Surface Water Ambient Monitoring Program

Using IDEXX For Fecal Indicator Bacteria Monitoring

Updated August 2009

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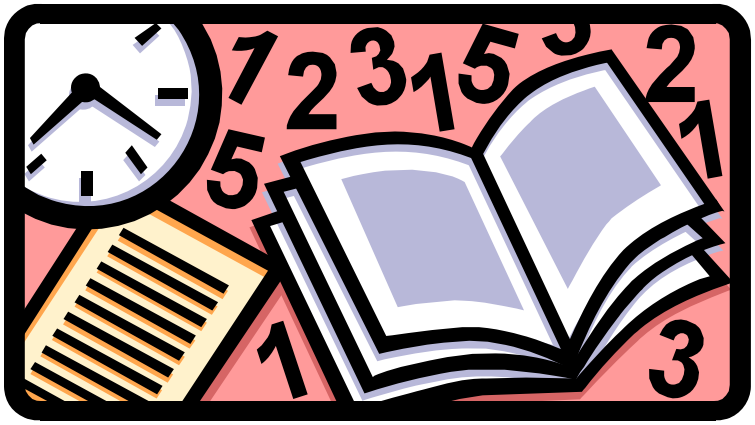
Water Boards

SWAMP
Surface Water Ambient Monitoring Program

Introduction



The goals of this document are to give Citizen Monitors basic information on fecal indicator bacteria monitoring. This document can also be used as a source for Citizen Monitoring Leaders to prepare presentation or training documents.

It has been organized into several basic sections:

Why do we use fecal bacteria as indicators and which fecal bacteria are used as indicators?

An overview of water quality criteria and criteria that use fecal indictor bacteria.

Citizen monitoring for FIBs; Sample collection methods; Testing for fecal indicator bacteria using Colilert and Enterolert methodologies.

Why Monitor for Fecal Indicator Bacteria (FIB)?

Why Monitor for Fecal Indicator Bacteria (FIB)?

Pathogenic micro-organisms are associated with fecal waste and can cause a variety of diseases (typhoid, cholera, hepatitis…) either through the ingestion of contaminated water or the consumption of contaminated shellfish. Since these pathogens tend to occur in very low numbers and are very small it is very difficult to measure them directly.

Instead monitoring for pathogens uses “indicator” species—so called because their presence indicates that fecal contamination may have occurred.

These bacteria are also easy to grow in a lab and all will be present if there is fecal contamination.

What are fecal bacteria and why are they important?

Members of two bacteria groups, **coliforms** and **fecal streptococci**, are used as **indicators** of possible sewage contamination because they are commonly found in human and animal feces. Although they are generally not harmful themselves, they indicate the possible presence of **pathogenic** (disease-causing) **bacteria**, viruses, and protozoans that also live in human and animal digestive systems. Therefore, their presence in streams suggests that pathogenic microorganisms might also be present and that swimming and eating shellfish might be a health risk.

Since it is difficult, time-consuming, and expensive to test directly for the presence of a large variety of pathogens, water is usually tested for coliforms and fecal streptococci instead. **Sources** of fecal contamination to surface waters include wastewater treatment plants, on-site septic systems, sewage infrastructure; human feces; livestock, pet and wild animal manure; and storm runoff.

Indicator bacteria types and what they can tell you…

The most commonly tested fecal bacteria indicators are

- Total coliforms
- Fecal coliforms
- *Escherichia coli*
- Enterococci.

All but *E. coli* are composed of a number of species of bacteria that share common characteristics such as shape, habitat, or behavior; *E. coli* is a single species in the fecal coliform group.

Total Coliforms

Total coliforms are a group of bacteria that are widespread in nature. All members of the total coliform group can occur in human feces, but some can also be present in animal manure, soil, and submerged wood and in other places outside the human body. Thus, the usefulness of total coliforms as an indicator of fecal contamination depends on the extent to which the bacteria species found are fecal and human in origin.

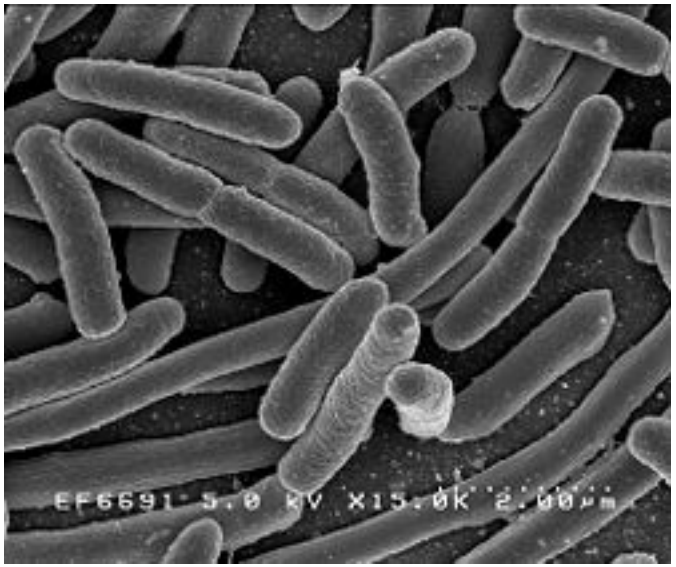
Public health agencies have used total coliforms and fecal coliforms as indicators since the 1920's.

For recreational waters, total coliforms are no longer recommended as an indicator. For **drinking water**, total coliforms are still the standard test because their presence indicates contamination of a water supply by an outside source.

Fecal Coliforms

Fecal coliforms, a subset of total coliform bacteria, are more fecal-specific in origin. However, even this group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* are commonly associated with textile and pulp and paper mill wastes. Therefore, if these sources discharge to your stream, you might wish to consider monitoring more fecal and human-specific bacteria. For recreational waters, this group was the primary bacteria indicator until relatively recently, when EPA began recommending *E. coli* and enterococci as better indicators of health risk from water contact. **Fecal coliforms are still being used in many states as the indicator bacteria.**

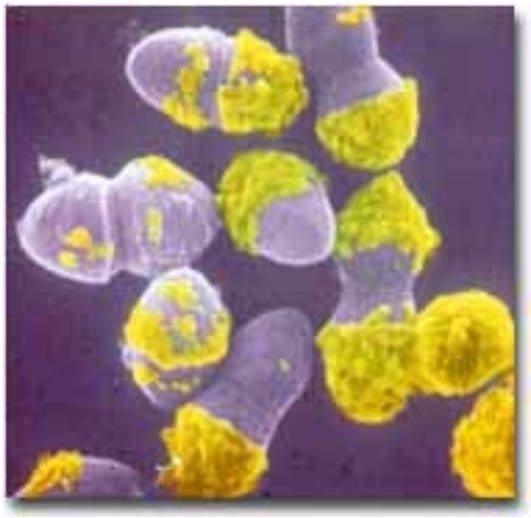
E. coli



E. Coli image from Wikkipedia.com

E. coli is a type of fecal coliform bacteria commonly found in the intestines of warm blooded animals and humans. *E. coli* is short for *Escherichia coli.* **The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination.** Sewage may contain many types of disease-causing organisms.

Enterococci



Enterococcus image from http://w3.ouhsc.edu/enteroccus

Enterococci are a subgroup within the fecal streptococcus group. Enterococci are distinguished by their ability to survive in salt water, and in this respect they more closely mimic many pathogens than do the other indicators. Enterococci are typically more human-specific than the larger fecal streptococcus group. EPA recommends enterococci as the best indicator of health risk in **salt water** used for recreation and as a **useful indicator in fresh water as well**.

Which Bacteria Should You Monitor?

Which bacteria is tested for depends on what question is being asked.

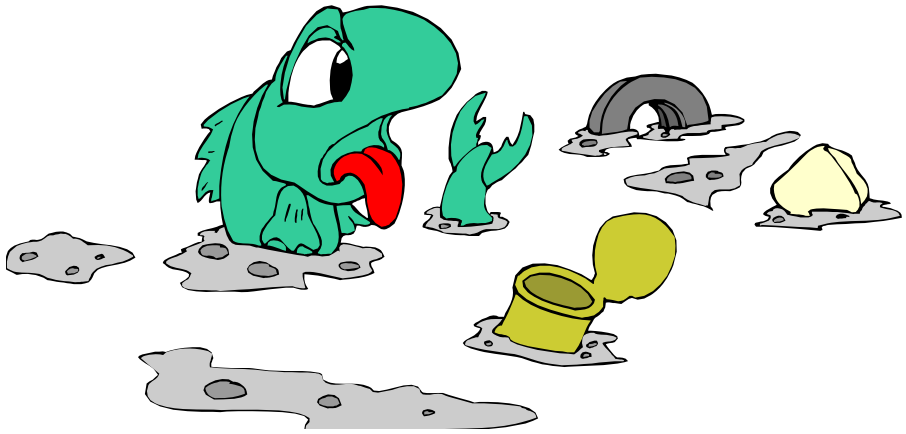
- Do you want to know whether swimming in your stream poses a health risk?

- Do you want to know whether your stream is meeting state water quality standards?

Consult with your Regional Water Quality Board's basin plan and staff especially if you expect them to use your data.

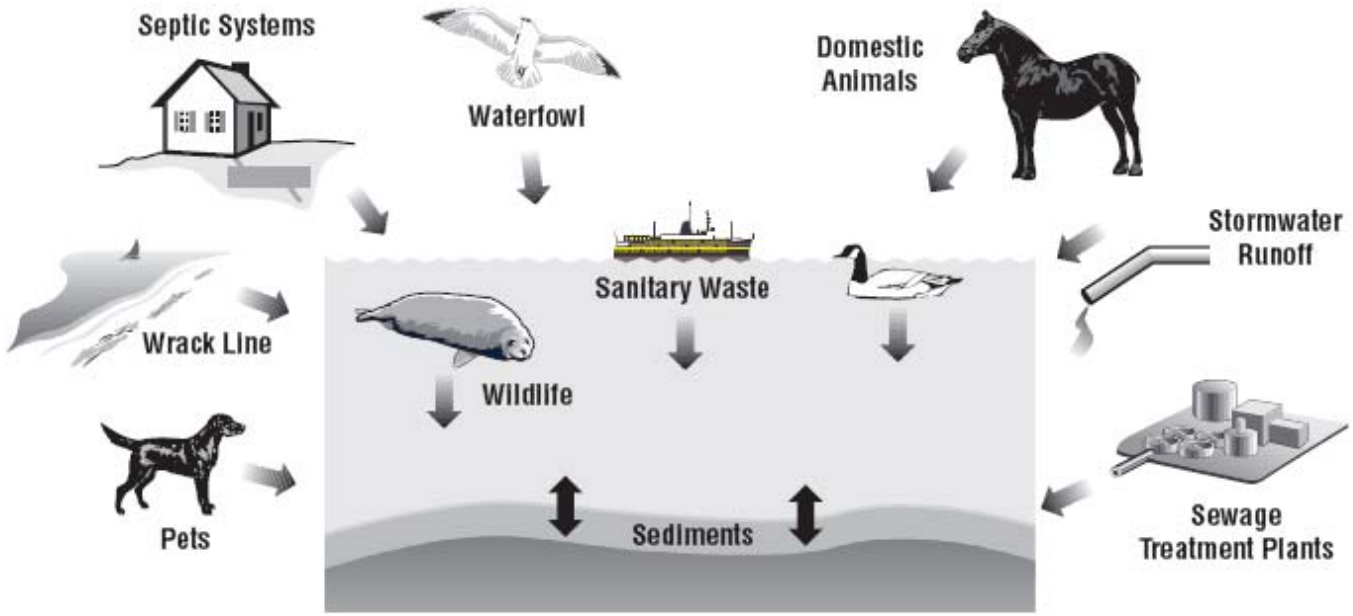
REMEMBER: For **salt water**, enterococci are the best. The occurrence of digestive system illness at swimming beaches suggest that the best indicators of health risk from recreational water contact in **fresh water** are *E. coli* and enterococci.

Additional Impairments Due to Fecal Bacteria



In addition to the possible health risk associated with the presence of elevated levels of fecal bacteria, they can also cause cloudy water, unpleasant odors, and an increased oxygen demand which may result in oxygen depleted water.

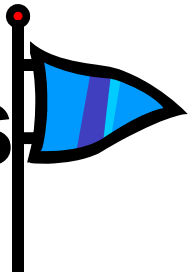
Sources of Contamination



Fecal Bacteria comes from human and animal wastes. During rainfalls, snow melts, or other types of precipitation, fecal bacteria may be washed into creeks, rivers, streams, lakes, or ground water. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, fecal bacteria may end up in drinking water. Breaks in sewage infrastructure and septic failures also can also lead to contamination.

Water Quality Standards

Water Quality Standards



The term **"water quality criteria"** is used in two sections of the Clean Water Act, section 304(a)(1) and Section 303(c)(2). The term has a different program impact in each section. In **section 304**, the term represents a **non-regulatory**, scientific assessment of ecological and public health effects. The criteria presented in this publication are such scientific assessments. Water quality **criteria associated with specific ambient water uses** when adopted as State water quality standards under **section 303** become enforceable maximum acceptable levels of a pollutant in ambient waters.

Water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations **States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards.** It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Coliform Bacteria and Drinking Water

Under the **Safe Drinking Water Act**, EPA requires public water systems to monitor for coliform bacteria. Systems analyze first for total coliform, because this test is faster to produce results. Any time that a sample is positive for total coliform, the same sample must be analyzed for either fecal coliform or *E. coli*. Both are indicators of contamination with animal waste or human sewage.

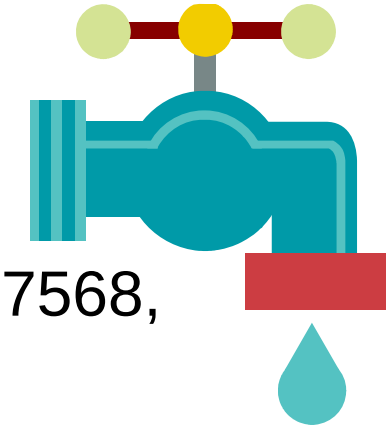
The largest public water systems (serving millions of people) must take at least 480 samples per month. Smaller systems must take at least five samples a month unless the state has conducted a sanitary survey – a survey in which a state inspector examines system components and ensures they will protect public health – at the system within the last five years.

Systems serving 25 to 1,000 people typically take one sample per month. Some states reduce this frequency to quarterly for ground water systems if a recent sanitary survey shows that the system is free of sanitary defects. Some types of systems can qualify for annual monitoring.

Systems using surface water, rather than ground water, are required to take extra steps to protect against bacterial contamination because surface water sources are more vulnerable to such contamination. At a minimum, all systems using surface waters must disinfect.

In 2006, EPA issued a new rule to ensure that systems using ground water sources take action to treat their drinking water to address microbial contamination if it is identified as a problem.

Total Coliform Rule



Total Coliform Rule (TCR): 54 FR 27544-27568, June 29, 1989, Vol. 54, No. 1241

Establishes a maximum contaminant level (MCL) based on the presence or absence of total coliforms, modifies monitoring requirements including testing for fecal coliforms or *E. coli*, requires use of a sample siting plan, and also requires sanitary surveys for systems collecting fewer than five samples per month.

The TCR applies to all public water systems.

TCR Provisions: Routine Sampling Requirements, Repeat Sampling Requirements, Additional Routine Sample Requirements, Routine Monitoring Frequencies, and Compliance Criteria

USEPA Limits for E. Coli

Recreation Water Bacterial Limits for *E. coli*, and enterococci

Ambient Water Quality Criteria For Bacteria - January 1, 1986

Water Type	Indicator	30 Day Geometric Mean
Fresh Water	*E. coli*	126/100ml
Fresh Water	Enterococci	33/100ml
Marine Water	Enterococci	35/100ml

The Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 requires all states with coastal recreation waters to adopt bacteria criteria that are as protective of human health as “1986 bacteria criteria”.

What is a Geometric Mean Log?

For samples taken over a 30-day period this would be the average of the logarithmic values of that data set converted back to a base 10 number.

Guidance on Calculating Geometric Means can be found at:

http://www.buzzardsbay.org/geomean.htm

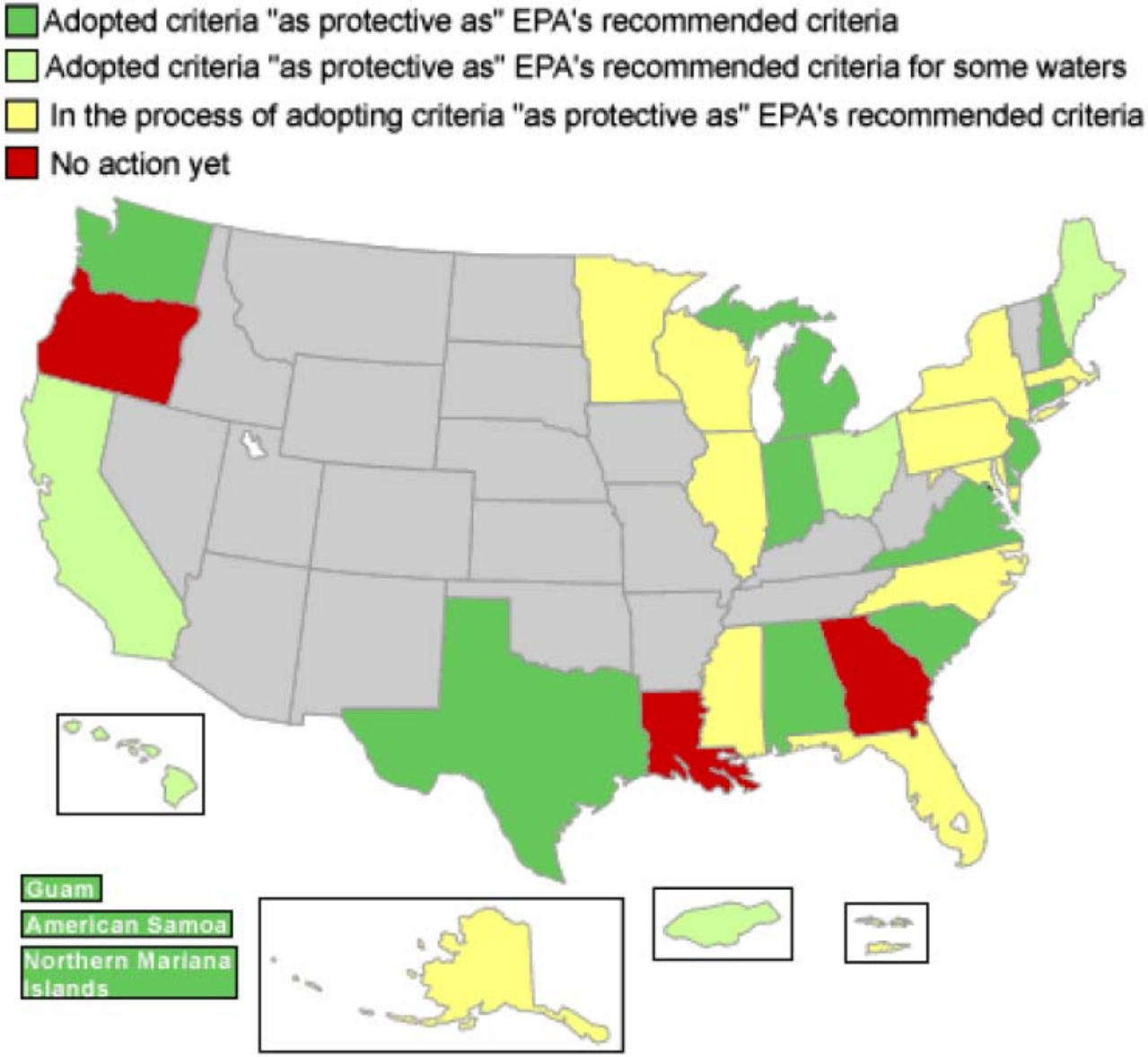
Single Sample State Standards

Total Coliform:
1,000 per 100 ml if Fecal/Total is >.1;
10,000 per 100 ml if Fecal/Total is <.1

Fecal Coliform: 400 per 100 ml

Enteroccoccus: 104 per 100 ml

Status of Standards Development for Coastal Recreation Waters
(as of November 8, 2004)



SWRCB & RWQCBs

There are nine **regional water quality control boards** statewide. The nine Regional Boards are semi-autonomous and are comprised of nine part-time Board members appointed by the Governor and confirmed by the Senate. Regional boundaries are based on watersheds and water quality requirements are based on the unique differences in climate, topography, geology and hydrology for each watershed. **Each Regional Board makes critical water quality decisions for its region, including setting standards, issuing waste discharge requirements, determining compliance with those requirements, and taking appropriate enforcement actions.**

Beneficial Uses

Examples of Beneficial Use Definitions: Some beneficial uses for waterbodies in the Los Angeles Region are listed and defined below. The uses are listed in no preferential order.

Presence of FIBs may impact this beneficial use.

- **Municipal and Domestic Supply (MUN)**

Uses of water for community, military, or individual water supply systems including, but not limited to, drinking water supply.

- **Agricultural Supply (AGR)**

Uses of water for farming, horticulture, or ranching including, but not limited to, irrigation, stock watering, or support of vegetation for range grazing.

- **Industrial Process Supply (PROC)**

Uses of water for industrial activities that depend primarily on water quality.

- **Industrial Service Supply (IND)**

Uses of water for industrial activities that do not depend primarily on water quality including, but not limited to, mining, cooling water supply, hydraulic conveyance, gravel washing, fire protection, or oil well re-pressurization.

- **Ground Water Recharge (GWR)**

Uses of water for natural or artificial recharge of ground water for purposes of future extraction, maintenance of water quality, or halting of saltwater intrusion into freshwater aquifers.

- **Freshwater Replenishment (FRSH)**

Uses of water for natural or artificial maintenance of surface water quantity or quality (e.g., salinity).

- **Navigation (NAV)**

Uses of water for shipping, travel, or other transportation by private, military, or commercial vessels.

- **Hydropower Generation (POW)**

Uses of water for hydropower generation

- **Water Contact Recreation (REC-1)**

Uses of water for recreational activities involving body contact with water, where ingestion of water is reasonably possible. These uses include, but are not limited to, swimming, wading, water-skiing, skin and scuba diving, surfing, white water activities, fishing, or use of natural hot springs.

- **Non-contact Water Recreation (REC-2)**

Uses of water for recreational activities involving proximity to water, but not normally involving body contact with water, where ingestion of water is reasonably possible. These uses include, but are not limited to, picnicking, sunbathing, hiking, beachcombing, camping, boating, tidepool and marine life study, hunting, sightseeing, or aesthetic enjoyment in conjunction with the above activities.

- **Commercial and Sport Fishing (COMM)**

Uses of water for commercial or recreational collection of fish, shellfish, or other organisms including, but not limited to, uses involving organisms intended for human consumption or bait purposes.

- **Aquaculture (AQUA)**

Uses of water for aquaculture or mariculture operations including, but not limited to, propagation, cultivation, maintenance, or harvesting of aquatic plants and animals for human consumption or bait purposes.

- **Warm Freshwater Habitat (WARM)**

Uses of water that support warm water ecosystems including, but not limited to, preservation or enhancement of aquatic habitats, vegetation, fish, or wildlife, including invertebrates.

- **Cold Freshwater Habitat (COLD)**

Uses of water that support cold water ecosystems including, but not limited to, preservation or enhancement of aquatic habitats, vegetation, fish, or wildlife, including invertebrates.

Inland Saline Water Habitat (SAL)

Uses of water that support inland saline water ecosystems including, but not limited to, preservation or enhancement of aquatic saline habitats, vegetation, fish, or wildlife, including invertebrates.

Estuarine Habitat (EST)

Uses of water that support estuarine ecosystems including, but not limited to, preservation or enhancement of estuarine habitats, vegetation, fish, shellfish, or wildlife (e.g., estuarine mammals, waterfowl, shorebirds).

Wetland Habitat (WET)

Uses of water that support wetland ecosystems, including, but not limited to, preservation or enhancement of wetland habitats, vegetation, fish, shellfish, or wildlife, and other unique wetland functions which enhance water quality, such as providing flood and erosion control, stream bank stabilization, and filtration and purification of naturally occurring contaminants.

Marine Habitat (MAR)

Uses of water that support marine ecosystems including, but not limited to, preservation or enhancement of marine habitats, vegetation such as kelp, fish, shellfish, or wildlife (e.g., marine mammals, shorebirds).

Wildlife Habitat (WILD)

Uses of water that support terrestrial ecosystems including, but not limited to, preservation and enhancement of terrestrial habitats, vegetation, wildlife (e.g., mammals, birds, reptiles, amphibians, invertebrates), or wildlife water and food sources.

Preservation of Biological Habitats (BIOL)

Uses of water that support designated areas or habitats, such as **Areas of Special Biological Significance (ASBS),** established refuges, parks, sanctuaries, ecological reserves, or other areas where the preservation or enhancement of natural resources requires special protection

Beneficial Uses With Potential FIB WQOs

Municipal and Domestic Supply (MUN) Uses of water for community, military, or individual water supply systems including, but not limited to, **drinking water supply**.

Water Contact Recreation (REC-1)
Uses of water for recreational activities involving **body contact with water**, where ingestion of water is reasonably possible. These uses include, but are not limited to, swimming, wading, water-skiing, skin and scuba diving, surfing, white water activities, fishing, or use of natural hot springs.

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Commercial and Sport Fishing (COMM)
Uses of water for commercial or recreational collection of fish, **shellfish**, or other organisms including, but not limited to, uses involving organisms intended for human consumption or bait purposes.

Aquaculture (AQUA)
Uses of water for aquaculture or mariculture operations including, but not limited to, propagation, cultivation, maintenance, or harvesting of aquatic plants and animals **(i.e. shellfish)** for human consumption or bait purposes.

Shellfish Harvesting (SHELL)
Uses of water that support habitats suitable for the collection of filter-feeding shellfish (e.g. clams, oysters, and mussels) for human consumption, commercial, or sports purposes.

Water Quality Objectives

Coliform Bacteria

Total and fecal coliform: In waters designated for water contact recreation (**REC-1**), the fecal coliform concentration shall not exceed a log mean of 200/100 ml (based on a minimum of not less than four samples for any 30-day period), nor shall more than 10% of total samples during any 30-day period exceed 400/100ml.

In waters designated **REC-2** only, the fecal coliform concentration shall not exceed a log mean of 2000/100ml (based on a minimum of not less than four samples for any 30-day period), nor shall more than 10% of total samples during any 30-day period exceed 4000/100ml.

In waters supporting shellfish harvest for human consumption (**SHELL**) the median fecal coliform concentration throughout the water column for any 30 –day period shall not exceed 70/100 ml, nor shall more than 10% of total samples during any 30-day period exceed 230/100ml.

FIB Water Quality Standards within the regional water quality control board's Water Quality Control Plans (aka "Basin Plans)…

FC = Fecal Coliforms

TC = Total Coliforms

EN = Enterococci

EC = *E. coli*

Bacterial Water Quality Standards by EPA Region

			Freshwater		Marine	
Region	**State**	**Class**	**Primary**	**Secondary**	**Primary**	**Secondary**
Region 9	**Arizona**		126 EC	126 EC		
			Single sample maximum is 235 for full body contact and 576 for partial body contact.			
	California	North Coastal Regional Board 1	50 FC		50 FC	
			No more than 10% of FC samples may exceed 400.			
		San Francisco Bay Regional Board 2	126 EC† 33 EN† 200 FC 240 TC	2000 FC	35 EN 200 FC 240 TC	2000 FC
			Marine waters: No sample may exceed 104 - 500 EN based on frequency of use. Fresh waters: No sample may exceed 61-151 EN or 235-576 EC based on frequency of use. No sample may exceed 4000 FC for secondary contact. No more than 10% of FC samples may exceed 400. No sample to exceed 10,000 TC.			
		Central Coast Regional Board 3	200 FC	2000 FC	200 FC	2000 FC
			No more than 10% of FC samples may exceed 400 for water contact recreation (REC-1) or 4000 for non-contact water recreation (REC-2).			
		Los Angeles Regional Board 4	126 EC 200 FC	2000 FC	35 EN 200 FC 1000 TC	2000 FC
			Marine: single sample maximum is 400 FC, 10,000 TC, and 104 EN. Fresh: single sample maximum is 235 EC and 400 FC.			
		Central Valley Regional Board 5	126 EC			
			Single sample maximum is 235 EC.			
		Folsom Lake (In Central Valley)	100 FC			
			No more than 10% of samples may exceed 200 FC.			
		Lahontan Regional Board 6	20 FC			
			No more than 10% of FC samples may exceed 40. No more than 10% of FC samples may exceed 75 for the Eagle Drainage Hydrologic Area. A log mean concentration exceeding 20/100 ml for any 30-day period shall indicate violation of this objective even if fewer than five samples were collected.			

Continued

FIB Water Quality Standards within the regional water quality control board's Water Quality Control Plans (aka "Basin Plans)

FC = Fecal Coliforms

TC = Total Coliforms

EN = Enterococci

EC = *E. coli*

Bacterial Water Quality Standards by EPA Region

			Freshwater		Marine	
Region	**State**	**Class**	**Primary**	**Secondary**	**Primary**	**Secondary**
Region 9 (cont'd.)	**California (continued)**	Colorado River Basin Regional Board 7	126 EC 33 EN 200 FC	630 EC 165 EN		
			No sample may exceed 100 EN and 400 EC for primary contact and 500 EN and 2000 EC for secondary contact. For the Colorado River, no sample may exceed 61 EN and 235 EC for freshwater primary contact. For secondary contact, no sample may exceed 305 EN and 1175 EC. No more than 10% of FC samples may exceed 400. Also maximum limits for EN and EC vary by level of use.			
		Santa Ana Regional Board 8	200 FC	2000 FC	200 FC	2000 FC
			No more than 10% of FC samples may exceed 400 for primary contact and 4000 for secondary contact; 100 TC maximum in lakes and streams designated as domestic water supply. The marine water criteria also apply to bays and estuaries.			
		San Diego Regional Board 9	126 EC 33 EN 200 FC	2000 FC	35 EN 200 FC	2000 FC
			For fresh water, no more than 10% of samples may exceed 400 FC for primary contact and 4000 FC for secondary contact. Single sample maximum ranges from 61 EN - 151 EN and 235 EC - 576 EC for fresh waters and 104 EN - 500 EN for marine waters based on frequency of use.			
		Ocean Plan			24 EN for 30 day period 12 EN for 6 month period 200 FC 1000 TC	
			No more than 20% of TC samples may exceed 1000 in bays and estuaries. No more than 10% of FC samples may exceed 400.			
	Comments:	Essentially all California waters are designated for primary contact recreation with the exception of the Colorado River Basin Region.				

AB 411 Ocean Water-Contact Sports Standards

In 1999, new bacteriological ocean water quality standards that are more protective of public health were added to the California Health and Safety Code. The new standards are informally called AB 411 Ocean Water-Contact Sports Standards.

AB 411 Requirements

Required testing of the waters adjacent to all public beaches for total coliform, fecal coliform, and enterococcus bacteria that are indicators of possible disease causing bacteria, viruses and protozoa.

Established **single sample standards** for total coliforms, fecal coliforms, and enterococci bacteria as follows:

Total Coliforms: 10,000 organisms per 100 milliliter sample.
Fecal Coliforms: 400 organisms per 100 milliliter sample.
Enterococci: 104 organisms per 100 milliliter sample.
Fecal:Total ratio: >1000 total coliforms if ratio exceeds 0.1.

Established **30-day geometric log mean standards** (of five weekly samples) for total coliforms, fecal coliforms, and enterococci bacteria as follows:

Total Coliforms: 1000 organisms per 100 milliliter sample.
Fecal Coliforms: 200 organisms per 100 milliliter sample.
Enterococci: 35 organisms per 100 milliliter sample.

When any waters adjacent to a public beach fail to meet any of the standards described above, the local health officer shall post the beach to restrict access. Weekly testing is required from April 1 to October 31 if all of the following apply: The beach is visited by more than 50,000 people annually; and The beach is located in an area adjacent to a storm drain that flows in the summer.

A.B. 411 Posted Warnings

The warning sign with the **yellow and black border** is posted near storm drains, creeks and rivers to advise the public of The contamination from urban runoff.

This warning sign with the **red and black border** is posted when a violation of the AB Standards occurs.

This **yellow** closure sign is posted when a release of raw sewage affects waters adjacent to a public beach.

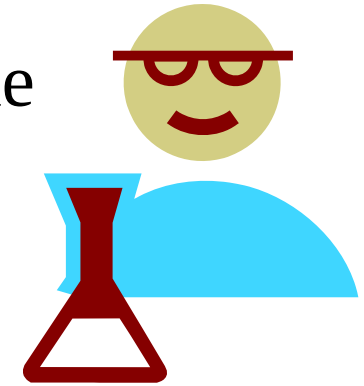
Citizen Monitoring for FIB’s

Fecal Indicator Bacteria Testing Methods

Why are Citizen Monitors looking at indicator bacteria?

Citizen Monitors usually decide to monitor for indicator bacteria because they are concerned with their watersheds, specific waterbodies and or public health.

It gives them further opportunities to involve the community and produce useable data.



EPA approved methods exist which can be performed by citizen monitors themselves, through which substantial monetary savings can be realized.

THE
Volunteer Monitor
THE NATIONAL NEWSLETTER OF VOLUNTEER WATERSHED MONITORING Volume 18, Number 1 • Winter 2006

volunteers conduct Bacteria Methods Comparison study

by Eric O'Brien

An interesting fact came to light at a 2002 strategic planning meeting for the Great Lakes region: out of the six states attending (Iowa, Indiana, Michigan, Minnesota, Ohio, and Wisconsin), only two had volunteer monitoring programs that included testing for bacteria. These were Iowa's IOWATER program, run by Iowa Department of Natural Resources (DNR), and Indiana's Hoosier Riverwatch, sponsored by Indiana DNR.

This discovery was the beginning of what would become the Citizens Monitoring Bacteria Project, a multiyear, multistate undertaking.

Soon after the meeting, representatives from Iowa DNR, Indiana DNR, Purdue University, Michigan State University, the University of Minnesota, the Ohio State University, and the University of Wisconsin formed a workgroup to encourage more bacteria monitoring by volunteer programs in the region. We decided that our first step should be to conduct a study to compare several different bacteria testing methods. Recognizing the potential value of our efforts, not only in our region but around the country, we applied for and received a grant from USDA Cooperative State Research, Education, and Extension Service (CSREES).

Iowa and Indiana took the lead in designing and carrying out the first year of the study while researchers in Wisconsin worked on creating survey questionnaires to determine the volunteers' opinions of the different methods. Michigan, Minnesota, and Ohio were charged with developing training and outreach materials.

We began the comparison study in 2004, expecting that at the end of a year we would have a clearcut "winner"—but it didn't quite work out that way, as we shall see.

"Real world" conditions

It's important to emphasize that our project was not a pure method-comparison study in which other variables besides the methods themselves are strictly controlled. To the contrary, we intentionally kept the "messiness" in. Our goal was to compare the performance of the different methods in the hands of actual volunteer monitors, sampling at their own monitoring sites and performing the analyses in their own homes. The volunteers' opinions and perceptions were also taken into account in evaluating the different methods.

Choosing methods for the study

All the methods we studied were for enumerating the indicator *E. coli*, which is, or soon will be, the indicator of choice for all the states in our region for ambient freshwater monitoring. In selecting the methods, we kept in mind the different needs and resources of different volunteer monitoring programs. Pro-

continued on page 3

Indiana volunteers at training workshop review protocols for 3M Petrifilm, Coliscan Easygel, and Coliscan MF.

Bacteria Monitoring

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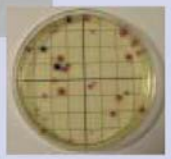
The Simple Methods

Coliscan Easygel

Water sample mixed with liquid Coliscan medium is poured into the Coliscan plate, which is coated with ingredients that cause the mixture to gel.



To make colony counting easier, the volunteers placed Easygel plates on a paper marked with a grid pattern.

3M Petrifilm

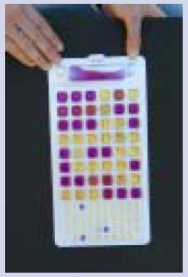
The sample is added directly to dehydrated medium on the film. The top layer of film traps gas bubbles produced by coliform bacteria (including *E. coli*).

The More Sophisticated Methods

Coliscan MF

The water sample is filtered, then the filter is placed on Coliscan medium.

IDEXX Colisure and Colilert



A special sealer must be used to seal off the individual wells in the IDEXX Quanti-Tray.

IDEXX Colisure. Wells with a red or magenta color plus fluorescence are positive for *E. coli*.

Membrane Filtration

http://ga2.er.usgs.gov/bacteria/pictures/SummaryQualityPetri1.jpg

The **membrane filtration** method involves filtering several different-sized portions of the sample using filters with a standard diameter and pore size,placing each filter on a selective nutrient medium in a petri plate, incubating the plates at a specified temperature for a specified time period, and then counting the colonies that have grown on the filter. This method varies for different bacteria types (variations might include, for example, the nutrient medium type, the number and types of incubations, etc.).

Multiple-Tube Fermentation

The **multiple-tube fermentation** method involves adding specified quantities of the sample to tubes containing a nutrient broth, incubating the tubes at a specified temperature for a specified time period, and then looking for the development of gas and/or turbidity that the bacteria produce. The presence or absence of gas in each tube is used to calculate an index known as the **Most Probable Number** (MPN).

Enterolert & Colilert

Enterolert™ reagent is used for the detection of enterococcus bacteria (enterococci) such as *E. faecium* and *E. faecalis* in fresh and marine water. This product is based on Defined Substrate Technology® (DST™) and utilizes a nutrient indicator that fluoresces when metabolized by enterococci. When the reagent is added to the sample and incubated, bacteria down to one MPN (most probable number) in a 100ml sample can be detected within 24 hours.

Colilert: measures coliform bacteria for freshwater

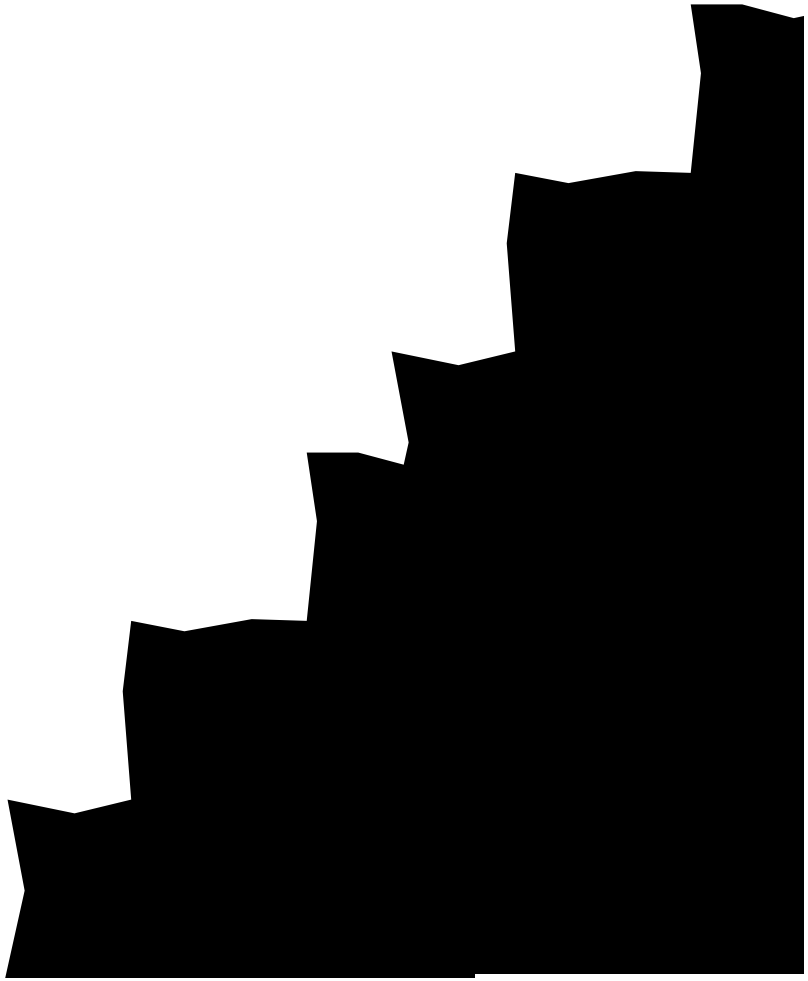
Colilert-18 Measures coliform bacteria for marine waters

Enterolert: measures enterococcus bacteria

Colilert-18 is used for the simultaneous detection and confirmation of total coliforms and *E. coli* in fresh and marine waters. It is based on IDEXX's patented Defined Substrate Technology® (DST®). When total coliforms metabolize Colilert-18's nutrient-indicator, ONPG, the sample turns yellow. When *E.coli* metabolize Colilert-18's nutrient indicator, MUG, the sample fluoresces. Colilert-18 can simultaneously detect these bacteria at 1 MPN/100ml within 18 hours even with as many as 2 million other heterotrophic bacteria cells per 100ml present

FIB Monitoring
Basic Steps Using IDEXX

- Field Preparation
- Collecting Samples
- Transporting Samples
- Delivering Samples
- Lab Preparation
- Preparing Sample
- Incubating Sample
- Reading Sample
- Disposing Sample
- Data Management
- Data Interpretation



Field Preparation

Field Collecting Equipment:

- Field Data Sheets
- Sampling Poles
- Sterile Gloves
- Sterile Sample Containers
- Ice Chest
- Ice Packs

Images from Forestry Suppliers and Cape Ice products.

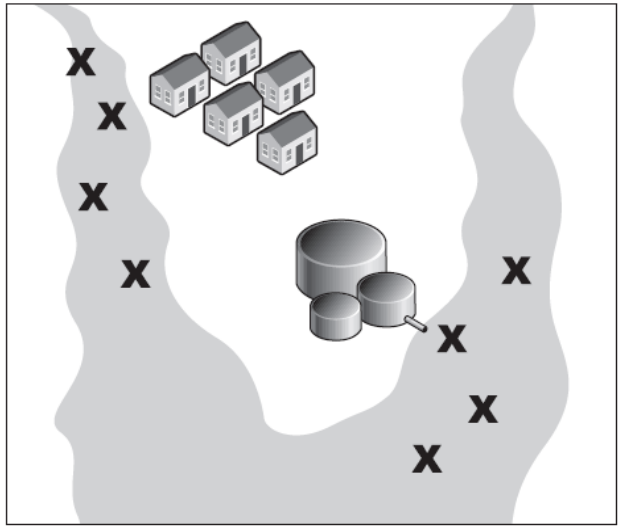
Collecting a Sample

Geographic and temporal selection of monitoring sites (near septic fields, adjacent to sewers, swimming areas…) and collection times (weekly, monthly, during storms…) is dependent on your monitoring questions.

Ensure that you will be able to collect samples that will yield data which will help you answer your monitoring questions.

Obtain a Representative Samples.

Avoid Contamination:Stay clear of algal blooms, surface debris, avoid agitating sediments, wear proper gloves…



Compliance vs. Ambient Monitoring

If bacteriological samples are to be used for **regulatory compliance** purposes, then samples must be kept at 4°C (dark) and transported to the laboratory so that the analysis begins within 6 hours of collection.

If bacteriological samples are **non-regulatory** in nature (ie, non-drinking water samples analyzed for non-compliance purposes), after collection samples can be tested within 24 hours of collection if the samples were stored in the dark and kept at 4°C until analysis.

Sample Collection Containers

• Sample containers should be cleaned and sterilized using procedures described in Standard Methods 9030 and 9040 (APHA *et al.* 1998). In most cases, these containers are provided by the laboratories conducting the analyses. Alternatively, sterile bottles or Whirl-pak type bags may also be used, per protocol

• For waters suspected to contain a **chlorine residual**, sample bottles should contain a small amount of **sodium thiosulfate** (Na2S2O3) sufficient to neutralize bactericidal activity. In most cases, bottles provided by contract laboratories already contain the sodium thiosulfate as a precautionary measure. For water containing high concentrations of copper or zinc, sample bottles should contain sufficient EDTA solution to reduce metal toxicity. *Note*: These conditions are rare in surface waters.

• Sample bottles may be glass or plastic (e.g. polypropylene) with a capacity of at least 100 ml., or again, Whirl-pak bags. After sterilization, sample bottles should be kept closed until they are to be filled.

FIB Sample Collection Containers and Holding Times Continued

Parameters for Analysis	Recommended Containers (all containers pre-cleaned)	Typical Sample Volume (ml)	Initial Field Preservation	Maximum Holding Time (analysis must start by end of max)
Bacteria and Pathogens in Water Samples				
E. Coli	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 ml sterile plastic (high density polyethylene or polypropylene) container	100 ml volume sufficient for both E. coli and Enterococcus analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 4°C; dark.	STAT: 6 hours at 4°C, dark for regulatory data use; lab must be notified well in advance. Possibly 24hr hold time at 4C dark, if non-regulatory data use.
Enterococcus	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 ml sterile plastic (high density polyethylene or polypropylene) container	100 ml volume sufficient for both E. coli and Enterococcus analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 4°C; dark.	STAT: 6 hours at 4°C, dark for regulatory data use; lab must be notified well in advance. Possibly 24hr hold time at 4C dark, if non-regulatory data use.
FECAL COLIFORM	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 ml sterile plastic (high density polyethylene or polypropylene) container	100 ml volume sufficient for both fecal and total coliform analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 4°C; dark.	STAT: 6 hours at 4°C, dark for regulatory data use; lab must be notified well in advance. Possibly 24hr hold time at 4C dark, if non-regulatory data use.
TOTAL COLIFORM	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 ml sterile plastic (high density polyethylene or polypropylene) container	100 ml volume sufficient for both fecal and total coliform analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 4°C; dark.	STAT: 6 hours at 4°C, dark for regulatory data use; lab must be notified well in advance. Possibly 24hr hold time at 4C dark, if non-regulatory data use.

Clean/Decontaminate Field Equipment & Use Sterile Collecting-Storage Items

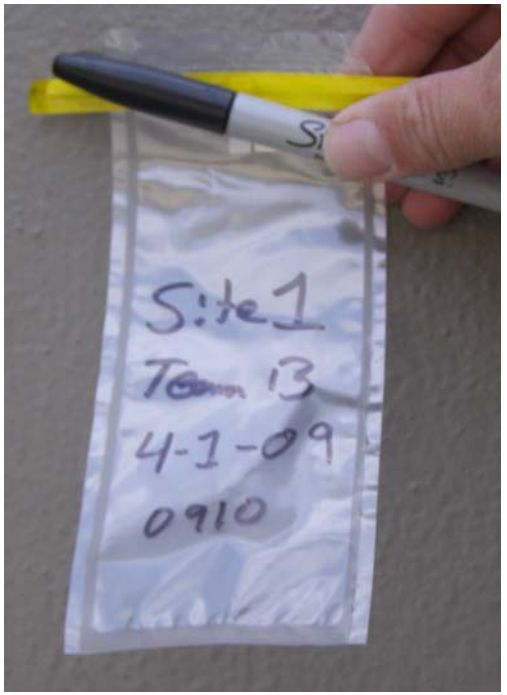
Many Citizen Monitors Utilize Sterile WhirlPak Bags for the Collection of Sample Water

- These bags are sterile.
- These bags are easily labeled prior to filing with water.
- If the sample water is suspected to contain chlorine bags can be obtained that contain sodium thiosulfate (neutralizes the chlorine so that it does not kill and bacteria that might be in the sample).

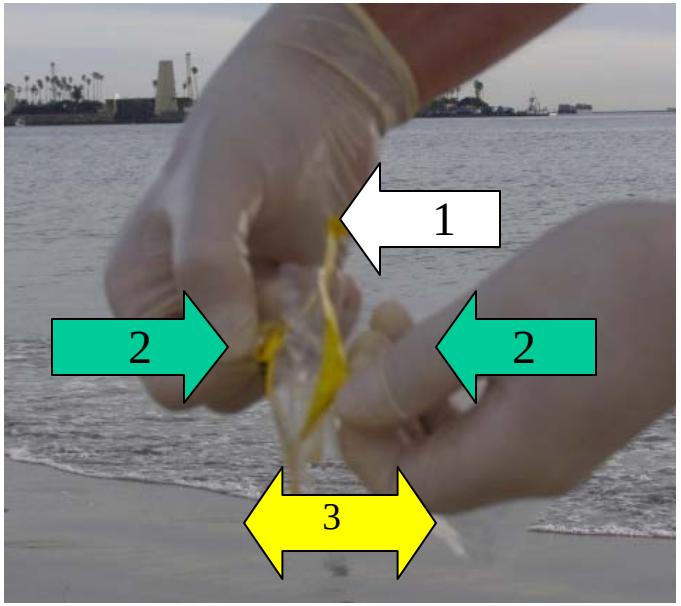
Images from http://www.coleparmer.com/Catalog/product_index.asp?cls=3727

Label the Bag <u>Prior</u> to Filling

- Site Name or Code
- Field Operator's Name
- Sample Date
- Sample Time



Correct Use of Whirl-Pak Bags



1- Remove perforated top

2 Grip tabs located on the side of the Whirl-pak bag.

3- Pull apart to open the bag.

The sides of the bag will stick together. Do not worry about this. The bag will open up when being filled with sample water.

Proper Sampling

Sediment not effected.

Gloves worn.

Equipment decontaminated.

Sampling vessels are sterile.

Samples obtained subsurface.

Improper “Bad” Sampling

No gloves.

Sampler may have re-suspended sediment.

Water may have washed the sampler prior to being sampled.

Improper “Bad” Sampling – Using a Sampling-pole

No gloves.

Sampler may have decontaminated sampling-pole.

Closing the Bag

1. Whirl the bag closed
2. Inspect Air Space
3. Twist the ties together

Improper “Bad” Samples & “Good” Sample

Fill the bag about 2/3 full.

Leave an inch of air-space.

Bad- No air space

Bad- Sediment

Bad- Not enough sample water

Transporting Samples

Holding Times: Within 6 Hours

Storage Temperatures: 1-4 °C

Protect Your Samples: Do not allow melted ice water to submerge your sample.

Be sure that you include a **temperature blank**.

A **temperature blank** is a container containing only water. It is placed into the ice chest at the same time as the first water sample. When the samples are released to the lab, they will check the temperature of the temperature blank as a surrogate for a water sample

Delivering Samples

With each hand-off, to a delivery person or to a lab, **Chain of Custody** is signed.

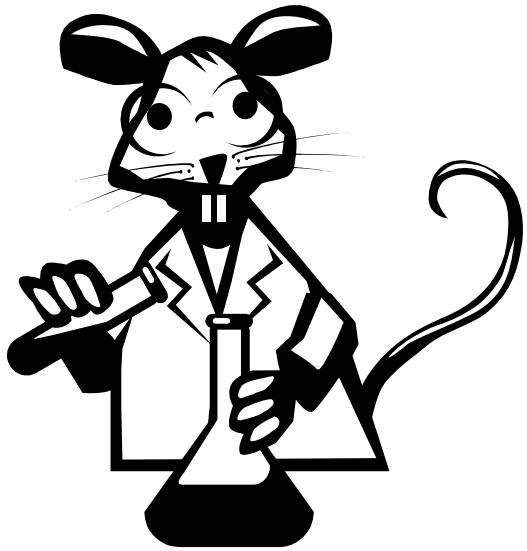
Temperature of the sample is taken my measuring the temperature of a Temperature Blank before the sample is processed in the lab.

CHAIN OF CUSTODY (COC) RECORD

Project Name: _____ Date/ Time: _____
Watershed Name:_____ : Lab: _____

Sample Number	Lab Number	Sample Date	Sample Description
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Processing Samples



Colilert Quanti-Tray Enumeration Method

Lab Procedures

1) Different types of water samples require different types of preparation as follows:

a) For sterile (blank) water or relatively clean fresh water pour 100 ml of sterile water or sample directly into the sterile 100 ml mixing bottle (by filling to the 100 ml line) and add one package of the reagent. Cap and shake until dissolved.

b) For fresh water that is suspected to contain contamination, pour 50 ml of sterile distilled water into the mixing bottle and add one package of the reagent. Cap and shake until dissolved. Then, after the foam subsides, using a sterile pipette add 10 ml of sample and top off with 40 ml (to the 100 ml line). Cap and shake again. This is a 1:10 dilution.

c) For all marine or estuarine water samples (salinity greater than 5 ppt), pour 50 ml of sterile distilled water into the mixing bottle and add one package of the reagent. Cap and shake until dissolved. Then, using a sterile pipette add 10 ml of sample and top off with 40 ml (to the 100 ml line). Cap and shake again. This is a 1:10 dilution.

2) Make sure there is little or no foam left in the headspace of the mixing bottle prior to moving on to the next step.

3) Pour sample/reagent mixture from the mixing bottle into a quanti-tray and seal in the IDEXX Sealer.

4) Place the sealed tray in a 35±0.5°C incubator for a minimum of 18 hours and a maximum of 22 hours (includes warming time). This is the incubation period.

Enterolert Quanti-Tray Enumeration Method

Lab Procedures

1) Different types of water samples require different types of preparation as follows:

a) For sterile (blank) water or relatively clean fresh water pour 100 ml of sterile water or sample directly into the sterile 100 ml mixing bottle (by filling to the 100 ml line) and add one package of the reagent. Cap and shake until dissolved.

b) For fresh water that is suspected to contain contamination, pour 50 ml of sterile distilled water into the mixing bottle and add one package of the reagent. Cap and shake until dissolved. Then, after the foam subsides, using a sterile pipette add 10 ml of sample and top off with 40 ml (to the 100 ml line). Cap and shake again. This is a 1:10 dilution.

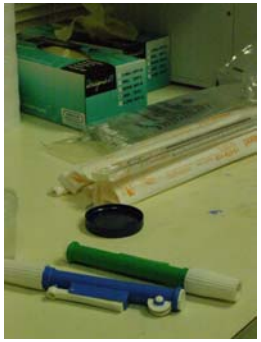
c) For all marine or estuarine water samples (salinity greater than 5 ppt), pour 50 ml of sterile distilled water into the mixing bottle and add one package of the reagent. Cap and shake until dissolved. Then, using a sterile pipette add 10 ml of sample and top off with 40 ml (to the 100 ml line). Cap and shake again. This is a 1:10 dilution.

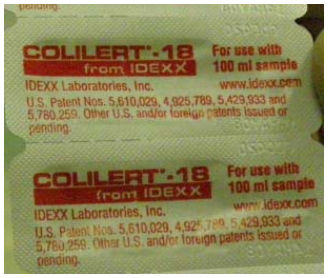
2) Make sure there is little or no foam left in the headspace of the mixing bottle prior to moving on to the next step.

3) Pour sample/reagent mixture from the mixing bottle into a quanti-tray and seal in the IDEXX Sealer.

Place the sealed tray in a 41° ± 0.5° C incubator for a minimum of 24 hours and a maximum of 28 hours (includes warming time). This is the incubation period

Lab Preparation: General Procedures





Clean Lab Surfaces

Organize Supplies

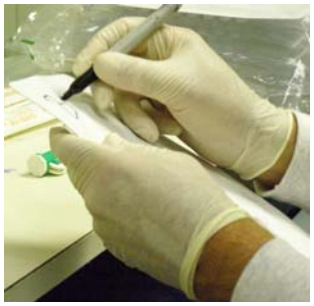
- DI Water
- Sterile Pipettes & Pipette Tips
- Sterile Bottles
- Sterile Quanti-trays
- Media (Colilert/Enterolert)

Turn on IDEXX Sealer

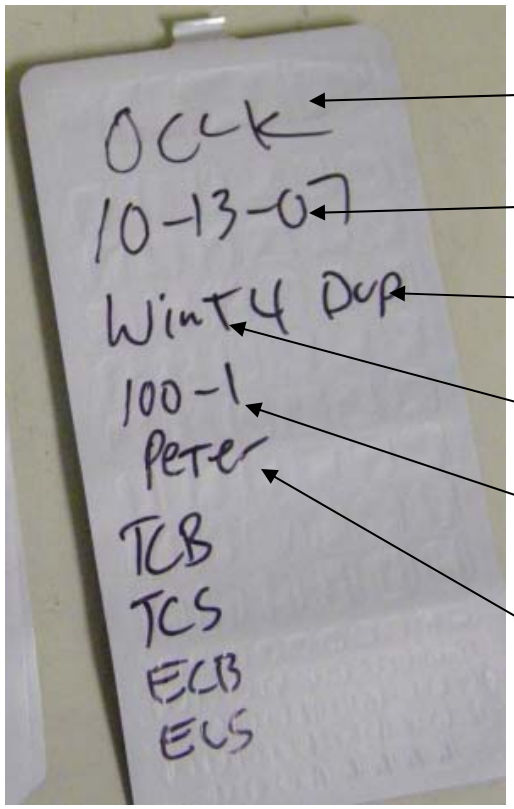
Turn on Incubator and Set to Proper Temperature

Organize Samples

- Start with samples collected earliest that day.

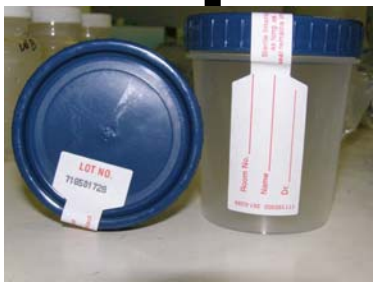


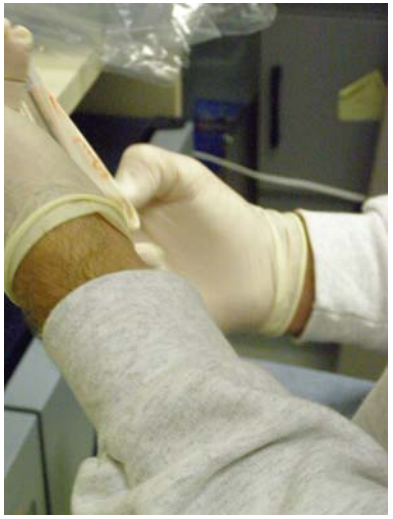
Lab Preparation: Labeling Quanti-Trays

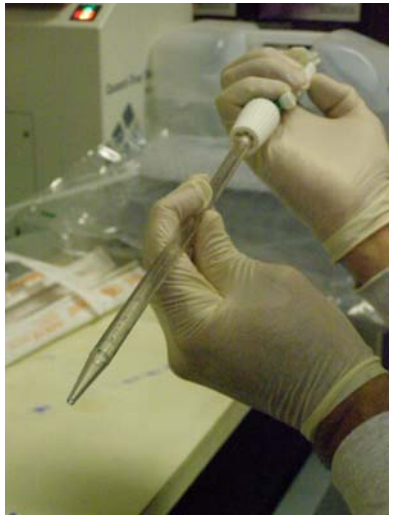


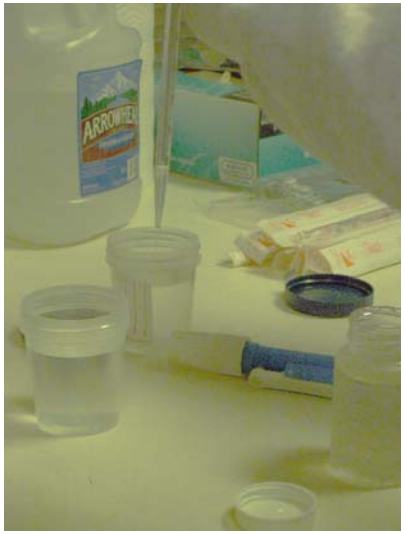
- Lab Name
- Date
- Q/A Note: Duplicate, Blank…..
- Sampled Location
- Dilution Rate: 10-1, 100-1, 1000-1
- Field Sampler

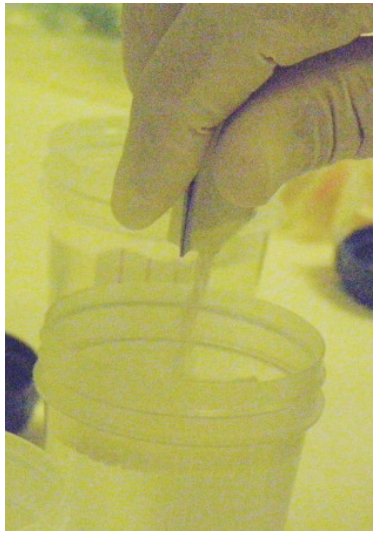
Preview: Preparing Sample, Sample Dilution & Adding Media

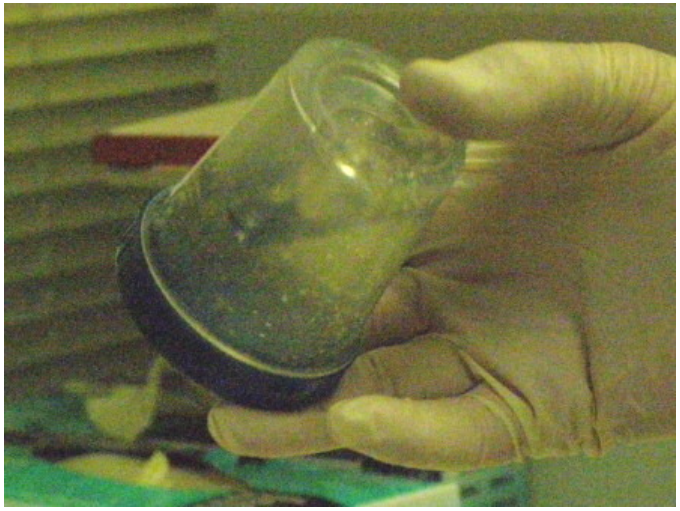












Preparing Sample- Dilution & Adding Media- 1

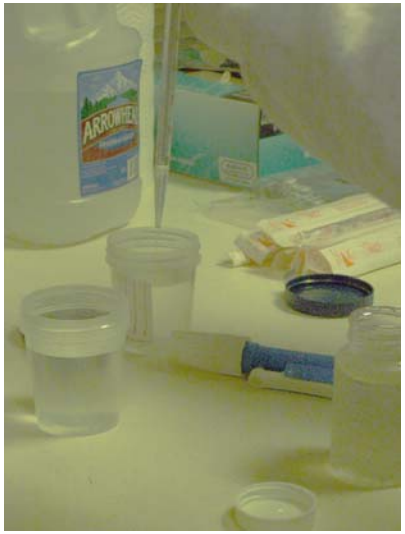
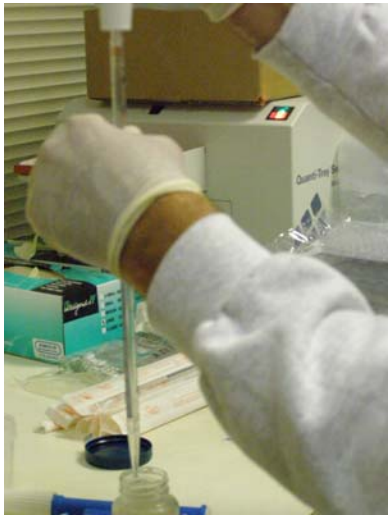
Start with a sterile mixing container and sterile water.

Preparing Sample- Dilution & Adding Media- 2

Pour sterile water into the mixing container.

Preparing Sample- Dilution & Adding Media- 3

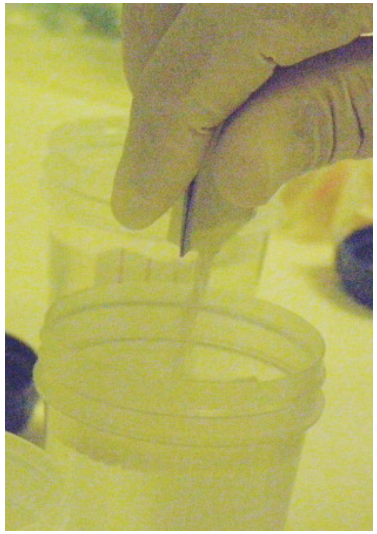


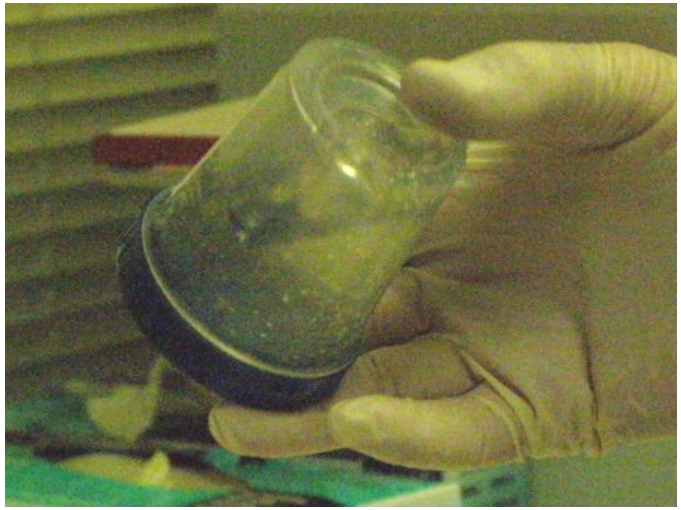



Prepare a sterile pipette and take some of the sample water (10ml or 1ml depending on Dilution rate desired).

Place this into the sterile mixing container.

Preparing Sample- Dilution & Adding Media- 4





Pour media (Colilert, Colilert-18 or Enterolert) into the sterile mixing container.

Add foam preventative additive if needed. (Colisure)

Shake mixture.

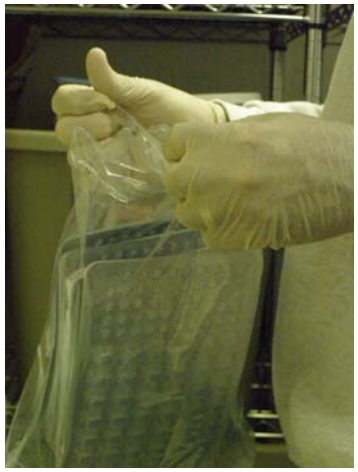
Allow foam to settle.

Preparing Sample- Dilution & Adding Media- 5

Fill the rest of the 100ml. sterile mixing container with sterile water.

Filling the Quanti-Tray

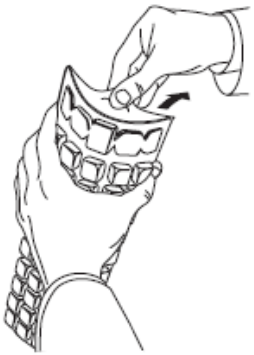
1



2

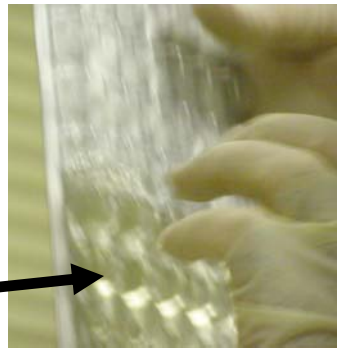
3

4



5

6



1-Begin with sterile Quanti-Trays

2-Use one hand to hold the Quanti-Tray upright with the well side facing the palm (bubble side).

3-Squeeze the upper part of the Quanti-Tray so that tray bends towards the palm.

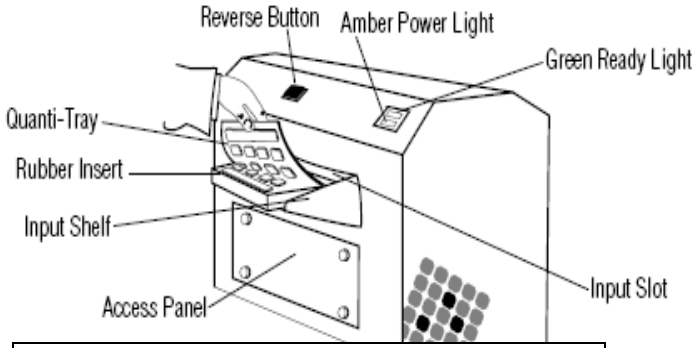
4-Open the Quanti-Tray by Pulling the foil tab away from the well side. (Do not touch inside of the foil or tray.)

5-Pour the reagent/sample mixture directly into the Quanti-Tray avoiding contact with the foil tab.

6-Tapping the Quantitray can help remove bubble. Allow the foam to settle.

Sealing the Quanti-Tray

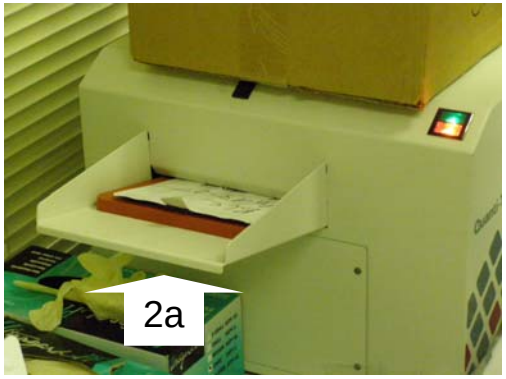
1 Set the Quanti-Tray within the proper rubber insert.



The Quanti-Tray Sealer

2 Place the Quanti-Tray/Inset onto the input shelf and feed it into the sealer.

2a Note:If the tray/insert will not feed and it was feed into the sealer correctly, gently lift up on the input shelf's outside lip during feeding.



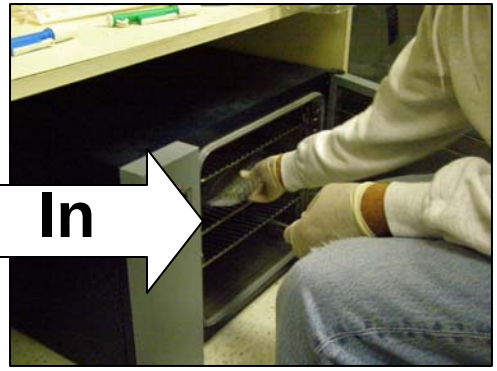
3 Remove Quanti-Tray and insert. If they were inserted crooked you may stop and reverse the insertion and re-insert them.

4 Sealed Quanti-Tray removed from the rubber insert.

Incubating Sample

Incubate Colilert at 35 °C for 24 hours (18 hours for Coliert-18)

Incubate Enterolert at 41 °C for 24 hours.

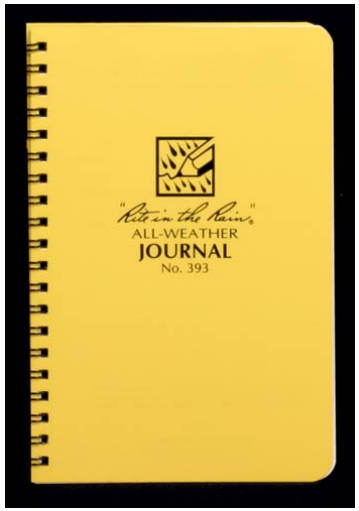


It is recommended that you post a note on the incubator door showing the time and date when the samples need to be read.

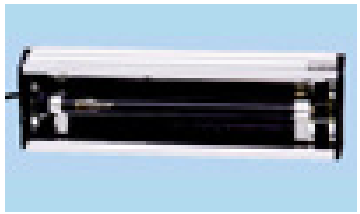
QA/QC Note:

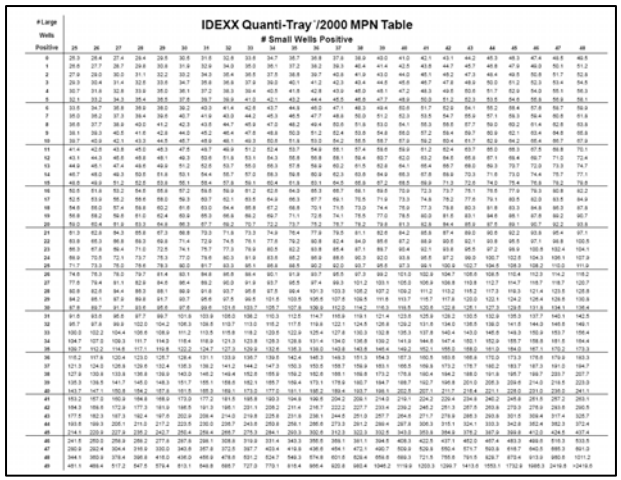
Check your incubators thermostat and thermometers against a NIST certified and traceable thermometer.

Preparation for Reading the Sample



- Results Notebook
- UV Lamp
- Anti-UV Goggles
- MPN Table

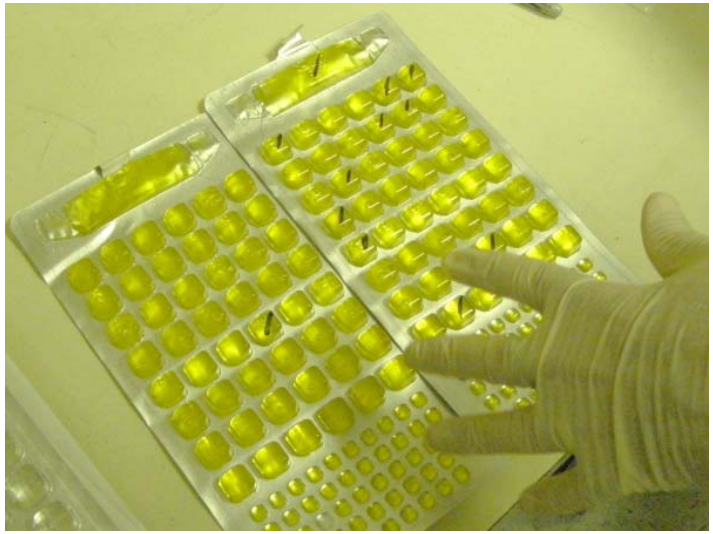


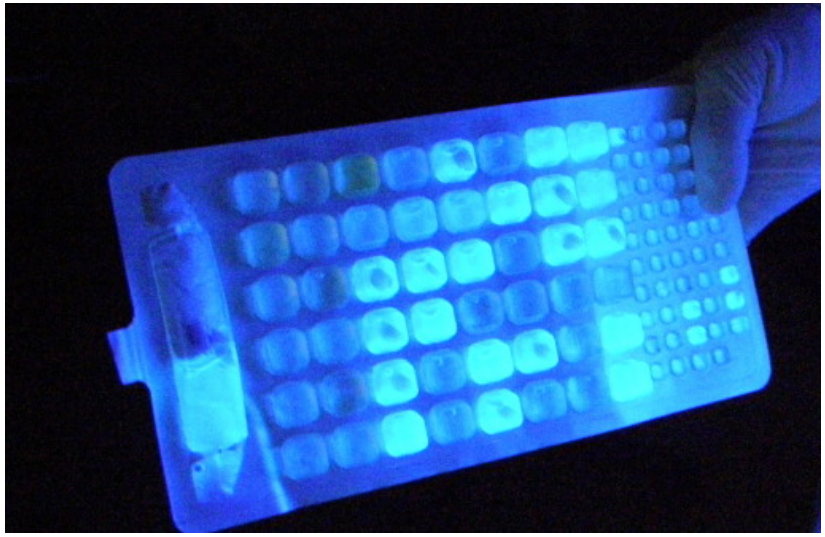


IDEXX Quanti-Tray®/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																								
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.5	33.6	34.7	35.7	36.8	37.9	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.6	50.7
1	25.6	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	46.9	47.9	49.0	50.1	51.2	52.3
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	53.9
3	29.9	30.4	31.4	32.5	33.6	34.7	35.8	36.9	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	55.6
4	30.7	31.6	32.6	33.6	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	57.4
5	32.1	33.2	34.3	35.4	36.6	37.8	39.7	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	33.6	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.9	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	43.6	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.6	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	51.9	53.1	54.3	55.6	56.9	58.1	59.4	60.7	62.0	63.2	64.6	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	50.6	51.9	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	68.6	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	58.6	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	54.5	55.9	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.8	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	56.6	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	81.6	83.1	84.6	86.1	87.6	89.2	90.7	
20	59.0	60.4	61.8	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	71.9	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	93.9	95.4	97.1	
22	63.9	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	67.9	69.4	71.0	72.6	74.1	75.7	77.3	79.0	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	99.0	100.8	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	93.6	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	74.5	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	84.8	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	107.6	109.6	111.7	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	129.6	131.9	134.1	136.4	
31	91.5	93.5	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	97.9	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	138.9	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	117.9	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.6	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	127.6	130.6	133.6	136.6	139.6	142.8	146.0	149.2	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	225.9	231.0	236.0	241.1	
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.9	190.3	194.8	199.4	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	164.9	169.6	173.9	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	263.9	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	231.9	238.1	244.5	251.0	257.7	264.5	271.7	278.9	286.3	293.9	301.6	309.4	317.4	325.7	
44	193.8	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.6	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4	
45	214.1	220.3	227.0	233.2	240.7	250.4	254.4	261.7	270.3	284.1	293.0	302.6	312.3	322.3	332.5	343.0	353.8	364.8	376.2	387.8	399.8	412.0	424.5	437.4	
46	241.8	250.0	258.6	269.2	277.8	287.8	298.1	308.6	319.6	331.4	343.3	355.6	368.1	381.1	394.5	408.3	422.5	437.1	452.2	467.4	483.3	499.6	516.3	533.5	
47	280.8	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.6	616.7	640.5	665.0	690.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.6	629.4	658.6	689.3	721.5	755.6	791.6	829.7	870.4	913.6	960.6	1011.2	
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.6	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.8	>2419.8	

Reading the Sample





Count the yellow cells that are positive, and mark the cell with a “Sharpie”.

Use a 6-watt 365nm UV light within 5 inches of the sample in a dark environment and count the positive cells.

Wear anti-UV glasses/goggles.

Determining the Most Probable Number of *Enterococci* Cells Per 100ml of Sample

1) Following the incubation period observe and count the number of positive (fluorescent) wells. For enterococci look for blue fluorescence with a 6 watt, 365nm, UV light within 5 inches of the sample. Face light away from your eyes and towards the sample. . The fluorescence intensity of positive wells may vary.

2) Wells that fluoresce yellow or yellow-green are **false positives**.

3) Refer to the MPN table (provided by IDEXX) specific to the type of quanti-tray used (51 well or 97 well type of quanti-tray) to obtain a Most Probable Number per 100 ml of sample.

4) If a dilution was performed, after obtaining the initial MPN result from the table, multiply that result by the dilution level to obtain the final result (e.g., if a 1:10 dilution was employed, multiply the result from the MPN table by 10 to get the final result in MPN/100 ml).

5) If the sample is inadvertently incubated over 28 hours without observation, the following guidelines apply: Lack of fluorescence after 28 hours is a valid negative test. Fluorescence after 28 hours is an invalid result. In other words, only positive results obtained using the proper incubation period (24-28 hours) are valid.

Determining the Most Probable Number of *Coliform* Cells Per 100ml of Sample

1) Following the incubation period, observe and count the number of positive wells. For *E. coli* look for fluorescence with a 6 watt, 365nm, UV light within 5 inches of the sample. Face light away from your eyes and towards the sample.

2) For total coliform and *E. coli* use the following **Result Interpretation Table**:

Note: Fluorescent wells that are not yellow (i.e., wells which are not positive for total coliforms) cannot be considered positive for *E.coli*. **In other words, these are false positives for *E. coli*.**

3) Refer to the MPN table (provided by IDEXX) specific to the type of quanti-tray used (51 well or 97 well type of quanti-tray) to obtain a Most Probable Number per 100 ml of sample.

Result Interpretation Table:

Appearance	**Result**
Colorless or slight tinge	negative for total coliforms and *E. coli*
Yellow equal to or greater than the comparator (supplied by IDEXX)	positive for total coliforms
Yellow equal to or greater than the comparator and fluorescence	positive for *E. coli*

4) If a dilution was performed, after obtaining the initial MPN result from the table, multiply that result by the dilution level to obtain the final result (e.g., if a 1:10 dilution was employed, multiply the result from the MPN table by 10 to get the final result in MPN/100 ml).

5) Samples are negative if at any time after the incubation period is complete there is no yellow or yellow/fluorescence. Yellow or yellow/fluorescence observed before 18 hours is a valid positive. However, after 22 hours from inoculation, heterotrophic bacteria may overwhelm Colilert-18's inhibition system. Therefore, yellow or yellow/fluorescence first observed after 22 hours from inoculation is not a valid positive.

Most Probable Number (MPN) Tables

IDEXX Quanti-Tray®/2000 MPN Table

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

51-Well Quanti-Tray MPN Table

No. of wells giving positive reaction per 100 ml sample	Most Probable Number	95% Confidence Limits Lower	Upper
0	<1	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	26.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.0
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	387.6
51	> 200.5	146.1	infinite

Sample Disposal

Quanti-trays that have been read can then be disposed of. Since the Quanti-Trays are now filled with bacteria, they are to be treated as hazardous waste.

Used Quanti-Trays can be sterilized by putting them in an autoclave after which they can be treated as normal waste.

Field Quality Assurance/Quality Control

These should be collected at 5 percent of your sample sites along with the regular samples.

Field Blanks. Sterile water in sterilized containers should be sent out with selected samplers. At a predetermined sample site, the sampler fills the usual sample container with this sterile water. This is labeled as a regular sample, but with a special notation (such as a "B") that indicates it is a field blank. It is then analyzed with the regular samples. Lab analysis should result in "0" bacteria counts for all blanks. Blanks are used to identify errors or contamination in sample collection and analysis.

Field Quality Assurance/Quality Control 2

Internal Field Duplicates. A field duplicate is a duplicate stream sample collected at the same time and at the same place either by the same sampler or by another sampler. This is labeled as a regular sample, but with a special notation (such as a "D") that indicates it is a duplicate. It is then analyzed with the regular samples. Lab analysis should result in comparable bacteria counts per 100 mL for duplicates and regular samples collected at the same site. Duplicates are used to estimate sampling and laboratory analysis precision.

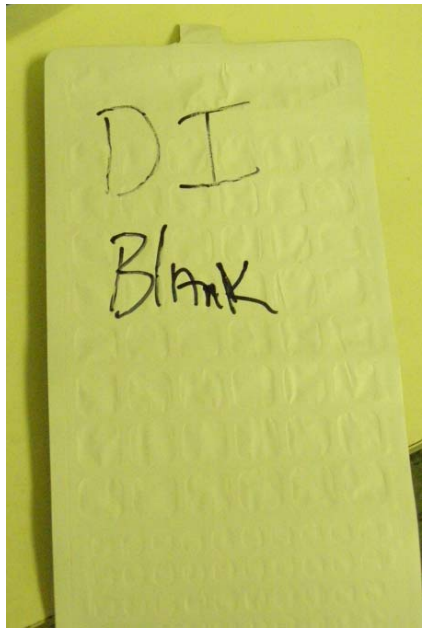
Field Quality Assurance/Quality Control 3

External Field Duplicates. An external field duplicate is a duplicate stream sample collected and processed by an independent (e.g., professional) sampler or team at the same place at the same time as regular stream samples. It is used to estimate sampling and laboratory analysis precision.

Lab QA/QC

Keep thing organized and sterile.

Use proper blanks and control media.



Lab QA/QC- 2

External Reference Samples: A **positive control** is a sample prepared in the lab to contain a known approximate concentration of enterococcus bacteria. An external reference sample is a positive control prepared and provided by a professional laboratory. The external reference sample is split. You should analyze the split external reference and compare your results to the professional lab. At least two external reference samples must be run per year.

Laboratory Custody Log

Laboratories shall maintain custody logs sufficient to track each sample submitted and to analyze or preserve each sample within specified holding times. A sample is considered under custody if:

- it is in actual possession;
- it is in view after in physical possession;
- it is placed in a secure area (accessible by or under the scrutiny of authorized personnel only after in possession)

Field Log

Field crews shall be required to keep a field log for each sampling event. The following items should be recorded in the field log for each sampling event:• time of sample collection;
• sample ID numbers and unique IDs for any replicate or blank samples;
• the results of any field measurements (temperature, D.O., pH, conductivity, turbidity) and the time that measurements were made;
• qualitative descriptions of relevant water conditions (e.g. color, flow level, clarity) or weather (e.g. wind, rain) at the time of sample collection;
• a description of any unusual occurrences associated with the sampling event, particularly those that may affect sample or data quality.

The field crews shall have custody of samples during field sampling. Chain of custody forms will accompany all samples during shipment to contract laboratories. All water quality samples
will be transported to the analytical laboratory directly by the field crew or by overnight courier.

Use Fresh Sterile Tools

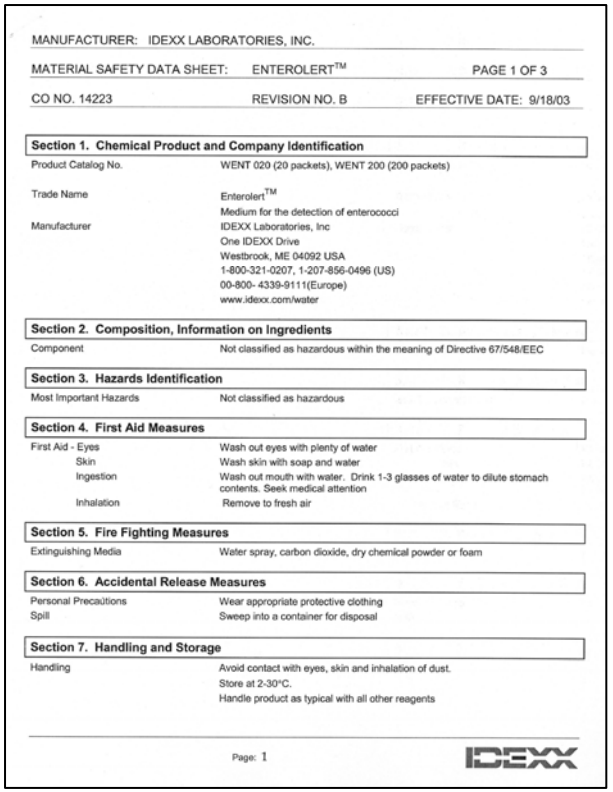
Your final results are valuable data. It is best to use new sterile items with each sample to be processed. Do not compromise your results by reusing items.

Health & Safety

All field and laboratory personnel must be made aware of all potential hazards. Exposure to hazards should be minimized. Appropriate personal protective equipment must be worn. Additional safety equipment and emergency response plans should also be provided

Health & Safety 2

Read and post all MSDS (Material Safety Data Sheets).



MANUFACTURER: IDEXX LABORATORIES, INC.

MATERIAL SAFETY DATA SHEET: ENTEROLERT™ PAGE 1 OF 3

CO NO. 14223 REVISION NO. B EFFECTIVE DATE: 9/18/03

Section 1. Chemical Product and Company Identification

Product Catalog No.	WENT 020 (20 packets), WENT 200 (200 packets)
Trade Name	Enterolert™ Medium for the detection of enterococci
Manufacturer	IDEXX Laboratories, Inc One IDEXX Drive Westbrook, ME 04092 USA 1-800-321-0207, 1-207-856-0496 (US) 00-800- 4339-9111(Europe) www.idexx.com/water

Section 2. Composition, Information on Ingredients

Component	Not classified as hazardous within the meaning of Directive 67/548/EEC

Section 3. Hazards Identification

Most Important Hazards	Not classified as hazardous

Section 4. First Aid Measures

First Aid - Eyes	Wash out eyes with plenty of water
Skin	Wash skin with soap and water
Ingestion	Wash out mouth with water. Drink 1-3 glasses of water to dilute stomach contents. Seek medical attention
Inhalation	Remove to fresh air

Section 5. Fire Fighting Measures

Extinguishing Media	Water spray, carbon dioxide, dry chemical powder or foam

Section 6. Accidental Release Measures

Personal Precautions	Wear appropriate protective clothing
Spill	Sweep into a container for disposal

Section 7. Handling and Storage

Handling	Avoid contact with eyes, skin and inhalation of dust. Store at 2-30°C. Handle product as typical with all other reagents

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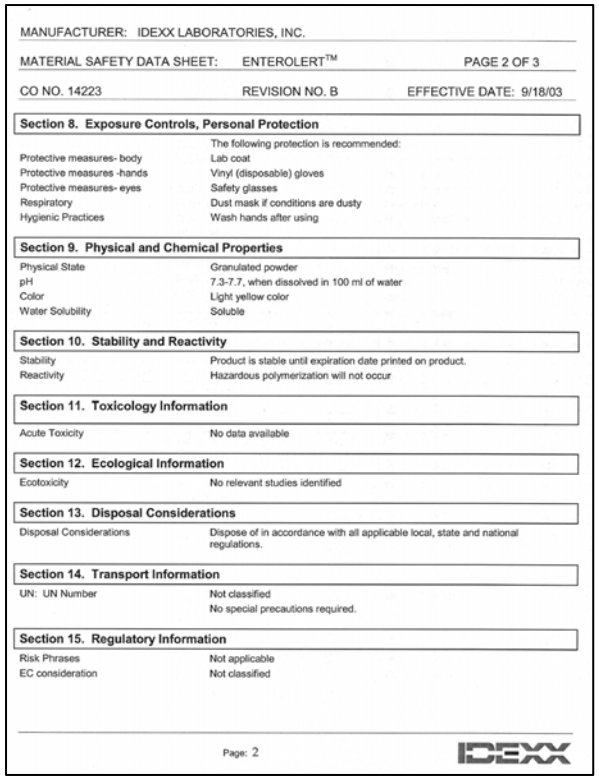

MANUFACTURER: IDEXX LABORATORIES, INC.

MATERIAL SAFETY DATA SHEET: ENTEROLERT™ PAGE 2 OF 3

CO NO. 14223 REVISION NO. B EFFECTIVE DATE: 9/18/03

Section 8. Exposure Controls, Personal Protection

	The following protection is recommended:
Protective measures- body	Lab coat
Protective measures -hands	Vinyl (disposable) gloves
Protective measures- eyes	Safety glasses
Respiratory	Dust mask if conditions are dusty
Hygienic Practices	Wash hands after using

Section 9. Physical and Chemical Properties

Physical State	Granulated powder
pH	7.3-7.7, when dissolved in 100 ml of water
Color	Light yellow color
Water Solubility	Soluble

Section 10. Stability and Reactivity

Stability	Product is stable until expiration date printed on product.
Reactivity	Hazardous polymerization will not occur

Section 11. Toxicology Information

Acute Toxicity	No data available

Section 12. Ecological Information

Ecotoxicity	No relevant studies identified

Section 13. Disposal Considerations

Disposal Considerations	Dispose of in accordance with all applicable local, state and national regulations.

Section 14. Transport Information

UN: UN Number	Not classified No special precautions required.

Section 15. Regulatory Information

Risk Phrases	Not applicable
EC consideration	Not classified

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Example of MSDS

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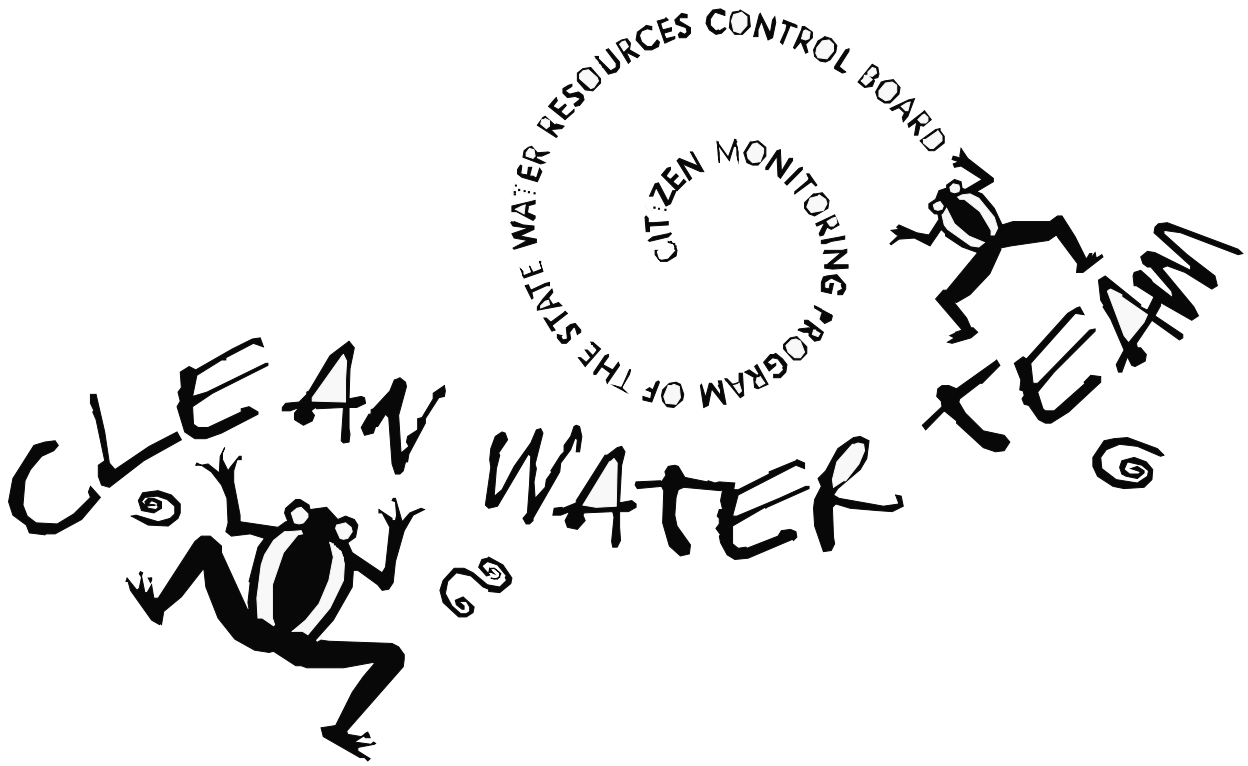
END



http://www.waterboards.ca.gov/water_issues/programs/swamp/cwt_volunteer.shtml