# Blue Water Task Force

## Coastal Water Quality Monitoring Manual

June 2003

Version 1.4

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#### **Chapter1: Introduction**

Since the Blue Water Task Force was established in 1991, the program has been very successful for the Surfrider Foundation. Currently the program is running in over 25 Surfrider chapters nationwide. Thank you to our chapters that have contributed to this success. To our new chapters we look forward to your involvement in this important program. As an organization we have made great strides in educating people about the pollution along our coastlines and the need to seek solutions to the pollution. Urban runoff and "non-point source pollution" are now common topics. Unfortunately, as we have learned in places such as Huntington Beach, California our job has just begun. Not only does Surfrider need to continue testing and educating people about their right to know if the ocean is clean, we also often need to move up into the watersheds to seek solutions to the problems.

Water quality issues consistently rank highest as our chapters' primary concern. Continual updates to the Blue Water Task Force Manual as targeted in our strategic plan, should assist chapters to continue to perform routine monitoring and improve the program on many fronts. This guide explains our most common test procedures. Further upgrades to the manual with your help and input will occur as needed. We hope this manual is helpful and that you keep up the good work to protect surfers, swimmers and other ocean enthusiasts around the country.

Thank you to the Surfrider Foundation chapters that have been testing the coastal waters for the last twelve years. Your experience and expertise are essential to making the Blue Water Task Force a success.

**PLEASE** don't be daunted by the size of the document. It includes a wealth of information and should be used as a reference document. Find the sections you need and read the others if interested.

Thanks,

Chad Nelsen
Environmental Director
Surfrider Foundation

Rick Wilson
Coastal Management Coordinator
Surfrider Foundation

#### **Chapter 2: Why Test the Water?**

The Surfrider Foundation Blue Water Task Force is a volunteer driven water quality-monitoring program. It is designed to take advantage of the daily presence of beach goers and surfers in the water, who can serve as coastal watchdogs. It is a hands-on program with the following goals:

The right to know: The Blue Water Task Force will collect data that will give beach goers information to make an informed decision as to where it is safe to swim and surf.

Add to existing testing: Limited resources mean that government agencies are not adequately testing the water along our coastline. When they do, they often concentrate on "point sources" that have a large ocean "outfall" pipe. Much of the coastal pollution on our beaches is caused from street run-off or "non-point sources." The Blue Water Task Force hopes to fill in the holes that government agencies leave untested.

**Watchdog:** A large percentage of existing testing is "self-monitoring" data. "Correcting your own homework" doesn't always work. Some dischargers find it convenient to test during the cleanest periods of the day. The Blue Water Task Force will serve as a critical third party by monitoring other people's data.

**Consistency:** The Blue Water Task Force takes advantage of our member's frequent contact with the water. If official testing is done only monthly or weekly, many contamination episodes will be missed.

**Public Education:** Once data is collected and pollution sources are identified, we will alert the authorities and launch a community education campaign to clean up the problem.

**Visibility**: Chapters have the opportunity to use a database driven website to input and display water quality data. This site is made available through Surfrider's homepage at <a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a> and serves to facilitate organizing, displaying and storing data.

This manual will help our activists find the areas of greatest coastal pollution; it will help them find other people's water quality data; it will help them accumulate their own data; and it will direct them on how to take steps to clean up our pollution problems.

Best of Luck and Keep Surfing.

#### **Chapter 3: Find the Sources of Water Pollution**

Before starting a water quality-monitoring program, you must first determine where the water should be tested. Coastal pollution breaks down into two classifications: (1) point sources and (2) non-point sources. A point source discharge comes from a well-defined, discrete spot along the shoreline. A typical point source is the outfall pipe of a sewage treatment plant or a factory. A non-point source arrives at the beach at a single point, but it originates from many different points. Non-point source pollution is run off from the streets and run off from the land. It arrives at the shoreline in a number of ways: (1) storm drains, (2) streams and creeks, (3) seaside parking lots, (4) drainage ditches and (5) roof and wall gutters. Non-point source pollution comes from a wide variety of sources, including: (1) fallout from air pollution, (2) mobile sources, such as boats and ships with pass-through plumbing, (3) wild and domestic animals, (4) agricultural run off, (5) fluid leaks from vehicles, (6) irrigation run off, (7) car washing, (8) beach litter and (9) contaminated ground water that seeps into the ocean.

Since non-point source pollution is the greatest cause of surf zone pollution in most areas, you must first find the areas where pollution enters the ocean. Nautical charts and Coast Guard charts can be quite helpful. Find the major streams and lagoons. Visit the area. If these ocean inlets have stagnant water with green or pink algae, suspect pollution. These foul smelling bodies of water are "over-nutrified." Rotting organic matter causes bacteria and algae to grow and "bloom." This type of "nutrification" may be good for algae, bacteria and virus, but it is very bad for water quality. If you see bubbles rising from the floor of the lagoon, although this may be normal, this may be a bad sign. It means that there is anaerobic decay (rotting in the absence of oxygen). The gas that is released is hydrogen sulfide. It smells like rotten eggs. This would qualify the area for a Blue Water Task Force sampling location.

#### FIND THE STORM DRAIN OUTLETS

Non-point source pollution frequently enters the coast through storm drains. The origin of this water is primarily from street side catch basins or street "gutters." Paving and other "impervious surfaces" prevent water from soaking into the ground. The higher the population density, the more drain outlets. Storm drains are designed to transfer water as quickly as possible from inland areas to the coast. These are flood control devices. Water quality is rarely a concern to a county flood control department. For this reason, public works departments may not even know where their outfall pipes empty into the ocean.

If your chapter has not implemented Beachscape, now is the time. It will be necessary for your group to physically walk the beach to look for the presence of pipes, ditches and culverts along the shoreline. Since many of these are hidden by vegetation, the best time to look for them is shortly after a rain. When you see muddy water flowing to the beach, you can trace the origin of the flow. Use the Beachscape protocols (<a href="www.surfrider.org/beachscape">www.surfrider.org/beachscape</a>) to map the storm drains. On the map, identify the storm drain by a number and then on another piece of paper, write a description of the drain. (The San Diego Chapter copyrighted their storm drain catalog and sold it to the city. The city

needed it for their NPDES storm water permit.) Knowing where the storm drains are, especially the flowing storm drains, will give you an idea where the most polluted spots are.

Another way to determine where to test is by finding out where surfers, wind surfers, divers and swimmers are reporting illnesses. If there are a large number of reports of illness in the same location, the water in this area should be tested.

Pollutants coming from a discharging storm drain will tend to stay near the drain. When waves break at an angle to the beach, a "longshore current" moves down the beach. This current will dilute pollutants. Sediment deposited in an offshore area may create sand bars, making the waves in front of a storm drain a popular surfing area with well-shaped waves. All Surfrider Foundation members should know, and spread the word, that surfing in front of a storm drain within 72 hours of a rainstorm is not recommended.

#### **Chapter 4: Existing Data**

There is a tremendous amount of water quality data being produced every day. The Federal Clean Water Act has mandated that permits be given to those who discharge water into our nation's bodies of water. These permits are called National Pollution Discharge Elimination System (NPDES) permits. A point source discharger is given a permit from the regional EPA that lists the levels of pollutants that he is allowed to place into the river, lake or ocean. It is the responsibility of the discharger to monitor the water quality to insure that the limits of the permit are not exceeded. The data is sent to regional water boards. However, these boards are usually severely understaffed. They have a great difficulty enforcing every permit. It is primarily a "correct your own homework" honor system.

The first step is to get the water quality data that has been produced by all the major dischargers in the area. The ones that do the most testing are sewage treatment plants. Also, power plants and large industrial plants have NPDES permits. Much of this information can now be found on the Internet or by calling your local regional water quality control board or county health department. They can help you. Call your local agencies and ask them for their test data.

Check your phone book for the addresses of all the sewage treatment plants in your area. Look under the city and county Department of Public Works, Sanitation District, Sewer Agency or Wastewater Authority. Whenever you write any agency, write them care of the Office of Environmental Monitoring. Ask if your group could have a tour of the facility. Tell them that you are an activist in the Surfrider Foundation, a 37,000 member environmental group that is concerned with coastal water pollution. Before the tour, ask them if they would bring a copy of their NPDES permit, their recent water test data, and a copy of their annual report. Be friendly and cooperative. Most sewage treatment plants are not responsible for surf-zone pollution and they know it. They will be glad to educate you on the causes of surf-zone pollution (non-point source pollution). If you find someone helpful, invite him or her to speak at your local chapter meeting.

#### A WORD ABOUT ENVIRONMENTAL MONITORING DEPARTMENTS

These are the people who went to school so that they could find a career that would benefit our environment. They often are very cooperative with environmentalists. They are usually environmentalists themselves. You may find an exception if they have had a bad experience in the past, or sometimes they might start out acting defensively. You will get a tremendous amount of information from these folks if you approach them in a friendly and cooperative manner. You will learn a lot more from interviewing them than you will by thumbing through stacks of their statistics.

#### POINT SOURCE DISCHARGERS

#### SEWAGE TREATMENT PLANT TOURS

After you have identified your major storm drain outlets, the next step is to evaluate your local point source dischargers, especially sewage treatment plants. The encyclopedia has a good explanation of sewage treatment. The more homework you do, the more you will learn. There are three levels of sewage treatment. Primary treatment is a physical process that removes up to about 60% of the solids from the sewage. Advanced primary is a process that removes 80 to 85% of the solids by adding chemicals. Secondary treatment is a biological treatment of the leftover liquid and small particles of solids. After full secondary treatment, 80 to 90% of the bacteria should be killed and 80 to 90% of the suspended solids should be removed.

The 1972 Clean Water Act mandated that all sewage plants that discharge into a body of water have full secondary treatment. Today, decades later, there are still some ocean-side plants that don't have full secondary treatment. Most of these plants have either advanced primary treatment or what is called *partial secondary treatment*. That means that they mix secondary effluent with primary effluent. They then discharge this mix into the ocean.

Ask about the length and depth of the outfall pipe. To promote mixing, and since sewage is fresh water, the discharge pipe often has a series of holes in the side called diffusers and the discharge is typically done in water at least 90 feet deep. If the pipe is in deep water, the discharge may stay below a layer of cold water called a "thermocline."

If the plant is not currently performing full secondary treatment, it is not the fault of the people leading your tour. There is no sense getting into it with them. You are trying to get data on the surf-zone water quality. Sewage plants test the surf-zone to make sure that the effluent that is discharged out of their ocean outfall pipe does not come back to the beach. You are there to collect information, not to argue the merits of the Clean Water Act.

#### TERTIARY SEWAGE TREATMENT

Tertiary treated sewage is water that has been cleaned of the remaining particles and bacteria. Tertiary treated sewage can be recycled to water lawns and golf courses, or even for use as drinking water. If the tertiary treated water is allowed to soak into the ground it can help restore depleted groundwater aquifers, rivers and wetlands. If the plant you are touring has an ocean outfall pipe, it is probably not sending clean, tertiary treated water into the ocean. Outfalls are for primary and secondary treated sewage. Someday, the Surfrider Foundation would like to see an end to all ocean outfall pipes.

#### **READING NPDES DATA**

Sewage plants generally do the most extensive testing of any dischargers into the ocean. The purpose of this testing is to establish whether they are in compliance with their NPDES permit and to prove that they are not harming the coastal environment.

When you get their permit, it will list the concentrations and mass emission limits of what they are allowed to discharge. Compare the monthly data sheets in their reports against the maximum daily and monthly levels allowable under the conditions of their NPDES permit. If there seem to be violations, call them and ask them about it. If their answers don't make sense, contact the permitting agency (using a regional water board or the EPA), seek expert advice in your area or call the national office.

We are interested in their shore station (beach) monitoring. If their only tests are far from shore, near their outfall pipes, the data will not be that useful to us. There are three tests that we focus on: total coliforms, fecal coliforms, and enterococcus. See Appendix D for California's standards. Acceptable levels for single samples are below 10,000 MPN per 100ml of water for total coliform, below 400 MPN for fecal coliform, and below 104 MPN for enterococcus. Not all agencies perform the more expensive test for enterococcus, as California does. The safe level for this bacterium is 35 MPN per 100ml for a 30-day log mean and 104 for single sample. When the numbers are higher than this, the water is not safe for human contact. (MPN stands for most probable number. This is to signify that the test is a general average.)

If the data consistently shows levels that are over the acceptable limits, contact your local NPDES permit agency and ask them what they are doing to ensure permit compliance. Frequently, these agencies are so over burdened and understaffed that this data just sits in their files.

#### **Chapter 5: Collecting Your Own Data**

Now that you have learned something about water pollution and found out about existing water quality data, you may find that you are still interested in doing some volunteer water quality testing to educate students or your community, testing places that aren't being tested, or checking up on the testing agency in your area. The Surfrider Foundation Blue Water Task Force provides several water quality testing options, which are listed below.

#### 1. Colilert 18 Method:

This has been the most common and popular volunteer water quality method (a slight variation from the old Colilert-MW method). This method tests for both Total Coliform and E. Coli, two very common bacteria strains that are associated with health disorders. The E. Coli reading can be converted to fecal coliform using an estimation method. The Colilert method can be performed using the 5 or 15 test tube dilutions, the Quanti-Tray sealer or a simple presence/absence test. (This will be explained in more detail later.) EPA and other agencies have recently recommended testing for enterococci instead of total coliform and E. Coli for salt water testing.

#### 2. Enterolert Test: Tests for Enterococci

Your chapter can also test for Enterococci. Enterococcus is another common health indicator bacteria. If your chapter has invested in the Quanti-Tray sealer this procedure is simple. Enterococcus can also be tested for using the 5 or 15 test tube method.

#### 3. Millipore Test ("the paddle test")

This test is used by students for science projects and is NOT used by the chapters. The test is simple to use but the results are too unreliable for chapter use in fighting for clean water. Although this test is not EPA certified, some states do use them for water quality measurements. They can be used as a screening method to identify problem areas and as education tools. These test kits are available from Surfrider's mail order catalog or online at <a href="https://www.surfrider.org/store">www.surfrider.org/store</a>

#### **Quanti-Tray:**

IDEXX, the company that supplies our water quality testing materials, has established a new testing tool called the Quanti-Tray Sealer that improves accuracy, reduces sample contamination and eliminates the need for the expensive and wasteful use of disposable test tubes. This is an EPA approved method. The sealer is expensive (\$3500) but can save money on supplies over the long run, as well as reduce the rate of error. If your chapter is serious about water quality testing and has or can raise sufficient funds, a sealer is probably a good investment.

#### **Chapter 6: Materials**

This chapter lists the materials you will need to create your water quality testing lab and begin testing the water.

#### **Test-tube Method Lab Materials:**

- Whirl-pak sample bags
- 35 degrees Celsius Incubator
- 365nm UV Lamp
- Test tube rack
- Sterile pipettes
- Pipette Pump
- 10 ml test tubes (non-fluorescing) with caps
- · Sterile deionized or distilled water
- Colilert-18 Presence/Absence packets
- Antibacterial Hand Soap
- Laboratory Notebook
   Disinfectant spray for counter tops

#### **Quanti-tray Sealer Method Lab Materials:**

- 35 degree Celsius Incubator
- UV Lamp
- Sterile pipettes
- Pipette Pump
- Sterile plastic bottles
- Sterile deionized or distilled water
- Colilert-18 reagent
- Enterolert reagent

- Quanti-tray sealer trays
- 115V Model 2X Quanti-tray Sealer
- Colilert Comparator
- 97-well rubber insert for Sealer
- Antibacterial Hand Soap
- Laboratory Notebook
- Disinfectant spray for counter tops

#### **Sampling Materials (needed for all testing methods):**

- Cooler
- Blue ice
- Whirl-pak sample bags

- Sharpie pen for writing site location name on sample bottle/whirl-pak
- Sample sheet and pen
- Tide Book

For budgeting purposes the initial expenses to start up a test-tube lab are approximately \$1,600 plus some additional expenses for basic household items, such as anti-bacterial hand soap. This includes 500 test tubes and 200 Colilert packs and therefore should be enough for 100 tests using the 15 test tube method and 400 tests using the 5 test tube method for coliform and E. Coli. Once the initial start up cost is met, the cost of testing materials is approximately \$6.00 per sample collected.

The initial cost for setting up a lab using the IDEXX equipment is approximately \$5,200. This includes all materials for 200 tests of coliform and E. Coli. Once the initial start up cost is met, the testing materials are approximately \$5.60 - \$6.20 per sample collected.

See table on next page for specific costs.

Materials Table: pricing and quantity for materials that must be ordered:

Description	VWR Catalog #	Unit	Quantity	Total
1-10ml sterile pipette	14672-922	Case of 200	1	84.00
Test tube rack	66023-534	1 rack	1	16.54
Pipette Pump 10ml	53502-233	1 pump	1	16.50
Whirl-pak, Collection Bags	11216-012	Pack of 500	1	45.30
125 ml sterile plastic vessels	16129-006	200 pack	1	90.00
Disposable sterile test tubes	60819-375	Case of 500	1	180.90
35 Degree Incubator	IDEXX	1 incubator	1	389.00
Colilert-18 P/A reagent	IDEXX	200 pack	1	720.00
Colilert-18 P/A reagent	IDEXX	20 pack	1	86.00
6 watt UV lamp 110 volt	IDEXX	1 lamp	1	89.00
115V Model 2X Quanti-tray Sealer	IDEXX	1 sealer	1	3500.00
Enterolert – P/A reagent	IDEXX	200 pack	1	800.00
Enterolert – P/A reagent	IDEXX	20 pack	1	97.00
97-Well Rubber Insert for Quanti-tray sealer	IDEXX	1 insert	1	40.00
Quanti-tray IDEXX Training Video	IDEXX	1 video	1	25.00
Colilert Comparator for Quanti- tray sealer method	IDEXX	1 comparator	1	6.00
97- Well Quanti-trays/2000 trays	IDEXX	100 trays	1	110.00

(January 2003)

All of these items can be ordered through Surfrider National by phone or email: 1-800-743-7873 or rwilson@surfrider.org

#### **Chapter 7: General Steps**

This chapter outlines the general steps in establishing your own water quality-monitoring program in your Surfrider Foundation chapter. Specific instructions for the lab work and sampling are found in Chapters 8, 9 & 10.

1. Lab Set Up: Once you collect samples at the beach, they must be prepared (inoculated) and put in the incubator within six hours of being collected. For this reason it is a good idea to have your lab set up before you begin sampling.

#### 2. Sample Collection:

- The first step in collecting samples is to establish the location of your sampling stations. These should be areas where water quality problems are suspected or places that are of high recreational use (see Chapter 4).
- Samples are then collected at the beach and a sample site sheet is filled out for each sample (see Sampling Procedures, Chapter 8 and Sample Form).
- The samples are then put in cold storage and brought to the lab. Sample must be prepared within 6 hours from the time it is collected.

#### 3. Lab Work:

The samples are then prepared in the lab (see Lab Work Instructions, Chapter 9) and put into the incubator for 18-22 hours. Three options are available depending on your budget and time allotment. A five test tube method (1:100 dilution) provides more generalized results but is quicker and less expensive. The 15 tube method will lead to more precise results but is more time intensive and expensive. The IDEXX Quanti-tray method is easy and accurate but requires the purchase of an expensive tray sealer.

#### 4. Reading Results:

Check for yellow color and florescence and establish Coliform and E. Coli levels using the MPN table (see Chapter 9). If testing for Enterococcus, check for florescence and establish the Enterococcus level using the IDEXX MPN number table (Chapter 9).

#### 5. Publish Your Results:

Your results can be published in a number of ways (see what to do with your results, Chapter 10). Many chapters publish them in their local newspaper, their web site, or on posters in surf shops. Results can also be posted on the Surfrider National Water Quality Website at <a href="https://www.surfrider.org/BWTFoutput.htm">www.surfrider.org/BWTFoutput.htm</a> or you can contact Rick Wilson at <a href="mailto:rwilson@surfrider.org">rwilson@surfrider.org</a> to set-up your chapter data pages.

#### **Chapter 8: Volunteers**

The volunteers that gather the weekly water samples and do the lab work are really the core component of a successful water sampling program. Volunteer samplers come from all parts of the community: high school and college students to working adults. They are drawn to the program for various reasons and will have varying degrees of enthusiasm. Typically they are motivated individuals and have many other activities and responsibilities going on in their lives. It is very important to remember that we are all volunteers and that we must understand and respect of each other's commitments to our personal lives.

The Chapter Volunteer Coordinator organizes the volunteers to insure that important test sites are covered at regular intervals. They also play a key role in keeping a high level of enthusiasm among the volunteers. If the Volunteer Coordinator can be at the lab when the volunteers bring in the samples, it goes a long way toward keeping them motivated. Together with the Lab Coordinator, they should endeavor to keep the atmosphere in the lab upbeat and fun - a place where the volunteers look forward to coming.

The coordinators should take time to talk to the volunteers and ask them if they have any questions, concerns or new ideas. Demonstrating and explaining the testing process will give the volunteers a broader understanding of what's going on in the lab and will increase their interest. Praise and thanks to the volunteers for their efforts will also keep them psyched and coming back with more water samples.

The Santa Cruz chapter is an example of a chapter that has a coordinator who is highly motivated and keeps the volunteers interested and dedicated. Their Coordinator calls the current volunteer samplers each week to remind them to take samples and confirm which sites they will be sampling. To achieve accurate results it is important to keep the same volunteers sampling specific sites consistently over at least several months. Having volunteers come and go by the week leads to inconsistent sampling and coordinating difficulties. The Coordinator also notifies samplers if the time to bring in the samples must be changed due to a holiday or other reason.

Publishing the test results in the local media not only informs the public but also has the effect of motivating the volunteers, and recruiting new volunteers. The volunteer samplers can see the published results and feel a sense of accomplishment, ownership and importance in the work they are doing.

#### **Chapter 9: Water Sampling Collection Instructions**

Equip	ment Check list:
	Sample location list Cooler Blue ice Whirl-pak sample bags Sharpie for writing site location name on sample bottle/whirl-pak Sample sheet and pen Tide Book
	Tide Book

#### Procedure:

- 1. Before approaching each site to take the sample, fill out the site log sheet. Use site location names from the master list. It is very important for record keeping that we all use the same names for record keeping purposes. See Appendix B for sample sheet.
- 2. Mark the whirl-pak or sample bottle with the site location name, and the date and time the sample is taken.
- 3. Gather a sample from a specific site using a sterile Whirl-pak sample bag or the sterile sample bottle. Open container just before taking sample to prevent contamination. Take the sample approximately six inches below the surface of the water in ankle to knee deep water from an area where waves are breaking or water is agitated. Take the sample during an incoming surge of water. Try to avoid taking the sample from an area where the water is not moving.
- 4. Do not fill to the top. An air space should be left to allow the contents to be shaken in the lab.
- 5. Close the sample tightly and immediately place the sample in a cooler with blue ice to keep sample cold.
- 6. The sample must be kept cool (in the cooler or refrigerated) and must be inoculated within six hours from the time it is collected.
- 7. Turn samples in at the lab. Remember to pick-up clean sample bottles or Whirl-paks at the lab for next week.

#### **Chapter 10: Lab Work Instructions (Procedures Manual)**

#### SURFRIDER FOUNDATION Standard Operating Procedure for

Bacteriological Analysis of Marine Waters Most Probable Number Method Utilizing Colilert – 18 and Enterolert Media

Revised 3-10-03

#### A. GENERAL DISCUSSION

Colilert-18 and Enterolert are approved methods for the bacteriological analysis of marine and estuarine waters. The purpose of this analysis is to give an estimation of the bacteriological density of coliform, E. Coli and/or enterococcus bacteria within the water in question. The quality of any analysis is highly dependent upon the integrity of the sample and the methods of testing. Proper sample collection practices are an integral part of any analysis. Please refer to the Standard Operating Procedure for Bacteriological Sampling of Recreational Waters for specific sampling requirements. It is important that good aseptic techniques (keeping everything sterile and uncontaminated) are maintained throughout the procedures in order to achieve good reliable data. The Most Probable Number (MPN) Method detailed below centers around using five or fifteen individual closable tubes or an IDEXX quanti-tray prepared with specific dilutions of sample and then referencing a graph, which will yield the final result.

#### **B.** REFERENCES

- 1. <u>Standard Test Methods for the Examination of Water and Waste Water</u>, 19<sup>th</sup> or 20<sup>th</sup> Edition, APHA, AWWA, WEF
- 2. IDEXX Colilert-18 or Enterolert Package Insert

#### C. PROCEDURE FOR TEST TUBE METHOD:

Prepare test tube rack by placing five test tubes per row for each dilution to be tested. Label the rack with an identification number (or name) of sample location. Additionally, label each row of tubes with dilutions to be tested (e.g. 1:10, 1:100, 1:1000, etc.).

If testing for coliform using the five test tube method use the 1:100 dilution instructions, if testing for enterococcus using the five test tube method use the 1:10 dilution instructions.

Traditionally, bathing beach waters require testing 10, 1, 0.1 and 0.01 ml volumes; the latter two volumes being delivered as a dilution of the original sample.

For an **extremely** specific test of known polluted waters (e.g., around creeks or stormwater outlets) the initial sample inoculations might be 0.1, 0.01, 0.001, 0.0001...0.00001 ml of the original sample delivered as dilutions into successive rows each containing five replicate volumes. This series of sample volumes will yield determinate results from as low as 200 to a high of 16,000,000 organisms per 100 ml.

#### **D. PREPARATION OF REAGENT (test tube method):**

## It is best to prepare the reagent immediately before you inoculate your samples with beach water. Do not prepare more than 4 hours prior to the start of testing.

To a sterile vessel containing 100 ml of either sterile deionized or distilled water, aseptically add 1 packet of Colilert-18 reagent (for coliform testing) or 1 packet of Enterolert reagent (enterococcus testing) to the vessel. Close vessel and mix to dissolve. There will be some foaming, but it will subside.

Aseptically dispense 9 ml of reagent water into all test tubes (whether 15 or 5 test tubes). (If using the 5 test-tube method, the 100ml vessel of distilled water with reagent should yield enough reagent water to perform 2 beach tests, since only 45 ml will be used for each 5 tubes test).

#### 1:10 DILUTION: (used for Enterococcus testing)

Inoculate the 1:10, 5-tube row (which already contains 9ml of water and reagent mixture) with 1 ml of your sample water. Cap and mix well.

#### (If using the 5 test tube method skip down to E. Incubation)

#### 1:100 DILUTION: (used for Coliform testing)

Prep a 1:10 dilution by dispensing 9ml of the well-mixed distilled water and reagent into another test tube.

Dispense 1.0 ml of the sample beach water into the tube.

This will create enough 1:10 dilution of the sample for all five test tubes.

Cap and mix well.

Take 1 ml of this 1:10 dilution and add to each of the five test tubes in the 1:100 row of test tubes (which already contain 9ml of the Colilert-18 reagent).

Cap and mix well.

This will create a set of 5 test tubes with a 1:100 dilution (including the reagent)

#### (If using the 5 test tube method skip down to E. Incubation)

#### 1:1000 DILUTION:

Shake the 1:10 dilution (from above) vigorously about 25 times before using.

Prep a 1:100 dilution by dispensing 1.0 ml of the well-mixed 1:10 dilution into another test tube containing 9 ml of either sterile deionized or distilled water. This will create a 1:100 dilution.

Cap and shake.

Pipette 1.0 ml of this well mixed sample (1:100) into each of the five tubes labeled 1:1000 containing 9 ml of reagent. This will create your 1:1000 dilution.

Cap and mix well.

#### **ADDITIONAL DILUTIONS:**

Please follow the outline above for any further dilutions required.

Prepare test tube rack by placing five test tubes per row for each dilution to be tested. Label the rack with an identification number (or name) of sample location. Additionally, label each row of tubes with dilutions to be tested (e.g. 1:10, 1:100, 1:1000, etc.).

Traditionally, bathing beach waters require testing 10, 1, 0.1 and 0.01 ml volumes; the latter two volumes being delivered as a dilution of the original sample.

For an **extremely** specific test of known polluted waters (e.g., around creeks or stormwater outlets) the initial sample inoculations might be 0.1, 0.01, 0.001, 0.0001...0.0001 ml of the original sample delivered as dilutions into successive rows each containing five replicate volumes. This series of sample volumes will yield determinate results from as low as 200 to a high of 16,000,000 organisms per 100 ml.

#### E. Procedure for IDEXX Quanti-Tray Sealer Method

<u>It is best to prepare the reagent immediately before you inoculate your samples with beach</u> water. Do not prepare more than 4 hours prior to the start of testing.

Collect water sample in the same manner as discussed in Chapter 9. Be sure to label the whirlpak bag before collection, as well as keep cooled during transport to lab.

**All seawater samples** must be diluted to at least 1:10 as described below. This is because there are many types of bacteria and other microorganisms called heterotrophs that naturally live in seawater. If dilution is not done, these organisms may overwhelm the bacteria that you are interested in and it is possible to get "false positives." This will invalidate your results.

Freshwater samples do not have to be diluted, but you can.

#### F. PREPARATION OF REAGENT

#### **1:10 DILUTION** (recommended dilution for enterococcus testing)

- 1. Using a sterile pipette, measure 90 milliliters of distilled or deionized water into one 125 ml sterile vessel for each water sample you will be testing.
- 2. Pop open the reagent pack (either Colilert-18 or Enterolert) and pour entire contents into vessel with sterile water. Close vessel and shake until reagent is thoroughly dissolved. There will be some foaming, but it will subside.
- 3. Using a different sterile pipette, measure 10 milliliters of the sample beach water and put it into one of the vessels with the 90 ml of reagent and water.
- 4. Cap and shake gently.

#### **Quanti-tray:**

- 5. With a black magic marker label the Quanti-tray with sample location, date, time, the reagent you used, and the dilution used.
- 6. Hold the Quanti-tray in one hand with foil seal up. Squeeze it over lengthwise in your hand and pull the seal away from the tray to open it at one end.
- 7. Pour entire contents of sample/reagent into the tray. Place tray in rubber insert so that it fits snugly, being careful not to spill out any water.
- 8. Slowly slide tray and insert into the sealer until the mechanism catches and begins to draw them in. Remove tray from rubber insert after it comes out other side of sealer.
- 9. Repeat for each sample. By doing them sequentially like this rather than simultaneously you will prevent confusion or mislabeling of the samples.

#### **1:100 DILUTION**

- 1. Using a sterile pipette, measure 99 milliliters of distilled or deionized water into one 125 ml sterile vessel for each water sample you will be testing.
- 2. Pop open the reagent pack (either Colilert-18 or Enterolert) and pour entire contents into vessel with sterile water. Close vessel and shake until reagent is thoroughly dissolved. There will be some foaming, but it will subside.
- 3. Using a different sterile pipette, measure 1 milliliter of the sample beach water and put it into one of the vessels with the 99 ml of reagent and water.
- 4. Cap and shake gently.
- 5. Follow steps 5-9 above.

#### SEE APPENDIX G FOR IMAGES ON HOW TO USE REAGENTS.

#### G. INCUBATION FOR TEST-TUBES:

If testing for coliform incubate all inoculated tubes at  $35 \pm 0.5^{\circ}$ C. for 18 hours (see package insert for instructions). If testing for enterococcus incubate all inoculated tubes or trays at 41 degrees Celsius for 24 hours.

#### H. INCUBATION FOR QUANTI -TRAY METHOD:

Note: Colilert-18 and Enterolert <u>cannot</u> be incubated at the same time in the same oven; they have to incubate at different temperatures and for different lengths of time.

Use the table below for incubation time and temperature:

Reagent	Temperature	Time
Colilert-18	35° C	18 hrs
Enterolert	41° C	24 hrs

#### I. DETERMINATION OF RESULTS FOR TEST-TUBE METHOD

When testing for coliform observe for yellow color after 18-hour incubation. A yellow color indicates a positive result for the presence of coliform bacteria. For those that are only moderately yellow use the comparison tube provided by the manufacturer to assist in judging the result. Tubes or cells, which have produced a moderate to partial reaction, should be incubated for an additional 4 hours to a total incubation time of up to 22 hours. Count the total number of positive test tubes.

For E. coli check for fluorescence using a 6 watt, 365nm, long wave UV lamp.

Calculate *E. coli* densities on the basis of number of positive (fluorescent) tubes, using the table of most probable numbers (MPN).

For Enterococcus check for fluorescence using a 6-watt UV lamp, after 24 hours incubation at 41 degrees Celsius. Calculate the enterococcus densities on the basis of positive (fluorescent) tubes, using the MPN table provided. Multiply the value from the table by 10 to get the true MPN number (assuming you used a 1:10 dilution).

**New!** -See Appendix C & E for explanation on converting *E. coli* to Fecal Coliform and determining an MPN number.

(<u>Standard Methods for the Examination of Water and Wastewater</u>, 19<sup>th</sup> or 20<sup>th</sup> Edition, APHA, AWWA, WEF)

#### J. DETERMINATION OF RESULTS FOR QUANTI-TRAY METHOD:

#### Colilert-18:

**Total Coliform:** Observe individual wells in Quanti-tray for yellow coloration after 18 hours of incubation at 35° C. Use the comparator to see what the yellow should look like. Count the number of positive yellow wells. Use the 97-well MPN table supplied to determine the MPN based on the number of positive small wells versus the number of positive large wells. Multiply the value from the table by 10 to get the true MPN number (assuming you used a 1:10 dilution). (**See Appendix C for MPN Table**)

**E.Coli:** Check for fluorescence using a 6-watt UV lamp. *Point the lamp away from your eyes.* Count positive wells and calculate the MPN using the same method as above.

#### **Enterolert:**

**Enterococcus**: Check for fluorescence after 24 hours of incubation at 41° C. Use the 97-well MPN table supplied to determine the MPN based on the number of positive small wells versus the number of positive large wells. Multiply the value from the table by 10 to get the true MPN number (assuming you used a 1:10 dilution).

The Quanti-trays can be incubated for an additional 4 hours if logistics don't allow you to read them at the specified time or if only a light coloration appears. If you incubate your Colilert-18 sample for more than 22 hours or Enterolert samples for more than 28 hours, you can get "false positives" and your results will be invalid.

Compare your MPN numbers with state standard levels in Appendix D to determine if levels are safe for surfing and swimming.

#### I. QUALITY ASSURANCE / QUALITY CONTROL (QA/QC)

Each Surfrider Foundation chapter should review their specific needs and requirements in regard to reporting results and the need for extensive QA/QC. Some of these considerations may be outside of the scope of individual Surfrider Foundation chapters due to monetary and/or safety reasons.

Performing QA/QC measures can give analytical validity to any testing being performed. This is generally required for any analysis that will be reported to a governing body or agency. Bacteriological quality control is most often performed by conducting replicate analysis of the same sample (for precision) and analysis of a known culture (for bias).

Precision is a measure of the closeness with which multiple analysis of a given sample agrees with each other. Precision is performed on 10% of all samples within each batch set-up or once per occurrence. Bias is a measure for systematic error and has two components: one due to the method and the other due to the laboratory's use of the method. Chapters splitting a given

sample and then comparing their results can evaluate bias. The overall accuracy is a combination of the precision and bias.

Other QA/QC measures that may be implemented include the use of temperature recorders on incubators or sterility checks on glassware. A simple min/max thermometer will replace the need for a recorder and only using pre-sterilized and (unfortunately) disposable glassware will minimize any need for checking sterility.

Another way to evaluate the accuracy of your results is to split your samples with a professional lab and then compare results.

#### **Chapter 11: What to Do with Your Results**

Now that you have your water quality results, it is time to use them to warn surfers of polluted waters and/or educate your community on the quality of your local water. Many of our chapters have employed successful techniques to spread the word. Here are some suggestions:

- 1. Call your local paper and ask if they will publish your results. You can fax them a graph or table of the test locations and the results.
- 2. Check your state standards and include them with your report. Highlight any results that are above the standards (see Appendix D).
- 3. Talk to surf shops and ask if they will post your results in their shop if you fax the results to them. Some chapters have made a map of their sample locations and the coastline on a white board so the shop owners can just write the numbers in.
- 4. Post your results on your chapter web page. Surfrider National has a website in which results and data can be published. Contact Surfrider at 1-800-743-SURF to find out or email rwilson@surfrider.org for further details.
- 5. Bring your results to the next city council meeting and explain what you are doing and how you think they should make water quality a priority. Chances are that if you live in a coastal town, tourism is one of the biggest economic engines. Tell them dirty water is bad business. Just ask any shop owner in Huntington Beach, California.

#### **Chapter 12: Solutions**

#### IT'S TIME TO GET INVOLVED IN LOCAL POLITICS

The solutions to urban run-off are both technical and educational. The technical solutions will have to be done through local government. The educational work can be done by your chapter, often working with other environmental or civic groups.

#### **EDUCATIONAL SOLUTIONS**

One of the best solutions is source control. This means preventing pollutants from getting into the storm drains in the first place. The Surfrider Foundation Storm Drain Stenciling Program is a great public education program. If your city's storm drains are not labeled, go to your city council staff and ask to get a spot on the council meeting agenda. Educate the council about urban run-off pollution. Tell them that your chapter would like to donate the labor to label all the street sewer/catch basins in the area. Ask the city to donate the paint brushes, paint and stencils. Explain to them that the city's public affairs department can publicize the project to educate the citizens not to dump waste into the storm drain system. Contact the Surfrider Foundation HQ for stencils.

Contact local car repair and Tune-Up chains. Ask them to start an oil recycling program. Offer to help publicize it. This will prevent people from dumping their old motor oil into the street sewers. More oil comes down Michigan storm drains per year than the entire Exxon Valdez oil spill. Most of this oil is dumped by uninformed, thoughtless citizens. (An oil recycling program is mandatory in the state of California.)

One important activity is to contact beachside businesses that use "throw-away" Styrofoam containers. Try to get them to use less packaging. Wrapping food in paper will save them money, and it will keep the beach cleaner.

Another important practice that cities need to undertake is frequent vacuum street sweeping. Also, cities need to clean out garbage-clogged street gutter catch basins on a regular basis. This is another thing to take up with your local government.

Ask your city council to set up an area for toxic chemical and household hazardous waste disposal. This way people will be less likely to dump paint thinners and pesticides, etc. into storm sewers. Usually, your city's sanitation department will help with this.

#### PUBLIC HEALTH WARNINGS

Another important educational activity is to educate media weather forecasters and surf reporters. They should inform the public that urban run-off pollution makes it unsafe to go in the

water near a storm drain or a river within 72 hours after a storm. Write a letter to all the local weather-people in your area explaining the problem.

Consider contacting local large gardening services and golf courses to get them to commit to limiting the amount of chemicals they use. Also, get them to commit to avoid overwatering so that water does not run off into the street. If they agree, call a press conference and praise them highly in the media.

#### **TECHNICAL SOLUTIONS**

These are expensive, governmental projects that raise people's taxes. Much educational work needs to be done before politicians will do anything. The best time to influence public works projects is when they are first being built. Therefore, new developments are subject to the most influence. Flood control and drainage control should also include pollution control. There are now NPDES discharge permits for run-off discharges. Some of our chapters have met with developers and city officials and had a very strong influence on the drainage system of some large coastal developments.

#### WATERSHED MANAGEMENT

Many of our natural rivers and wetlands have been covered with concrete. The goal of the Blue Water Task Force is to restore our rivers and wetlands to as close to their natural state as possible. If water soaks into the ground, there is no run-off.

The basic concept of run-off pollution control is to slow the flow and increase the drainage area so that the water may soak into the ground. Instead of smooth concrete channels that shoot water to the coast, channels could have a natural bottom or be rock-lined. There should be waterfalls or turbulent flow areas that oxygenate the water and wetlands populated with plants that purify the water. This will absorb some pollutants and kill some of the bacteria before the water enters the ocean.

Another important concept is the water retention basin. These are small reservoirs or lakes that trap and retain the run-off. The water then soaks into the ground to replenish groundwater aquifers. The retention basin water may also be used for irrigation. An important building reform is minimizing the amount of pavement and using permeable pavement, where possible, such as for parking lots, walkways and driveways.

#### WETLANDS vs. PAVEMENT

Wetlands allow polluted run-off to be cleaned naturally. Our Blue Water Task Force advocates wetland protection and wetland restoration. This is an important weapon in the war against coastal pollution.

#### **COMBINED SEWERS**

In areas that have combined sewers there is less problem with dry weather run-off because the storm drain system (street sewers) is connected to sewage treatment plants. These plants treat

bacteria and remove solids. They are not equipped to remove dissolved toxic chemicals. A combined sewer sends treated polluted run-off far off shore through the ocean outfall pipe. A significant downside of combined sewers is that during a rain, the sewage treatment plant is often so overwhelmed with water that it overflows and raw sewage goes straight into the ocean. Not all outfall pipes are long enough or in deep enough water. Raw sewage carries many disease-producing bacteria and virus. It is very dangerous to human health.

If the sewer system is separate from the storm drain system (as is the case in most west coast cities except San Francisco), street sewer catch basins go straight to the beach with any treatment at all.

#### THE LAST RESORT: "END-OF-THE-PIPE" SOLUTIONS

In some areas it may be appropriate to demand an "end-of-the-pipe" solution. If a particular storm drain outlet is chronically polluted, it may be appropriate to divert the polluted water into a sewage plant (feasible during dry weather only) or to build a treatment facility at the end of the pipe before it hits the beach. This was done in the Los Angeles area as a result of the political work done by a Surfrider Foundation chapter and another environmental group, *Heal the Bay*. These types of solutions are now being implemented at many locations throughout California.

#### NETWORK WITH OTHER ENVIRONMENTAL GROUPS

There are many groups that have done some very good work around this issue. Most notable among these is the Natural Resources Defense Council (NRDC). Their web site is: <a href="https://www.nrdc.org">www.nrdc.org</a> Also contact Clean Ocean Action, P.O. Box 505, Highlands, NJ 07732. Another excellent group is Heal the Bay, 2701 Ocean Park Blvd., Santa Monica, CA 90405. Their web site is: <a href="https://www.healthebay.org">www.healthebay.org</a>

Don't do it alone. In unity there is strength.

#### **Chapter 13: Future Steps**

As we mentioned in the introduction, this manual is just the start of our revamping the Blue Water Task Force. Since the inception of the program in 1991, the science has changed and technology, such as the Internet, has made information sharing easy. Also, our efforts and the efforts of others for the last twelve years have raised the awareness about water quality. Many states and counties now regularly test their local waters. In order to make the Blue Water Task Force more effective, we have developed an Internet site for data entry, storage and publication of all water quality findings by chapters. Contact <a href="mailto:rwilson@surfrider.org">rwilson@surfrider.org</a> or <a href="mailto:mbabski@surfrider.org">mbabski@surfrider.org</a> to get started. Please take a look at our demo site: <a href="mailto:www.surfrider.org">www.surfrider.org</a>/BWTFoutput.htm

We also plan to keep you updated on new testing procedures, and developing tools to improve access to agency water quality results, such as Surfrider's new daily updated program available at <a href="https://www.rashguard.org">www.rashguard.org</a>

We look forward to working with you as we improve the program. Please send us feedback on the program and on this manual, so that changes, updates and corrections can be made. Keep up the good work.

#### Appendix A: Sampling Procedures for Labs with an Autoclave

(This only applies to labs that re-use glassware rather than using disposable plastic.)

#### SURFRIDER FOUNDATION STANDARD OPERATING PROCEDURE

## MICROBIOLOGICAL SAMPLE COLLECTION

**REVISED 8-12-99** 

#### A. General Discussion

The collection of samples for microbiological analysis requires care and adherence to certain procedures to prevent contamination. Contamination of these samples could result in compromising the integrity of the sample, leading to the invalidation of all the analyses.

#### **B. References**

Standard Method for the Examination of Water and Wastewater, 18th Edition, Section 9020 B., p. 9-18 through 9-20.

#### C. Apparatus and Materials

Polypropylene or polymethylpentene wide mouth bottles, 250ml or larger

Autoclave AND Sterility Tape

Presterilized Colilert® Bottles

#### **D.** Reagents

Sodium Thiosulfate solution, 10% - Dissolve 50g sodium thiosulfate, ACS, into 500ml with deionized water.

Tryptic Soy Broth (TSB) - Dissolve 30g dehydrated Tryptic Soy Broth in 1000ml deionized water. Divide into 250ml volumes in glass media bottles and autoclave for 12 minutes at  $121^{\circ}$ C and 15psi. Incubate one bottle for 48 hours at  $35 \pm 0.5$  C and check for bacteriological growth (turbidity). If any turbidity is present, both bottles of reagent must be discarded and a new batch prepared.

#### E. Bottle Preparation

Wash and rinse the sample bottles according to the SOP for washing microbiology glassware and plasticware. If there is reason to believe that there is chlorine within the water to be sampled add 2 drops sodium thiosulfate solution, 10%, to each bottle. Loosely place the caps on the bottles without engaging the threads. Place a piece of steam indicator tape on one of the bottle caps. Autoclave the bottles for 30 minutes at 121 °C and 15psi (dry cycle). Allow the bottles to cool to room temperature and then tighten the caps.

#### F. Quality Control

Sterility - To each set of bottles set aside the bottle with the indicator tape for quality control add approximately 50 ml TSB and incubate for 48 hours at  $35 \pm 0.5$  °C. Check the media for signs of bacterial growth (turbidity). Discard all bottles from an autoclave run if the sterility check fails for that batch.

#### G. Procedure

Sample bottles are sterile and the caps should be on tight to insure the integrity of the bottle. Do not use any bottle with a loose cap. Store the bottles in a clean environment and do not open the bottle until the sample is ready to be collected. Collect samples in such a way as to prevent potential contamination by the sampler. The location should provide a sample that is representative of the water being tested. Collect the bacteriological sample in either a sterile polypropylene or Collect<sup>®</sup> bottle as follows:

- 1. Open the valve and allow the line to flush for a minimum of three (3) minutes. Adjust the flow down to prevent splashing, but not so low that the water creeps up around the outside of the sample point.
- 2. Collect and analyze a sample for free and total chlorine using the HACH® Chlorine test kit. Collect other samples for physical or chemical analysis.
- 3. Hold the sterile bottle by the base and remove the cap. Hold the cap with the inside pointing down. Do not allow the interior surface of the bottle or cap to touch anything except the sample being collected.
- 4. Collect the sample by holding the bottle in the water stream. The bottle contains a declorinating agent, do not rinse, overfill or pour out excess sample. Do not allow the water to contact hands or external plumbing surfaces. Fill the Colliert® bottles to the 100ml line and fill the polypropylene bottles leaving 1/2" of headspace for mixing. Replace the cap immediately.

- 5. Place the bottle in an ice chest containing blue ice immediately after collection. Record all associated field result data on a LIMS Login sheet.
- 6. Do not bring any samples into the laboratory that may be contaminated.

Following the above procedures should insure that the laboratory data collected from these samples accurately reflects conditions in the field. If there is any question about the integrity of a bottle or sample it should be discarded and recollected.

Prepared by:	, Date:
•	
Reviewed by:	, Date:

#### **Appendix B: Sample Site Sheet**

Site Name: Lab Tech Name:

Date: Sample Date: Score Date:

Tide: Sample Time: Score Time:

_	Sample	Temp. (F) Time Depth (	Observations	Bacterial Level: MPN Number /				
	Location	Water	Air	Depth		(Number of Tubes or wells)		
					Surf:	Total	E. Coli (Fecal)	Entero- coccus
					Weather:		(	
					Recent Rain: (Y) or (N)			
					Surf:	Total	E. Coli (Fecal)	Enteroco ccus
					Weather:			
					Recent Rain: (Y) or (N)			
					Surf:	Total	E. Coli (Fecal)	Enteroco
					Weather:			
					Recent Rain: (Y) or (N)			
					Surf:	Total	E. Coli (Fecal)	Enteroco
					Weather:			
					Recent Rain: (Y) or (N)			

Surf: wave height (approx.)

Weather: Sunny, partly cloudy, overcast, fog, light rain, heavy rain, snow

Recent Rain: Check yes if it has rained in last 3 days

Total: MPN number or # of tubes or wells that are yellow

E. Coli or enterococcus: MPN number or # of tubes or wells that fluoresce

#### **APPENDIX C: MPN Numbers**

This Most Probable Number (MPN) chart is for quantitative results of the 5-tube marine water sample test. To use the chart take the number of positive tubes, follow it across to the MPN index number and multiply it by a factor equal to the dilution. For example, to calculate the MPN for a 1:100 dilution, count the number of positive tubes, find the MPN number and then multiply by 100.

MPN Index and 95% confidence limits for various combinations of positive and negative results when five 10ml portions are used.

See Appendix E for calculating the E. Coli count and/or Fecal Coliform count.

#### FIVE TEST TUBE METHOD

# of tubes giving positive reactions out of 5	MPN Index/ 100ml	95% Confidence Limits		
0	<2.2	0	0.06	
1	2.2	0.1	12.6	
2	5.1	0.5	19.2	
3	9.2	1.6	29.4	
4	16.0	3.3	52.9	
5	>16.0	8.0	infinite	

#### FIFTEEN TEST-TUBE METHOD MPN TABLE

TABLE 9221.IV. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 ML, 1.0 ML, 0.1 ML)

Combination MPN Index/ of Positives 100 mL	MPN Index/	95% Confid	dence Limits	(9)	(1940)	Combination	MPN Index/	95% Confid	lence Limits
		Lower	Upper		i Seta m	of Positives	100 mL	Lower	Upper
						4-2-0	22	9.0	56
0-0-0	<2	-	-			4-2-1	26	12	65
0-0-1	2	1.0	10		1	4-3-0	27	12	67
0-1-0	2	1.0	10			4-3-1	33	15	77
0-2-0	4	1.0	13		-11.	4-4-0	34	16	80
					1700	5-0-0	23	9.0	86
1-0-0	2	1.0	11		Hilling	5-0-1	30	10	110
1-0-1	and an in-	1.0	15		195 (31)	5-0-2	40	20	140
1-1-0	4	1.0	15		1000	5-1-0	30	10	120
1-1-1	6	2.0	18		100	5-1-1	50	20	150
1-2-0	6	2.0	18			5-1-2	60	30	180
2-0-0	4	1.0	17		100	5-2-0	50	20	170
2-0-1	7	2.0	20			5-2-1	70	30	210
2-1-0	7	2.0	21		2000.3	5-2-2	90	40	250
2-1-1	9	3.0	24			5-3-0	80	30	250
2-2-0	9	3.0	25			5-3-1	110	40	300
2-3-0	12	5.0	29			5-3-2	140	60	360
3-0-0	8	3.0	24			5-3-3	170	80	410
3-0-1	11	4.0	80.0		100	5-4-0	130	50	390
3-1-0	- 11	4.0	29		1.134	5-4-1	170	70	480
3-1-1	14	6.0	35		15	5-4-2	220	100	580
3-2-0	14	6.0	35		17.9	5-4-3	280	120	690
3-2-1	17	7.0	40		200	544	350	160	820
					100	5-5-0	240	100	940
4-0-0	13	5.0	38			5-5-1	300	100	1300
4-0-1	17.4	7.0	45		5.7	5-5-2	500	200	2000
4-1-0	17	7.0	46			5-5-3	900	300	2900
4-1-1	21	9.0	55		- 400	5-5-4	1600	600	5300
4-1-2	26	12	63		100	5-5-5	≥1600	11102	

For the Quantitray Method MPN Table, please see the accompanying web document:

http://www.surfrider.org/bwtf/quantitray-MPN-table.pdf

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#### **Appendix D: State Standards for Water Quality**

Unfortunately, because there are not yet any national standards for recreational waters in the United States, many states have different water quality standards. This will change with implementation of the BEACH Act. In order to compare your Blue Water Task Force results to your state standards, look them up on the Natural Resources Defense Council (NRDC) Testing the Waters web site: http://www.nrdc.org/water/oceans/default.asp

Thanks to Assembly Bill 411 that passed in 1998, California has some of the strictest standards in the nation. Here are the California standards.

#### Standards and Testing for California:

Required Indicator Organisms:

Total coliform, fecal coliform, enterococcus, and the ratio of total coliform to fecal coliform.

#### Standards:

- (1) Most probable number (MPN) of total coliform organisms less than 1,000/100 ml, provided that not more than 20 percent of the samples at any station, in any 30-day period, shall exceed 1,000/100 ml, and provided further that no single sample, when verified by a repeat sample taken within 48 hours, shall exceed 10,000/100 ml.
- (2) Standard of the geometric mean of 200 fecal coliform/100 ml in five samples in a 30-day period or 400/100ml in a single sample used by counties that choose to test for fecal coliform.
- (3) Ratio of fecal coliform to total coliform may not be greater than 0.1 when total coliform is 1,000 or greater.
- (4) Logarithmic mean of 35 enterococci per 100 ml in five samples taken over 30 days or 104/100ml for a single sample.

Testing Methods:

Most Probable Number, Membrane Filtration for enterococci.

#### Appendix E: E. Coli vs. Fecal Coliform, Calculating the MPN

Using the Colilert-18 testing method we are testing for *E. coli* (this is the indicator that makes the tube fluoresce). Most state standards for water quality do not have a standard for *E. coli*, instead they have a standard for **Fecal Coliform**. Fortunately, *E. coli* can be used as a substitute for Fecal Coliform (with some caveats of course!).

For Surfrider's purposes we recommend the Precautionary conversion (see below) formula:

(E. coli MPN number) x (1.25) = Fecal Coliform MPN number

#### **Explanation:**

*E. coli* is a member of the Fecal Coliform family. A number of other bacteria in combination comprise the Fecal Coliform group. In other words *E. coli* is not a direct substitute for Fecal Coliform. *E. coli* can compromise anywhere from 5% to 90% of the Fecal Coliform in the water and usually ranges from 80 - 90% of the Fecal Coliform. This means that when we get an *E. coli* MPN number, that number is usually 80 –90% of the Fecal Coliform MPN number. Knowing this we can perform a conversion to approximate a Fecal Coliform number to compare to a state standard. Not everyone agrees on the best way to do the conversion (there is no definitive study or paper). Here are our suggestions:

#### **Precautionary Technique:**

We call this the precautionary technique because it will result in an MPN number on the high side. This technique assumes that the *E. coli* makes up 80% of the Fecal Coliform and therefore the conversion factor is 1.25. By multiplying the *E. coli* MPN number by 1.25 you will get the Fecal Coliform MPN.

This is the method that is used by Heal the Bay for their Beach Report Card.

#### **Conservative Technique:**

The conservative technique is assumes that *E. coli* make up 90% of the Fecal Coliform and therefore the number will tend to estimate a lower Fecal Coliform MPN. The conversion for 90% is 1.1. By multiplying the *E. coli* MPN number by 1.1 you will get the Fecal Coliform MPN.

#### **Most Precise Technique:**

The most precise technique will give you the best number, however it requires communication and coordination with a lab that is testing for Fecal Coliform directly.

The best way is compare your results with someone else who is testing directly for Fecal Coliform using the same sample. By comparing your *E. coli* counts with someone else's Fecal Coliform counts over time a conversion number can be calculated. This calculation should be repeated every few months to ensure accurate results.

#### **Information Sources:**

The information for this addendum was accumulated through conversations with IDEXX customer service representatives, Orange County Sanitation District Microbiologists, Surfrider Environmental Issues Team members experienced in water quality testing (Thanks Chris Kinner and Don Schulz), a microbiologist for the Californa Department of Health Services, Orange County Health Care Agency staff and <u>Standard Methods for the Examination of Water and Wastewater 20<sup>th</sup> Edition.</u>

#### **APPENDIX F: More on Indicator Bacteria**

The Surfrider Foundation's Blue Water Task Force (BWTF) – volunteer water quality monitoring program tests for up to three indicators of bacteria in coastal waters: Total Coliform, Fecal Coliform (which can be converted to *E.Coli* count – see below), and Enterococcus. The Surfrider Foundation has decided to use these three indicators until there is a clear consensus of scientific opinion that there is no additional health risk to the public as a result of limiting monitoring to one indicator. A review of the many research reports cited in the U.S. Environmental Protection Agency DRAFT Implementation Guidance for Ambient Water Quality Criteria for Bacteria-1986 (Document EPA-823-D-00-001), clearly indicates that the choice of an appropriate indicator organism may very well be site dependant. Thus, results of an epidemiological study performed over 15 years ago on the East Coast may not be applicable everywhere.

The Surfrider Foundation strongly encourages the EPA to conduct and support any additional epidemiological studies necessary in order to establish that both the indicator organisms and numerical limits decided upon, are sufficient to insure that the public health and safety are protected in all regions of U.S. recreational waters.

The BWTF is implemented nationally by Surfrider Foundation chapters. In order to provide a program that encompasses the large variance in water quality standards nationwide, we recommend that our chapters investigate all three indicators.

Below are descriptions of the indicators:

#### Coliform Bacteria

The quality of marine and other recreation waters is usually determined through testing for the presence of indicator bacteria. The indicator bacteria that are most commonly examined are called "Coliforms". Coliform bacteria originate from soils, plants and human and animal wastes. Although not all coliforms are harmful to humans the presence of high numbers of coliforms in a water body is a good indicator that the water is polluted with harmful microorganisms and viruses.

Used together, total coliform and fecal coliform bacteria levels are an important tool that help scientists determine whether it is safe to surf and swim at the beach. Surfrider's Blue Water Task Force also performs beach water tests for total coliform and fecal coliform to determine if it safe to surf or swim.

The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. The historical definition of the group has been based on the method used for detection (lactose fermentation) rather than on the tenets of systematic bacteriology. Accordingly, when the fermentation technique is used, the group is defined as all facultative, anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose gas and acid formation within 18 hours at 35 degrees C.

In more common terms, Total Coliform is a huge family of bacteria that includes many different families. Fecal Coliform is a subset of the Total Coliform. Total Coliform occurs naturally in both marine and terrestrial systems in soils, microbial mats, etc. Total Coliform may not a good indicator for water quality. A high Total Coliform count could result from agriculture, microbial mats in stagnant water, etc.

#### **Fecal Coliform Bacteria**

Fecal Coliform are a specific kind of coliform bacteria that are found primarily in the intestinal tracts of mammals and birds. These bacteria are released into the environment through human and animal feces. One species of fecal coliform bacteria is the infamous *E.coli* bacteria, which has been linked to numerous food born illness outbreaks in the United States.

Used together, total coliform and fecal coliform bacteria levels are an important tool that help scientists determine whether it is safe to surf and swim at the beach.

Fecal coliform are enteric bacteria that normally inhabit the intestinal tract of humans and animals. The presence of enteric bacteria is an indication of fecal pollution, which may come from stormwater runoff, pets and wildlife, and human sewage. If they are present in high concentrations in recreational waters and are ingested while swimming or enter the skin through a cut or sore, they may cause human disease, infections or rashes.

#### E. Coli

E. coli is a subset of the Fecal group called *Escheriachia coli* (*E. coli*). *E. coli* is a member of the indigenous fecal flora of warm-blooded animals. The occurrence of *E. coli* is considered a specific indicator of fecal contamination and the possible presence of enteric pathogens. In other words, finding *E. coli* indicates that human or animal waste is in the water. The source could be a human, a dog, a bird, or another animal.

*E. coli* is a member of the Fecal Coliform family. A number of other bacteria in combination comprise the Fecal Coliform group. In other words *E. coli* is not a direct substitute for Fecal Coliform. *E. coli* can compromise anywhere from 5% to 90% of the Fecal Coliform in the water and usually ranges from 80 - 90% of the Fecal Coliform. This means that when we get an *E. coli* MPN number, that number is usually 80 –90% of the Fecal Coliform MPN number. Knowing this we can perform a conversion to approximate a Fecal Coliform number to compare to a state standard. Not everyone agrees on the best way to do the conversion (there is no definitive study or paper). Here are our suggestions:

#### **Precautionary Technique:**

We call this the precautionary technique because it will result in an MPN number on the high side. This technique assumes that the *E. coli* makes up 80% of the Fecal Coliform and therefore the conversion factor is 1.25. By multiplying the *E. coli* MPN number by 1.25 you will get the Fecal Coliform MPN

(E. coli MPN number) x (1.25) = Fecal Coliform MPN number

#### **Enterococcus**:

The enterococcus group is a subgroup of the fecal streptococci. The enterococci portion of the streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters. Studies in marine and fresh water bathing beaches indicated that swimming associated gastroenteritis is related directly to the quality of the bathing water and that enterococci are the most efficient bacterial indicator of water quality, especially for salt water.

**Helpful Links:** <a href="http://www2.nrdc.org/water/oceans/ttw/sumcal.pdf">http://www2.nrdc.org/water/oceans/ttw/chap1.asp</a><a href="http://www.nrdc.org/water/oceans/ttw/titinx.asp">http://www.nrdc.org/water/oceans/ttw/titinx.asp</a><a href="http://www.nrdc.org/water/oceans/ttw/titinx.asp">http://www.nrdc.org/water/oceans/ttw/titinx.asp</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.nrdc.org/water/oceans/ttw/titinx.asp</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.nrdc.org/water/oceans/ttw/titinx.asp</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a><a href="http://www.surfrider.org/BWTFoutput.htm">http://www.surfrider.org/BWTFoutput.htm</a></a>

#### **APPENDIX G:**

## HOW TO USE COLILERT-18 / AND OR ENTEROLERT REAGENT WITH TRAYS

Quantification

Step 1.

Add reagent to sample.

**Step 2.** Pour into Quanti-Tray®



**Step 3.**Seal in <u>Quanti-Tray<sup>®</sup> Sealer</u> and place in 35°C incubator for 18 hours.



**Step 4.** Read results:

- Yellow wells = total coliforms
- Fluorescent wells = E. coli
- If using Enterolert Flourescent wells = Enterococcus

