**Citizen Science QAPP**
**Title and Approval Page**

**Citizen Science Pathogen Monitoring**
**Effective Date of Plan: May 26, 2016**

HEP Project Manager:

5/3/2016

Signature/Date
Ariane Giudicelli/NY-NJ HEP

IEC Project Manager/QA Officer:

5/3/2016

Signature/Date
Evelyn Powers/IEC

EPA Project Officer:

Richard P. Winfield 5/3/2016

Signature/Date
Rick Winfield/EPA

EPA QA Officer:

5/3/16

Signature/Date
Kathryn Drisco/EPA

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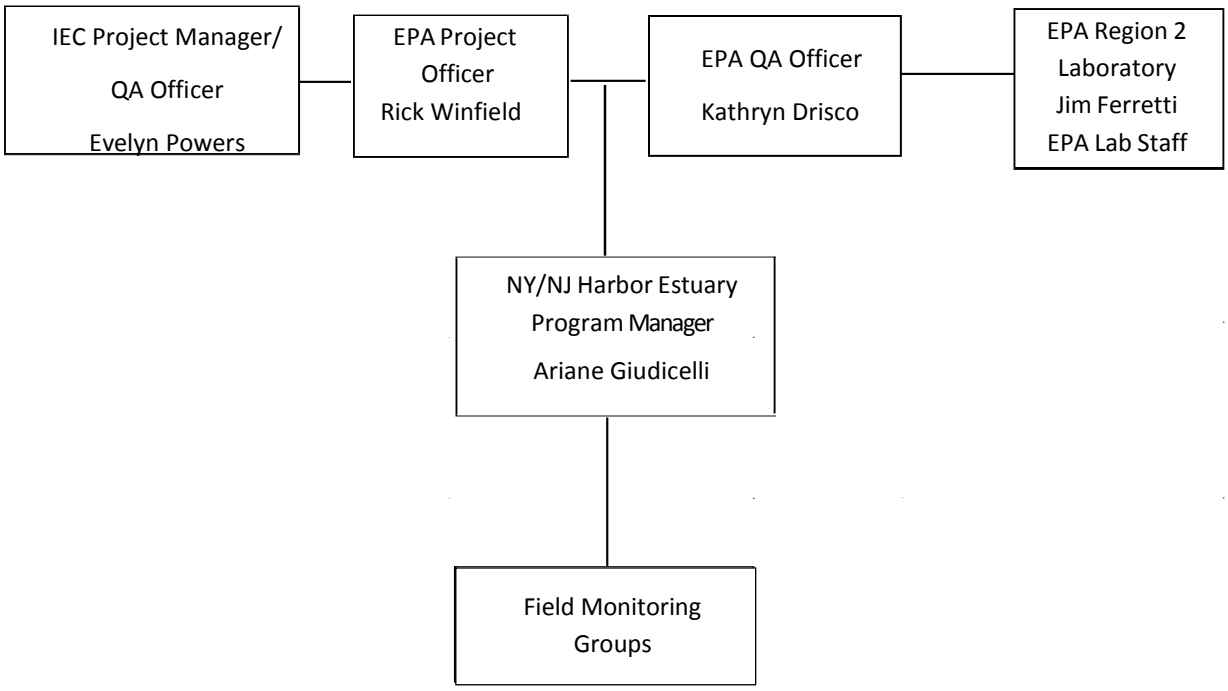
## Project Organization Chart




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

Project Distribution List

| Name/Title | Contact Information |
|---|---|
| Ariane Giudicelli<br>NY-NJ HEP | Email: ariane@harborestuary.org<br>Phone: 212-483-7667 |
| Evelyn Powers<br>IEC Project Manager/ QA Officer | Email: epowers@iec-nynjct.org<br>Phone: 718-982-3792 |
| Rick Winfield<br>EPA Project Officer | Email: winfield.richard@epa.gov<br>Phone: 212-637-3432 |
| Kathryn Drisco<br>EPA Quality Assurance Officer | Email: drisco.kathryn@epa.gov<br>Phone: 732-906-6800 |
| Jim Ferretti EPA<br>Laboratory | Email: ferretti.jim@epa.gov<br>Phone:732-321-6728 |
| Stan Stephansen<br>EPA Data Management | Email: stephansen.stanley@epa.gov<br>Phone: 212-637-3776 |
| Bob Schuster<br>NJDEP | Email: Robert.Schuster@dep.nj.gov<br>Phone: 609-748-2017 |
| Jeff Myers<br>NYSDEC | Email: jeff.myers@dec.ny.gov<br>Phone: 518-402-8166 |
| Beau Ranheim<br>NYCDEP | Email: beaur@dep.nyc.gov<br>Phone: 518-402-8179 |

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

In addition, any agencies, organizations, and individuals not listed here may obtain a copy of this Quality Assurance Project Plan upon request.

## Project/Task Organization

| Name | Title | Organizational Affiliation | Responsibilities (specific to this project) |
|---|---|---|---|
| Ariane Giudicelli | Project Manager | NY-NJ HEP | Coordinate with contractors and partners; update QAPP; review contractor addendums; provide grant oversight; QA checks; assist with data upload |
| Rick Winfield | EPA Project Officer | EPA | Provide grant oversight; review QAPP |
| Kathryn Drisco | EPA Quality Assurance Officer | EPA | Review the QAPP |
| Evelyn Powers | IEC Project Manager/ QA Officer | IEC Laboratory | Review QAPP; Coordinate/review Enterolert results; Run Membrane Filtration on subset of samples; provide training for field equipment and sampling; perform field assessments; QA checks |
| Jim Ferretti | EPA Team Leader, Sanitary Chemistry and Biology Team | EPA Laboratory | Provide training for the IDEXX Enterolert analyses and equipment distributed through EPA's equipment loan program (YSI meter & GPS); review Enterolert results |
| Stan Stephansen | EPA Data Management | EPA | Will provide data management training and assist with uploading data into STORET; Data QA checks |

# Problem Definition and Project Objectives

## Problem Definition

This project builds on the framework of the 2014 Citizen Science Monitoring for Pathogen Indicators in NY-NJ Harbor Tributaries while shifting focus to public access locations in the more open waters of the Harbor. It provides an opportunity for community groups to collaborate with HEP, Interstate Environmental Commission (IEC) and USEPA to gather water quality data in 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 waters. At the same time, HEP, USEPA, and the state environmental departments will obtain valuable datasets that may indicate areas needing follow-up monitoring and further attention, among other potential uses. 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 help paint a more complete picture of recreational water quality in the Harbor.

## Project Objectives

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 two contractors to conduct sampling at public access locations in saline and tidally influenced waters in a limited region of the NY-NJ Harbor Estuary (see Project Location) within reasonable travel distance of the IEC's lab on Staten Island .

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 agencies, at their discretion, for a variety of purposes, including but not limited to screening, complementing available data to confirm state findings, enhancing information on sources and extent of impairments, and uncovering areas that require direct action.
- 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, and by providing access to data in a user-friendly format.
- Goal 6: Collect water quality data in an area not currently covered by citizen science pathogen monitoring and where there is no regular recreational season monitoring conducted by state or city agencies.

## Data Users

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 NJDEP and NYSDEC. The state environmental departments can obtain valuable datasets that could serve to fill in data gaps, allow for more focused and targeted monitoring, and guide restoration efforts among other potential uses.

# Background and History

## Background/History

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.

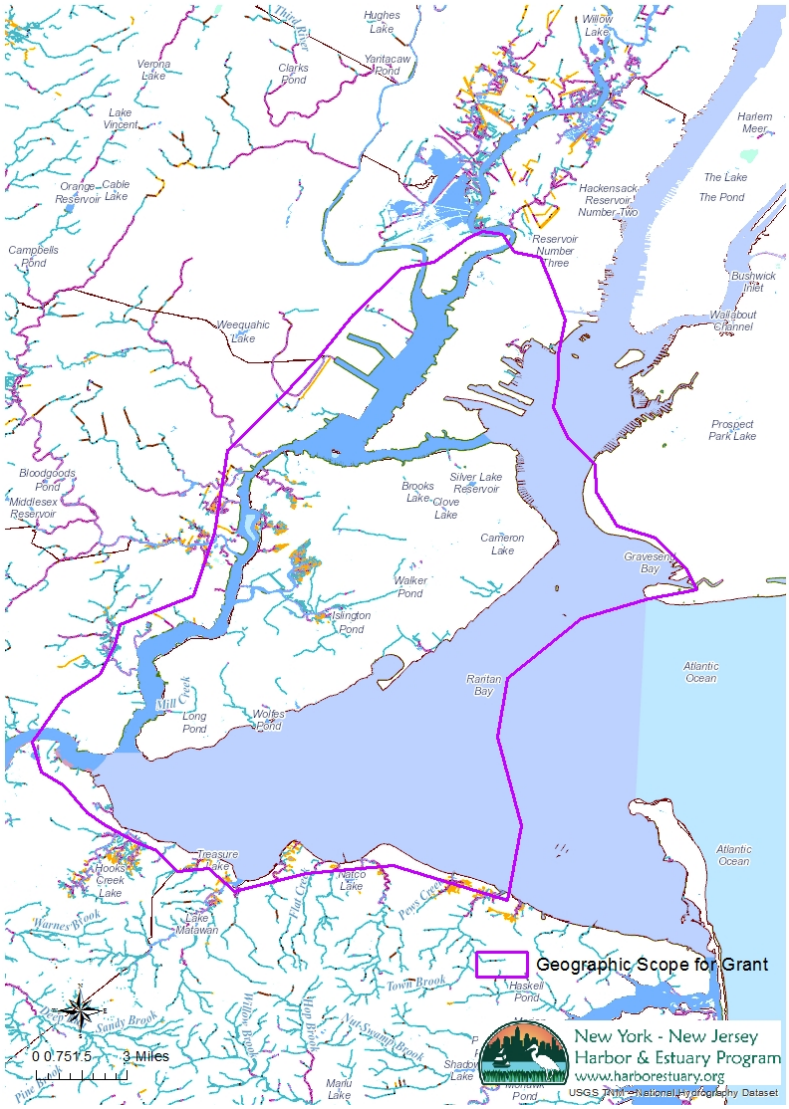
The Hudson River Foundation (HRF) seeks to make science integral to decision-making with regard to the Hudson River and its watershed and to support competent stewardship of this extraordinary resource. This purpose is pursued through support of scientific research; communication to expand knowledge about the river among the scientific community, policy makers, and the public at large; initiatives to enhance management of the Hudson ecosystem; education about the River; and physical improvements to the riverfront. The Hudson River Foundation is the host of HEP and the Foundation is the contracting entity for this project.

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 result 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 reliable water quality data from water bodies that have an impact in their communities.

# Project Location

## Project Location

The project will be conducted at publicly accessible locations of the NY-NJ Harbor Estuary, within the area highlighted the figure below. Acceptable sampling locations include locations along the shore and within tidal creeks. In New Jersey, preferred locations are areas in the Raritan Bay not covered by current citizen science efforts and where there is currently no pathogen indicator monitoring or inconsistent monitoring by city or state agencies but where there may be boating and other recreational activities occurring, as well as locations where recreation is desired but not currently feasible. There is special interest in locations in proximity to tidal creek outlets in the Raritan Bay. In New York preferred locations are areas on Staten Island where there is currently no pathogen indicator monitoring or inconsistent monitoring. Each contractor will provide specific proposed geographic locations including GPS coordinates in Appendices 6 and 7.



# Project Schedule

| Activities | Organization/Group responsible for activity completion | Timeframe work will be done |
|---|---|---|
| Approval of QAPP and Supplemental Addendums | EPA Quality Assurance Officer/IEC QA Manager | April-May 2016 |
| Field Instrument and Sample Collection Training | EPA/IEC | May 2016 |
| Laboratory and Analytical Training | IEC Laboratory/EPA | May 2016 |
| Data Management Training | EPA | May 2016 |
| Sample Collection | Contractor | May-September 2016 |
| Sample Analysis | Contractor w/IEC Lab assistance | May –September 2016 |
| Data Management | Contractor w/EPA assistance | May – December 2016 |
| Data Evaluation | Contractor w/IEC Lab assistance | June- December 2016 |
| Submission of Final Report(s) | Contractor | December 15, 2016 |

# Existing Data

| 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.

# Quality Objectives

## 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.

In addition, a duplicate water sample will be taken at one sampling location per event. For each sampling event, the duplicate sample will be taken at a different sampling location, following the above criteria. The duplicate water sample will be collected at the same location as the duplicate YSI profile.

Laboratory – the acceptable criteria for turbidity deviation between duplicate samples is 10% RPD.
The acceptable deviation for Enterolert is 30% RPD (see Appendix 1).

## Bias

Field – This project sampling design is a judgmental design and considered a biased sampling approach. The geographic extent of where sampling may take place was influenced by a number of factors, including travel distance to the partner laboratory and locations of publicly accessible areas believed to be under sampled during the recreational season. There will also be a seasonal influence on the data since sampling will occur during May through September. The seasonal variations in temperature, UV radiation and rainfall are known to influence enterococcus numbers. Variations in rainfall, wind speed and direction, as well as tides and currents will also influence project parameters.

Representativeness

Field- All sampling locations will be in tidally influenced or saline waters of the NY-NJ Harbor and Estuary where pathogen indicator levels are unknown.

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 decimal degrees (DD.DDDDD) format. The field samplers will also use standard water sample collection methods which are described under Data Collection Methods.

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. A subset of samples will be analyzed using both the Enterolert method and membrane filtration. The membrane filtration method, including all quality control protocols, is outlined in IEC's SOP: *Enterococci In Water By Membrane Filtration Using Membrane-Enterococcus Indoxyl-β-D- Glucoside Agar (mEI)* (IEC SOP ID XID, Effective Date February 2015) and is based on EPA Method 1600. All current IEC SOP's are maintained in the laboratory's SOP manual. In addition, hard copies of SOP's applicable to this project will be available to citizen scientists in the field.

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

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

Laboratory - The detection limit for undiluted samples for Enterolert is 1 MPN/100 mL of sample and the reporting limit is 10 MPN/100 mL. The reporting limit for analysis on *Enterococci* using membrane filtration is <10 CFU/100mL.

The reporting limit for turbidity is 1 nephelometric turbidity units (NTU).

| Instrument | Range | Sensitivity |
|---|---|---|
| **YSI Pro Plus Multimeter** | | |
| Temperature | -5 to 65°C | 0.1°C |
| Salinity | 0 to 70 0/00 (PPT) | 0.01 0/00 (PPT) |
| Conductivity | 0 to 200 mS/cm | 0.0001 mS/cm |
| DO | 0 to 20mg/L | 0.01 mg/L |
| pH | 0 to 14 units | 0.01 units |
| | | |
| **YSI 556 MPS*** | | |
| Temperature | -5 to 45°C ± 0.15°C | 0.1°C |
| Salinity | 0 to 70 0/00 ± 0.1 0/00 | 0.01 0/00 (PPT) |
| 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 Pro Plus Multimeter and YSI 556 MPS Range and Sensitivity provided in the above table are manufacturer specified ranges.**

# Data Collection Methods

## Sampling Design

### Sampling Schedule

Sampling will occur May through September 2016. There will be 5 sampling events in a 4 week period/per month. Sampling will occur once per week with an additional floating day per month which will be used to capture a wet weather event in the case that one of the other sampling days did not. Sampling will occur on a fixed schedule with one targeted wet-weather sampling event (defined as at least ¼ inch of rain). One sample will be collected per sampling location. The sampling day of the week will be determined in advance by each group with the IEC lab. No weekend days are allowed for sampling and laboratory use. All groups must sample on Monday, Tuesday, Wednesday, or Thursday.

### 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/or purchased by HEP /grantee 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 ProPlus Multi- Parameter meter and the YSI 556 Multi Parameter Sonde (MPS)(one group will use the YSI ProPlus meter & the one group will use the YSI 556 MPS). Temperature, salinity/conductivity, DO, and pH parameters will be measured at each station. Water quality measurements will be taken, ideally between 0.5 to 1 meter below the surface of the water, taking care not to disturb the sediment if the sample collector is walking in the water. . 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.

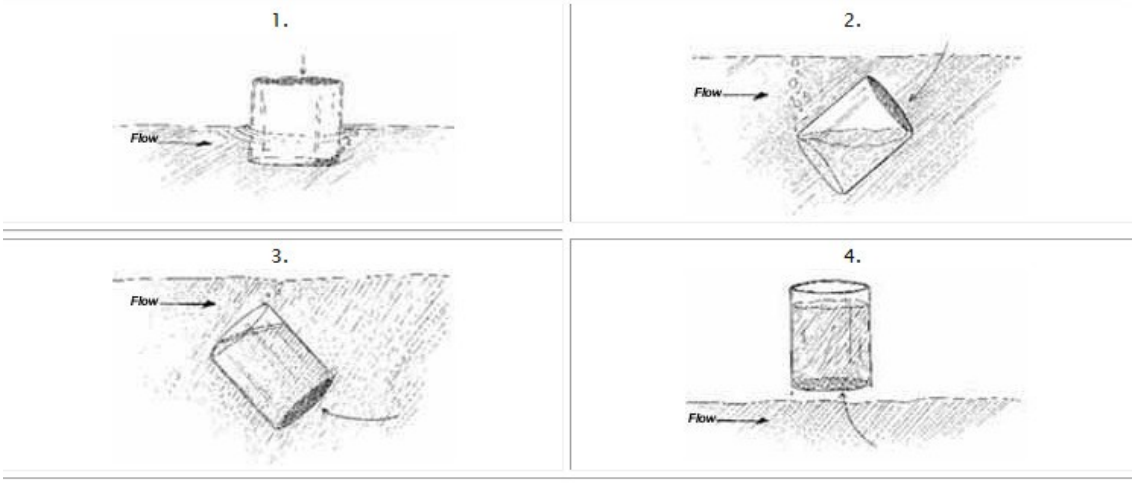
Water samples will then be collected using one of the following methodologies. The method used will be dependent on what the water depth and field/site conditions are when the sampling teams arrive at the station. Samples will be collected into sterilized 120 ml HDPE plastic bottles for Enterococcus analysis and turbidity testing. **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 sterile 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. *Dock/Shoreline using 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 upstream in current) 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 an 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 (typically the 100 mL mark on the bottle if applicable). 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 – followed by -D if Duplicate sample
- Sampling Time
- Analysis: Enterolert/Turbidity
- Preservation: Ice

Example:
Citizen Science Pathogen Monitoring
06-23-2016
NNB 01 062316(-D if Duplicate)
1030
Enterolert/Turbidity
Ice

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

Ex. NNB 01 062316-: This sample was collected by the NY/NJ Baykeeper at station 1 on June 23, 2016.

The contractor designations are as follows:
NY/NJ Baykeeper: NNB
Gotham Whale: GW

Samples will be transported to the laboratory immediately after the sampling event is complete. Samples will be put into ziploc bags and sealed before placing them in the cooler. Samples will be transported in a cooler on ice, accompanied by a temperature blank. The temperature blank is a sample bottle filled with deionized water, the temperature of which is read and recorded upon arrival in the laboratory. Samples should ideally be received at 0>6ºC. If an infrared thermometer is available, a separate temperature blank bottle is not required. Samples that are delivered to the laboratory immediately after collection may not meet this criteria. In this case, the samples shall be considered acceptable if there is evidence that the chilling process has begun, such as arrival on ice.

| 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 | One duplicate collected per sampling event | One sample per location per event, up to 20 sampling events for the project | QAPP or IEC/EPA Provided SOP, see Analytical Methods | Determine pathogen indicators |
| Water | To be determined by the contractor, see addendum | 1 | Turbidity | One duplicate collected per sampling event | One sample per location per event, up to 20 sampling events for the project | IEC SOP, See Analytical Methods | Determine turbidity |
| 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 IEC/EPA Provided SOP, see Analytical Methods | Record water quality parameters in the selected study area |

Water samples collected for enterococcus will be analyzed at the designated lab using IDEXX Enterolert with Quantitray and the procedure described in the Enterolert SOP (see Appendix 1). Samples will also be analyzed for turbidity using IEC Turbidity SOP ID XXVI, Revision No. 10, Effective Date January 2015, which is based on EPA Method 180.1 Revision 2.0.

A subset of samples will be analyzed for Enterococcus using the Membrane Filtration method *Enterococci In Water By Membrane Filtration Using Membrane-Enterococcus Indoxyl-ß-D-Glucoside Agar (mEI)* (IEC SOP ID XID, Effective Date February 2015) and is based on EPA Method 1600 by IEC lab staff in order to compare results and further validate the IDEXX method. The volume of sample collected for the IDEXX analysis is sufficient to run both methods on the chosen sampling dates.

# Equipment List and Instrument Calibration

## EPA/IEC/HEP Supplied Field Supplies/Equipment List

| |
|---|
| Garmin Montana 650 T GPS |
| YSI ProPlus meter |
| YSI 556 MPS |
| 120 mL sterilized plastic bottles |
| 1 Liter sterilized plastic bottles |
| Sampling poles |
| Weighted sampling device with nylon rope |
| Calibration standards for YSI ProPlus meter & YSI 556 MPS |

## Instrument Calibration and Maintenance

| Instrument/Equipment | Calibration Frequency | Maintenance Requirements |
|---|---|---|
| Multi-parameter YSI meters | 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 ProPlus meter and YSI 556 MPS units 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 ProPlus or 556 MPS units. 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 IEC Project QA Manager will be responsible for maintaining these records.

Analytical Methods

| 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 | IEC Laboratory, Staten Island, NY |
| Water | Enterococcus | 1 CFU/100mL | 10 CFU/100mL | Enterococci In Water By Membrane Filtration Using Membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI) (IEC SOP ID XID, Effective Date February 2015). Based on EPA Method 1600 | 100mL | 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 | IEC Laboratory, Staten Island, NY |
| Water | Turbidity | 0.01NTU | 1NTU | IEC Turbidity SOP ID XXVI, Revision No. 10, Effective Date January 2015, which is based on EPA Method 180.1 Revision 2.0. | 100mL | 120 mL sterile HPDE sample containers (the same bottle will be used for both Enterolert & Turbidity analyses) | Store on ice after collection and during transport to the laboratory | Analyzed within 48 hours of sample collection | IEC Laboratory, Staten Island, NY |

## Field Data Sheets

The following data sheets to be utilized for this project are provided in the Appendices as listed below:
Field Observation Datasheet – Appendix 3
Field Instrument Calibration Sheet – Appendix 4
Laboratory and Field Data Chain of Custody Datasheets – Appendix 5

## Training and Specialized Experience

### Training

| 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 ProPlus multimeter and YSI 556 MPS, GPS unit and water sampling equipment. Instruction on lab analyses. | Session prior to 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 prior to beginning of the sampling season |

Training will be hosted at the EPA Region 2 Facility in Edison, New Jersey on May 26, 2016. The tentative schedule for the training is as follows:

| **Morning Session** |
|---|
| YSI 556 MPS and GPS Units |
| Field Sampling Techniques |
| |
| **Afternoon Session** |
| Data Review & Formatting for WQX/STORET |
| Lab Overview/Enterolert Training DOC |

### Specialized 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 |

Assessments and Oversight

| 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 | Beginning of sampling season and mid-season | Field sampling teams | IEC lab staff | Re-train if necessary and reassess |
| Technical System Assessments | During the first 3 weeks of sampling | Sample Collection and use of YSI, Laboratory assessment | IEC Lab and Field Staff | Field correction at time of assessment. Results discussed with HEP PM to determine if necessary action (e.g. retraining). |

## Data Management

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 Contractor Project QA Manager will review 10% of the data to verify the accuracy of the data entered into the data templates/spreadsheets from the field, COC and laboratory datasheets/forms. All data will be assessed for completeness and reasonableness, including the correct qualification of data based upon quality assurance procedures identified in this QAPP. The updated data template will then be emailed, along with a certification that the data has been assessed and quality-assured, to the appropriate contacts at IEC, HEP, and EPA. The designated contacts at the IEC, HEP, or EPA will inform the Project QA manager of any outstanding data issues and the data template will be corrected and resubmitted to the IEC, HEP, and EPA. At the conclusion of the project and after all data has been finalized, the project data will be loaded into Storet for sharing with the public and other agencies. Monitoring groups with existing Storet Organization Ids will load the data into Storet (assistance is available from the Storet Support Team at **1-800-424-9067** or STORET@epa.gov.); HEP/EPA will load the data into Storet for groups without existing Storet Ids. The original datasheets will be stored by the Project Leader for a period of 5 years after the completion of the project in the project file. Copies of the field/lab datasheets will be sent to HEP as part of the progress and final reports.

## Data Review and Usability Determination

### 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 | |

Data uses will depend on the objectives of the project, whether sampling is conducted in fresh or marine water and the established designated uses of the project water body. Listed below is an example of a chart to interpret data based on a project's individual results using *Enterococcus* in freshwater and marine waters and national water quality standards for primary contact recreation. A rationale based on individual project objectives should be used to develop different numerical designations, if applicable.

| Category | Range | Description |
|---|---|---|
| Low | < 61 cfu/100 mL<br>< 33 or 35 cfu/100mL, calculated as a GeoMean of 5 samples over 30 days | 61 is the lowest criterion for both Freshwater and Marine Enterococcus WQS Single Sample Maximum Concentration (SSMC) for Primary Contact Designated Beach Site (using 1986 WQS Enterococcus); 33 for Freshwater or 35 for Marine waters are the lowest criteria for geometric means. |
| Moderate | 61 – 104 cfu/100 mL | 104 is the SSMC for Marine waters and 61 is the SSMC for Freshwater Primary Contact Designated Beach Site |
| High | 105 – 500 cfu/100 mL | 500 is the SSMC for Marine waters for Infrequently used Full Body Contact Recreation (575 for freshwater) |
| Very High | > 501 cfu/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.

## Reporting

### Reports

Project Leaders will submit a progress report, due July 15, to the HEP Project Manager containing:

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

A final report to HEP should including the following:

- Summary of Major Project Components
- Data Use and Recommendations
- Project Conclusions
- Data in Excel Format that is compatible with upload to Storet/WQX

The above project-related materials will be kept by HEP/HRF for as long as possible and for a minimum of three years from the date of submission of the final expenditure report, as stipulated by HRF's Document Retention and Destruction Policy.

HEP reports progress to EPA Region 2 in its annual workplan every federal fiscal year. A final progress and financial report will be submitted by 9/30/2018 (three years after the start of the grant).

APPENDIX 1
Enterolert Guidance

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

## REVISION 5


**U.S. Environmental Protection Agency**
**Region 2 Laboratory**

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

February 2015

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*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***

DISCLAIMER: THIS DOCUMENT IS INTENDED ONLY AS GUIDANCE AND TECHNICAL ASSISTANCE FROM USEPA REGION 2. STUDY DESIGN, QUALTIY CONTROL SAMPLES, DATA MANAGEMENT AND DATA INTERPRETATION IS THE SOLE RESPONISIBILITY OF THE CITIZEN SCIENCE ORGANIZATION

## 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$^{o}$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, 21$^{st}$ 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 (especially in marine samples), there may be site specific situations where an 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.

5. Wear long pants and closed toe shoes in case of any spills or drops in the laboratory.

## 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)

*NOTE: The equipment listed above is available through EPA's Equipment Loan Program.

### 6.2 ENTEROLERT REAGENTS – For 200 Sample Analyses

- Sample Collection Vessel, 120 mL, Sterile (IDEXXWV120-200, $124.26)
- Quanti-Tray 2000 (IDEXX, WQT-2K, 2pcks of 100, $189.00 per pack; $378.00 per 200)
- Enterolert Reagent/Snap Packs (WENT200, $1109)

### 6.3 STERILE DEIONIZED WATER FOR SAMPLE DILUTIONS AND QUALITY CONTROL

- Deionized Water, 90 mL, Sterile One Use (Hardy Diagnostics, #D090, Pck of 50, $104.49)
- Deionized Water, 99 mL, Sterile One Use (Hardy Diagnostics, #D099, Pck of 50, $124.05)
- Deionized Water, 1L, Sterile (Hardy Diagnostics, #U284, Pck of 10, $260.23)

*NOTE: 90 mL for sample dilution (10%) and 99 mL for QC Blank and Positive control samples.
**If using 1L sterile DI water, the measuring vessel used **must be sterile**. It is

recommended using a sterile 25mL pipette to transfer either 90mL or 100mL DI water into the sterile IDEXX vessel before sample is added. There is also a greater risk of contamination by doing this so make sure a negative control is performed with every batch.

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 1).

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.

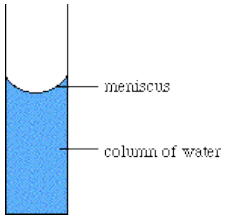
11. Gently invert the bottles containing the test samples 25 times to suspend anything that may have settled to the bottom of the sample before making dilutions.

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 1).

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 1). Record your name and the time and date that the tray results were read and recorded on the data sheet (Attachment 1).

6. Dispose of used trays in biohazard bags/containers and dispose of in accordance with local requirments. Remember to wash hands before and after and change disposable gloves often to minimize chances of cross contamination..

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. These QC requirements are listed for information purposes. Your choice of Quality Control samples will depend on your project objective, data*

*uses, and Quality Assurance Project Plan.*

***There are three types of Quality Control Samples that should be analyzed on each batch of samples tested (up to 20 samples) each day samples are tested***

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 LABORATORY SAMPLE DUPLICATE ANALYSIS

90 mL sterile deionized water + IDEXX reagent + 10 mL from one sample in your daily batch.

*The laboratory duplicate will provide precision information. Subsample the laboratory duplicate from the sample bottle that the original sample was analyzed. It is presumed that all test samples will be run as 10% dilutions so sample volume will not be an issue.* ***Please reshake your sample again when preparing duplicate sample.*** *If the Relative Percent Difference (RPD) between the duplicate samples varies by more than 30%, then note on the data sheet that the Duplicate RPD was >30%. The equation to calculate RPD between duplicates is as follows:*

$$\text{Precision (as RPD)} = \frac{(A - B) \times 100\%}{(A + B)/2}$$

Where: A = MPN from aliquot A and
B = MPN from aliquot B (Duplicate Sample)

*For Example:*
*Enterococcus Results (MPN/100)*
*Sample1 = 144*
*Sample 1 Duplicate = 201*

$$RPD = \frac{(201-144)}{((201+144)/2)} \times 100$$

$$= 33\% \text{ RPD}$$

RPD is a statistic that shows the precision between sample duplicates. If an RPD is >30%, please evaluate test procedures, homogenization, reagents etc. prior to next set of samples. If RPD is > 30, it means that the precision was not as tight between two samples, and the associated sample should be qualified with the letter "J" (see section 12.1)

## 11.4 OTHER QC ACTIVITIES: Negative Controls, Sterility Checks, and Media pH Checks

A blank, positive control, and sample duplicate are the three main quality control tests that should be conducted with every batch of 20 samples. There are a few other QC type of activities that are recommended but would be considered outside the scope of a some citizen science monitoring protocols. These additional QC activities may not be needed based on the use of your data.

*11.4.1* 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.

Negative Controls

1. Gram (+) 100 mL sterile deionized water, IDEXX reagent, *Staphylococcus aureus*
2. Gram (-) 100 mL sterile deionized water, IDEXX reagent, *Escherichia coli*

11.4.2 Sterility Checks- It is recommended that sterility checks be performed on each batch of sterile products and media. Sterility checks are performed to ensure individual lots of bottles, media, and Quanti-Trays are sterile prior to use. Check the sterility of sample bottles and Quanti-Trays using a nonselective media such as Tryptic Soy Broth (TSB). Aseptically pour the TSB into the sample bottle or the Quanti-Tray and seal. Incubate at 35°C for 48 ± 3 hours and check for any sign of growth. If growth is present, the media will look turbid.

11.4.3 pH- The pH on each lot of Enterolert media should be checked. This is done to ensure appropriate conditions for testing.

## 11.5 DEMONSTRATION OF CAPABILITY (DOC)

It is recommended that the analyst practice the method before running real project samples to ensure that they are comfortable running the method and are able to correctly perform aseptic technique. To ensure this, 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. Laboratories 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.

- The Relative Percent Difference between the test sample and duplicate was greater than 30% (see section 11.3)

- 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 Water Quality Standard for a minimum of 5 samples over a 30 day period is 35 MPN / 100 mL for *Enterococcus* in saltwater and 33 MPN / 100 mL in freshwater (1986 Water Quality Standards).

> 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 the classification of the water body designated by your State, Commonwealth, Territory, or Tribal Nation. Ultimately, your project's designations will be based on the type of bacteria that was tested (i.e., *Enterococcus*, E. coli, or fecal coliform etc) and the objectives of your project and the state classification of your waterbody tested and its designated uses.

## 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 1- Example of citizen science chain of custody and laboratory data form.**

| Station ID | Sample ID | Laboratory Parameter(s)/ Bottle Size/Bottle Material | Sample Collection Date | Sample Collection Time | *Water Temp. ($^oC$)* | *pH (units)* | *Dissolved $O_2$ (mg/L)* | *Conductivity (uSiemens)* | *Salinity 0/00* | *Date/ Time Water Quality* |
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|---|---|---|---|---|---|
| Samples iced immediately after collection? | ☐ Yes ☐ No | Samples collected approx. 1 ft. below surface? | ☐ Yes ☐ No | Sample Bottle Lot #: | Sample Temp $^oC$ @ 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** |
| | | | | | |

**Comments:**

| 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 | | | | | |
| **Duplicate** | QC3 | | | | | | 10 | | | | | |
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| | QC3 Duplicate | | | | | | 10 | | | | | |

**Positive Control Result within manufacturers Acceptance Limits? Yes or No**

| **Incubator** | Manufacturer:<br>Serial #: | Temp 40.5 – 41.5 °C at start of incubation? Y or N<br>Temp 40.5 – 41.5 °C at end of incubation? Y or N | If No, Specify:<br>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** | |

## ATTACHMENT 2- IDEXX-Quanti-Tray 2000 MPN Table

## *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 |

09-63235-01

**Attachment 3: Examples of some Youtube Videos Demonstrating Idexx Enterolert and Colilert Procedures**

**https://www.youtube.com/watch?v=J-ejkKjrQ2g**
**You Tube Video - Fecal Indicator Bacteria Enumeration Using IDEXX's Colilert, Colilert-18, Enterolert (Total Coliform, E. Coli, Enterococcus, Fecal Coliform) by IDEXX**

**https://www.youtube.com/watch?v=B5w_zPduf8U**
**Enterolert® Test Overview by IDEXX**

**https://www.youtube.com/watch?v=xRbO-hrR_NE**
**Colilert® Test Overview and How To Instructions by IDEXX**

# APPENDIX 2
# Pathogens as Indicators of Water Quality

# Pathogens as Indicators of Water Quality

## REVISION 1

**U.S. Environmental Protection Agency**
**Region 2 Laboratory**


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

February 2015

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.

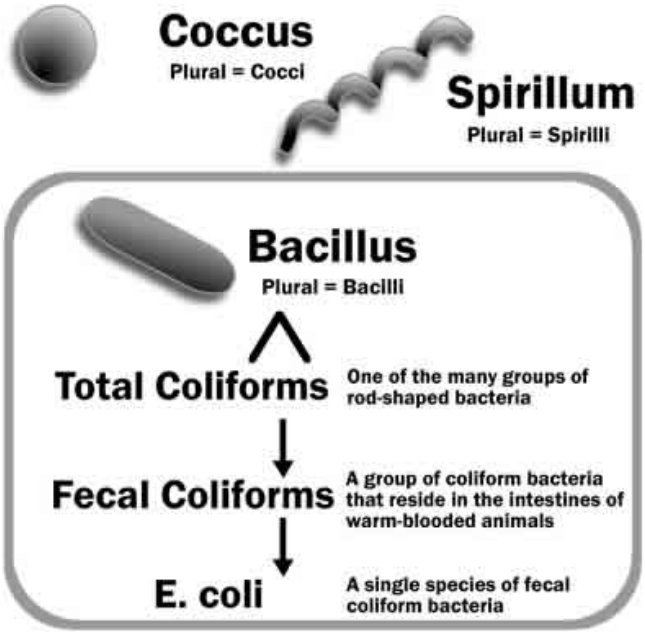


**Figure 1. Commonly Used Indicator Organisms for Pathogen Water Quality Assessments and their relationships**

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.

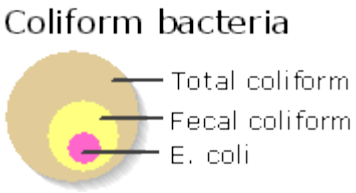


**Figure 2. Relationship among Total Coliform, Fecal Coliform, and *E. Coli***

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 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.

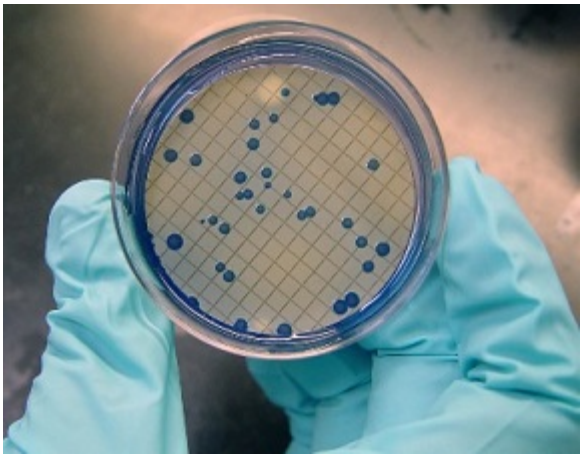

**Figure 3. Petri Dish containing blue fecal coliform colonies after 24 hour incubation on m-FC media.**

**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.

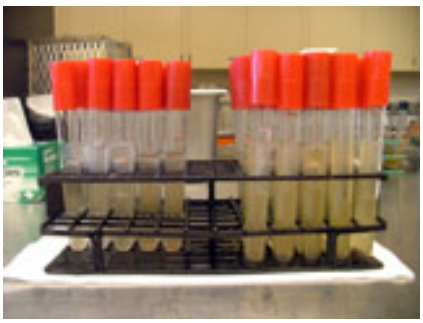

**Figure 4. Test tube rack containing Lauryl Tryptose Broth used to measure bacteria growth and gas from coliform bacteria.**

**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.

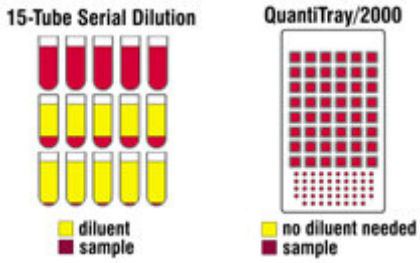


**Figure 5. Comparison of Multiple Tube Fermentation Test and Enterolert w/ Quant-Tray 2000**

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.

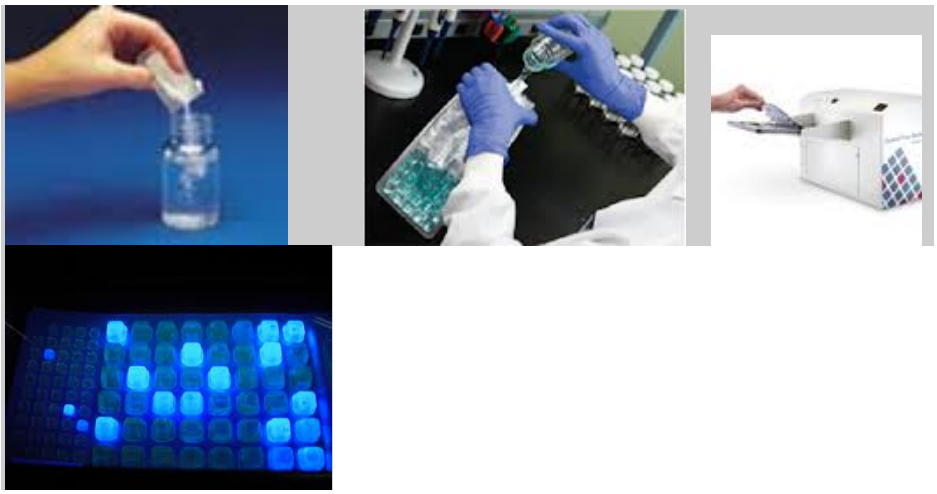

**Figure 6. Major Steps in measuring *Enterococcus* using Enterolert.**

# APPENDIX 3
# 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 | | | |
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| | | | | | | | |
| Data Sheet Completed By: (Sign and Date) | | | | | | | |

# APPENDIX 4
# 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 5
## Laboratory and Field Data Chain of Custody Datasheets

| Sample ID | Station ID | | 100% Sample | | | | 10% Sample | | | | Final MPN/100 mL | Qualifier Needed? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Large Wells | Small | Table | | Large | Sma | Table MPN | | Table MPN x | U, J, or R? |
| Blank | QC1 | 1 | | | | Sterile DI Water 99 mL | | | | | | |
| Positive Control | QC2 | 1 | | | | Sterile DI Water 99 mL +Enterococcus QC | | | | | | |
| | | | | | | 10 | | | | | | |
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Positive Control Result within manufacturers Acceptance Limits? Yes or No

| Incubator | Manufacturer:<br>Serial #: | Temp 40.5 – 41.5 °C at start of incubation?<br>Y or N<br>Temp 40.5 – 41.5 °C at end of incubation?<br>Y or N | If No, Specify:<br>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)* |
|---|---|---|---|---|---|---|---|---|---|---|
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|---|---|---|---|---|---|
| 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 |
| | | | | | |