bottle number and/or site number on the appropriate field data sheet. This is important because it tells the lab coordinator which bottle goes with which site.
7. Seal the sample bottle in a plastic bag and place the sample in the cooler on ice.

Sample Labeling, Custody and Transport
Samples will be labeled using the following format:

- Project Name
- Sampling Date
- Station ID
- Sampling Time
- Analysis: Enterolert
- Preservation: Ice

The Station ID will have the following format:
Contractor Designation – Station Number – Month Day Year
The station number must be 2 digits and the month, day and year will also each be 2 digits.

Ex. BXA-01-062314: This sample was collected by the Bronx River at station 1 on June 23, 2014.

The contractor designations are as follows:
Bronx River Alliance: BXA
Friends of the Bonsal Preserve: FBP
Sparkill Creek: SPC
NY/NJ Baykeeper: NNB

Samples will be transported to the laboratory immediately after the sampling event is complete. Samples will be put into gusset bags and sealed before placing them in the cooler. Samples will be transported in a cooler on ice at <10ºC.

Complete all required information in the table below, using additional rows/columns, if necessary. Only a short reference back to the project objective is necessary in the table.

- In the **Matrix** section, state what kind of matrix (air, water, soil, animal/organism) is being sampled during the project.
- In the **# of Sampling Location(s)** section, provide the number of sampling locations.
- In the **# of Samples per Location** section, state if multiple efforts will be made at one location, such as sampling at different depths or taking repeated measurements over a given amount of time (i.e. once/quarter).
- In the **Parameter** section, state what substance will be measured/sampled.
- In the **Field QC Samples** section, state how many and what type of quality control samples will be collected.
- In the **Total Number of Samples** section, state the total number of samples that will be collected for each sampling event or total project including field QC samples.
- In the **Sampling SOP Reference** section, state what specific methods will be used for the sample/monitoring data collection. Attach any SOPs as necessary.
- In the **Project Objective for Sampling and Analysis or Monitoring** section, state why the data will be collected at the particular location, frequency and time.

Matrix	# of Sampling Locations	# of Samples per Location	Parameter	Field QC Samples	Total Number of Samples/ Measurements	Sampling SOP Reference	Project Objective for Sampling and Analysis or Monitoring
Water	To be determined by the contractor, see addendum	1	Enterococcus	None	Up to 15 per sampling event, 15 sampling events for the project	QAPP or EPA Provided SOP, see Template 11	Determine pathogen indicators
Water	To be determined by the contractor, see addendum	1 set of measurements	Temperature, pH, DO, Salinity, Conductivity	1 duplicate set of readings per sampling event	1 set of measurements multiplied by # of sampling locations per sampling event	QAPP or EPA Provided SOP, see Template 11	Record water quality parameters in the selected study area

Attach all SOPs as an appendix to this document.

Water samples collected for enterococcus will be analyzed at the designated lab using IDEXX Enterolert and the procedure described in the Enterolert SOP (see Appendix 1).

Citizen Science QAPP Template #10B
Equipment List and Instrument Calibration

EPA Supplied Field Supplies/Equipment List

Generate a list of all field equipment that will be used for the project.

Garmin Montana 650 T GPS
YSI 556 MPS
120 mL sterilized plastic bottles
1 Liter sterilized plastic bottles
Weighted sampling device with nylon rope
Calibration standards for YSI 556 MPS

Instrument Calibration and Maintenance

In the table below, fill in any calibration or maintenance requirements for the equipment that will be used during the project. State how the calibration information will be documented.

Instrument/Equipment	Calibration Frequency	Maintenance Requirements
Multi-parameter YSI sonde	Calibrate before each use per manufacturer's instructions. Check calibration at the end of each day after use.	As per manufacturer's instructions
Handheld GPS Units	N/A	As per manufacturer's instructions

All calibrations for this project will be documented. Calibration records will be documented on the calibration data sheet provided in Appendix 3. The calibration for the YSI 556 MPS unit will be performed each sampling day before the sampling teams leave for the field in accordance with the manufacturer's instructions. A calibration check will be performed at the end of the day to see if any drift occurred in the YSI 556 MPS unit. Calibration records will include date, time, name of individual doing calibration, and the calibration results themselves. Acceptance criteria for calibration checks will be included on the data sheets. Any data that does not meet the acceptance criteria will be qualified. The Project QA Manager will be responsible for maintaining these records.

Citizen Science QAPP Template #11
Analytical Methods*

Identify all laboratory organization(s) that will provide analytical services for the project. Group by matrix, analytical group/parameter, reporting limit, detection limit, analytical/preparation method SOP, sample volume, containers, preservation requirements, maximum holding time and the laboratory contact information. ***This table only needs to be completed when sample analysis by a laboratory is applicable to the project.**

Matrix	**Analytical Group/Parameter**	**Reporting Limit**	**Detection Limit**	**Analytical & Preparation Method/ SOP Reference**	**Sample Volume**	**Containers (*number, size, type*)**	**Preservation Requirements (*chemical, temperature, light protected)***	**Maximum Holding Time (*preparation/ analysis)***	**Laboratory used for Analysis**
Water	Enterococcus	10 MPN/100 mL	1 MPN/100 mL	IDEXX Enterolert w/Quanti-Tray; ASTM D6503 – 99 (Reapproved 2009) and Budnick et al. 1996; Citizen Science Enterolert Guidance Document, EPA Region 2, 2014	100 mL	120 mL sterile HPDE sample containers	Store on ice after collection and during transport to the laboratory	Analyzed as soon as possible and all samples incubated within 8 hours of collection	USEPA Region 2 Laboratory, Edison, NJ And Rocking the Boat 812 Edgewater Road, Bronx NY

Citizen Science QAPP Template #12
Field Data Sheet

If a field data sheet will be used for the project, attach it below.

The following data sheets to be utilized for this project are provided in the Appendices as listed below:

Field Observation Datasheet – Appendix 2

Field Instrument Calibration Sheet – Appendix 3

Field Data and Chain of Custody Datasheet– Appendix 4

Laboratory Datasheet – Appendix 4

Citizen Science QAPP Template #13
Training and Specialized Experience

Training

In this section, state any required training that an individual involved with the project would need. Also include any refresher trainings that may be conducted.

- In the **Personnel/Group to Be Trained** section, state who will need the specific training and how many people will be trained.
- In the **Description of Training** section, state who will perform the training and what kind of information the trainee will learn.
- In the **Frequency of Training** section, state how many times the training will be conducted during the project.

Personnel/Group to be Trained	Description of Training	Frequency of Training
Contractors (Individuals trained will be listed in the QAPP addendums provided by the contractors)	Proper use of YSI 556 MPS, GPS unit and water sampling equipment. Instruction on lab analyses	Session at the beginning of the sampling season
Contractors (Individuals trained will be listed in the QAPP addendums provided by the contractors)	Data Management and upload of data to WQX/STORET	Session at the beginning of the sampling season

Training will be hosted at the EPA Region 2 Facility in Edison, New Jersey on May 14 and 15, 2014. The tentative schedule for the training is as follows:

Day 1	Day 2
Morning Session	**Morning Session**
YSI 556 MPS and GPS Units	Data Management, Data Review
Field Sampling Techniques	Uploading Data to WQX/STORET
Afternoon Session	**Afternoon Session**
Enterolert Training Part 1 and DOC	Enterolert Training Part 2 and DOC

Specialized Experience

If any individuals have specialized experience that will be utilized by the project please complete the specialized experience table. State who the individual is, what specialized experience they have related to the project and their years of experience.

Person	Specialized Experience	# of Years of Experience
This information is located in the QAPP addendums provided by the individual contractors	This information is located in the QAPP addendums provided by the individual contractors	This information is located in the QAPP addendums provided by the individual contractors

Citizen Science QAPP Template #14
Assessments and Oversight

Assessments and project oversight include various reviews to identify shortcomings or deviations from the project. For each type of assessment, describe procedures for handling QAPP and project deviations encountered during the planned project assessments. Fill in all necessary information.

Assessment Type	Frequency of Assessment	What is Being Assessed	Who will Conduct the Assessment	How Issues or Deviations will be Addressed
Data Checks and Assessments	Biweekly	Field data entries into spreadsheet and WQX/STORET database	Contractors with verification by Project QA/QC Manager for at least 10% of data	Verify with sampling team
On-Site Field Inspection	2 weeks into sampling season and mid-season	Field sampling teams	Project QA/QC Manager	Re-train if necessary and reassess
Technical System Assessments	During the first 3 weeks of sampling	Sample Collection and use of YSI, Laboratory assessment	EPA Region 2 Lab and Field Staff	Field correction at time of assessment, Results discussed with EPA PO and NEIWPCC PM to determine if necessary action (e.g. retraining).

NEIWPCC may implement, at their discretion, various audits or reviews of this project to assess conformance and compliance to the quality assurance project plan in accordance with the NEIWPCC Quality Management Plan.

Citizen Science QAPP Template #15
Data Management

Data Management

Describe the data management processes used throughout the life of the project. Data management includes: recording and transcribing field notes, logging and retrieval of instrument data, transmittal of automated field and laboratory results, data transformation and reduction procedures, compilation of survey results, and data storage, retrieval and security uses throughout the project. Describe the way data handling errors will be controlled (i.e. spot checks for transcription and calculation errors).

All data will be collected on the field/calibration/laboratory datasheets. After each field sampling event, the data will be checked for completeness, missing information or questionable data. The individual responsible for data entry will contact the field sampling team for the missing data and have the team clarify any discrepancies with the data. The data will be entered into preformatted electronic spreadsheets. The preformatted electronic spreadsheets will be supplied by EPA Region 2.

The Project QA Manager will review 10% of the data to verify accuracy of the data entered into the spreadsheet from the field datasheets. The validated and completed electronic spreadsheets will be emailed to Stanley Stephansen of EPA Region 2, or the designated contact, by the Project QA Manager approximately every 2 weeks. Mr. Stephansen or the designated contact will aggregate the data and inform the contractor of any obvious data problems requiring correction. Mr. Stephansen will assist the contractors in testing and loading the project data into WQX/Storet during the initial phases of the project as the data is verified and approved by the Project QA Manager. At the completion of the data collection phase of the project, assistance and training will be provided to the contractor in loading data from the electronic spreadsheets into the WQX/Storet database. The contractor will load all data from the electronic spreadsheets into the WQX/STORET database and confirm the completeness and accuracy of the project data in the WQX/Storet database so that the data may be shared with the public and regulatory agencies. The original datasheets will be stored by the Project Leader for 5 years after the completion of the project in the project file. Copies of the field/lab datasheets will be sent to NEIWPCC as part of the quarterly reports.

Citizen Science QAPP Template #16
Data Review and Usability Determination

Include in this section the types of checks that will be performed at the end of the project to determine if the data collected is usable for achieving the goals of the project. Examples of data checks are provided in the table below.

Data Checks

Field/Lab	Data Management
Monitoring performed per SOPs or QAPP	Data entry and transcription errors
Measurements performed correctly	Calculation/reduction errors
Calibrations performed correctly	Proper data and document storage
Data meets acceptance criteria	Missing data documented
Holding times met	
Evaluate any deviations from QAPP or SOPs to determine the impact to the data and project objectives	

Describe the process used to determine the usability of your project data. If your data review, based on the table above, does not uncover any issues and all of your QC criteria are satisfied, then your data will be assumed to be usable for the intended project objective. However, this is not always the case and so you will need to lay out a process for determining data usability in the event that all QC criteria are not met.

The impact and how you will interpret your results should be addressed in your QAPP using Template #16 Data Review and Usability Determination. Uses will depend on the objectives of your project, if you are in fresh or marine water and established designated uses of your project water body. Listed below is an example of a chart to interpret data based on a project's individual results using *Enterococcus* in marine waters. A rationale based on your project objectives should be used to develop your own categories. In the example below, national water quality standards for primary contact recreation were tied in to the different levels. Your Citizen Science Pathogen Project Your Citizen Science Pathogen Project may use different numerical designations to indicate relevance, but you should have a rationale for selecting the levels.

Category	Range	Description
Low	< 61 MPN/100 mL	61 is the lowest criteria for both Freshwater and Marine Enterococcus WQS Single Sample Maximum Concentration (SSMC) for Primary Contact Designated Beach Site (using 1986 WQS Enterococcus)
Moderate	61 – 104 MPN/100 mL	104 is the SSMC for Marine SSMC and 61 is the SSMC for Freshwater Primary Contact Designated Beach Site
High	105 – 500 MPN/100 mL	500 is the SSMC for Marine SSMC for Infrequently used Full Body Contact Recreation (575 for freshwater)
Very High	501 & > MPN/100 mL	501 would not meet any SSMC for any full body contact in marine water (575 for freshwater)

All data issues identified by the Project QA Manager, including but not limited to the items stated in the Data Checks table above, will be discussed with the Project Leader to determine data usability on a case by case basis. All decisions to allow data that did not fully comply with QC criteria or QAPP requirements will be explained, and any resultant limitations on data use fully discussed in the final project report.

Citizen Science QAPP Template #17
Reporting

Reports

Specify the frequency of all reports, the names of the originators and to whom they will be issued. Itemize what information and records must be included in the report(s). This might include but is not limited to the following:

- Sample collection records
- QC sample records
- Equipment calibration records
- Assessment reports
- Data reconciliation results and associated recommendations/limitations
- Final report of results

Note: If your project will include posting data to a website for public access, state in your description information about how data limitations will be conveyed.

Project Leaders will submit quarterly progress reports to the NEIWPCC Project Manager containing

- Status information for individual tasks, including completed project activities and any outstanding issues that require resolution
- Sample collection records
- QC sample records
- Equipment calibration records
- Data reconciliation results and associated recommendations/limitations.

A final report to NEIWPCC should including the following:

- Summary of Major Project Components
- Data Use and Recommendations
- Project Conclusions

The above **project**-related materials will be kept by NEIWPCC for as long as possible and for a minimum of three years from the date of the final Financial Status Report to EPA, as stipulated by 40 CFR § 31.42.

NEIWPCC Reporting to EPA Region 2

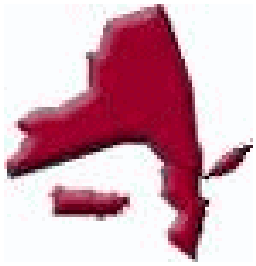
The NEIWPCC Project Manager is responsible for submitting quarterly reports to the EPA Project Officer. The quarterly reports will provide a status update for the project and will include the quarterly reports provided to NEIWPCC by the contractors, which will include sample collection records, QC sample records, equipment calibration records, and data reconciliation results and associated recommendations/limitations. The final report will summarize the quality assurance information provided by the contractors. The rationale for the use of any data that do not fully comply with the quality criteria requirements of the approved QAPP will be fully explained in the final report. The final report will also include any additional documentation required by the EPA grant conditions.

APPENDIX 1
Enterolert Guidance

GUIDANCE DOCUMENT FOR CITIZEN SCIENCE PATHOGEN MONITORING OF ENTEROCOCCI USING IDEXX ENTEROLERT WITH QUANTI-TRAY® 2000

REVISION 3.2

U.S. Environmental Protection Agency
Region 2 Laboratory



Division of Environmental Science & Assessment
2890 Woodbridge Avenue
Edison, NJ 08837

March 2014

Table of Contents (note- page numbers refer to stand alone document)

NOTE: THE MENTION OF BRAND NAMES DOES NOT CONSTITUTE RECOMMENDATION OF A SPECIFIC COMPANY OR PRODUCT BY USEPA, NOR DOES THIS DOCUMENT IMPLY ANY REGULATORY REQUIREMENTS.

GUIDANCE DOCUMENT FOR CITIZEN SCIENCE PATHOGEN MONITORING
ENTEROLERT QUANTI-TRAY® FOR ENTEROCOCCI

1.0 SCOPE AND APPLICATION

Enterolert® is a commercially available enzyme-substrate medium (IDEXX Laboratories, Inc., Westbrook, Maine). This Most Probable Number (MPN) type method is facilitated by use of a specially designed disposable incubation tray called the Quanti-Tray®. When enterococci bacteria utilize an enzyme to metabolize Enterolert's nutrient indicator, the sample fluoresces if *Enterococcus* is present. After 24 hours of incubation at 41°C, an enterococci-positive result causes a blue fluorescence under long-wave ultraviolet light (365 nm).

The Enterolert method is approved for use with fresh and marine ambient waters. The Idexx Enterolert test requires the use of a heating unit called a Quanti-Tray Sealer that seals the incubation tray (Quanti-Tray). Quanti-Tray provides counts from 1 to 200 MPN/100 mL. Quanti-Tray/2000 provides counts from 1 to 2,400 MPN/100 mL. Higher counts can be obtained by diluting the sample.

The detection limit for this method is 1 Most Probable Number (MPN)/100 mL for undiluted samples. This methodology is based on IDEXX product inserts, Burdick et al. 1996, ASTM D6503-99 (Reapproved 2009) and Standard Methods, 21st Ed., 2005, Method 9230D, Approved 1993.

2.0 METHOD DESCRIPTION

This method involves diluting your water sample with sterile deionized water to avoid interference from non-target organisms, adding the Enterolert reagent, sealing the sample mixture in a Quanti-Tray 2000 and placing in an incubator at 41°C for 24-28 hours. After incubation, positive reactions between the reagent and any *Enterococcus* bacteria in your sample are determined by shining a UV light on your sample trays and counting any fluorescing wells. Test results are expressed as Most Probable Number (MPN) of *Enterococcus* CFU per 100 ml by using the IDEXX Quanti-Tray/2000 MPN Table. Special care should be made to conduct this procedure in a suitable environment using aseptic technique (no contamination). A blank should be run with every set of 20 samples processed to ensure that your dilution water, reagent and supplies have not been contaminated. *Enterococcus* measurements using Enterolert are performed as 100 mL samples. If using an undiluted 100 mL sample, the maximum number of bacteria that can be detection is 2420 MPN/ 100 mL. Higher concentrations of *Enterococcus* can be detected by diluting the sample prior to testing. Enterolert can be used on both marine and freshwater samples. For most environmental samples, a 10% sample concentration mixed with 90% sterilized deionized water will provide an adequate test result range (10 – 24200 MPN/ 100 mL). Although a 10% sample volume will be the standard recommended in this procedure, there may be site specific situations where no dilution or even higher sample dilutions are better suited for a particular sampling site.

3.0 DEFINITIONS

1. **Enterococci** are defined as gram-positive bacteria possessing the enzyme ß-D-glucosidase, which cleaves the nutrient indicator and produces fluorescence under a long wavelength (365nm) ultraviolet (UV) light. The presence of this microorganism in water is an indication of fecal contamination and the possible presence of enteric pathogens. Attachment 1 provides additional background information on pathogen indicating bacteria and types of tests used to measure them.

2. **Most Probable Number (MPN)** is a statistical method for determining bacterial density based on the Poisson distribution.

3. **Enterolert** is a product of IDEXX's patented Defined Substrate Technology (DST). The Enterolert test is also referred to as a chromogenic/fluorogenic substrate test. Enterolert can be used as either a presence/absence test or for enumeration of Most Probable Number (MPN) per 100 ml. Enumeration is possible using either multiple tubes (as in a traditional test) or using IDEXX's Quanti-Trays.

4.0 INTERFERENCES

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

5.0 LABORATORY SAFETY AND HAZARDS

1. Each chemical and environmental sample should be regarded as a potential health hazard and exposure to these compounds should be minimized by good laboratory practices, e.g. wear proper protective equipment, safety glasses, gloves and lab coat as the minimum standard for laboratory safety.

2. Always clean all surfaces used in the processing of samples with an anti-bacterial solution (e.g., Chlorox, etc.) before setting up samples, once the Quanti-trays are in the incubator, after a spill of a sample, and at the end of processing samples. Change gloves often to avoid cross contamination.

3. All spent Quanti-Trays containing live bacterial cultures (positive, yellow wells) must be autoclaved prior to disposal**. Develop a partnership with a college, laboratory, public health agency or hospital that can accept and autoclave the spent Quanti-Trays.**

4. Wash hands frequently and make sure your work area is clear and clean.

6.0 EQUIPMENT, SUPPLIES, CONSUMABLES CHECKLIST

Prices for the major equipment items are approximations as of January 2014 to provide information for groups that need to manage budgets for projects using Enterolert. Excluding major equipment, the approximate costs for supplies to run one Quanti-Tray 2000 test (including QC samples) is approximately $8.00 - $10.00 per sample.

6.1 EQUIPMENT

- Incubator, Microbiological, 110V, Target Temperature 40.5 to 41.5 º ($2000)
- Certified incubator thermometer (spirit filled or digital) with 0.1 0C increments ($75.00)
- UV Lamp, 6 Watt, 110 volt (IDEXX WL160, $144.00)
- UV Viewing Cabinet (IDEXX WL160, $205.00)
- Quanti-Tray Sealer Model 2X (IDEXX, WQTS2x-115, $4200)
- Quanti-Tray 97 Well Rubber Sealing Insert (IDEXX, WQTSRBR-2K $74.00)
- Automated Pipette (VWR, 37001-856, $378.00) + extra filters and Pipette Bulb
- Infrared Thermometer to measure sample temperature($100)

6.2 ENTEROLERT REAGENTS – For 200 Sample Analyses

- Sample Collection Vessel, 120 mL, Sterile (IDEXXWV120-200, $110.00)
- Quanti-Tray 2000 (IDEXX, WQT-2K, 2pcks of 100, $173.00 per pack; $346.00 per 200)
- Enterolert Reagent/Snap Packs (WENT200, $1046)

6.3 STERILE DEIONIZED WATER FOR SAMPLE DILUTIONS AND QUALITY CONTROL

- Deionized Water, 90 mL, Sterile One Use (Hardy Diagnostics, #D099, Pck of 50, $114.70)
- Deionized Water, 99 mL, Sterile One Use (Hardy Diagnostics, #D099, Pck of 50, $96.61)
  Note: 90 mL for sample dilution (10%) and 99 mL for QC Blank and Positive control samples.

6.4 QUALITY CONTROL SAMPLES (Refer to Section 11 for more detailed information on QC terms and requirements)

- ***Enterococcus faecalis*, 1000 cfu** (Sigma Aldrich, RQC01777, Pack of 10 pellets, 41.50)
  - Used for positive controls and demonstration of capability testing of analysts using Enterolert Test (Performed on each batch of samples)

- ***Staphylococcus aureus*, 50 cfu** ATCC 6538 (Sigma Aldrich, RQC13002, Pack of 10 Pellets, $41.50)
    - Used as negative control for a gram + bacteria for Enterolert Testing; (Performed on each lot of bottles, reagents, and/or Quanti-Trays)

- ***Escherichia coli*, 1000 cfu**, ATCC 11775 (Sigma Aldrich, RQC01707, Pack of 10 Pellets,$41.50)
    - Used as negative control for a gram – type of bacteria for Enterolert Testing. (Performed on each lot of bottles, reagents, and/or Quanti-Trays)

(As an alternative, IDEXX sells a "QC PACK" for *Enterococcus.* It contains a positive control and two types of negative control organisms (UN3373-WQC-ENT). A set of 3 organisms in triplicate is approximately $153.00.

6.5 CONSUMABLES, LAB SUPPLIES, SAFETY EQUIPMENT

- Chlorox Disinfecting Spray
- Paper Towels
- Pipettes, 10 mL Sterile, Individually Wrapped, One Time Use
- Pipettes, 25 mL Sterile, Individually Wrapped, One Time Use
- Biohazard Bags
- Contamination Label/Autoclave Tape
- Data Sheet
- Pen with black ink, Indelible Marker, Waterproof Laser Labels
- Lab Coat (Disposable is fine), Safety Glasses, Disposable Gloves
- Sample Bottle Outer Plastic Bags (i.e. Ziplock bags)
- Cooler and Ice

7.0 SAMPLE HANDLING, PRESERVATION, STORAGE AND HOLDING TIME

Samples must be collected in pre-sterilized containers. For most projects, a 120 mL non fluorescing plastic bottle (IDEXX WV120 or equivalent) is sufficient. Samples should be placed on ice as soon as possible after collection. Fill the sample bottle to the neck (just before the threads) to allow for mixing the sample by shaking in the laboratory. Immediately after collection, a plastic bag (Ziploc will work) should be placed around each sample bottle to prevent contamination from other sample bottles, or from ice or the cooler itself prior to placing on ice.

Temperature should be maintained at <10 ^{o}C and measured upon arrival to the testing facility using an infrared thermometer. If the commute time to the testing facility is short, evidence of icing is sufficient. Samples should be returned to the laboratory within 6 hours of collection and must be placed in the incubator within 8 hours of collection. Always start analysis with the sample collected earliest in the day.

If residual chlorine is expected (but should not be present in ambient surface water samples) use sterile sample bottles preloaded with sodium thiosulfate (powder or tablet) to neutralize the residual chlorine.

8.0 PREPARING THE ENTEROLERT REAGENT AND TEST SAMPLES

Please refer to Figure 1 for a graphic representation of some of the steps listed below.

1. Turn on the Quanti-Tray sealer to warm it up. This takes approximately 10 minutes.

2. Make sure the temperature of your incubator is 40.5 °C – 41.5° C (read certified incubator thermometer in chamber, do not rely on any digital display on the unit).

3. Wipe down your workspace with disinfectant spray.

4. Wash your hands and put on disposable gloves.

5. Label a sterile deionized water sample bottle that contains 90 mL (test sample) of sterile deionized water bottles that corresponds to each sample collected.

6. Label a sterile deionized water sample bottle that contains 99 mL of sterile deionized water, IDEXX reagent and *Enterococcus* faecalis (positive control), refer to Section 11 for more details, and another 99 mL of sterile deionized water for use as your blank

7. Label a Quanti-Tray 2000 to match each location at which a sample was collected and your two QC samples. If not using labels, write only on the back of the trays with a sharpie permanent marker to keep from puncturing tray. The information on the back of the trays (or label) should include Station ID, Analysis Date, Dilution, or any QC designation (Positive Control or Blank). **It is recommended that water proof labels be prepared in advance to expedite sample processing.**

8. Fill out Laboratory Bench Sheet (Attachment 2).

9. Remove samples from cooler and arrange on bench in order of collection time (start with the earliest time). This is the order that the samples should be recorded on the Laboratory Bench Sheet as well. Work with one sample at a time.

10. Arrange your test sample bottles and sterile deionized dilution bottles in the order that you will prepare dilutions and analyze the samples. When handling sample bottles and sterile deionized water bottles, never touch the top of the bottle or inside the lid with your fingers after the lid has been removed. Only the outside of lid and the sides and bottom of the bottle can be touched.

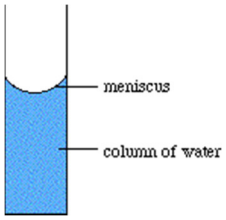
11. Gently invert the bottles containing the test samples 25 times to suspend anything that may have settled to the bottom of the sample before making dilutions.

12. Sample Dilutions: After mixing approximately 25 times, open the lid of the sample container (if using whirl-pak sample bags open by bending the yellow tabs out and unroll the top. Then pull on the white tabs only to open the top of the bag. Do not touch the bag at the top or inside the top.)

    a. Tear open or peel back the protective sleeve from the **top** of the pipette (do not touch the tip with your fingers or have the tip come in contact with the bench top).

    b. Insert the pipette into the automated pipette or pipette bulb, then place the tip into the water sample (this should be done immediately after inverting your water sample approximately 25 times to ensure even distribution of the potential bacteria in the sample). You should practice picking up and dispensing until you are comfortable using the automated or manual pipette prior to actually working with a real sample.

    c. Extract exactly 10 ml of the water sample into the pipette with the bottom of meniscus at 10 ml mark (see diagram to the right).



    d. Place the pipette inside of the top of the opened deionized water bottle and discharge the sample water into the sterile 90 mL mixing bottle of deionized water.

    e. Close the lid on the deionized water with 10 mLs of sample and remix 25 times. This is your 10% sample dilution.

    f. Dispose of pipette into a dedicated waste container and repeat above steps until all sample sites have been completed (remember to analyze to samples in the order of collection (early to later). Pipettes must only be used once and then disposed.

    g. As a reminder, all test samples are prepared in 90 mLs of sterile deionized water while the Blank and Positive Control will be prepared in a 100 mL sterile deionized water container (99 mL nominal).

13. Add one packet of Enterolert reagent to each sample bottle including the control and positive control. Hold the snap packs facing away from your face and pop open the top.

    a. Snap the bottle lids back on and gently shake or invert in a gentle arc until all the powder dissolves and the bubbles disappear. The Enterolert reagent has a typical light yellow color. Your sample is now ready for placement into the Quanti-Tray 2000 heating unit.

9.0 PREPARING THE QUANTI-TRAYS WITH THE REAGENT/SAMPLE MIXTURE

1. After mixing sample by inverting the bottle approximately 25 times, pour the reagent/sample mixture into its corresponding Quanti-Tray while avoiding contact with the foil tab.

2. Use one hand to hold a Quanti-Tray 2000™ upright with the well side facing the palm. Squeeze the upper part of the Quanti-Tray 2000™ so that it bends towards the palm in a U-Shape. Gently pull the foil tab to separate the foil from the tray. Avoid touching the inside of the foil or tray.

3. After adding sample, tap the end of the Quanti-Tray with the small wells closest to your bench top to release any air bubbles. Allow foam to settle for a few seconds.

4. Place the sample-filled tray into the rubber insert of the Quanti-Tray Sealer with the plastic well side facing down. Check that the Green Light is lit on the sealer indicating that the proper temperature of the sealer is adequate.

5. Place the rubber sealer form with the tray on top the inlet hopper of the sealer machine with the large reservoir cell placed the farthest away from the machine and the **small cells** entering the machine first.

6. Gently feed the tray and holder into the machine gently. The sealing machine will automatically grab the rubber form and tray and draw them through the sealer.

7. Retrieve the sealed tray on the other side of the machine (don't pull on it).

8. Check to see that all plastic wells on the tray are filled with the sample. A well is considered full if it is at least ½ full. Up to 2 wells can even be completely empty and the tray can still be used as the results will still be statistically valid.

9. If there is a problem sealing the tray or there are more than 2 empty cells you will have to discard tray and prepare a new sample/reagent mixture from the reserved sample and pour into a new tray.

10. Set aside your sealed Quanti-Trays and place all together into the incubator at 41°C. It is best to minimize the number of times you open the incubator door so as not to disturb the temperature setting.

11. The trays can be stacked but should be spread out as much as possible in the incubator.

12. Note on your data sheet the time you placed the trays into the incubator. They will stay there for a MINIMUM of 24 hours but no longer than 28 hours.

13. Dispose of gloves, wrappers, pipettes, and sample bottles into a biohazard bag and seal with autoclave tape and contamination tape.

14. Wash hands with anti-bacterial soap and wipe down the lab bench with disinfectant spray.

15. Turn off the Quanti-Tray Sealer

Figure 1. 120 ML sample bottle and major components to prepare Quanti-Tray 2000

10.0 READING THE RESULTS (24-28 HOURS LATER)

1. Take the Quanti-Trays out of the incubator 24 hours after they were put in (but no later than 28 hours). Turn UV light on and darken the room if possible. If using the IDEXX UV light with housing, a darkened room is not necessary. Enterolert results are definitive at 24–28 hours. In addition, positives observed before 24 hours and negatives observed after 28 hours are also valid

2. Shine UV light on each tray or place in UV light holder box available from IDEXX. You are looking for wells fluorescing blue. Wells that are not blue or do not fluoresce ARE NOT counted as a positive result. It may help to look at your blank if you are having difficulty determining what is a positive result in some of your test samples.

3. If any wells in the Blank are positive, then there was a contamination of your lab procedure or reagents. Results should be qualified due to blank contamination. Blanks are considered contaminated if results are greater than 10 MPN/mL if associated sample concentrations are prepared as 10% solutions.

Count large and small positive wells that:

a. Fluoresce under a long-wave ultraviolet light as enterococci.
b. A permanent marker may be used to place a slash over each positive well to be used as a confirmation of initial counts under the UV light source.
c. Off-color fluorescence is not counted as positive results.
d. The large overflow well at the top of the tray is counted as a large well.
e. The Quanti-Tray 2000 has 49 large wells **(including the one large well at the top of the tray)** and 48 small wells on the opposite side of the tray (Attachment 3). Record 2 numbers for each tray on the data sheet:- # of Large Wells Positive + # Small Wells positive on the data sheet (Attachment 2).

4. Refer to the MPN table (Attachment 3) to obtain results. For a 1:10 dilution, multiply the MPN table result by 10 to obtain the final MPN/100 mL.

5. Record the results on the data sheets and record the blank and positive control results as well (Attachment 2). Record your name and the time and date that the tray results were read and recorded on the data sheet (Attachment 2).

6. Dispose of used trays in biohazard bags/containers; wash hands.

7. Review Data Sheet

11.0 QUALITY ASSURANCE AND QUALITY CONTROL FOR EACH BATCH OF SAMPLES

Quality control (QC) is a set of procedures intended to ensure that collected data adheres to a defined set of quality criteria and data uses. QC elements of this guidance are in place to ensure data is reliable, collected in adherence to established methods and meet the objectives of the Quality Assurance Project Plan.

There will be two types of Quality Control Samples that must be analyzed by the Citizen Scientists on each batch of samples tested (up to 20 samples)

11.1 BLANK

100 mL (99 mL nominal of commercially prepared sterile deionized water and IDEXX reagent)

The blank will provide information regarding aseptic techniques and materials. There should be no fluorescing wells after the incubation period or the associated test data must be qualified as an estimated value or invalidated if above the reporting limit.

11.2 POSITIVE ANALYTICAL QUALITY CONTROL SAMPLE

100 mL (99 mL nominal volume of commercially prepared sterile deionized water, IDEXX reagent + *Enterococcus faecalis* QC pellet)

We are going to quantify our positive control, sometimes referred to in the laboratory as a Laboratory Fortified Blank (LFB), Blank Spike (BS) or Analytical Quality Control Sample (AQC). This sample will also serve as our Positive Control. The positive control is deionized water, IDEXX reagent, and a purchased certified pellet of Enterococcus *bacteria with an expected density after hydrating in 100 mLs of water. The positive control should yield fluorescing wells after incubation. This control indicates the ability to detect/measure the target organism in the test sample. If there are no fluorescing wells then the associated sample data is invalid. The manufacturer of the positive control will provide the true value and acceptance range for the Enterococcus sample. The % Recovery of the Positive Control should be between 50 – 200% of the true value.*

Percent Recovery Formula for Positive Analytical Quality Control Sample:

$$\% \text{ Recovery} = \frac{\text{Measured Result}}{\text{True Value}} \times 100$$

11.2.1 How to prepare Positive Control Sample

1. Remove the positive control *Enterococcus* bacterial pellet from the freezer (-20 to -70 ^{o}C).

2. Do not touch the pellet but merely open packaging and let the pellet drop into the 99 mL sterile Deionized water with the IDEXX reagent already dissolved.

3 Swirl the sample and allow to stand for 10 – 15 minutes. The pellet should completely dissolve.

4 After pellet is dissolved, invert the sample approximately 25 times to completely mix and pour into Quanti-Tray and seal.

5 The QC samples should be placed in Quanti-Trays within 30 minutes of hydration. Record Vendor, Lot number and expiration date of the positive control and lot number and expiration date of sterile deionized water on your data sheet (Attachment 2). Also, record the true value and recovery limits from the certificate of analysis for the *Enterococcus faecalis* positive control on the data sheet.

11.3 OTHER QC ACTIVITIES

There are a few other quality control activities that will need to be performed on a less frequent basis. These include checking the sterility of sample bottles and Quanti-Trays using a Tryptic Soy Broth Solution, pH of IDEXX Enterolert media, and Negative Controls using two types of bacteria as Negative Controls. All of these QA/QC requirements will be performed by USEPA and they only need to be performed on any new LOT of the IDEXX supplies and reagents. Listed below is a summary of other QC activities that will be performed by the USEPA Region 2 Laboratory and shared with the appropriate Citizen Science Organizations.

Negative Controls

1. Gram (+) 99 mL sterile deionized water, IDEXX reagent, *Staphylococcus aureus*
2. Gram (-) 99 mL sterile deionized water, IDEXX reagent, *Escherichia coli*

The negative controls are used to document the effect of non- Enterococcus bacteria with the IDEXX reagent. The negative controls should not have any fluorescing wells or the test must be repeated and/or new reagent purchased.

3. Sterility Checks and pH measurement on IDEXX media

The sterility checks of sample bottles, IDEXX trays, and pH of IDEXX media will also be performed on each lot of new items by USEPA Region 2. Sterility checks are performed to ensure individual lots of bottles, media, and Quanti-Trays are sterile prior to use. The IDEXX reagent pH is checked on each lot to ensure appropriate conditions for testing.

11.4 DEMONSTRATION OF CAPABILITY (DOC)

A Demonstration of Capability (DOC) should be conducted by any person analyzing samples for *Enterococcus* using Enterolert. The DOC is used to demonstrate proficiency in performing a method properly and is conducted by analyzing four quantitative positive control samples (preparation of each DOC samples are the same procedure as highlighted in Section 11.2.1 above). The percent recovery of the average of the four quantitative positive controls is divided by the true value and then multiplied by 100 to calculate the % recovery. The true value is provided by the manufacturer of the Enterococcus bacteria. An acceptable percent recovery should be 50-200% of the true value. If your DOC is outside this range, the SOP should be reread, the reason for the excursion should be evaluated, and the DOC repeated.

A successful DOC should be performed before any actual test samples are analyzed. A DOC will be provided to anyone that will be part of the laboratory team for each grantee during training before the summer sampling. Also, a citizen scientist can prepare Enterolert samples if they are under the direct supervision of someone who has a valid DOC on file.

12.0 DATA EVALUATION

- Review your data sheet/field data sheet for completeness.
- Make sure the results and dilutions are accounted for correctly in the results
- Evaluate the blank and make sure contamination is not introduced
- Did E*nterococcus* grow in your positive control and was that growth within the acceptance limits provided by the manufacturer?

- Was the test performed in accordance with your established procedures? If not, note the deviation on the data sheet.

- Were there any other deviations during sampling and analysis which would affect the quality of the data?

- The most important thing to do is to DOCUMENT, DOCUMENT, DOCUMENT! Observations that may not seem important at the time of sampling or analysis may be crucial when it comes to evaluating your data.

12.1 MY SAMPLING EVENT IS COMPLETED AND MY DATA IS COLLECTED, NOW WHAT?

Your data sheet should be evaluated for completeness and any deviations to your SOP/QAPP should be identified and noted on the data sheet. Laboratory's use qualifiers as a means to rapidly indicate confidence and usability of a data to your data users (especially helpful for electronic reporting of data). Qualifiers are typically letter designations which are attached to individual data points to relay some additional information regarding the usability of the data. There are potentially many data qualifiers, but for the purpose of Citizen Science, there are 3 main ones that should be used at a minimum.

"U" Qualifier
This qualifier is attached to a result to indicate that the organism was not detected at the prescribed reporting limit of the method. For E*nterococcus*, using Enterolert 2000, the reporting limit for a 10% test sample is"10 MPN/100 mL". Therefore, if no wells fluoresce, then your result is not zero, but "10U". Sample dilution will affect your reporting limit. If you dilute your sample to 1%, then the reporting limit would correspond with a change from 10 to 100 MPN/100 mL.

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

- *Missed holding times* – Methods are developed with maximum holding times. If exceeded, then the confidence in the data is not very good.

- *Blank contamination greater than the reporting limit* – If you have *Enterococcus* growing in your blank, then reassess your procedures and improve your aseptic technique before your next set of samples.

- *No growth in your positive control* – Positive controls should show some growth. If there is no growth at all, then reassess procedures and evaluate your *Enterococcus* positive control culture to make sure it is viable.

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

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

- There is blank contamination, but it is below the laboratory reporting limit.
- The positive control was positive but the percent recovery measured during the test was either above or below the QC acceptance limits for percent recovery.
- Incubator temperature was 41.6 ^{o}C, or 0.1 ^{o}C above the specified maximum range. Of course major deviations of incubator temperature may require an "R" designation.
- Samples not stored on ice and/or temperature above 10.0 ^{o}C. Extreme variations in temperature may require the data be rejected as well.
- Other SOP deficiencies or method anomalies.

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

12.2 SINGLE MAXIMUM CONCENTRATION VERSUS A GEOMETRIC MEAN

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

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

How do you calculate a geometric mean? The easiest way to think of the geometric mean is that it is **the average of the logarithmic values, converted back to a base 10 number.**

However, the actual formula and definition of the geometric mean is that it is the *n*th root of the product of n numbers, or:

Geometric Mean = n-th root of $(X_1)(X_2)...(X_n)$.

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

Let's use some *Enterococcus* values from an area we will call Station 1 which was sampled five times for the Month of June.

June 1: 15 MPN/100 mL
June 8: 120 MPN/100 mL
June 15: 1 MPN/100 mL
June 22: 300 MPN/100 mL
June 29: 70 MPN/100 mL

What is the geometric mean of these five observations?

Geometric Mean = 5th root of (15)(120)(1)(300)(70)

= 5th root of 37,800000
= 32.8 *Enterococcus* MPN/100 mL

So the geometric mean for these five values from Station 1, in June is 32.8 MPN/100 mL. This value can then be compared to the WQS or project specific Geometric Mean identified for your QAPP. If you obtain a Non Detect in one of your individual measurements, i.e. 10 MPN/100 mL, use 10 as your result for the purpose of the GM calculation.

There are many "root" calculators on line that you can use to calculate your geometric mean. Excel can be used as well. It is a good idea to validate your results by a manual calculation initially to check the accuracy of your Excel spreadsheet or online calculator values.

12.3 INTERPRETATION OF DATA AND IMPACTS

The impact and how you will interpret your results should be addressed in your QAPP. Uses will depend on the objectives of your project, if you are in fresh or marine water and established designated uses of your project water body. Listed below is an example of a chart to interpret data based on a projects individual results using *Enterococcus* in marine waters. A rationale based on your project objectives should be used to develop your own categories. In the example below, national water quality standards for primary contact recreation were tied in to the different levels. Your citizen science pathogen project may use different numerical designations to indicate relevance, but you should have a rationale for selecting the levels. The colored dots can be used to indicate relative concentrations when plotting on a map.

<61 CFU/100 mL	LOW	61 is Lowest Criteria for both Freshwater and Marine Enterococcus WQS Single Sample Maximum Conc. (SSMC) for Primary Contact Designated Beach Site (using 1986 WQS Enterococcus)
61-104 CFU/100 mL	MODERATE	104 is the SSMC for Marine and 61 is the SSMC for Freshwater Primary Contact Designated Beach Site
105-500 CFU/100 mL	HIGH	500 is the SSMC for Marine SSMC for Infrequently used Full Body Contact Recreation (575 for freshwater)
501 & > CFU/100 mL	VERY HIGH	501 would not meet any SSMC for any full body contact in marine water (575 for freshwater)

12.4 QUESTIONS REGARDING THIS GUIDANCE DOCUMENT?

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ferretti.jim@epa.gov

13.0 REFERENCES:

1. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 2007, Standard methods for the analysis of water and wastewater: Washington, D.C. American Public Health Association, Section 9230D.

2. Budnick, G.E., Howard, R.T., and Mayo, D.R. 1996. Evaluation of Enterolert for Enumeration of Enterococci in Recreational Waters. Applied and Environmental Microbiology, Vol 62, No. 10 pp 3881 – 3884.

3. Standard Test Method for Enterococci in Water Using Enterolert. ASTM D6503 – 99 (Reapproved 2009).

4. "Enterolert from IDEXX" product instructions (most recent)

5. "Quanti-Tray/2000Enterolert from IDEXX" product instructions (most recent)

ATTACHMENT - Pathogens as indicators of water quality

Pathogenic microorganisms are associated with fecal waste and can cause a variety of diseases (typhoid, cholera, Cryptosporosis, etc) either through ingestion/contact with contaminated water or ingestion of shellfish. There are many different types of pathogens that are dangerous to humans, including bacteria, viruses, and protozoa. Measuring all of these potential harmful organisms is not practical, cost effective, or methods are complicated. Instead, specific surrogate bacteria (i.e., Fecal Coliforms, *E. coli, and Enterococcus* sp) that can be cultured or detected easily and can be related to risk of human illness are used as "indicator" bacteria, because their presence indicates that fecal contamination may have occurred.

Members from two bacteria groups, coliforms and fecal streptococci are commonly used as indicators of possible fecal contamination because they are commonly found in human and animal intestines and ultimately waste or fecal material. Although these indicator bacteria are generally not harmful to any great degree themselves, they are used to correlate with the possible presence of more harmful, pathogenic microorganisms. The higher the number of indicator bacteria would increase the risk of finding increasingly more harmful assemblages of pathogenic or disease causing organisms associated with fecal contamination.

CLASSIFICATION OF BACTERIA

Bacteria can be classified into 3 basic types based on structure (Figure 1):

Cocci – Round

Spirilli – Corkscrew

Bacillus – Rod shaped

Gram staining is a method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative). The term "Gram" was named after the scientist who discovered the staining technique to differentiate between the two types of membranes which determine whether a bacterium is gram negative or gram positive. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique.

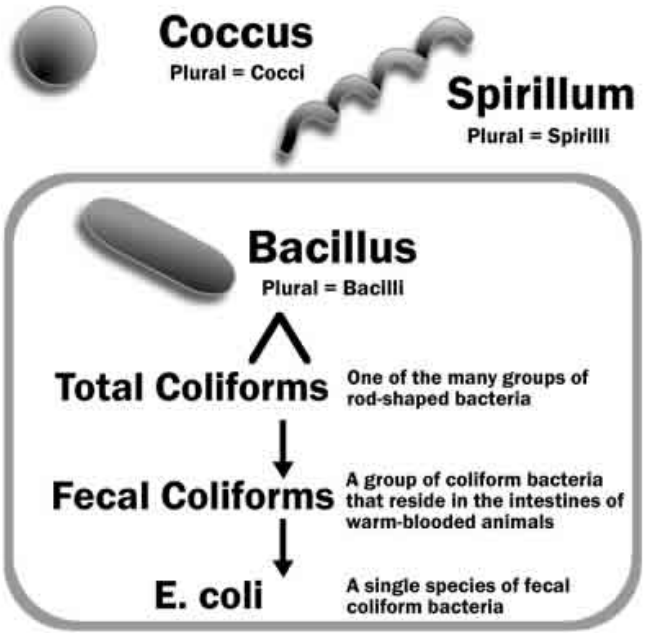

Figure 1. Commonly Used Indicator Organisms for Pathogen Water Quality Assessments and their relationships

The most commonly tested fecal bacteria indicators are total coliforms, fecal coliforms, Escherichia coli (or *E. coli*) and *Enterococcus* (a type of fecal streptococci) (Figure 2).

Total coliforms are a group of bacteria that are widespread in nature. All members of the total coliform group can occur in human feces, bust some can also be present in animal manure, soil, submerged wood and even outside the human body. The usefulness of total coliforms as an indicator of fecal contamination depends on the extent to which the bacteria species found are fecal and human in origin. Public health offices have used total coliforms and fecal coliforms as indicator organisms since the 1920's. For recreational waters, including bathing beaches, total coliforms are no longer recommended as an indicator.

Fecal coliforms are a subset of total coliform bacteria and are more fecal specific in origin. However, even this group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* are commonly associated with textile and pulp and paper wastes. Fecal coliform measurements are still used in many state standards for pathogens based on the designated use of the water body (primary contact, secondary contact, etc.)

Escherichia coli or *E. coli* is a type or subset of fecal coliform, or a specific fecal coliform commonly found in the intestines of warm blooded animals, including humans. The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination.

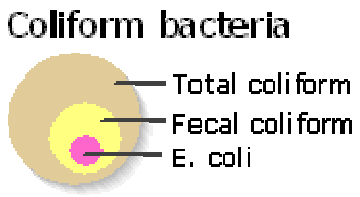

Figure 2. Relationship among Total Coliform, Fecal Coliform, and *E. coli*

Enterococci are a subgroup within the fecal streptococcus group (not a coliform). Enterococci are distinguished by their ability to survive in salt water and they have been found to more closely mimic many pathogens than do the other indicator organisms. Enterococci are typically more human-specific than the larger fecal streptococcus group. Enterococci are an indicator organism in national and many state water quality standards in marine and freshwater recreational waters.

States are allowed to adopt the indicator organism which works best for their applications. National Water Quality Standards are established for these indicator organisms and levels are based on standards and the designated uses of a water body. These values vary state by state. There are many possible classifications for waters in your state. Drinking water supplies and primary contact recreation will have more stringent WQS than a water body that is classified for navigation or non-contact recreation.

The indicator bacteria you choose (Total coliforms, Fecal Coliforms, *E. coli,* or *Enterococcus*) will depend on what is the uses of your data and the scope of your project. If you want to know whether your water body is meeting its state specified water quality standards, then mimicking those bacteria prescribed by that state would be appropriate. If you want to know whether swimming or other contact with a water body poses a health risk, then most of the indicator organisms described above would be appropriate. Nationally, EPA recommends enterococci as the best indicator of health risk in salt water used for recreation and as a useful indicator in freshwater as well. Typically states will designate a use for parts or all of a water body and have numerical standards and a specific indicator organism.

Enterococcus is a fecal indicating bacteria that lives in the intestines of humans and other warm-blooded animals. *Enterococcus* ("Enterococci") counts are useful as a water quality indicator due to their abundance in human sewage, correlation with many human pathogens and low abundance in sewage free environments. The United States Environmental Protection Agency (EPA) reports Enterococci counts as colonies (or cells) per 100 mL of water.

There are multiple factors that determine public health risk to people who have primary contact with water, such as swimmers. For this project, using enterococci, we have based assessment of acceptable water quality on the 2012 Federal Recreational Water Quality Criteria from the US EPA.

The National Water Quality Standard for *Enterococcus* in Primary Contact waters is a single sample maximum of 104 colony forming units/ per 100 mL sample (CFU/100 mL) in marine water and 61 CFU/100 mL in freshwater. Water Quality Standards are adopted based on 'acceptable risk". The enterococci water quality standard is based on an illness rate of 32 per 1000 swimmers. The single sample maximum should not be exceeded or there is a greater risk of pathogens. This does not mean you will contract a disease, but EPA has established risk levels

based on indicator bacteria and correlation with human health data. Standards are commonly calculated as geometric means over a longer period of time as a target not to exceed value. For example, for primary contact, a geometric mean of five individual tests conducted over a monthly period should not exceed 35 CFU/100 in marine samples and 33 CFU/100 mL in freshwater. What is a Geometric Mean you ask?

Means are mathematical formulations used to characterize the central tendency of a set of numbers. Most people are familiar with the "arithmetic mean", which is also commonly called an average. Geometric mean is different than an arithmetic mean or average. A geometric mean in mathematical terms is the average of the logarithmic values of a data set, converted back to a base 10 number.

For bacterial water quality standard evaluation, a geometric mean is a popular metric for regulators who monitor swimming beaches and shellfish areas. In addition to single maximum concentrations of a bacteria, often, the data must be summarized as a "geometric mean" (a type of average) of all the test results obtained during a reporting period. Typically, public health regulations identify a precise geometric mean concentration at which shellfish beds or swimming beaches must be closed.

A geometric mean, unlike an arithmetic mean, tends to dampen the effect of very high or low values, which might bias the mean if a straight average (arithmetic mean) were calculated. This is helpful when analyzing bacteria concentrations, because levels may vary anywhere from 10 to 10,000 fold over a given period.

The numerical water quality standards will be slightly different for different organisms (i.e., Single Sample Maximum for *E. Coli* in Freshwater primary contact waters is 126 CFU/100 mL), so it's important to know which organisms you are using to compare to the correct water quality standard.

COMMON METHODS TO MEASURE INDICATOR BACTERIA

There are three common types of methods to measure pathogen indicator bacteria:

Membrane Filtration, Multiple-Tube Fermentation, and Defined Substrate MPN Tests (Enterolert and Colilert).

Membrane Filtration involves filtering 10 to 100 mL of sample through a 0.45 micron filter and placing the filter on a selective nutrient medium in a petri dish and incubating the petri dishes at a specific temperature for approximately 22-26 hours. Selective media is media that has chemicals that will only allow the target bacteria of interest to grow. This is a type of culture method. After 24 hours, if your target organism is in the sample, a round, color specific colony will grow (Figure 3). The individual colonies are counted after 24 hours and results are expressed as colonies per 100 mL of sample.

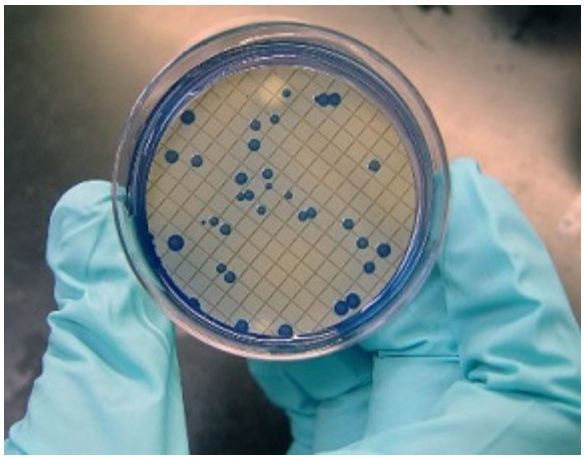

Figure 3. Petri Dish containing blue fecal coliform colonies after 24 hour incubation on m-FC media.

Multiple-Tube Fermentation involves adding specified quantities of the test sample to tubes containing a nutrient broth and incubated at a specific temperature for approximately 24 hours and then looking for the development of gas bubbles and growth indicating a positive presence of your target organism (Figure 4). A statistical formula is used to develop a table to calculate the result as the Most Probable Number per 100 mL (MPN/100 mL). This technique is the most time consuming of the three discussed here.

Figure 4. Test tube rack containing Lauryl Tryptose Broth used to measure bacteria growth and gas from coliform bacteria.

Defined Substrate Tests (i.e., Enterolert) is used for the detection of enterococci bacteria (Enterococci) species such as *E. faecium* and *E. faecalis* in either fresh or marine waters. This technology utilizes a nutrient indicator that fluoresces when metabolized by enterococci. The prepackaged reagent is added to a 100 mL water sample and then sealed into a tray with large and small wells and incubated for 24-28 hours. The number of blue fluorescing wells from the tray is recorded and a concentration of Enterococci is calculated from a table. This type of test can be used to measure total coliforms and E. coli as well (Colilert).

IDEXX's Quanti-Tray/2000 is based on the same statistical model as the traditional 15-tube serial dilution (Multiple Tube Fermentation Test) but is much more rapid to complete than a

standard Multiple Tube Fermentation test by automatically distributing the sample into 97 wells of two different sizes (Figures 5 and 6). Quanti-Tray/2000 yields a counting range of 1–2,419 MPN/100 mL. This counting range can be raised by diluting the test sample before testing.

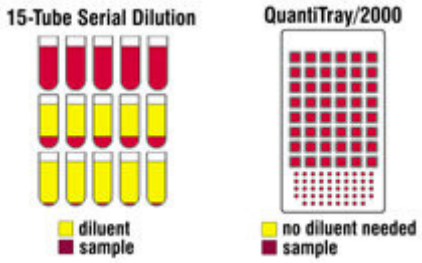

Figure 5. Comparison of Multiple Tube Fermentation Test and Enterolert w/ Quant-Tray 2000

There is no right or wrong method in most cases to measure indicator bacteria. This Guidance describes Enterolert, which is the Defined Substrate Test. Although each method described above has its own beneficial applications, the choice of which method should be based on the objectives or your project, data needs (i.e. specific organism needed or in your states WQS), budget, and other factors such as ease of use should be evaluated. Defined Substrate Tests such as Enterolert or Colilert are very well suited to citizen groups due to ease of use, shelf life of reagents, clear endpoints, and minimal sample handling. The biggest drawback is the one time cost of the tray sealer.

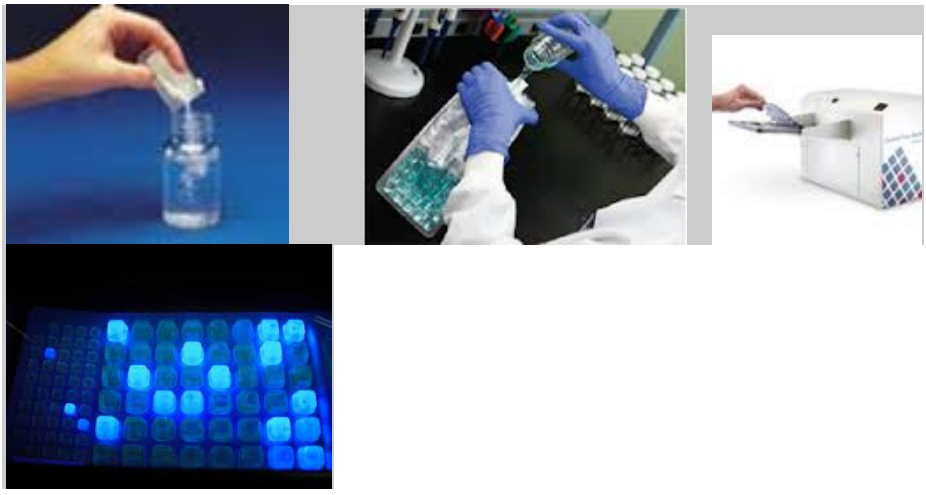

Figure 6. Major Steps in measuring *Enterococcus* using Enterolert.

ATTACHMENT Example of citizen science chain of custody and laboratory data form.

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Station ID	Sample ID	Laboratory Parameter(s)/ Bottle Size/Bottle Material	Enterolert Sample Collection Date	Enterolert Sample Collection Time	*Water Temp. (°C)*	*pH (units)*	*Dissolved O_2 (mg/L)*	*Conductivity (mS/cm)*	*Specific Conductance (uS/cm)*	*Salinity o/oo*	*Water Quality Date/ Time*
											/
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Samples iced immediately after collection?	☐ Yes ☐ No	Samples collected approx. 1 ft. below surface?	☐ Yes ☐ No	Bottle Lot #:		Sample Temp °C @ Lab Arrival:

Custody Signatures:

Person Assuming Responsibility for Samples:	Time	Date			
Relinquished By:	**Time**	**Date**	**Received by:**	**Time**	**Date**
Relinquished by:	**Time**	**Date**	**Received by:**	**Time**	**Date**

Project Name:		Project/Sampling Event #:									
Sample ID	**Station ID**	**DF**	**100% Sample**			**DF**	**10% Sample (specify if different DF used)**			**Final MPN/100 mL**	**Qualifier Needed?**
			Large Wells	Small Wells	Table MPN		Large Wells	Small Wells	Table MPN	(Table MPN x DF)	U, J, or R?
Blank	QC1	1				Sterile DI Water 99 mL					
Positive Control	QC2	1				Sterile DI Water 99 mL +Enterococcus QC					
						10					
						10					
						10					
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						10					

Positive Control Result within manufacturers Acceptance Limits? Yes or No

Incubator	Manufacturer: Serial #:	Temp 40.5 – 41.5 °C at start of incubation? Y or N Temp 40.5 – 41.5 °C at end of incubation? Y or N	If No, Specify: If No, Specify:	Therm. #:

Reagent/Supply	**Manufacturer**	**Cat #**	**Lot #**	**Expiration Date**	**Notes**
Enterolert Reagent	IDEXX				
Quanti Tray 2000	IDEXX				
Sample Bottle					
Sterile DI Water 90 mL					
Sterile DI Water 99 mL					
Enterococcus QC Bacteria					**True Val: 1000 Accept Limits 500-2000 MPN 100 mL:**
Test set up started by:	**Date/Time Test Startup Began**	**Date/Time Incubated**		**Results Read by Date & Time**	

IDEXX-Quanti-Tray 2000 MPN Table

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IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

09-63235-01

IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6

APPENDIX 2
Field Observation Datasheet

Citizen Science Pathogen Monitoring Field Observation Datasheet

Station ID:				**Date:**				
Sampling Team:							**Time:**	
GPS Coordinates in DD.DDDDD			**Lat:**			**Long:**		
GPS #:				**Sonde #:**				
Weather								
Cloud Cover (Circle One)				**Precipitation**		**YES/NO**	**Comment**	
CLEAR		**BROKEN**		**Rain Now?**				
SCATTERED		**OVERCAST**	**OBSCURE**	**Rain past 24 hrs?**				
Comment:				**Rain past 48 hrs?**				
Tide at time of Sampling				**Water Discoloration Y/N**				
Tide Stage (Check One)				**Apparent Color**				
Daily High (DH)		**EBB**		**Blue**		**Black**		
Daily Low (DL)		**FLOOD**		**Green**		**Orange**		
Not Tidal (NT)				**Brown**		**Other**		
Comment:				**Comment:**				
Odor - Adverse or Offensive: Y/N (If Yes check box for those that apply, Use Comments)								
Musty		**Petroleum**		**Other/Comments**				
Sewage		**Decay**						
Chlorine		**Sulfide**						
Other Observations (Check box for those that apply, include details/severity in Comments)								
	Y/N	**Comment**			**Y/N**	**Comment**		
Foam/Suds				**Floating Debris**				
Oil/Grease				**Floating Sewage**				
Potential Pollution Sources (Check box for those that apply, include details, number/amount in Comment)								
	Y/N	**Comment**			**Y/N**	**Comment**		
Wildlife				**Outfall Pipe / Drainage Ditch**				
Livestock				**Active Discharge**				
Domestic Pets				**Other**				
Observed Activities in Waterbody at time of sampling (Check box for those that apply, include details, number/amount in Comment)								
	Y/N	**Comment**			**Y/N**	**Comment**		
Swimming				**Fishing**				
Boating				**Other**				
Photos Taken?		**Circle: Y/N**						
Photo ID		**File Name**		**Description**				
Data Sheet Completed By: (Sign and Date)								

APPENDIX 3

Field Instrument Calibration Datasheet

Citizen Science Daily Field Instrument Calibration Datasheet

YSI Daily Pre-Sampling pH Calibration						
YSI Meter/Serial Number:						
pH Brand	NIST Certified (Y/N)	pH Buffer	Lot No.	Expiration Date	Calibration pH (SU)/Temp.(°C)	Calculated Buffer Standard from Buffer Bottle pH (SU)/Temp.(°C)
Name: Printed/Signature				Date / Time:		

YSI Daily Post-Sampling pH Calibration Check Using pH 7 Buffer		
pH Buffer	pH Meter Reading __(SU)@Temp___(°C)	pH Buffer Standard ____ (SU)@Temp ____(°C)
7.00		
Did meter read within ± 0.2 SU of labeled buffer value? (circle) Yes No		
If Yes, meter maintained calibration		
If No, pH data should be qualified		
Name: Printed/Signature	Date / Time:	

YSI Daily Pre-Sampling Specific Conductance Calibration				
YSI Meter/Serial Number:				
Specific Conductance Brand	NIST Certified (Y/N)	Specific Conductance Concentration (uS)	Lot No.	Expiration Date
Name: Printed/Signature		Date / Time:		

YSI Daily Post-Sampling Specific Conductance Calibration Check against Original Concentration Standard
Specific Conductance Meter Reading ____ (uS)@____Temp(°C)
Did meter read within ± 500uS of Specific Conductance value? (circle) Yes No
If Yes, meter maintained calibration
If No, Specific Conductance/Conductivity data should be qualified
Name: Printed/Signature Date / Time:

YSI Daily Pre-Sampling Dissolved Oxygen Sensor Calibration
YSI Meter/Serial Number:
Calibration Method: Percent Saturation
Barometric Pressure: _____ mmHg
Name: Printed/Signature Date / Time:

APPENDIX 4

Laboratory and Field Data/Chain of Custody Datasheets

Project Name:		Project/Sampling Event #:									
Sample ID	Station ID	DF	100% Sample			DF	10% Sample			Final MPN/100 mL	Qualifier Needed?
			Large Wells	Small Wells	Table MPN		Large Wells	Small	Table MPN	Table MPN x DIL	U, J, or R?
Blank	QC1	1					Sterile DI Water 99 mL				
Positive Control	QC2	1					Sterile DI Water 99 mL +Enterococcus QC				
						10					
						10					
						10					
						10					
						10					
						10					
						10					
						10					
						10					
						10					
						10					
						10					
						10					
						10					

Positive Control Result within manufacturers Acceptance Limits? Yes or No

Incubator	Manufacturer: Serial #:	Temp 40.5 – 41.5 °C at start of incubation? Y or N Temp 40.5 – 41.5 °C at end of incubation? Y or N	If No, Specify: If No, Specify:	Therm. #:

Reagent/Supply	Manufacturer	Cat #	Lot #	Expiration Date	Lot Checked?
Enterolert Reagent	IDEXX				Y N NA
Quanti tray 2000	IDEXX				Y N NA
Sample Bottle					Y N NA
Sterile DI Water 90 mL					Y N NA

Qualifiers:

"U" –Undetected
When,
no wells fluoresce

"J"- Estimated Value
When,
Minor deviation from protocol occurs that would not likely affect usability of data. Ex. Cooler temperature 12 °C or incubator temperature off by a small amount.

Also we will use "J" when there may be bias from the Positive Control . i.e. Pos. Control Result < or > acceptable recovery

"R" – Rejected
When,
Major deviation from protocol or very high blank contamination occurs; i.e. Missed holding times; If blank contamination is < Reporting limit than no qualifier needed.

COMMENTS:

Sterile DI Water 99 mL					Y N NA
Enterococcus QC Bacteria					True Val: Accept Limits:
Test Conducted B, Date and Time	Acceptable DOC performed? Yes No	Date/Time Incubated		Results Read by Date & Time	

Station ID	Sample ID	Laboratory Parameter(s)/ Bottle Size/Bottle Material	Collection Date	Collection Time	*Water Temp. (°C)*	*pH*	*Dissolved O_2 (mg/L)*	*Conductivity (mS/cm)*	*Specific Conductance (µS/cm)*	*Salinity (0/00)*

Samples iced immediately after collection?	☐ Yes ☐ No	Samples collected approx. 1 ft. below surface?	☐ Yes ☐ No	Sample Bottle Lot #:	Sample Temp °C:

Custody Signatures:

Person Assuming Responsibility for Samples:	Time	Date			
Relinquished By:	**Time**	**Date**	**Received by:**	**Time**	**Date**
Relinquished by:	**Time**	**Date**	**Received by:**	**Time**	**Date**