

# Quality Assurance Project Plan

## Lower Hudson Community Science Water Quality Testing Program

May-October 2017



1 Bronx River  
Parkway  
Bronx, NY 10462



PO Box 272  
Delmar, NY 12054



20 Secor Road  
Ossining, NY 10562



35 Alexander Street  
Yonkers, NY 10701

Lower Hudson Community Science Water Quality Testing Program  
2017 QAPP

**Title and Approval Page**

Riverkeeper, Inc.

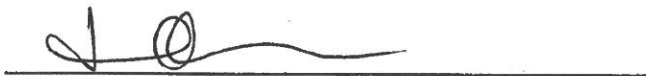
Community Science Water Quality Testing Program

Effective Date of Plan: January 2016-March 2018



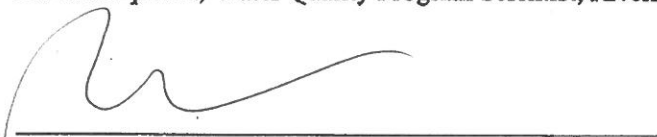
Michelle Luebke, Ecology Director, Bronx River Alliance

Date: 27 April 2017



Jennifer Epstein, Water Quality Program Scientist, Riverkeeper

Date: 4/28/17



Ryan Palmer, Director, Center for the Urban River at Beczak

Date: 4/28/17

Barbara E. Spinweber

Barbara Spinweber, EPA Project Officer

Date: May 10, 2017



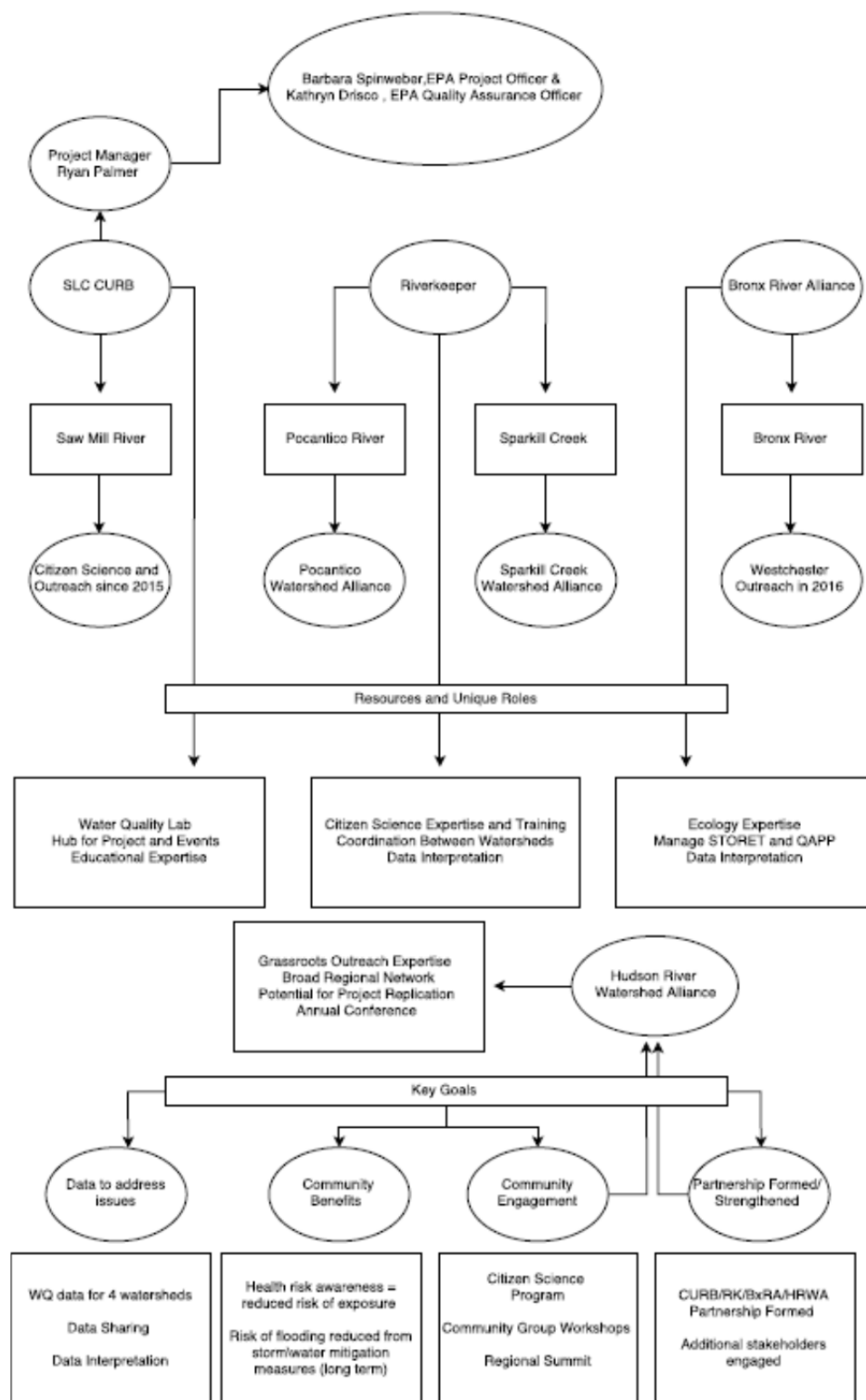
Kathryn Drisco, EPA Quality Assurance Officer

Date: 4/27/17

## Table of Contents

1	Project Organization Chart .....	4
2	Document Distribution List .....	5
3	Project and Task Organization .....	5
4	Problem Definition and Project Objectives .....	6
5	Background and History .....	8
6	Project Location.....	9
7	Project Schedule .....	13
8	Quality Objectives .....	17
9	Data Collection Methods .....	21
10	Equipment List and Instrument Calibration.....	22
11	Analytical Methods.....	25
12	Field Data Sheet .....	28
13	Training and Specialized Experience.....	29
14	Assessments and Oversight.....	31
15	Data Management .....	32
16	Data Review and Usability Determination .....	32
17	Reporting.....	34

## 1 Project Organization



This sampling program will be conducted as part of a partnership between the Sarah Lawrence College Center for the Urban River at Beczak (CURB), Bronx River Alliance (BxRA), Hudson River Watershed Alliance (HRWA), and Riverkeeper, under an EPA Urban Waters Small Grant. Our organizations will collaboratively investigate water quality; build a grassroots network of water quality stewards; engage communities through hands-on science; provide trainings, workshops and forums; and strengthen relationships among local and regional stakeholders. The sampling and other activities cover four watersheds: Bronx River, Pocantico River, Saw Mill River and Sparkill Creek.

## 2 Document Distribution List

Name/Title	Contact Information
Jennifer Epstein QA Manager, Sparkill Creek & Pocantico River Sampling Coordinator	jepstein@riverkeeper.org
Ryan Palmer Project Manager, Lab Manager, Saw Mill River Sampling Coordinator	rpalmer@sarahlawrence.edu
Michelle Luebke STORET Manager, Bronx River Sampling Manager	michelle.luebke@parks.nyc.gov
David Jofat Laboratory Technician	djofat@sarahlawrence.edu
Community Scientists	Various

In addition being directly distributed to the individuals above, this document will be posted on the Riverkeeper website where it may be downloaded by the general public.

## 3 Project and Task Organization

Name	Title	Responsibilities
Jennifer Epstein	QA Manager, Sparkill Creek & Pocantico River Sampling Coordinator	Manage online database; Manage sampler training; Coordinate community scientists for Sparkill Creek and Pocantico River; Maintain the QA Project Plan; Review data QA checks and data analysis

Ryan Palmer	Project Manager, Lab Manager, Saw Mill River Sampling Coordinator	Manage laboratory and sample processing; Train and supervise laboratory staff; Conduct public policy and outreach activities; Purchase supplies and equipment; Coordinate community scientists for Saw Mill River
Michelle Luebke	STORET Manager, Bronx River Sampling Manager	Manage STORET data uploads; Recruit community scientists; Conduct data analysis, public policy, and outreach activities; Coordinate community scientists for Bronx River
David Jofat	Laboratory Technician	Laboratory operation and equipment checks; Sample processing; Conduct data QA checks and data analysis
Community Scientists		Sample collection

## 4 Problem Definition and Project Objectives

### 4.1 Problem Definition

The NYS Department of Environmental Conservation (DEC) characterizes New York State’s wastewater infrastructure as “deteriorating” and has declared that the system is “in crisis.”<sup>1</sup> Many of the wastewater treatment plants (WWTPs) in New York State are currently operating past their design life span or with outdated technology. NYS has documented over \$28 billion in need for improvements, repairs and expansions of its wastewater treatment facilities and collection systems.<sup>2</sup>

These issues impact all types of collection and treatment systems, and 27 municipalities in the Hudson River Watershed are served by combined sewer systems that, by design, discharge untreated or partially treated sewage directly into the Hudson River and its tributaries during rain events.<sup>3</sup> When these combined systems were designed and constructed, watersheds had less impervious surface coverage—and therefore less stormwater runoff—and storm events that would trigger overflows were less frequent. In addition, disrepair results in dry-weather discharges that may go undetected.

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<sup>1</sup> NYS DEC. “A Gathering Storm – New York Wastewater Infrastructure in Crisis”  
<http://www.dec.ny.gov/chemical/48803.html/infrastructurereport>

<sup>2</sup> US EPA, 2016. Clean Watersheds Needs Survey 2012: Report to Congress. EPA-830-R-15005.

<sup>3</sup> Riverkeeper, 2015. How’s the Water? 2015 Fecal Contamination in the Hudson River and its Tributaries.

As of 2014, municipal wastewater was a major or contributing factor in nearly 40% of waterbody impairments throughout New York State. In addition, 19% of lesser impacts were caused by municipal wastewater.<sup>4</sup> Aging wastewater infrastructure is expected to further degrade New York State's waters in the future.<sup>5</sup> The Lower Hudson Community Science Water Quality Testing Program addresses the following questions:

1. Where is fecal contamination present in the Bronx River, Pocantico River, Saw Mill River and Sparkill Creek?
2. What is the frequency and severity of fecal contamination?

## 4.2 Project Objectives

The objective of the program is to collect water samples to analyze *Enterococcus* concentrations ("Enterococcus counts") at fixed points in waterways, including upstream and downstream of potential sewage pollution sources, at tributary confluences, and at public access points.

## 4.3 Data Users

Riverkeeper rates the water quality of each sample in comparison to the U.S. Environmental Protection Agency's (EPA) 2012 Recreational Water Quality Criteria.<sup>6</sup> The Enterococcus count and rating are uploaded to our website (<http://www.riverkeeper.org/water-quality/citizen-data>) after each sampling event. The site explains the sampling method and rating system that we use. We also rate the long-term water quality at each sampling site according to the same criteria.

Riverkeeper and our partners urge participants in the sampling program, and the wider public, to use data from this program to inform themselves and others about water quality conditions in their communities and to pursue solutions to local pollution problems. Riverkeeper staff use the water quality data to advocate for increased investment in wastewater infrastructure, better enforcement of existing water quality protections, more frequent water quality sampling, and better prediction and public notification of sewage contamination.

Periodically we release reports summarizing data and findings. These reports explain our methods, highlight key findings, and suggest ways for citizens to take action. Riverkeeper's reports include guidance about how our water quality data should be interpreted. The reports are available on the Riverkeeper website for viewing or download, and are available in print format upon request.

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<sup>4</sup> NYS DEC, 2014. Top Ten Water Quality Issues in New York State.  
[http://www.dec.ny.gov/docs/water\\_pdf/305btopten10.pdf](http://www.dec.ny.gov/docs/water_pdf/305btopten10.pdf)

<sup>5</sup> NYS DEC. "A Gathering Storm – New York Wastewater Infrastructure in Crisis"  
<http://www.dec.ny.gov/chemical/48803.html/infrastructurereport>

<sup>6</sup> EPA, 2012. Recreational Water Quality Criteria.

## 5 Background and History

### 5.1 Background

In 2006, Riverkeeper started testing for *Enterococcus* (“Entero”), an indicator of fecal contamination, at fixed locations in the Hudson River. We observed that fecal contamination occurred most frequently at tributary confluence sampling sites (compared to mid-channel, nearshore and WWTP outfall sites). While our data show that elevated Entero counts in our study area are often triggered by wet weather, some tributary streams exhibited high counts during dry weather, making them chronic sources of sewage contamination to the Hudson.

This finding also caused concern among people who live and recreate in these tributary watersheds, and Riverkeeper in 2010 began coordinating sampling by community members in select Hudson River tributaries. This community science water quality testing takes place alongside our Hudson River main stem water quality testing program, which is conducted entirely by Riverkeeper staff and science partners.

Our community science program has continually expanded in response to growing public awareness about the problem of fecal contamination. In 2015, Riverkeeper began partnering with the Sarah Lawrence College for the Urban River at Beczak to strengthen the water quality steward community and streamline sample processing in three tributary watersheds of the Lower Hudson River: Pocantico River, Saw Mill River and Sparkill Creek. In 2016, the Bronx River Alliance entered into this collaboration, adding another major tributary in the region.

### 5.2 History

Despite the Clean Water Act’s goal of “swimmable” water, there is currently insufficient testing, or modeling and prediction, of water quality to properly assess whether it is safe to swim in the Hudson and its tributaries. Tributaries not only influence water quality near Hudson River bathing beaches, but also contain popular swimming holes well inland from the Hudson’s shoreline. People also come in contact with the water while kayaking, canoeing, boating, tubing, and wading in tributaries.

The majority of beach closings and advisories in the United States are due to high levels of sewage contamination.<sup>7</sup> In the Hudson watershed, aging wastewater infrastructure and overburdened sewage systems are major causes of stream and river impairment.<sup>8</sup> Riverkeeper’s water quality testing results clearly demonstrate that some tributaries are chronically

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<sup>7</sup> Dorfman, M. and K.S. Rosselot. Testing the Waters: A Guide to Water Quality at Vacation Beaches, Natural Resources Defense Council, New York, NY, 2010.

<sup>8</sup> NYS DEC, 2014. Section 305(b) Water Quality Report. <http://www.dec.ny.gov/chemical/66532.html>



contaminated with sewage throughout their courses.<sup>9</sup> In others, contamination is locally or temporally constrained.<sup>10</sup>

In the Hudson River Watershed, there is no comprehensive program in place to monitor sewage contamination, or to identify its sources. Regular sampling is largely limited to official bathing beaches, despite the prevalence of informal swimming access, and many of these beaches are sampled only a handful of times per month during the bathing season (May to October), although we know that sewage contamination varies from day to day. Riverkeeper's sampling program seeks to raise public awareness about the need for better water quality monitoring and provide information that will help communities resolve water quality problems.

## 6 Project Location

Our sampling program will include 63 sites located in the Bronx, Pocantico, Saw Mill and Sparkill watersheds. Sites were selected by soliciting input from community watershed groups, state and local environmental agency staff, members of sports and outdoor associations, and other people with local knowledge. Ease of access, permanence of access, and volunteer safety were also considered during sample site selection. If a site becomes inaccessible, relocation will be considered on a case-by-case basis. From time to time, we also test water quality at additional, exploratory sites. Sampling sites in the Bronx, Pocantico, Saw Mill and Sparkill Rivers are listed below, grouped by watershed.

Site ID	Site Name	Approx. Latitude	Approx. Longitude	Sampling Frequency
Bronx River				
WC 10	Highclere Lane, Valhalla, NY	41.0901	-73.7792	1x/month
WC 8	South Kensico Avenue, Pat Henry Field, Valhalla	41.0669	-73.7737	1x/month
WC 7	Westchester County Center, White Plains	41.0371	-73.7780	1x/month
WC 6	Greenacres Avenue, Hartsdale	41.0133	-73.7928	1x/month
WC 4	Bronx River Parkway at Leewood Drive, Eastchester	40.9691	-73.8173	1x/month
WC 3	Dewitt Avenue/ Paxton Avenue at the confluence with the Grassy Sprain Brook, Bronxville	40.9388	-73.8377	1x/month
SWS-02	Bronx River Parkway between McLean Avenue and Wakefield Avenue, Yonkers	40.9010	-73.8602	1x/month
SWS-21	Muskrat Cove Pipe	40.9012	-73.8600	1x/month
SWS-06	Burke Avenue Bridge	40.8717	-73.8728	1x/month
SWS-11	182nd Street, River Park	40.8435	-73.8765	1x/month
SWS-14N	Starlight Park North Dock	40.8323	-73.8830	1x/month

<sup>9</sup> Riverkeeper, 2015. How's the Water? 2015 Fecal Contamination in the Hudson River and its Tributaries.

<sup>10</sup> Ibid.

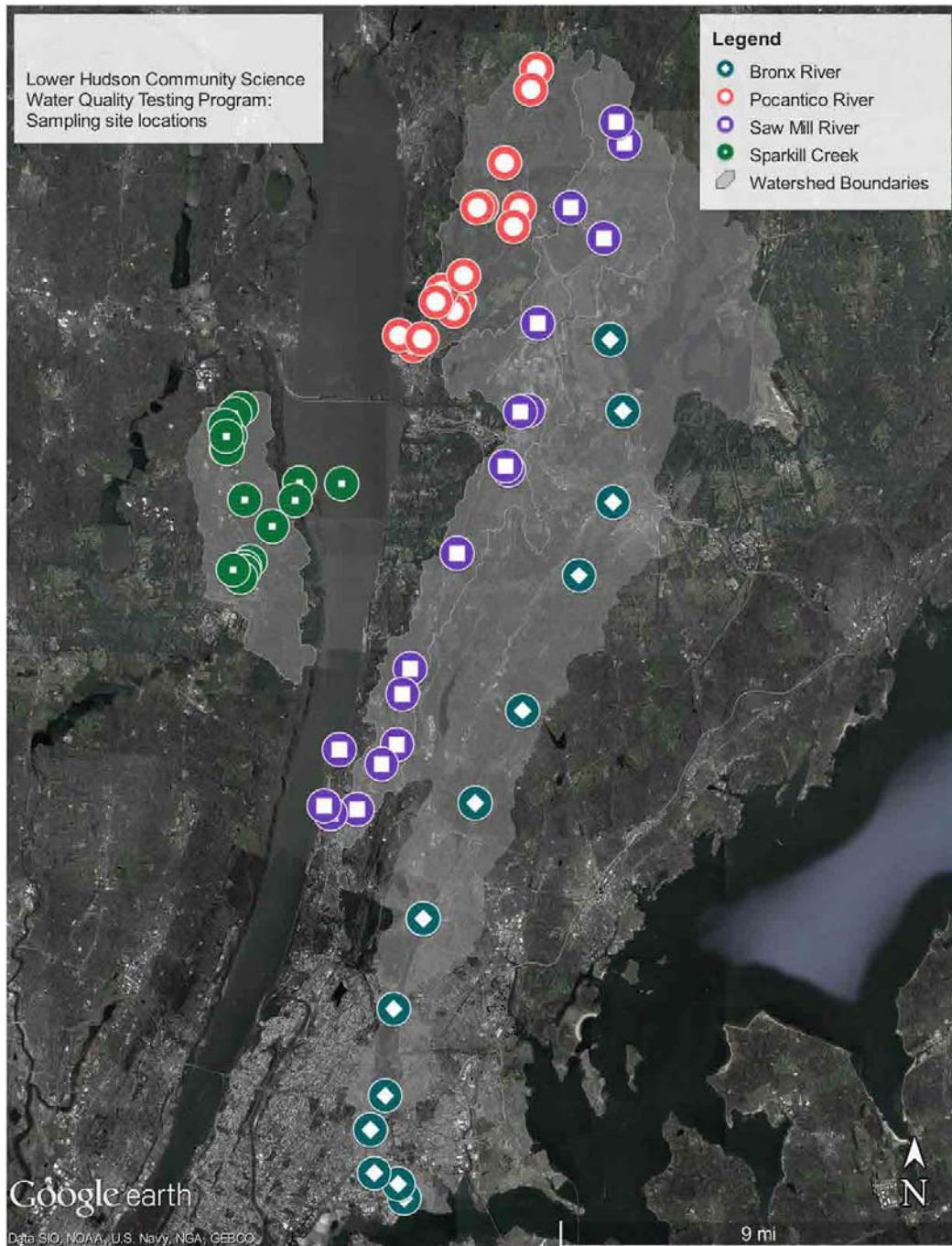
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2017 QAPP

Site ID	Site Name	Approx. Latitude	Approx. Longitude	Sampling Frequency
SWS-16	Hunts Point Riverside Park	40.8180	-73.8813	1x/month
SWS-01	Soundview Park South, mouth of River	40.8097	-73.8682	1x/month
BxR-009	Soundview Park, HP-009 outfall	40.8144	-73.8708	1x/month
<i>Pocantico River</i>				
28E	Kingsland Point Park- Pocantico River	41.091540	-73.870680	1x/month
PR-0.57	Sleepy Hollow- Below Philipsburg Manor	41.088163	-73.864206	1x/month
PR-0.82	Sleepy Hollow Cemetery	41.090418	-73.860620	1x/month
PR-1.75	Sleepy Hollow- Rockefeller Park	41.102461	-73.854664	1x/month
PR-GB-0.16	Sleepy Hollow- Gory Brook tributary	41.106175	-73.852076	1x/month
PR-RB-0.11	Sleepy Hollow- Rockefeller Brook tributary	41.099465	-73.846899	1x/month
PR-2.56	Sleepy Hollow- DEP Spillway	41.102658	-73.844596	1x/month
PR-3.19	Briarcliff Manor- Below Pocantico Lake	41.111004	-73.842689	1x/month
PR-CB-0.77	Briarcliff Manor- Caney Brook tributary	41.133567	-73.834981	1x/month
PR-4.22	Briarcliff Manor- Unnamed tributary at pump house	41.133230	-73.836779	1x/month
PR-5.11	Briarcliff Manor- Above Pocantico Lake	41.127108	-73.821193	1x/month
PR-5.61	Briarcliff Manor- Long Hill Road	41.133054	-73.818568	1x/month
PR-6.78	Briarcliff Manor- North County Trail	41.147989	-73.825023	1x/month
PR-8.63	Briarcliff Manor- Stone Creek Lane	41.172049	-73.813696	1x/month
PR-9.09	New Castle- Echo Lake	41.178675	-73.811209	1x/month
<i>Saw Mill River</i>				
SMR-HR-20	Yonkers- JFK Marina boat launch	40.956308	-73.896446	2x/month
SMR-HR-18.5	Yonkers Paddling and Rowing Club	40.937874	-73.902977	2x/month
SMR-0.19	Yonkers- Saw Mill River, day lighted section	40.935345	-73.900253	2x/month
SMR-1.11	Yonkers- Walsh Road	40.936766	-73.888764	2x/month
SMR-2.44	Yonkers- Torre Place	40.951595	-73.878123	2x/month
SMR-4.22	Yonkers- Hearst Street	40.974530	-73.869078	2x/month
SMR-4.87	Hastings- South County Trail at Farragut Avenue	40.982927	-73.865711	2x/month
SMR-7.90	Ardsley- V. E. Macy Park ballfields	41.020558	-73.845492	2x/month
SMR-RB-0.13	Elmsford- Rum Brook tributary	41.047660	-73.823337	2x/month
SMR-10.41	Elmsford- Rum Brook Park ballfields	41.048950	-73.824620	2x/month
SMR-MB-0.15	Elmsford- Mine Brook tributary	41.067079	-73.814898	2x/month
SMR-11.82	Elmsford- Above Mine Brook	41.066618	-73.818228	2x/month
SMR-14.88	Hawthorne- Saw Mill River Road	41.095474	-73.810700	2x/month
SMR-NB-0.07	Hawthorne- Nannyhagen Brook tributary	41.123230	-73.781832	2x/month
SMR-18.84	Pleasantville- Pleasantville Road	41.133241	-73.796458	2x/month
SMR-TB-0.34	Chappaqua- Tertia Brook tributary	41.154473	-73.772892	2x/month
SMR-21.18	Chappaqua- Duck Pond spillway	41.161397	-73.776373	2x/month

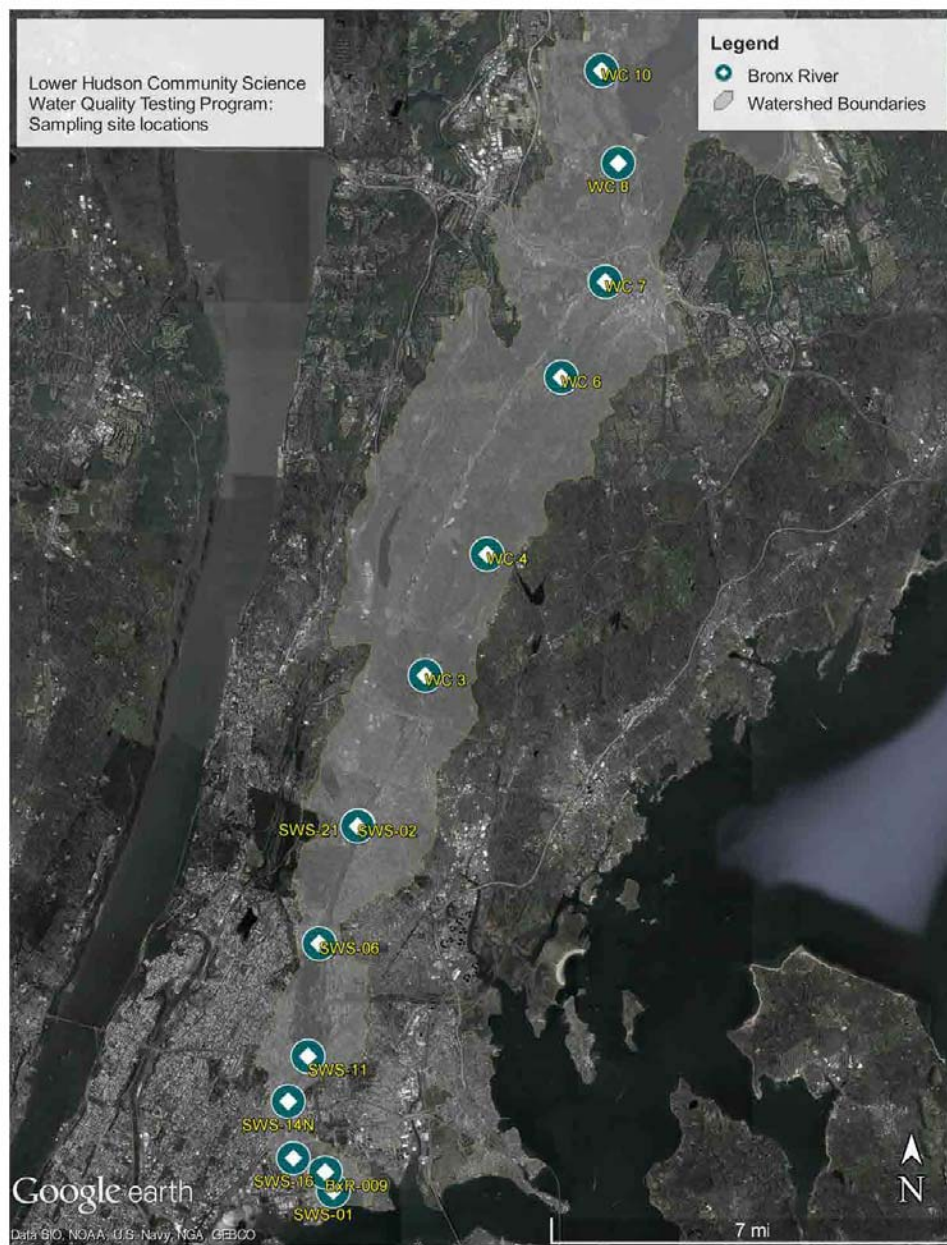
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2017 QAPP

Site ID	Site Name	Approx. Latitude	Approx. Longitude	Sampling Frequency
<i>Sparkill Creek</i>				
26.2W	Piermont- Pirelli Park	41.043417	-73.9137	1x/month
26.1W	Piermont Pier	41.04316	-73.89554	1x/month
SC-0.71	Piermont- Old Draw Bridge	41.03772	-73.915585	1x/month
SC-1.50	Piermont- Skating Pond	41.029292	-73.925459	1x/month
SC-4.94	Orangeburg- Orangetown STP	41.037839	-73.937329	1x/month
SC-BA-0.02	Blauvelt- Blauvelt Arm tributary	41.054489	-73.945322	1x/month
SC-6.35	Blauvelt- Clausland Arm	41.054588	-73.944913	1x/month
SC-TT-0.12	Blauvelt- Tackamack trib at Greenbush Rd Bridge	41.058766	-73.943657	1x/month
SC-2.45	Tappan- Moturis	41.017918	-73.934915	1x/month
SC-SB-0.14	Rockleigh, NJ- Sparkill Brook tributary	41.012596	-73.939081	1x/month
SC-2.96	Tappan- State Line	41.015142	-73.942295	1x/month
SC-6.62	Blauvelt- Spruce Street	41.058629	-73.945322	1x/month
MOTEXP3	Moturis- US of commercial & sewer main	41.0149	-73.938117	1x/month
MOTEXP1	Moturis- DS of Oak Tree Rd Br	41.014694	-73.937631	1x/month
MOTEXP2	Moturis- First east bank trib	41.0160449	-73.9373714	1x/month
SPREPX3	Spruce- US of sewershed	41.062091	-73.945912	1x/month
SPREXP1	Spruce- Reservoir outfall	41.067762	-73.938798	1x/month
SPREXP2	Spruce- US of wooded reach	41.06588	-73.941641	1x/month

## Site Map- All Watersheds



## Site Map- Bronx River Sites





## Site Map- Pocantico River Sites



## Site Map- Saw Mill River Sites





## Site Map- Sparkill Creek Sites





## 7 Project Schedule

Activities	Person Responsible	Timeframe
Program Design	Project Team (Riverkeeper, BxRA, CURB)	January-April 2017
Training and Introductory Event	Project Team	April 2017
Water Quality Sampling	Community Scientists Project Team Laboratory Staff	May-October 2017
Web Posting of Raw Data	Riverkeeper Water Quality Program Scientist	May-October 2017
Knowledge Sharing Event	Project Team	October-November 2017
STORET Input	BxRA Ecology Director	November 2017-January 2018
Data Analysis and Interpretation	Project Team	November 2017-January 2018
Regional Summit	Project Team	January-March 2018
Final Report to EPA	Project Team	April 2018

## 8 Quality Objectives

### 8.1 Precision

#### *Field:*

One duplicate sample will be processed from each tributary watershed on each sampling date by collecting two grab samples consecutively. Duplicate sites will be selected randomly at the start of the season. Duplicate results will be used to understand environmental variation, as background information for data analysis, and for comparison with methodological variation. Because these controls are collected solely for information, there are no acceptance criteria for these samples.

#### *Laboratory:*

One sample will be processed in replicate on each sampling date by dividing one grab sample into separate sample processing bottles in the laboratory. Replicate results will be used to better understand methodological (i.e., Enterolert) variation.

Replicate sites will be selected randomly at the start of the season, except for the Pocantico watershed. All Pocantico replicates will be run on samples from Kingsland Point Park (site ID 28E), because samples from this brackish water site are routinely diluted, while samples from the freshwater sites upstream of here are not routinely diluted, and therefore sample volumes are not large enough to allow replication.

The Relative Percent Difference between the replicate samples will be calculated using the following formula:<sup>11</sup>

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

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

If an RPD is >30%, we will evaluate sample processing procedures such as sample homogenization, reagent dispensing and pipetting; reagent expiration date; storage and condition of consumables (e.g., reagent, containers, pipette tips); and pipettor volume calibration prior to the next sampling date.

## 8.2 Bias

### *Field:*

Our study is intended to detect fecal contamination at specific locations and times. Sampling points are distributed to provide insight into possible sewage exposure by river users, to locate contamination hot spots, and to identify potential pollutant sources. The full subset of samples may not describe general waterway conditions.

Instructions on how to avoid introduction of bias during sample collection will be given during sampler training. First, samplers will be instructed to always access the water at the same point (insofar as natural changes in water level and bank configuration allow). Second, samplers will be instructed to contact the appropriate Sampling Coordinator before leaving the site if they encounter suspicious or unusual conditions. In this type of case, the Sampling Coordinator will direct the sampler to avoid biasing the routine sample collection, while also collecting additional information if possible. Finally, samplers will be given precise access point information for their assigned sampling sites.

### *Laboratory:*

One laboratory blank will be processed alongside field samples on each sampling date. The blank will be prepared by pouring sterile water into the mixing vessel in place of stream water. If a blank has a positive *Enterococcus* measurement (1 cell/100 mL or greater for non-diluted samples; 10 cells/100 mL or greater for diluted samples), the sample results since the last acceptable blank will be qualified, and lab procedures will be reviewed to determine the source of contamination. Additional blanks will be processed until the source of contamination has been eliminated.

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<sup>11</sup> EPA Region 2 Laboratory, 2015. Guidance Document for Citizen Science Pathogen Monitoring of Enterococci Using IDEXX Enterolert with Quanti-Tray 2000 (Revision 5). Division of Environmental Science & Assessment, Edison, NJ. Attached.

One set of positive and negative control cultures (*Enterococcus faecalis*, *Escherichia coli*, *Streptococcus bovis*) will be analyzed for each lot of IDEXX reagent prior to the start of the sampling season. The Percent Recovery of the positive control (*E. faecalis*) will be calculated using the following formula:<sup>12</sup>

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

The Percent Recovery should be between 50-200% of the expected value cited by the manufacturer. The negative controls (*E. coli* and *S. bovis*) should yield a count of <1 cell/100 mL (<10 cells/100 mL if dilution is required). If the acceptance criteria are not met, the Lab Manager will first contact IDEXX to inquire about known problems with the reagent batch. If no problems exist, processing procedures will be reviewed and reagents will be examined for potential problems. Additional positive/negative controls will be processed until problems are identified and corrected.

### 8.3 Representativeness

#### *Field:*

Community scientists will sample once (Bronx River, Pocantico River, and Sparkill Creek) or twice (Saw Mill River) monthly from May to October, the time of year when most people get in the water. The data is not meant to be representative of average conditions for the recreational season, nor is it to be used to predict conditions at a specific time and place along a waterway. The data should not be used to draw conclusions about conditions during the winter months.

### 8.4 Comparability

#### *Field:*

The Hudson River Estuary and its tributaries include saltwater, freshwater, and brackish environments. We use *Enterococcus* as an indicator of fecal contamination because it can be used in all salinities, allowing us to compare results directly among all of our sampling sites.

#### *Laboratory:*

We follow the manufacturer's procedures for Enterolert, an EPA-approved method for pathogen indicator testing (see attachment). Sample handling and processing procedures are standardized

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<sup>12</sup> EPA Region 2 Laboratory, 2015. Guidance Document for Citizen Science Pathogen Monitoring of Enterococci Using IDEXX Enterolert with Quanti-Tray 2000 (Revision 5). Division of Environmental Science & Assessment, Edison, NJ. Attached.

across labs to ensure comparability of results. We rate water quality in comparison to EPA guidelines for safe swimming so that our results can be compared with results nationally.<sup>13</sup>

## 8.5 Completeness

### *Field:*

Community scientists will collect and analyze samples from the sites identified above monthly or twice monthly from May to October, totaling 486 samples. We intend to analyze 90 QC samples, not including positive and negative controls.

Sampling will be conducted unless weather conditions make access unsafe for samplers. If a sampling event must be cancelled or rescheduled, we will sample when conditions are clear, as scheduling permits, and QC samples will be included in the altered schedule. If the community scientist(s) assigned to a particular site are unavailable, for planned or unplanned reasons, the sampling coordinator will attempt to find a replacement sampler.

Since this project is a long-term monitoring study, rare gaps in sample coverage will not present a great challenge to the project. Data to be collected in 2017 are an extension of existing data sets, so they will provide information about average watershed conditions and site-by-site trends as long as data gaps are occasional and spread out in space. At an individual site, loss of 50% of samples or more, or loss of two months' worth of consecutive samples, will pose a challenge to interpreting trends at that site, and will make it difficult to compare that site's seasonal average with other sites. From time to time, changes in site access (e.g., road construction) may require a site to be dropped temporarily, moved or replaced. All available data will be posted on the Riverkeeper website, regardless of data discontinuities. However, discontinued sites and sites with data gaps may be excluded from data summary reports and watershed data analyses, or footnoted with a disclaimer about data limitations.

## 8.6 Sensitivity

### *Laboratory:*

The IDEXX Quanti-Tray 2000 Enterolert Most Probable Number (MPN) method allows detection of 1 colony forming unit per 100 mL in undiluted samples. As per standard methods, samples collected in saline or brackish water are diluted tenfold, making the lower limit 10 colony forming units per 100 mL. The MPN method can quantify up to 2,419.6 Enterococci per 100 mL without dilution (24,196 with a tenfold dilution).

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<sup>13</sup> EPA, 2012. Recreational Water Quality Criteria. Office of Water 820-F-12-058.  
<https://www.epa.gov/sites/production/files/2015-10/documents/rwqc2012.pdf>

## 9 Data Collection Methods

### 9.1 Site Names

Each site is assigned an ID consisting of the waterway initials and approximate river mileage (e.g., “WR-18.64”). In the case of secondary tributaries, the initials of both the main and secondary tributary are included, and the approximate river mile recorded is the location within the subtributary (e.g., “RC-BK-0.14”). Each site is also assigned a name usually consisting of the municipality in which the site is located, followed by a descriptor (e.g., “Ellenville- Beer Kill tributary”).

### 9.2 Sampling Design

Each community scientist will receive sampling guidelines (attached) at the time of training.

Sampling will occur approximately monthly from May to October. Water samples will be collected for *Enterococcus* using sterile containers. Samples will be taken from the stream banks with minimal disturbance to sediments. Samplers will wear disposable gloves to avoid contamination.

Samplers will record the location and time of sampling on the bottle or on waterproof labels (U-Line item #S-16643 or similar) with permanent marker. Both types of labeling have been tested over several sampling season and label disintegration and smudging have not presented problems in the past. The lab technician will check bottles for sampling times upon receipt at the lab, so problems with labels can be addressed immediately.

Samples will be immediately placed on ice in a dark container for transport to the laboratory, located at CURB. Upon sample delivery, laboratory staff will note on the Chain of Custody spreadsheet the time of receipt and the name of the sampler for each sample. Sample processing will be conducted following IDEXX instructions (attached).<sup>14</sup>

Maximum holding time will be six hours. In each watershed, there will be a designated sampling start time and lab drop-off time to ensure that the maximum holding time will not be exceeded. The sampling sites will be grouped into routes that a sampler or sampling team can visit within the designated time frame, with time to reach the drop-off point and time to spare. The site groupings will be determined prior to the start of the sampling season.

Each watershed will have a designated pool of community scientists who are committed to sampling throughout the season. In most cases, the same community scientists will visit the same site or group of sites repeatedly throughout the season. Exceptions may occur due to absence.

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<sup>14</sup> <https://www.idexx.com/water/products/enterolert.html>

People with knowledge of the site locations (staff or experienced samplers) will orient new samplers in person when possible, and with maps otherwise. Samplers will not be directly supervised in the field.

Laboratory replicates and field duplicates will be assigned prior to the start of the sampling season. One laboratory replicate and one field duplicate will be processed per watershed per sampling day. One laboratory blank will be processed on each sampling day. The blank will be prepared by pouring sterile water into the mixing vessel in place of stream water.

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	63	6/ 12	<i>Enterococcus</i>	1 field duplicate/ sampling date/ watershed 1 laboratory replicate/ sampling date/watershed 1 laboratory blank/ sampling date	486 samples/year 90 QC samples/year	EPA, 1997. Volunteer Stream Monitoring: A Methods Manual. Chapter 5, Task 2: Collecting Samples, For Screw Cap Bottles	Meets Objective 1

### 9.3 Non-Direct Measurements

We will use precipitation data from Weather Underground ([www.wunderground.com](http://www.wunderground.com)) as background information during data interpretation and analysis. We will calculate total precipitation as cumulative precipitation up to the time of sampling on the day of sampling, plus cumulative precipitation for the three days prior to sampling. Precipitation data will be posted alongside Entero counts on the Riverkeeper website. Samples may be classified as “wet-weather” (0.25+ inches of cumulative rainfall) or “dry-weather” (<0.25 inches) for data analysis and communications.

A primary, secondary and tertiary reference weather station will be designated for each sampling site prior to the start of the sampling season. From time to time, weather station data may be unavailable from a particular weather station. In the event that the primary weather station is not reporting at the time of sampling, the secondary and tertiary stations will be consulted. The station used will be noted in the data master spreadsheet.

At the close of the season, weather station data will be checked alongside Entero data. Data from secondary or tertiary stations will be updated with data from primary stations, where available. Weather stations will be evaluated for reporting gaps, and stations with frequent problems will be replaced.

## **10 Equipment List and Instrument Calibration**

### **10.1 Equipment**

#### *Field:*

- Cooler with ice
- Sample bottles
- Nitrile or latex gloves
- Marker
- Watch
- Sampler instruction sheet

#### *Laboratory:*

- IDEXX 100-mL sealed sterile disposable plastic bottles with sodium thiosulfate
- IDEXX Enterolert reagent powder snap packs
- IDEXX Quanti-Tray 2000 incubation trays
- Incubator
- Quanti-tray sealer
- Quanti-tray holder
- UV viewing cabinet
- IDEXX MPN table
- Sterile, deionized water
- Chain of Custody sheet
- Data Reporting sheet

### **10.2 Instrument Calibration and Maintenance**

Equipment will be inspected monthly by CURB staff and documented on a check list.

- Quanti-Tray Sealer Model 2x will be visually inspected ensuring trays are sealing
- WCM10 UV viewing cabinet bulb will be inspected
- SR-L4110W Laboratory Refrigerator temperature is at 4°C
- WI600, Large Incubator temperature will be measured with an additional FRIO-Temp thermometer to confirm incubator temperature (41°C)
- VWR Signature Ergonomic High-Performance Pipettor set volumes will be confirmed using a graduated cylinder

### **10.3 Inspection/Acceptance of Supplies and Consumables**

- IDEXX 100-ml sealed sterile disposable plastic bottles will be visually inspected
- IDEXX 100-ml sealed sterile disposable plastic bottles with sodium thiosulfate will be visually inspected
- IDEXX Enterolert reagent powder snaps will be checked for expiration date or any broken packs

Community scientists will obtain supplies in two ways. Those people who deliver samples directly to CURB will pick up replacement supplies each time they drop off samples. Those people who hand off samples for delivery by a driver will be mailed replacement samples prior to the next sampling date.



## 11 Analytical Methods

Matrix	Analytical Group/Parameter	Reporting & Detection Limits	Analytical & Preparation Method/ SOP Reference	Sample Volume	Containers	Preservation Requirements	Max Holding Time	Lab Used for Analysis
Stream Water	<i>Enterococcus</i>	<p><b>Detection Limits:</b>  <b>Undiluted</b>  Lower limit 1 Entero/100 mL, upper limit 2,420 Entero/100 mL  <b>Diluted</b>  Lower limit 10 Entero/100 mL, upper limit 24,196 Entero/100 mL</p> <p><b>Project Specific Ratings:</b>  Beach Action Value 60 Entero/100 mL  Statistical</p>	<p>IDEXX  Enterolert  Product Insert</p> <p>Riverkeeper  Protocols for  Enterolert  Sampling and  Processing</p>	100 mL	IDEXX mixing containers	On ice in dark	6 hours	Sarah Lawrence College Center for the Urban River at Beczak (CURB), 35 Alexander Street, Yonkers NY

Matrix	Analytical Group/Parameter	Reporting & Detection Limits	Analytical & Preparation Method/ SOP Reference	Sample Volume	Containers	Preservation Requirements	Max Holding Time	Lab Used for Analysis
		<p>Threshold Value 110 Entero/100 mL Geometric Mean 30 Entero/100 mL</p> <p><b>Replicate Limits:</b> Relative Percent Difference 30% or less</p> <p><b>Duplicate Limits:</b> Gives information about naturally occurring environmental variation; no duplicate limit</p>						

Lower Hudson Community Science Water Quality Testing Program  
2017 QAPP

Matrix	Analytical Group/Parameter	Reporting & Detection Limits	Analytical & Preparation Method/ SOP Reference	Sample Volume	Containers	Preservation Requirements	Max Holding Time	Lab Used for Analysis
Sterile Water	<i>Enterococcus</i>	<b>Blank Limits:</b> Entero counts >1 cell/100 mL	Riverkeeper Protocols for Enterolert Sampling and Processing	100 mL	IDEXX mixing containers	N/A	N/A	Sarah Lawrence College Center for the Urban River at Beczak (CURB), 35 Alexander Street, Yonkers NY

## 12 Field Data Sheet

Esopus Creek Raw Sampling Data:												EXAMPLE	
Site ID	River Mile	Sample Site Name	Date collected	Time collected	Start incubation	Time & date read	Sample (ml)	Dilution (ml)	# Small (max 48)	# Large (max 49)	Count (MPN) per 100 ml	Comments	
Esopus Creek													
102-EC1		Esopus Creek Entrance											
EC-1.57	1.57	Saugerties Village Beach											
EC-4.55	4.55	Mt Marion- USGS Streamgage											
EC-PC-0.53	6.32	Lake Katrine- Plattekill Creek tributary											
EC-8.37	8.37	Lake Katrine- Leggs Mill Bridge											
EC-SC-0.26	9.44	Lake Katrine- Sawkill Creek tributary											
EC-11.50	11.5	Lincoln Park- Orlando Park											
EC-14.26	14.26	Kingston- Washington Avenue Bridge											
EC-18.04	18.04	Hurley- Wyncoop Rd Bridge Fire Dept intake											
EC-21.38	21.38	Marbletown- Route 209											
EC-24.22	24.22	Marbletown- Tongore Park swimming beach											
		DUPLICATE											
		REPLICATE											
		BLANK											

## 13 Training and Specialized Experience

### 13.1 Training

Personnel/Group to be Trained	Description of Training	Frequency of Training
Community scientists	How to avoid sample contamination How to handle samples How to obtain consistent samples	Prior to first sampling trip
Lab Technician	Sample processing Reading Enterolert trays	Prior to sampling season

Field personnel will be trained in sample collection and handling by project staff listed in section 13.2, or by experienced samplers. The QA Manager will keep a list of trained samplers. Training will include information about how to prevent contamination of samples, how to handle samples, and how to obtain consistent samples. Samplers will receive a copy of sampling instructions for reference (attached).

Samples will be processed by the Lab Technician, with the Lab Manager as a substitute in case of last-minute scheduling conflicts. The Lab Technician was trained by the Lab Manager and QA Manager in 2016 and demonstrated capability by working alongside the Lab Manager until proficiency was reached (two sampling days). To demonstrate capability reading Enterolert trays, the Lab Manager, QA Manager, and Lab Technician cross-calibrated readings of one blank tray and multiple trays with positive results. We intend for the existing Lab Technician to process all samples in 2017. If a new Lab Technician must be hired, the Lab Manager will train him or her according to the procedures described above.

### 13.2 Specialized Experience

Person	Specialized Experience	# of Years' Experience
Jennifer Epstein	M.S., Biological Sciences Collecting and analyzing water samples for multiple parameters Coordinating community science-based water quality monitoring Data management and QA/QC	9
Ryan Palmer	Stream monitoring, biodiversity assessment, watershed management and planning Coordinating community science-	12

Lower Hudson Community Science Water Quality Testing Program  
2017 QAPP

	based water quality monitoring Collection and analysis of water samples for pathogen indicators	
Michelle Luebke	M.S., Conservation Ecology & Sustainable Development M.A., Environmental Conservation Education Managing citizen science-based ecological programs Teaching about environmental science	10
David Jofat	M.S. Candidate, CUNY Queens College. Teaching and lab assistant with Dr. Gregory O'Mullan Graduate work focused on fecal indicator bacteria, including use of IDEXX Enterolert system	3

## 14 Assessments and Oversight

<b>Assessment Type</b>	<b>Frequency of Assessment</b>	<b>What Is Being Assessed</b>	<b>Who Will Conduct the Assessment</b>	<b>How Issues or Deviations Will Be Addressed</b>	<b>How Results Will Be Documented</b>	<b>Who Results Will Be Reported To</b>
Sampling protocol	Each sampling date	Sample container and transport Sample volume Data recording (sampling time, location)	Laboratory Technician	Personal communication with community scientists	Affirmative checks (i.e., no problems found) will not be recorded Negative checks will be noted on chain of custody sheet	QA Manager
Data transcription	Each sampling date	Completeness and accuracy of online data Accuracy of water quality rating	QA Manager	Correct errors	End-of-season report	EPA Project Officer
Data quality check	End of season	Completeness and accuracy of master data sheet	QA Manager	Correct errors	End-of-season report	EPA Project Officer

## 15 Data Management

The Laboratory Technician will record Enterococcus counts from the IDEXX MPN table after accounting for dilution. Results will be written on Data Reporting sheets.

Scanned Data Reporting and Chain of Custody sheets will be emailed to the QA Manager. Enterococcus count results will be transcribed into a digital spreadsheet and emailed to the QA Manager, who will oversee uploading to the Riverkeeper website. A minimum of 10% of sites will be checked online for accuracy immediately after uploading.

## 16 Data Review and Usability Determination

### 16.1 Data Checks

Field/Lab	Data Management
<ul style="list-style-type: none"><li>•</li><li>• Lab blank results</li><li>• Lab replicate results</li><li>• Field duplicate results</li><li>• Positive/negative control results</li><li>• Sample holding times</li><li>• Sample handling</li></ul>	<ul style="list-style-type: none"><li>• Rating errors</li><li>• Transcription errors</li><li>• Data completeness by site</li><li>• Precipitation data gaps</li></ul>

#### *Data Checks Prior to Sampling Season*

Positive/negative control results will be reviewed at the time of processing. If positive/negative controls fail acceptability limits, the Lab Manager will first contact IDEXX to inquire about known problems with the reagent batches used during processing. If no problems exist, processing procedures will be reviewed and reagents will be examined for potential problems, and additional positive/negative controls will be processed until problems are identified and corrected.

#### *Data Checks During Sampling Season*

The lab technician will check for handling methods, sampling time recording and missed sampling sites as samples are delivered. Problems will be noted on the Chain of Custody sheet. Sample holding times will be evaluated when field sample results are recorded.

Results of QC samples (lab blanks, lab replicates and field duplicates) will be recorded by the Lab Technician in a dedicated QC sample spreadsheet at the same time that concurrent field sample results are recorded. The spreadsheet will contain formulas to automatically check acceptance criteria and flag samples that do not meet QC goals. The Lab Technician will report the results of QC sample checks to the QA Manager, Lab Manager and STORET Manager after each sampling date.



If QA criteria are not met, samples will be qualified as follows in the master spreadsheet:

- “HA” – Sample handling did not meet criteria (individual samples will be qualified)
- “HT” – Sample holding time was exceeded (individual samples will be qualified)
- “LB” – Lab blank sample result was 1 or greater (10 or greater for diluted samples) (all samples processed that day will be qualified)
- “LR” – Lab replicate RPD was greater than 30% (all samples processed that day will be qualified)

Except in severe cases (e.g., severe violation of sample handling criteria resulting in clear evidence of sample degradation, such as temperature), sample results will be posted on the Riverkeeper website and shared with samplers. These communications methods include background information to guide interpretation of results and prevent misapplication of data, including disclaimers that the study is designed to assess long-term trends and that sample data cannot be used to predict conditions at specific times and places.

#### *Data Checks at End of Season*

At the end of the sampling season, data from all four watersheds will be compiled. The number of data lines for the year will be checked against the number of samples planned to ensure that there are no missing data lines. Discrepancies will be referenced with Chain of Custody and Data Reporting sheets, and missing data will be added to the master spreadsheet. After all data have been compiled, 3% of data lines in the master spreadsheet (cumulative for all watersheds, to 2010) will be selected at random to check for data entry errors.

A data line contains the date, time, location Entero count and precipitation data recorded for an individual sample. The values in the master spreadsheet for each entry on the selected data lines will be verified against the Chain of Custody and Data Reporting sheets. If discrepancies are found, the data lines for all samples collected on that date will also be verified. Any errors will be corrected in the master spreadsheet and on the Riverkeeper website. The QC reports and QC sample spreadsheet will be reviewed for trends by the QC Manager, Lab Manager and STORET Manager at the end of the season. The team will also assess data record completeness site by site. The team will consider separately whether violations of acceptance criteria preclude data from being used for site-specific analyses and overall watershed analyses; data may be deemed useable for one purpose and not the other. If data are determined to be unusable the lines will be removed from the master spreadsheet and archived in the QC sample spreadsheet. Limitations on data usability will be noted in the master spreadsheet.

A summary of sampling statistics (number of sites sampled, total number of samples) and a qualitative explanation of data variability will be disseminated to the data users in post-season reports.

## 17 Reporting

Raw data will be uploaded to the Riverkeeper website (<https://www.riverkeeper.org/water-quality/citizen-data/>) after each sampling date. Scanned data and chain of custody sheets will be saved indefinitely on Riverkeeper's server. Enterococcus count spreadsheets will be saved in a shared Google Drive folder until they are transcribed to the master data sheet at end of the sampling season. The master data sheet will be saved indefinitely on Riverkeeper's server. Original paper copies will be stored indefinitely at CURB.

The project team (QA Manager, Lab Manager, STORET Manager) will collaborate on data usability determinations and data analysis during winter 2017-2018. The STORET Manager will oversee data upload to STORET in winter 2017-2018. A final report will be submitted to EPA in April 2018.



## GUIDELINES FOR WATER QUALITY SAMPLING

### Supplies Needed for Sampling:

- Cooler with ice to completely cover samples (no ice packs/blocks)  
\*If using a Styrofoam cooler, place samples inside a black bag inside the cooler
- Sterile sample containers
- Rubber gloves
- Permanent marker
- Watch

### By following the steps below you will minimize:

- Collecting sediment
- Contaminating the sample with your hands

### Steps to Follow when Sampling:

*Always sample from the same location!*

1. Write the location and time on the sample container
2. Put rubber gloves on both hands
3. Look for moving water. If you can't reach it from the bank, wade into the river (move carefully to avoid stirring up sediment) or step out on rocks until able to reach moving water
4. Remove the bottle cap
5. Submerge the bottle upside-down. While still underwater, tilt the bottle upwards until air escapes, with opening facing upstream
6. Discard the water downstream of your body (or elsewhere that avoids stirring up sediment)
7. Repeat for a total of three rinses
8. Using the same method, fill to the "shoulder" of the bottle
9. Cap the bottle
10. Immediately place the sample in the cooler and cover with ice
11. Deliver the sample directly to the lab

**If you see anything unusual while sampling** (e.g., outfall pipes flowing in dry weather, extensive algal growth), take close-up and wide-angle photos, and immediately contact Jen Epstein ([jepstein@riverkeeper.org](mailto:jepstein@riverkeeper.org)), Michelle Luebke ([michelle.luebke@parks.nyc.gov](mailto:michelle.luebke@parks.nyc.gov)), or Ryan Palmer ([rpalmer@sarahlawrence.edu](mailto:rpalmer@sarahlawrence.edu))



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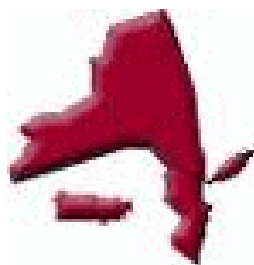
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**GUIDANCE DOCUMENT FOR CITIZEN SCIENCE PATHOGEN MONITORING  
OF ENTEROCOCCI USING IDEXX ENTEROLERT WITH QUANTI-TRAY<sup>®</sup> 2000**

**REVISION 5**



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



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

February 2015

## Table of Contents

1.0 SCOPE AND APPLICATION .....	3
2.0 METHOD DESCRIPTION .....	3
3.0 DEFINITIONS.....	4
4.0 INTERFERENCES.....	4
5.0 LABORATORY SAFETY AND HAZARDS.....	4
6.0 EQUIPMENT, SUPPLIES, CONSUMABLES CHECKLIST.....	5
6.1 Equipment	
6.2 Enterolert Reagents	
6.3 Sterile Deionized Water For Sample Dilutions And Quality Control	
6.4 Quality Control Samples	
6.5 Consumables, Lab Supplies, Safety Equipment	
7.0 SAMPLE HANDLING, PRESERVATION, STORAGE AND HOLDING TIME.....	7
8.0 PREPARING THE ENTEROLERT REAGENT AND TEST SAMPLES.....	7
9.0 PREPARING THE QUANTI-TRAYS WITH THE REAGENT/SAMPLE MIXTURE.....	9
10.0 READING THE RESULTS (24-28 HOURS LATER).....	111
11.0 QUALITY ASSURANCE AND QUALITY CONTROL FOR EACH BATCH OF SAMPLES .....	122
11.1 Blank	
11.2 Positive Analytical Quality Control Sample	
11.2.1 How To Prepare Positive Control Sample	
11.3 Laboratory Sample Duplicate Analysis	
11.4 Other Qc Activities	
11.5 Demonstration Of Capability (Doc)	
12.0 DATA EVALUATION .....	155
12.1 My Sampling Event Is Completed And My Data Is Collected, Now What?	
12.2 Single Maximum Concentration Versus A Geometric Mean	
12.3 Interpretation Of Data And Impacts	
13.0 REFERENCES:.....	189
ATTACHMENT 1- Example of citizen science chain of custody and laboratory data form.....	20
ATTACHMENT 2- IDEXX-Quanti-Tray 2000 MPN Table .....	23

*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<sup>®</sup> FOR ENTEROCOCCI***

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

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### **1.0 SCOPE AND APPLICATION**

Enterolert<sup>®</sup> 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<sup>®</sup>. When enterococci bacteria utilize an enzyme to metabolize Enterolert's nutrient indicator, the sample fluoresces if *Enterococcus* is present. After 24 hours of incubation at 41°C, an enterococci-positive result causes a blue fluorescence under long-wave ultraviolet light (365 nm).

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

The detection limit for this method is 1 Most Probable Number (MPN)/100 mL for undiluted samples. This methodology is based on IDEXX product inserts, Burdick et al. 1996, ASTM D6503-99 (Reapproved 2009) and Standard Methods, 21<sup>st</sup> 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 detected 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  $\beta$ -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 ° (\$2000)
- Certified incubator thermometer (spirit filled or digital) with 0.1 °C 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  $<10^{\circ}\text{C}$  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.

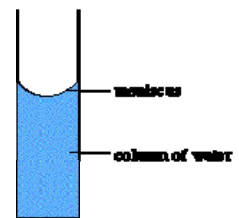
If residual chlorine is expected (but should not be present in ambient surface water samples) use sterile sample bottles preloaded with sodium thiosulfate (powder or tablet) to neutralize the residual chlorine.

## 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  $40.5^{\circ}\text{C} - 41.5^{\circ}\text{C}$  (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 requirements. 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 °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 *Enterococcus* grow in your positive control and was that growth within the acceptance limits provided by the manufacturer?
- Was the test performed in accordance with your established procedures? If not, note the deviation on the data sheet.
- Were there any other deviations during sampling and analysis which would affect the quality of the data?
- The most important thing to do is to Document, Document, Document! Observations that may not seem important at the time of sampling or analysis may be crucial when it comes to evaluating your data.

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

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

#### **“U” Qualifier**

This qualifier is attached to a result to indicate that the organism was not detected at the prescribed reporting limit of the method. For *Enterococcus*, 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 °C, or 0.1 °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 °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 “ $n$ th” 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 37,800,000

= 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 projects 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.**

[illegible]

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

Positive Control Result within manufacturers Acceptance Limits?
Yes or No

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

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**

# Large Wells Positive	IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)																								
	# 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																	

# Large Wells Positive	IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)																							
	# 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				



## Enterolert\* Test Kit

### Introduction

Enterolert\* detects enterococci, such as *E. faecium* and *E. faecalis*, in fresh and marine water. It is based on IDEXX's patented Defined Substrate Technology\* (DST\*). When enterococci utilize their β-glucosidase enzyme to metabolize Enterolert's nutrient-indicator, 4-methyl-umbelliferyl β-D-glucoside, the sample fluoresces. Enterolert detects enterococci at 1 cfu per 100 mL sample within 24 hours.

### Storage

Store at 2–30°C away from light.

### Presence/Absence (P/A) Procedure

- Add contents of one pack to a 100 mL sample in a sterile, transparent, nonfluorescing vessel.
- Cap vessel and shake.
- Incubate at 41 ±0.5°C for 24 hours.
- Read results according to Result Interpretation table below.

### Quantı-Tray\* Enumeration Procedure

- Add contents of one pack to a 100 mL water sample in a sterile vessel.
- Cap vessel and shake until dissolved.
- Pour sample/reagent mixture into a Quanti-Tray\* or Quanti-Tray\*/2000 and seal in an IDEXX Quanti-Tray\* Sealer.
- Place the sealed tray in a 41 ±0.5°C incubator for 24 hours.
- Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

### Result Interpretation

Appearance	Result
Lack of fluorescence	Negative for enterococci
Blue fluorescence	Positive for enterococci

- Look for fluorescence with a 6–watt, 365 nm, UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.
- Enterolert results are definitive at 24–28 hours. In addition, positives for enterococci observed before 24 hours and negatives observed after 28 hours are also valid.

### Procedural Notes

- Marine water samples must be diluted at least tenfold with sterile fresh water. Multiply the MPN value by the dilution factor to obtain the proper quantitative result.
- Use only sterile, nonbuffered, oxidant-free water for dilutions.
- For comparison, a water blank can be used when interpreting results.
- This insert may not reflect your local regulations. For compliance testing, be sure to follow appropriate regulatory procedures.
- Enterolert can be run in any multiple tube format. *Standard Methods for the Examination of Water and Wastewater*<sup>1</sup> MPN tables should be used to find Most Probable Numbers.
- Enterolert is a primary water test. Enterolert performance characteristics do not apply to samples altered by any pre-enrichment or concentration.
- Aseptic technique should always be followed when using Enterolert. Dispose of in accordance with Good Laboratory Practices.

### Quality Control Procedures

- One of the following quality control procedures is recommended for each lot of Enterolert:
  - IDEXX-QC Enterococci<sup>2</sup>: *Enterococcus faecalis*, *Escherichia coli*, and *Streptococcus bovis*.
  - i. For each of the American Type Culture Collection (ATCC)<sup>3</sup> bacterial strains (*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862, and *Aerococcus veridans* ATCC 10400), streak the culture onto labeled TSA or Blood Agar plates and incubate at 35 ±2°C for 18–24 hours.
  - ii. For each bacterial strain, touch a sterile 1 µL inoculating loop to a colony and use it to inoculate a labeled test tube containing 5 mL of sterile deionized water. Close cap and shake thoroughly.
  - iii. For each bacterial strain, take a 1 µL loop from the test tube and use it to inoculate a labeled vessel containing 100 mL of sterile deionized water. These are your controls.
- Follow the P/A Procedure or Quanti-Tray Enumeration Procedure above.
- Results should match the Result Interpretation table above.

**NOTE:** IDEXX internal quality control testing is performed in accordance with ISO 11133:2014. Quality Control Certificates are available at [idexx.com/water](http://idexx.com/water).

## Kit d’analyse Enterolert\*

### Introduction

Enterolert\* permet la détection d’enterococci tels que *E. faecium* et *E. faecalis* dans l’eau douce et l’eau de mer. Ce test est basé sur la technologie brevetée Defined Substrate Technology\* (DST\*) d’IDEXX. Lorsque les enterococci utilisent leur enzyme β-glucosidase pour métaboliser l’indicateur de nutriants Enterolert, le 4-méthyl-umbelliféryl β-D-glucoside, l’échantillon devient fluorescent. Enterolert détecte les enterococci à 1 cfu par prélèvement de 100 mL en 24 heures.

### Conditions de conservation

Conserver entre 2–30°C à l’abri de la lumière.

### Procédure de présence/absence (P/A)

- Ajouter le contenu d’un sachet dans un prélèvement de 100 ml placé dans un récipient stérile, transparent et non fluorescent.
- Fermer le récipient et agiter.
- Incuber à 41 ±0.5°C pendant 24 heures.
- Interpréter les résultats en se référant au tableau d’interprétation des résultats ci-dessous.

### Procédure de numération Quanti-Tray\*

- Ajouter le contenu d’un sachet dans un prélèvement de 100 ml d’eau placé un récipient stérile.
- Fermer le récipient et agiter jusqu’à dissolution.
- Verser le mélange prélèvement/réactif dans un Quanti-Tray\* ou Quanti-Tray\*/2000 et fermer hermétiquement dans un IDEXX Quanti-Tray\* Sealer.
- Placer le plateau hermétiquement fermé dans un incubateur à 41 ±0.5°C pendant 24 heures.
- Interpréter les résultats en se référant au tableau d’interprétation des résultats ci-dessous. Compter le nombre de puits positifs et se référer au tableau MPN fourni avec les plateaux pour obtenir le Chiffre le plus probable (MPN).

### Interprétation des résultats

Aspect	Résultat
Absence de fluorescence	Négatif pour enterococci
Fluorescence bleue	Positif pour enterococci

- Évaluer la fluorescence dans l’obscurité, avec une ampoule UV de 6 watts et 365 nm placée à 13 cm du prélèvement. Orienter la lumière vers le prélèvement, dans la direction opposée à celle des yeux de l’opérateur.
- Les résultats de Enterolert doivent être lus entre 24–28 heures. En outre, les résultats positifs pour enterococci notés avant 24 heures, de même que les résultats négatifs notés après 28 heures, sont également valides.

### Remarques concernant la procédure

- Le prélèvement d’eau salée doit être dilué au moins au dixième avec de l’eau douce stérile. Multiplier la valeur MPN par le facteur de dilution pour obtenir le résultat quantitatif correspondant.
- Utiliser uniquement de l’eau stérile, non tamponnée et sans oxydant pour les dilutions.
- Pour comparer, il est possible d’utiliser une eau pure stérile (water blank) pour interpréter les résultats.
- Cette notice peut différer des réglementations en vigueur dans votre pays. Pour tout test de conformité, suivre les procédures réglementaires appropriées.
- Enterolert peut être effectué en format de tubes multiples. Utiliser des méthodes standard et les tableaux MPN pour le contrôle des eaux et des eaux usées<sup>1</sup> afin de déterminer les Chiffres les plus probables (MPN).
- Enterolert est avant tout un test pour eau. Les caractéristiques de performance d’Enterolert ne s’appliquent pas aux prélèvements altérés par tout enrichissement préalable ou toute concentration.
- Utiliser systématiquement des techniques aseptiques dans l’emploi d’Enterolert. Mettre au rebut conformément aux Bonnes pratiques de laboratoire.

### Le contrôle de la qualité

- L’une des procédures de contrôle qualité suivantes est recommandée pour chaque lot d’Enterolert:
  - IDEXX-QC Enterococci<sup>2</sup>: *Enterococcus faecalis*, *Escherichia coli* et *Streptococcus bovis*.
  - i. Pour chacune des souches bactériennes American Type Culture Collection (ATCC)<sup>3</sup> (*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862 et *Aerococcus veridans* ATCC 10400), placer la culture sur des plaques TSA ou de gélose au sang dûment étiquetées et incuber à 35 ±2°C pendant 18 à 24 heures.
  - ii. Pour chaque souche bactérienne, prélever 1 µL d’une colonie à l’aide d’une anse et inoculer un tube à essai dûment étiqueté contenant 5 ml d’eau désionisée stérile. Fermer le bouchon, puis secouer vigoureusement.
  - iii. Pour chaque souche bactérienne, prendre une anse de 1 µL du tube à essai et l’utiliser pour inoculer un récipient dûment étiqueté contenant 100 ml d’eau désionisée stérile. Il s’agit de vos contrôles.
- Suivre la procédure P/A ou la procédure de numération Quanti-Tray ci-dessus pour tester ces contrôles.
- Les résultats doivent correspondre avec le tableau d’interprétation des résultats ci-dessus.

**REMARQUE:** les tests de contrôle qualité internes d’IDEXX sont effectués conformément à la norme ISO 11133:2014. Les certificats de contrôle qualité sont disponible à l’adresse [idexx.fr/water](http://idexx.fr/water).

## Kit di analisi Enterolert\*

### Introduzione

Il kit Enterolert\* rileva gli enterococci come l’*E. faecium* e l’*E. faecalis* in acque dolci e marine. Il kit si basa sulla tecnologia brevettata IDEXX chiamata Defined Substrate Technology\* (DST\*). Quando gli enterococci utilizzano il loro enzima β-glucosidasi per metabolizzare il nutriente-indicatore di Enterolert, il 4-metil-umbelliferil β-D-glucoside, il campione diventa fluorescente. Enterolert rileva gli enterococci in concentrazioni di 1 cfu (unità formante colonia) per campione da 100 mL entro 24 ore.

### Conservazione

Conservare a 2–30°C lontano dalla luce.

### Procedura di presenza/assenza (P/A)

- Aggiungere il contenuto di una bustina (pack) ad un campione di 100 ml, in un recipiente sterile, trasparente e non fluorescente.
- Chiudere il recipiente ed agitarlo.
- Incubare a 41 ±0.5°C per 24 ore.
- Leggere i risultati in base alla Tabella di interpretazione dei risultati qui sotto.

### Procedura di enumerazione Quanti-Tray\*

- Aggiungere il contenuto di una bustina (pack) ad un campione di acqua di 100 ml, in un recipiente sterile.
- Chiudere il recipiente ed agitarlo fino alla dissoluzione.
- Versare la miscela campione/reagente in un Quanti-Tray\* o un Quanti-Tray\*/2000 e sigillarlo con un IDEXX Quanti-Tray\* Sealer.
- Mettere il vassoietto sigillato in un incubatore a 41 ±0.5°C per 24 ore.
- Leggere i risultati in base alla Tabella di interpretazione dei risultati qui sotto. Contare il numero dei pozzetti positivi e consultare la tabella MPN in dotazione con i vassoietti per ottenere il Numero più probabile (MPN).

### Interpretazione del risultato

Aspetto	Risultato
Mancanza di fluorescenza	Negativo per gli enterococci
Fluorescenza azzurra	Positivo per gli enterococci

- Individuare la presenza di fluorescenza con una luce a raggi ultravioletti da 6 watt, 365 nm a distanza di 13 cm, in ambiente oscuro. Rivolgere la luce in direzione opposta ai propri occhi e verso il campione.
- I risultati di Enterolert sono definitivi a distanza di 24–28 ore. Inoltre, sono validi anche i risultati positivi per gli enterococci osservati prima delle 24 ore ed i risultati negativi osservati dopo 28 ore.

### Note procedurali

- Il campione di acqua marina deve essere diluito almeno dieci volte con acqua dolce sterile. Moltiplicare il valore MPN per il fattore di diluizione per ottenere il risultato quantitativo giusto.
- Per le diluizioni usare solo acqua sterile, non tamponata e senza ossidanti.
- A scopi comparativi, quando si interpretano i risultati si può utilizzare un campione di acqua come modello.
- Questo inserto informativo potrebbe non riflettere i regolamenti locali dell’utente. Per i test di conformità, assicurarsi di seguire le appropriate procedure normative.
- Il test Enterolert può essere eseguito in qualsiasi formato con provette multiple. Per trovare i Numeri più probabili (MPN) vanno impiegate le tabelle MPN dei Metodi standard per l’analisi dell’acqua e delle acque di scarico (*Standard Methods for the Examination of Water and Wastewater*<sup>1</sup>).
- L’analisi Enterolert è un’importante analisi dell’acqua. Le caratteristiche di prestazione Enterolert non sono applicabili a campioni alterati da qualsiasi pre-aricchimento o concentrazione.
- Quando si usa Enterolert vanno sempre seguite tecniche asettiche. Eliminare seguendo le Buone pratiche di laboratorio.

### Procedure per il controllo di qualità

- Per ciascun lotto di Enterolert si consiglia una delle seguenti procedure di controllo della qualità:
  - Enterococchi<sup>2</sup> IDEXX-QC: *Enterococcus faecalis*, *Escherichia coli* e *Streptococcus bovis*.
  - i. Per ciascun ceppo batterico della American Type Culture Collection (ATCC)<sup>3</sup> (*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862 e *Aerococcus veridans* ATCC 10400), strisciare la coltura su piastre TSA o su piastre Agar sangue e incubare a 35 ±2°C per 18–24 ore.
  - ii. Per ciascun ceppo batterico, toccare una colonia con un’ansa sterile per inoculazione da 1 µL e utilizzarla per inoculare una provetta etichettata contenente 5 ml di acqua deionizzata sterile. Chiudere il tappo e agitare bene.
  - iii. Per ciascun ceppo batterico, prelevare un’ansa da 1 µL dalla provetta e utilizzarla per inoculare un contenitore etichettatocontenente 100 ml di acqua deionizzata sterile. Questi sono i controlli.
- Seguire la procedura P/A o la procedura di enumerazione Quanti-Tray di cui sopra.
- I risultati dovrebbero corrispondere alla tabella di interpretazione dei risultati di cui sopra.

**NOTA:** i test di controllo di qualità interni IDEXX sono condotti in conformità con ISO 11133:2014. I certificati di controllo qualità sono disponibili sul sito [idexx.it/water](http://idexx.it/water).

## Enterolert\* Testkit

### Einführung

Enterolert ist zum Nachweis von Enterokokken, wie z. B. *E. faecium* und *E. faecalis*, in Süß-und Meereswasser bestimmt. Der Test basiert auf der patentierten Defined Substrate Technology\* (DST\*) von IDEXX. Wenn die Enterokokken mit ihrem β-Glucosidase-Enzym den Nährstoff-Indikator 4-Methylumbelliferyl-β-D-Glucosid von Enterolert metabolisieren, fluoresziert die Probe. Enterolert weist Enterokokken im Bereich von 1 CFU (kB ; koloniebildende Einheit) pro 100 mL Probe innerhalb von 24 Stunden nach.

### Lagerung

Bei 2–30° C und vor Licht geschützt lagern.

#### Presence/Absence (P/A)-Test

- Den Inhalt einer Packung einer 100 ml Probe in einem sterilen, transparenten, nicht fluoreszierenden Gefäß hinzufügen.
- Gefäß verschließen und schütteln.
- 24 Stunden bei 41 ±0.5°C inkubieren.
- Das Ergebnis gemäß der nachstehenden Ergebnisauswertetabelle ablesen.

#### Quantı-Tray\* Auszählmethode

- Den Inhalt einer Packung einer 100 ml Probe in einem sterilen Gefäß hinzugeben.
- Gefäß verschließen und schütteln, bis sich das Präparat völlig aufgelöst hat.
- Die aus Probe und Reagenz bestehende Mischung in ein Quanti-Tray\* oder Quanti-Tray\*/2000 gießen und in einem IDEXX Quanti-Tray\* Sealer verschließen.
- Das verschlossene Tray 24 Stunden bei 41 ±0.5°C inkubieren.
- Die Ergebnisse anhand der nachstehenden Ergebnisauswertetabelle ablesen. Die Anzahl der positiven Probenvertiefungen zählen und die wahrscheinlichste Zahl (MPN; Most Probable Number) anhand der MPN-Tabelle, die den Trays beiliegt, ermitteln.

#### Ergebnisauswertung

Erscheinung	Ergebnis
Keine Fluoreszenz	Negativ für Enterokokken
Blaue Fluoreszenz	Positiv für Enterokokken

- Prüfung auf Fluoreszenz mit einer 6-Watt-, 365-nm-UV-Lampe aus einem Abstand von 13 cm in einer dunklen Umgebung. Dabei die Lampe nur auf die Probe, nicht auf die Augen, richten.
- Die Enterolert-Ergebnisse sind nach 24–28 Stunden definitiv. Außerdem sind vor Ablauf von 24 Stunden beobachtete positive und nach Ablauf von 28 Stunden beobachtete negative Reaktionen auf Enterokokken ebenfalls gültig.

#### Verfahrenshinweise

- Meereswasser muss mindestens zehnfach mit sterilem Süßwasser verdünnt werden. Dann ist der MPN-Wert mit dem Verdünnungsfaktor zu multiplizieren, um das richtige quantitative Ergebnis zu erhalten.
- Nur steriles, nicht gepuffertes, keine Oxidantien enthaltendes Wasser zur Verdünnung verwenden.
- Für Vergleichszwecke kann bei der Ergebnisauswertung eine Blindprobe (Wasser) herangezogen werden.
- Diese Packungsbeilage gibt möglicherweise nicht Ihre örtlichen Vorschriften wieder. Bei Qualitätstests sind die anwendbaren aufsichtsbehördlichen Verfahren zu befolgen.
- Das Enterolert-Verfahren kann in jedem Multiple-Tube-Format durchgeführt werden. Zur Ermittlung der MPNs (wahrscheinlichste Zahlen) sollten MPN-Tabellen für Standardverfahren zur Untersuchung von Wasser und Abwasser<sup>1</sup> herangezogen werden.
- Enterolert ist primär ein Wassertest. Die Leistungsmerkmale von Enterolert gelten nicht für Proben, die durch Voranreicherung oder Konzentration modifiziert wurden.
- Bei der Verwendung von Enterolert ist ein aseptisches Vorgehen vorgeschrieben. Entsorgung gemäß Standard-Laborpraktiken.

#### Qualitätskontrollverfahren

- Eines der folgenden Qualitätskontrollverfahren wird für jede Enterolert-Charge empfohlen:
  - IDEXX-QC *Enterococci*<sup>2</sup>: *Enterococcus faecalis*, *Escherichia coli* und *Streptococcus bovis*.
  - i. Für jeden American Type Culture Collection (ATCC)<sup>3</sup>-Bakterienstamm (*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862 und *Aerococcus veridans* ATCC 10400) die Kultur auf etikettierte TSA- oder Blutagarplatten streichen und 18-24 Stunden bei 35 ±2°C inkubieren.
  - ii. Für jeden Bakterienstamm eine Kolonie mit einer sterilen 1-µl-Impföse berührend ein etikettierten Probenröhrchens, das 5 ml steriles deionisiertes Wasser enthält, inokulieren. Kappe verschließen und gründlich schütteln.
  - iii. Für jeden Bakterienstamm mit einer 1-µl-Öse eine Probe aus dem Teströhrchen entnehmen und ein etikettiertes Gefäß, das 100 ml steriles deionisiertes Wasser enthält, damit inokulieren. Dies sind Ihre Kontrollen.
- Das oben beschriebene P/A-Verfahren oder das Quanti-Tray-Auszählverfahren anwenden.
- Die Ergebnisse sollten mit der Tabelle zur Ergebnisauswertung (siehe oben) übereinstimmen.

**HINWEIS:** Die internen Qualitätskontrollprüfungen von IDEXX werden im Einklang mit ISO 11133:2014 durchgeführt. Qualitätskontrollzertifikate sind unter [idexx.de/water](http://idexx.de/water) erhältlich.

<sup>[1]</sup> Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. Standard Methods for the Examination of Water and Wastewater. 21st ed. Washington, DC: American Public Health Association; 2005.

<sup>[2]</sup> IDEXX-QC Enterococci—IDEXX Catalog #UN3373-WQC-ENT

<sup>[3]</sup> American Type Culture Collection 1-800-638-6597 atcc.org

<sup>[\*]</sup>Enterolert, Defined Substrate Technology, DST and Quanti-Tray are trademarks or registered trademarks of IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries. Patent information: idexx.com/patents.

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<sup>[1]</sup> Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. Standard Methods for the Examination of Water and Wastewater. 21st ed. (Méthodes traditionnelles pour l’analyse de l’eau et des eaux usées). Washington, DC: American Public Health Association; 2005.

<sup>[2]</sup> Entérocoques d’IDEXX-QC - Catalogue IDEXX n° UN3373-WQC-ENT

<sup>[3]</sup> American Type Culture Collection 1-800-638-6597 atcc.org

<sup>[\*]</sup>Enterolert, Defined Substrate Technology, DST et Quanti-Tray sont des marques de fabrique ou des marques déposées d’IDEXX Laboratories, Inc. ou ses filiales aux États-Unis et/ou dans d’autres pays.

Information sur les brevets: idexx.com/patents.

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<sup>[1]</sup> Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. Standard Methods for the Examination of Water and Wastewater. 21st ed. Washington, DC: American Public Health Association; 2005.

<sup>[2]</sup> Enterococchi IDEXX-QC - Catalogo IDEXX N. UN3373-WQC-ENT

<sup>[3]</sup> American Type Culture Collection 1-800-638-6597 atcc.org

<sup>[\*]</sup>Enterolert, Defined Substrate Technology, DST e Quanti-Tray sono marchi di proprietà di, e/o registrati da, IDEXX Laboratories, Inc. o di suoi associate e protetti negli Stati Uniti e/o in altri paesi. Informazioni sui brevetti: idexx.com/patents.

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<sup>[1]</sup> Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. Standard Methods for the Examination of Water and Wastewater. 21st ed. (Standardverfahren für die Wasser- und Abwasseruntersuchung). Washington, DC: American Public Health Association; 2005.

<sup>[2]</sup> IDEXX-QC Enterococci – IDEXX Bestellnr. UN3373-WQC-ENT

<sup>[3]</sup> American Type Culture Collection 1-800-638-6597 atcc.org

<sup>[\*]</sup>Enterolert, Defined Substrate Technology, DST und Quanti-Tray sind Schutzmarken oder eingetragene Schutzmarken von IDEXX Laboratories, Inc. oder eines Tochterunternehmens von IDEXX in den Vereinigten Staaten und/oder anderen Ländern.

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IDEXX Water Quality Control Laboratory is accredited to ISO/IEC 17025:2005

## Kit de análisis Enterolert\*

### Introducción

Enterolert\* detecta enterococos tales como enterococos *E. faecium*, y enterococos *E. faecalis* en agua superficial de bano y agua de mar. Se basa en Defined Substrate Technology® (Tecnología de sustrato definido [DST®]), patentada por IDEXX. Cuando los enterococos utilizan su enzima β-glucosidasa para metabolizar el indicador de nutriente de Enterolert, 4-metil-umbeliferil β-D-glucosida, la muestra fluoresce. Enterolert detecta enterococos en una muestra de 1 ufc por 100 mL dentro de 24 horas.

### Almacenamiento

Almacenar a temperatura de 2–30°C, alejado de la luz.

### Procedimiento de presencia/ausencia (P/A)

- Añadir el contenido de un paquete a una muestra de 100 ml en un recipiente estéril transparente, no fluorescente.
- Tapar y agitar el recipiente.
- Incubar a 41±0,5°C durante 24 horas.
- Leer los resultados de acuerdo con el cuadro de interpretación de resultados, más abajo.

### Procedimiento de enumeración Quanti-Tray\*

- Añadir el contenido de un paquete a una muestra de 100 ml de agua, en un recipiente estéril.
- Tapar y agitar el recipiente hasta disolver.
- Verter la mezcla de muestra/reactivo en una Quanti-Tray\* o una Quanti-Tray\*/2000 y sellar en un IDEXX Quanti-Tray\* Sealer.
- Colocar la bandeja sellada en una incubadora a 41 ±0,5°C durante 24 horas.
- Leer los resultados de acuerdo con el cuadro de interpretación de resultados, más abajo. Contar el número de pocillos positivos y referirse a la table NMP proporcionadas con las bandejas para obtener el número más probable.

### Interpretación de resultados

Apariencia	Resultado
Ausencia de fluorescencia	Negativo para enterococos
Fluorescencia azul	Positivo para enterococos

- Buscar fluorescencia usando una luz ultravioleta de 6 vatios, 365 nm a distancia de unas 5 pulgadas (13 cm) de la muestra, en un entorno oscuro. Apuntar el haz de luz en dirección contraria a los ojos y hacia la muestra.
- Los resultados Enterolert son definitivos a las 24–28 horas. Además, los positivos para enterococos observados antes de las 24 horas y los negativos observados después de las 28 horas también son válidos.

### Notas sobre el procedimiento

- La muestra de agua de mar deberá diluirse por lo menos diez veces con agua superficial de bano estéril. Multiplicar el valor NMP por el factor de dilución para obtener el resultado cuantitativo apropiado.
- Utilizar solamente agua estéril, no tamponada, libre de oxidantes, para efectuar las diluciones.
- Para comparación, se puede utilizar blanco testigo de agua al interpretar resultados.
- Este prospecto tal vez no refleje sus reglamentaciones locales. Para realizar pruebas en cumplimiento, asegúrese de seguir los procedimientos reglamentarios apropiados.
- Enterolert puede procesarse en cualquier formato de múltiples tubos. Deben utilizarse métodos estándares para examen del agua y las tablas NMP de aguas residuales (*Standard Methods for the Examination of Water and Wastewater*<sup>1)</sup>) para encontrar el resultado de NMP.
- Enterolert es un a prueba primaria de agua. Las características de desempeño de Enterolert no se aplican a muestras alteradas por cualquier enriquecimiento o concentración previos.
- Siempre debe aplicarse una técnica aséptica cuando se utilice Enterolert. Se debe desechar en cumplimiento con las buenas prácticas de laboratorio.

### Procedimientos de control de calidad

- Se recomienda uno de los siguientes procedimientos de control de calidad para cada lote de Enterolert:

- IDEXX-QC Enterococci<sup>2)</sup>: *Enterococcus faecalis*, *Escherichia coli* y *Streptococcus bovis*.
- Para cada una de las cepas bacterianas de Colección Americana de Cultivos Tipo (American Type Culture Collection, ATCC)<sup>3)</sup>(*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862, y *Aerococcus veridans* ATCC 10400), siembre en estrías el cultivo sobre placas etiquetadas de agar sangre o agar tripticasa de soja (Trypticase Soy Agar, TSA) e incube a 35±2°C durante 18 a 24 horas.
  - Para cada cepa bacteriana, agregue 1 µl de asa de inoculación a una colonia y úsela para inocular una probeta etiquetada que contenga 5 ml de agua estéril desionizada. Cierre la tapa y agite energícamente.
  - Para cada cepa bacteriana, tome 1 µl de asa de la probeta y úsela para inocular un recipiente etiquetado que contenga 100 ml de agua estéril desionizada. Estos son los controles que debe realizar.
- Sega el procedimiento P/A o el procedimiento de enumeración de Quanti-Tray que se indica más arriba.
- Los resultados deben coincidir con la tabla de interpretación de resultados que se indica más arriba.

**NOTA:** Las pruebas de control de calidad interna de IDEXX se realizan según ISO 11133:2014. Los certificados de control de calidad se encuentran disponibles en [idexx.es/water](http://idexx.es/water).



## Enterolert\* (エンテロラート) テストキット

### はじめに

Enterolert\* は、水および海水中の*E. faecium*、*E. faecalis*などの腸球菌を検出します。検出方法は、IDEXXが特許を所有する特定酵素基質法 Defined Substrate Technology® (DST®) に基づいています。腸球菌がβ-グルコシダーゼ酵素によって Enterolert中の栄養指標である4-メチル-ウンベリフェリルβ-D-グルコシドを代謝すると、検体は蛍光を発します。Enterolertは、24時間以内に1 cfu/100mLの感度で腸球菌を検出することができます。

### 保管

直射日光を避け、2～30° Cで保管してください。

### 定性検査・操作手順

- 透明な滅菌容器に検体100 mlを入れ、スナップバック1個を加えてください。
- 容器の蓋を閉め、混ぜてください。
- 41±0.5°Cで24時間培養してください。
- 以下の「結果判定表」に従って結果判定してください。

### Quanti-Tray\* 定量法

- 滅菌容器に検体100 mlを入れ、スナップバック1個を加えてください。
- 容器の蓋を閉め、ゆっくり混ぜてください。
- Quanti-Tray\*/2000Iに注ぎ、IDEXX Quanti-Tray\*シーラーで密封してください。
- 密封したトレーを41±0.5°Cで24時間培養してください。
- 下記の「結果判定表」に従って結果を判定してください。陽性ウェルの数を数え、専用MPN表を参照して最確数 (MPN) を求めてください。

### 結果判定

培養液の状態	結果
蛍光なし	腸球菌陰性
青く蛍光発色	腸球菌陽性

- 暗所で検体から5インチ (13 cm) 以内の距離から6W・365nmのUVランプを使用して判定してください。光は目に向けないようにし、検体に向けてください。
- Enterolertの結果は24～28時間で判定してください。24時間以内で腸球菌が陽性となった場合、陽性判定が有効となり、28時間以上培養して陰性となった場合は、陰性判定が有効です。

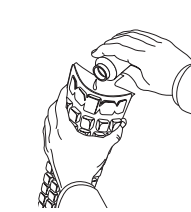
### 操作上の注意

- 海水検体は滅菌水で10倍以上に希釈してください。MPN値に希釈係数を掛けて適切な定量結果を求めてください。
- 希釈には、緩衝剤や酸化物質の入っていない、滅菌水のみを使用してください。
- 比較のため、結果判定時に水のみブランクを使用してください。
- 本使用説明書の内容は、各国の法律・条例に適合していないこともあります。法律・条例に準拠したテストをする為に、適切な規定の手順に必ず従ってください。
- Enterolertは5本法などの最確数法でも検査可能です。MPN表は、最確数 (MPN) を求めるためにご使用ください。
- Enterolertは水の一次検査です。Enterolertの性能特性として、増菌培地で培養または濃縮によって変質した検体に適用できません。
- Enterolertを使用しているときは、常に無菌操作を行ってください。GLPIに従って廃棄してください。

### 品質管理手順

- Enterolertを使用する場合、ロット毎に次の品質管理手順のいずれかを行うことをお勧めします。
  - IDEXX-QC腸球菌<sup>1)</sup>: *Enterococcus faecalis*、大腸菌、*Streptococcus bovis*。
  - 米国培養細胞系統保存機関 (ATCC)<sup>3)</sup>の各菌種 (*Enterococcus faecium* ATCC 35667、*Serratia marcescens* ATCC 43862および*Aerococcus veridans* ATCC 10400) については、ラベルを付けたTSA培地もしくは血液寒天培地上に菌を画線し、35±2°Cで18～24時間培養します。
    - 各菌種につき、1 µLの滅菌接種ループで、コロニーを滅菌脱イオン水 5 mLが入った試験管に接種します。蓋を閉め、十分に振ってください。
    - 各菌種につき、試験管から1 µLをループで取り、滅菌脱イオン水100 mLを入れた容器に接種します。これが対照群 (コントロール) となります。
- 上記の定性法 (P/A) 手順、またはQTLレイ定量法操作手順に従ってください。
- 結果は上記の結果解釈表と一致するはず です。

**注:** IDEXXの社内品質管理検査は、ISO 11133:2014に準拠して行われます。成績証明証 (品質管理認証) は [idexx.co.jp/water](http://idexx.co.jp/water) にて利用可能です。



# Enterolert\*

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IDEXX

**For Technical Support, please call:**

North/South America: 1 207 556 4496/1 800 321 0207

Europe: 00800 4339 9111

UK: +44 (0) 1638 676800

China: +86 21 61279528

Japan: +81 422 71 5921

Australia: 1300 443 399

IDEXX

IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092 USA

[idexx.com/water](http://idexx.com/water)

1. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. *Standard Methods for the Examination of Water and Wastewater*. 21st ed. (Métodos estándares para el análisis del agua y las aguas residuales). Washington, DC: American Public Health Association (Asociación Americana de Salud Pública); 2005.

2. IDEXX-QC Enterococci—IDEXX Catalog #UN3373-WQC-ENT

3. American Type Culture Collection 1-800-638-6597 atcc.org

\*Enterolert, Defined Substrate Technology, DST y Quanti-Tray son marcas o marcas registradas de IDEXX Laboratories, Inc. o sus filiales en los Estados Unidos de América y/o en otros países.

Información sobre la patente: [idexx.com/patents](http://idexx.com/patents).

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1. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. *Standard Methods for the Examination of Water and Wastewater*. 21st ed. Washington, DC: American Public Health Association; 2005.

2. IDEXX-QC 腸球菌—IDEXX カタログ番号 UN3373-WQC-ENT

3. American Type Culture Collection 1-800-638-6597 atcc.org

\*Enterolert Defined Substrate Technology, DST, 及びQuanti-Trayは、米国および他国のIDEXX Laboratories, Inc.またはその関連会社の、商標または登録商標です。

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