

# Surfrider Foundation Blue Water Task Force



## Laboratory Instructions for IDEXX Quanti-tray/ Enterolert method, 1:10 dilution

### General overview

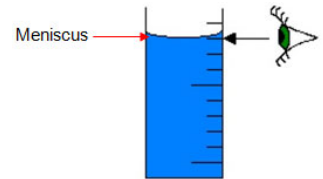
This method very basically involves diluting your water sample to avoid interference from non-target organisms, adding the Enterolert reagent, sealing the sample mixture in a Quanti-tray and placing in an incubator at 41°C for 24 hours. After incubation, positive reactions between the reagent and any Enterococcus bacteria in your sample are determined by shining a UV light on your sample trays and noting any fluorescent wells. Test results are expressed as Most Probable Number (MPN) of Enterococcus cfu (colony forming units) per 100 ml by using the [IDEXX Quanti-Tray/2000 MPN Table](#).

Special care should be made to operate this procedure in a sterile environment using aseptic technique (no contamination). **A blank control must be run with every set of samples processed to ensure that your dilution water and plastic ware, or glassware, have not been contaminated.**

### Preparing the Enterolert reagent/sample mixture

1. Turn on the incubator to warm it up.
2. Turn on the Quanti-Tray sealer to warm it up. It takes 10 minutes.
3. Make sure the temperature of your incubator is around 41°C.
4. Wipe down your workspace with disinfectant spray.
5. Put on gloves or wash your hands with anti-bacterial soap.
6. Label a water sample mixing bottle and a quanti-tray to match each sample location marked on the whirl-pak sample bags. Write only on the back of the trays with a sharpie permanent marker to keep from puncturing the tray. Add date to trays.
7. Label a set for the control.
8. Remove the first sample bag from the cooler and place in a cup, glass or other container for support. Work with one sample at a time.
9. Remove seal around the mixing bottle lid to open. To make sure this container remains sterile, never touch the top of the bottle or inside the lid with your fingers after the lid has been removed. Only the outside of the lid and the sides and bottom of the bottle can be touched.
10. Gently invert the sample bag 2-3 times to suspend anything that may have settled to the bottom of the sample.
11. Open the whirl-pak sample bag by bending the yellow tabs out and unroll the top. Then pull on the white tabs only to open the top of the bag. Do not touch the bag at the top or inside the top.

12. Get a 10 ml glass pipet out and insert it into the pipet-aid until it feels snug (hold from near the top of the tube to avoid breaking glass during insertion)
13. Do not touch the lower 1/2 of the pipet with your fingers or let it touch anything else in the lab.
14. Insert the pipet into the water sample and use the top button on the pipetaid to draw water and begin filling the pipet with sample water.
15. Extract exactly 10ml of the water sample into the pipet with the bottom of the meniscus at 10ml mark (see diagram).
16. Place the pipet inside of the top of the opened bottle and discharge the sample water into the sterile mixing bottle by pressing the bottom button on the pipetaid.
17. Re-seal the sample bag and store in case the sample is spilled or contaminated and you need to remix a new solution.
18. Place soiled pipet in the metal pipet box and repeat above steps until all sample sites have been completed.
19. Next, fill each jar to the 100 ml mark with distilled water. Kneel down to table level when pouring to ensure accuracy. Do this CAREFULLY & SLOWLY! Remember you can add more water, but you can't take water away!
  - a. If you purchase your distilled water in a 1 gallon jug, you may find it difficult to pour and stay on the 100 ml target. Alternatively, you can fill your sample bottles up to the 100 ml line with distilled water first. If you pour too much water in, you can pour some out if you only have distilled water in your bottle. Then use a pipet to remove 10 ml of distilled water from the filled bottle once it is on mark, and with the same pipet add 10 ml of your sample to the 90 ml of distilled water that is now in the bottle. Your final mixture will include 10 ml sample + 90 ml of distilled water for a total solution of 100 ml. Throw away your pipet and replace with a new one before working with your next sample.
20. Fill the control bottle with 100 ml of distilled water.
21. Add one packet of Enterolert to each sample bottle, including the control. Hold the snap packs facing away from your face and pop open the top.
22. Put the bottle lids back on and gently shake or swirl the contents until all the powder dissolves and the bubbles disappear.



### Filling the Quanti-trays with the reagent/sample mixture

1. Use one hand to hold a Quanti-Tray upright with the plastic well side facing your palm.
2. Squeeze the upper part of the Quanti-Tray so that the tray bends toward the palm making a U-shape.
3. Gently pull foil tab to separate the foil from the tray. Don't touch the inside of the foil or tray as this could contaminate it.
4. Pour the reagent/sample mixture into its corresponding Quanti-tray while avoiding contact with the foil tab.
5. Tap the small wells to release any air bubbles. Allow foam to settle.

6. Place the sample-filled tray into the rubber insert of the Quanti-Tray Sealer with the plastic well side facing down.
7. Place the rubber sealer form with the tray on top into the inlet hopper of the sealer machine with the large reservoir cell placed the farthest away from the machine and the small cells entering the machine first.
8. Push the form into the machine gently. It will automatically grab the rubber form and tray and draw them thru the sealer.
9. Catch the sealed tray on the other side of the machine.
10. Check to see that all plastic wells on the tray are filled with the sample. A well is considered full if it is at least ½ full. Up to 2 wells can even be completely empty and the tray can still be used as the results will still be statistically valid.
11. If there is a problem sealing the tray or there are more than 2 empty cells you will have to discard tray and prepare a new sample/reagent mixture from the reserved sample bag and pour into a new tray. *\*if this is a consistent problem see tips in Troubleshooting.*
12. Set aside your sealed Quanti-Trays and place all together into the incubator at 41°C. It is best to minimize the number of times you open the incubator door so as not to disturb the temperature setting.
13. The trays can be stacked 4 high, but should be spread out as much as possible to ensure airflow for each tray.
14. Note on your data sheet the time you placed the trays into the incubator. They will stay there for 24 hours.
15. Pour remaining water samples into toilet and flush.
16. Dispose of gloves, wrappers, and used whirlpaks into a trash can.
17. Wipe down all lab surfaces with disinfectant spray (1:10 mixture of bleach and water) and paper towels.
18. Wash hands with anti-bacterial soap.

### Reading the results (24 hours later)

1. Take the Quanti-Trays out of the incubator 24 hours after they were put in. Check the data sheet for the exact time and record the time of reading.
2. Turn UV light on and darken the room.
3. Shine UV light on each tray, looking for wells fluorescing blue. Wells that show a bright yellow color but do not fluoresce do not count as a positive result.
4. Note the number of large and small fluorescing wells on the data sheet.
5. **If any wells in the control fluoresce, then there was a contamination of your lab procedure somewhere, and the entire data set must be thrown out!**
6. Determine the MPN for each sample by plugging the number of positive wells into the [MPN table](#).
7. Multiply the MPN by 10 to account for the dilution to determine the number of Enterococcus colony forming units (cfu) in 100 ml of water.
8. Record the results on the data sheets and check that the control was zero.
9. Dispose of used trays.

10. Wash hands.
11. Now is the time to enter data into the computer!

### Post your results

1. Enter your data here: <http://bwtf.surfrider.org/>
  - a. [View data entry tutorial video](#)
  - b. Login: [volunteer@bwtf31.surfrider.org](mailto:volunteer@bwtf31.surfrider.org)
    - i. 3A==8yPZ
2. View your data: <https://bwtf.surfrider.org/report/31>

### References

1. Standard Test Methods for the Examination of Water and Wastewater, 19<sup>th</sup> & 20<sup>th</sup> Editions, APHA, AWWA, WEF <http://www.standardmethods.org/>
2. Enterolert Procedure, IDEXX <http://www.idexx.com/resource-library/water/enterolert-procedure-en.pdf>
3. Quanti-Tray 2000 Procedure, IDEXX <http://www.idexx.com/resource-library/water/quantitray-2000-procedure-en.pdf>
4. IDEXX Video: A step-by-step guide to using Enterolert. How To/Q&A tab at [http://www.idexx.com/view/xhtml/en\\_us/water/products/enterolert.jsf](http://www.idexx.com/view/xhtml/en_us/water/products/enterolert.jsf)

### Tips & troubleshooting

**Water Source.** Distilled water, readily available at grocery, drug & discount stores in gallon jugs, is the most commonly used water by BWTF labs for diluting seawater samples. Chapters partnering with local academic or government labs may be able to access other types of sterile water.

**Maintaining Incubator Temperature at 41°C.** Incubators should be set up on interior walls away from drafty windows and heat or AC vents for optimum performance. The incubator will lose heat every time the doors are opened, so it is best to place all sealed trays into the oven at the same time rather than one by one. It will take a few runs for you to find the optimum spot on the heat dial to maintain 41°C.

**Empty Wells in Sealed Quanti-Trays.** Check that your table and machine are level if your trays are coming out of the sealer *consistently* with empty wells in the same location, i.e. upper right hand or upper left hand corners. If the problem persists, you can gently run a ruler, or other straight edge, across the filled Quanti-tray, from the bottom to the top, to begin spreading the liquid into the cells before inserting it into the Sealer. Be careful to not let any of the sample come out of the top of the tray.

**Gloves vs. Hand Washing & Sanitizer.** It is very important to not contaminate your sample mixture with any bacteria that might be present on your hands or elsewhere in the lab. Thorough hand washing with anti-bacterial soap or wearing disposable gloves are both good options for avoiding contamination. You will need to decide which works best for your volunteers in your lab. Ultimately the most important caution is to not touch and keep your fingers, gloved or otherwise, out of the openings of your sample bags, bottles, pipets and trays!

**Blank Controls.** Blue Water Task Force labs must run a negative blank control with every batch of water samples processed together. If this proves too costly, \*depending on the intended use and audience for your data\*, after a new water testing program has established their lab procedure and all volunteers have acquired experience processing samples in the lab, it could be appropriate to drop the frequency of the control to monthly or bi-monthly.