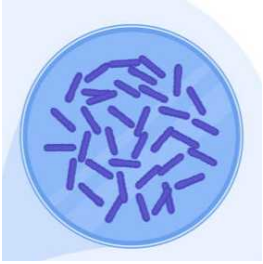
Mar 20, 2025

Detection of Shigatoxigenic *E. coli* O157:H7 & Enumeration of Fecal Indicators SOP



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We use this protocol and it's working

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Disclaimer

Abstract

To describe the procedures on how to set-up culture-based assays for the detection of Shigatoxigenic *Escherichia coli* O157:H7 (STEC O157) from a variety of animal and environmental sources; and the procedures for enumeration of generic *E. coli,* total coliforms, and enterococcus from those same sources.

Guidelines

Scope

This protocol describes the procedures for two commonly used culture-based laboratory assays. All culture work must be done on fresh samples that are kept cool and processed within 24-hours of collection (or 48-hours if sample is collected remotely and shipped).

The first procedure applies to immunomagnetic separation (IMS) of different serotypes of STEC. IMS is a technique that will isolate cells from an enriched sample containing background substance matter. IMS is used widely for many organisms; this SOP is specifically for STEC isolation. Dynabeads are used to separate the target cells from the rest of the sample. Target-specific antibodies attach to the surface of the magnetic Dynabead sphere, allowing the STEC cells to remain while the rest of the sample is discarded. The isolated cells are then plated onto selective media for incubation to determine the amount of *E. coli* cells in each sample. These grown colonies can then be saved for freezer isolation. *Escherichia coli* (*E. coli*) are a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhea, urinary tract infections, respiratory illness and pneumonia. Some *E. coli* cause disease by making a toxin called Shiga toxin. The bacteria that make these toxins are called “Shiga toxin-producing” *E. coli*, or STEC for short. Still other kinds of *E. coli* are used as markers for water contamination—so you might hear about *E. coli* being found in drinking water, which are not themselves harmful, but indicate the water is contaminated.

The second procedure applies to the IDEXX Quanti-Tray/2000 test. The IDEXX Quanti-Tray Colilert and Enterolert kits count and detect *E. coli*, total coliforms, and Enterococcus in a 100 mL sample. The Colilert test shows positive total coliforms as a yellow color, and positive *E. coli* as yellow and fluorescent. The Colilert test uses a proprietary Defined Substrate Technology to detect and quantify both total coliforms and *E. coli*. This technology uses two nutrient indicators – ONPG and MUG – as the major sources of carbon in the Colilert packets. These nutrients are metabolized by the enzyme β-galactosidase (coliforms) and β-glucuronidase (*E. coli*). Coliforms grown in Colilert metabolize ONPG using β-galactosidase, which changes it from colorless to yellow. The same is true for MUG. β-glucuronidase metabolizes MUG and creates a fluorescence. The MPN of *E. coli* can be found from the table based on the number of large and small wells that are yellow and fluoresce. The MPN for total coliforms can be found based on the number of small and large wells that are yellow with no fluorescence.

Enterolert testing involves the same procedure and a different proprietary Defined Substrate Technology. β-D-glucoside present in Enterococci binds with 4-methyl-umbelliferone in Enterolert to show fluorescence in a positive sample. As with Colilert, the MPN can be found using the table and the number of large and small fluorescent wells. (IDEXX Water Academy, 2020).

Responsibility

This SOP applies to all staff members and students. These individuals must be knowledgeable about the requirements set forth within this document. The lab manager or designee shall ensure that all staff and students know the proper techniques.

Materials

STEC O157 Enrichment

- 1.5X BGB media (274000EA, BD, Sparks, MD) (Recipe in **Appendix A Step 19**)
- STEC O157 positive control culture plate
- 50% glycerol tubes or freezer beads (G153-1, Fisher, Waltham, MA)
- Whirl-Pak® Filter Sterilized Bags **Whirl-Pak Catalog #**B01348, Pleasant Prairie, WI)
- Plastic beaker
- 100mL Cylinder
- Bunsen burner
- Scale
- Timer
- 1 gallon or quart zip freezer bag
- Spatulas, Disposable **VWR International (Avantor) Catalog #**80081-188, Radnor, PA)
- 2mL Microcentrifuge tubes (02-681-299, Fisherbrand, Pittsburgh, PA)
- Orbital Shaker (located in Durso lab OR Miller lab, 340 Filley Hall)
- Dynabeads™ anti-E. coli O157 **Thermo Fisher Catalog #**71003, Waltham, MA)
- DynaMag-2 - Magnetic Bead Separator (Dynal, Invitrogen, Carlsbad, CA)
- Disposable Inoculating Loops and Needles, Flexible Needle and Loop **Fisher Scientific Catalog #**22-363-600, Pittsburgh, PA)
- **TCA** media plates (Chromagar O157 + Potassium Tellurite) (EE222, Chromagar, DRG International, Springfield, NJ) (Recipe in **Appendix A Step 18**)
- Potassium Tellurite (3.5%) Solution (100427, MPBio, Santa Ana, CA)
- Syringe Filters - Sterile **Fisher Scientific Catalog #**09-720-005, Pittsburgh, PA)
- Water Bath (for tempering TCA agar)
- Appropriate pipettes and tips, ranging from 20µL to 1000µL
- Bead wash (Recipe in **Appendix A Step 17**)
- DrySpot™ E. coli O157 Latex Agglutination Test **Thermo Fisher Scientific Catalog #**DR0120M, Oxoid, Hampshire, United Kingdom)
- 37^{o}C incubator

Quanti-Tray/2000

- 15 mL PET Centrifuge Tubes **Corning Catalog #**430055., Corning, NY)
- 1X Phosphate Buffered Saline **Fisher Scientific Catalog #**BP3994, Waltham, MA) (Recipe in **Appendix A Step 20**)
- Automatic Pipettor/disposable pipettes
- Sharpie Markers

- Colilert Packs (WP200IPK, IDEXX, Westbrook, ME)
- Enterolert Packs (98-21374-00, IDEXX, Westbrook, ME)
- Whirl-Pak® Filter Sterilized Bags **Whirl-Pak Catalog #**B01348, Pleasant Prairie, WI)
- Plastic Beaker
- 100mL Cylinder
- Bunsen burner
- Micro Pipettor and tips
- Quanti-Tray Sealer (89-10894-04, IDEXX, Westbrook, ME)
- Quanti-Tray/2000 MPN Table (IDEXX, Westbrook, ME)
- Quanti-Tray/2000 Rubber Insert (WQTSRBR-2K, IDEXX, Westbrook, ME)
- Quanti-Tray/2000 Trays (WQT2KPK, IDEXX, Westbrook, ME)
- Quanti-Tray/2000 Colilert Comparator (98-09227-00, IDEXX, Westbrook, ME)
- Sterile polystyrene bottle (1156Z41, Thomas Scientific, Swedesboro, NJ)
- 37^{o}C and 42^{o}C incubator
- UV lamp/viewing cabinet (98-21322-00, IDEXX, Westbrook, ME)
- Sterile water (Milli-Q, distilled, or autoclaved)

STEC O157 Enrichment Procedure

14h 38m

1 Day 1: Sample Enrichment

1.1 If 1.5X BGB enrichment media has been refrigerated, warm to Room temperature.

1.2 Weigh out the sample: Using a plastic beaker to hold the filter whirl-pak bag, tare the scale, and add 10 g of sample with a clean disposable spoon or spatula to each labeled assay bag. Record all weights on a data worksheet.

1.3 Using a sterile 100mL plastic cylinder or sterile pipet, transfer 90 mL of Room temperature 1.5X BGB to each bag and homogenate gently by hand. Flame-sterilize the pipet between each sample.

1.4 Make a positive control. Add 9 mL 1.5x BGB to a whirl-pak bag or 15mL centrifuge tube. Using a loop, take a colony from the STEC O157 positive control plate and swirl around in the BGB to mix.

1.5 Place BGB bags and control in 37 °C incubator for 06:00:00, then chill to 4 °C until ready for next step (can be held overnight). Record the time into the incubator on a data worksheet.

6h

2 Day 2: Manual Immunomagnetic Separation (IMS)

2.1 Label and dry two TCA plates per sample and control. Keep labeled plates in an organized order.

> Note
>
> To dry plates: Turn on a BSL-2 hood. Place the petri dishes inside with their lids askew to allow moisture to evaporate. Let set for approximately 30 minutes and check. There should not be any visible moisture remaining on top of the agar. Agar needs to be at room temperature prior to using.

2.2 Using one 2mL microcentrifuge tube per sample, label the ID# on the top of the tube, and place tubes in DynaMag-2 holder without the magnetic bar.

2.3 Vortex Dynabeads Anti-*E. coli* Beads well to re-suspend beads into solution.

2.4 Add 20 µL of beads to each microcentrifuge tube.

2.5 Gently mix the sample bag and add 1 mL of enrichment sample into each labeled tube.

2.6 Vortex tubes to mix, and place tube back into the holder.

2.7 Still without the magnetic bar, put the holder and tubes in a one-gallon freezer bag and zip close. Place on shaker for 00:30:00 . 30m

2.8 After 30 minutes, remove holder from the shaker and bag. Put the magnetic bar in and wait 00:05:00 (lay holder flat on bench). 5m

2.9 Remove fluid with a pipette without disturbing the beads.

2.10 Remove magnetic bar and add 1 mL of bead wash to tubes.

2.11 Vortex, put magnetic bar back in, and wait 00:03:00 . 3m

2.12 Remove fluid and repeat washing steps **2.9 - 2.11**.

2.13 Remove fluid with pipette without disturbing the beads.

2.14 Remove the magnetic bar and re-suspend beads in 100 µL of bead wash. Vortex.

2.15 Pipette 50 µL of re-suspended beads onto each labeled TCA plate. Spread all over the plate with a sterile spreader.

2.16 Invert plates and incubate Overnight at 37 °C. Record the time into the incubator on a data worksheet.

8h

3 **Day 3: Read and Record Results**

3.1 Check each plate for suspect colonies. For STEC O157, suspect colonies will be mauve. See **Appendix B** go to step #21.1.

3.2 Pick and test up to three suspect colonies per plate.

- DrySpot tests can be used for immediate confirmation. See Appendix B go to step #21.2
- If plates are over-crowded, streak for isolation and do dry-spot after 24-hour incubation.

3.3 Record results on a data sheet.

Quanti-Tray/2000

4 **Pre-prep:** Set out Quanti-Tray trays and bottles for each sample and remove the shrink wrap from each bottle cap. Separate the 'Enterolert' (Ent) and 'Colilert' (TC/EC (Total Coliform/*E. coli*)) so that they don't get switched around.

4.1 Label the bottle lids and trays clearly with sample number and 'Colilert' or 'Enterolert.' Labels can be color-coded to distinguish between Colilert and Enterolert, and either printed or handwritten legibly.

Note

Only use a marker to label the trays. Stickers can get caught in the sealer.

5 Fill each bottle with sterile water with the correct amount for the labeled dilution according to **Table 2**. For this project we used 99 mL .

	A	B	C
	Final Dilution	**Amount from Dilution Tube**	**Quanti-Tray Bottle Amount**
	10^-1	100mL of dilution bag	From bag: 100mL PBS with 10g Sample
	10^-2	1mL of dilution bag	99mL sterile water
	10^-3	1mL 10^-1 dilution	99mL sterile water
	10^-4	1mL 10^-2 dilution	99mL sterile water
	10^-5	1mL 10^-3 dilution	99mL sterile water
	10^-6	1mL 10^-4 dilution	99mL sterile water
	10^-7	1mL 10^-5 dilution	99mL sterile water

Table 2. Quanti-Tray Dilutions – amount from dilution tubes that go into snap-pack bottles.

6 For Enteroloert - Empty an **Enterolert** Snap-Pack into each bottle. Tightly put the cap back on and shake the bottle to dissolve the Snap Pack powder.

7 For Colilert - Empty a **Colilert** Snap-Pack into each bottle. Tightly put the cap back on and shake the bottle to dissolve the Snap Pack powder.

8 Weigh out the sample: Using a plastic container to hold the filter whirl-pak bag, tare the scale, and add 10 g of sample with a clean disposable spoon or spatula to each labeled assay bag. Record all weights on a record worksheet.

9 Using a sterile 100mL plastic cylinder or sterile pipet, transfer 90 mL of Room temperature PBS to each bag and homogenate gently by hand. Flame-sterilize the pipet between each sample. This will create a 10^{-1} dilution.

10 Turn on the Quanti-Tray Sealer. When the sealer is ready, the green light will turn on at the top of the sealer.

11 Dilution tubes will be labeled and pre-filled with 9 mL PBS. Massage the PBS enriched sample bag to mix.

11.1 Pipette 1 mL from the dilution bag (10^{-1}) into the correspondingly labeled 9mL PBS dilution tube, creating a 10^{-2} dilution. Tighten the lid and vortex to mix.

11.2 Continue pipetting 1 mL, then vortex, from each consecutive tube until you reach the desired final dilution (**Table 1**).

	A	B
	Dilution Needed	**Use**
	10°	10g of Sample
	10^-1	90mL of PBS + 10g of Sample
	10^-2	9mL of PBS + 1mL of 10^-1 Bag
	10^-3	9mL of PBS + 1mL of 10^-2 Tube
	10^-4	9mL of PBS + 1mL of 10^-3 Tube
	10^-5	9mL of PBS + 1mL of 10^-4 Tube
	10^-6	9mL of PBS + 1mL of 10^-5 Tube

Table 1. Dilution tube set-up for solid samples.

11.3 Keep dilution tubes cool. Place On ice or in 4 °C until ready for use.

12 Vortex each dilution tube right before use and add the correct amount of the dilution to corresponding sample bottle(s) (see **Table 2**), tightly screw on lid and gently shake or invert to mix well.

	A	B	C
	Final Dilution	**Amount from Dilution Tube**	**Quanti-Tray Bottle Amount**
	10^-1	100mL of dilution bag	From bag: 100mL PBS with 10g Sample
	10^-2	1mL of dilution bag	99mL sterile water
	10^-3	1mL 10^-1 dilution	99mL sterile water
	10^-4	1mL 10^-2 dilution	99mL sterile water
	10^-5	1mL 10^-3 dilution	99mL sterile water
	10^-6	1mL 10^-4 dilution	99mL sterile water
	10^-7	1mL 10^-5 dilution	99mL sterile water

Table 2. Quanti-Tray Dilutions – amount from dilution tubes that go into snap-pack bottles.

13 Pour contents of the inoculated bottle into Quanti-Tray/2000 Trays.

14 Place the tray in Quanti-Tray/2000 Rubber Insert and run through the sealer (well side down). You may have to push the tray until the sealer begins to pull the tray through.

15 Check that all wells have liquid in them, **face the trays up** (wells on top), and place in incubator no more than 10-trays high. Record the time into the incubator on a data worksheet.

- Colilert: 37 °C for 24-28 hours.
- Enterolert: 42 °C for 24-28 hours.

16 After 24 hours, the trays can be removed from the incubator, and the results read. Mark all positive *E. coli* wells with a permanent marker. See **Appendix B** go to step #22 for additional information on reading results.

- Positive TC – Yellow Well
- Positive EC – Yellow and Fluorescing Well
- Positive Entero – Fluorescing Well

Appendix A – Recipes

45m

17 **Bead Wash Solution Recipe (1x PBS with 0.05% Tween 20)**

17.1 **Directions to make 1L:** 15m

- Add 500 µL Tween 20 to 1 L 1X PBS in a media bottle.
- Stir until mixed
- Autoclave 00:15:00 at 121 °C with lid on loosely.
- Cool to Room temperature before use.
- Label and store in the refrigerator.

18 **TCA (Chromagar O157 + Potassium Tellurite) Recipe**

18.1 **Directions to make Potassium Tellurite (3.5%) Solution:**

- Weigh 0.35 g Potassium Tellurite. Clean the scale and turn it off.
- Add weighed Potassium Tellurite to 10 mL distilled water in a sterile beaker.
- Filter-sterilize using a 0.45µM filter

1. Open and attach the filter to the syringe, using the luer lock
2. Remove the syringe plunger and pour the Potassium Tellurite solution into the open end of the syringe.
3. Place the filter over a new, sterile labeled 15mL centrifuge tube and depress the filter until all the solution passes through the filter.

- Refrigerate any extra for up to 30 days.

18.2 **Directions to make TCA Plates:**

- Weigh 14.6 g Chromagar O157 powder. Clean and turn off the scale.
- Add 14.6 g Chromagar O157 powder to 500 mL distilled water in a 1L bottle.
- Heat while stirring and bring just to a boil. **DO NOT AUTOCLAVE!**
- Cool to 45 °C - 50 °C in a water bath.
- Add 18µL / L (or 9µL/500mL) of 3.5% Potassium Tellurite Solution
- Swirl to mix media.

- Pour or pipette in 15 mL increments into sterile petri dishes and let cool until solid.
- Label and store in the refrigerator and use within 30 days.

19 **1.5X Brilliant Green Bile Broth (BGB) Recipe**

19.1 **Directions to make 1L: (Make in 500mL batches)** 15m

- Weigh 60 g BGB powder. Clean and turn off the scale.
- Add BGB powder to 1 L distilled water in a media bottle.
- Heat while stirring until mixed.
- Autoclave for 00:15:00 at 121 °C with lid on loosely.
- Cool to Room temperature before use.
- Label and refrigerate if not being used the same day.

19.2

Note

BGB powder is an irritant to the lungs. Take care to avoid inhalation of the powder while weighing or it will cause coughing. Can wear a mask to minimize impact.

20 **1x Phosphate Buffered Saline (PBS) Recipe**

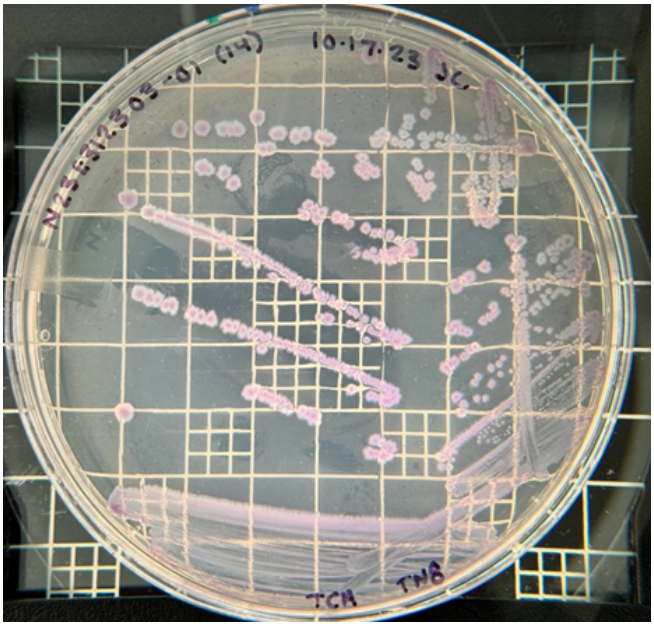
20.1 **Directions** 15m

- Measure 1800 mL of distilled water and pour into a 2L bottle.
- Measure 200 mL of 10X PBS.
- Pour the 200 mL of 10X PBS into the bottle with the 1800 mL of water.
- Autoclave at 121 °C for 00:15:00 .
- Cool to Room temperature before use.
- Label and store in the refrigerator.

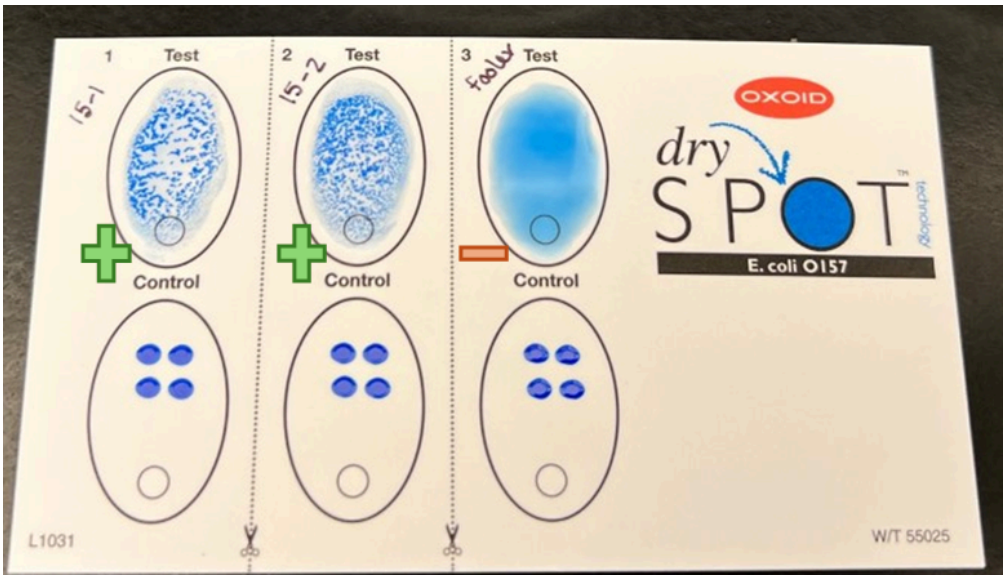
Appendix B – Reading Results

21 **STEC O157**

21.1 Positive and suspect colonies are a mauve color.



21.2 Positive colonies will coagulate on the DrySpot test, while negative colonies will not react and be smooth.



22 **Quanti-Tray/2000**

22.1

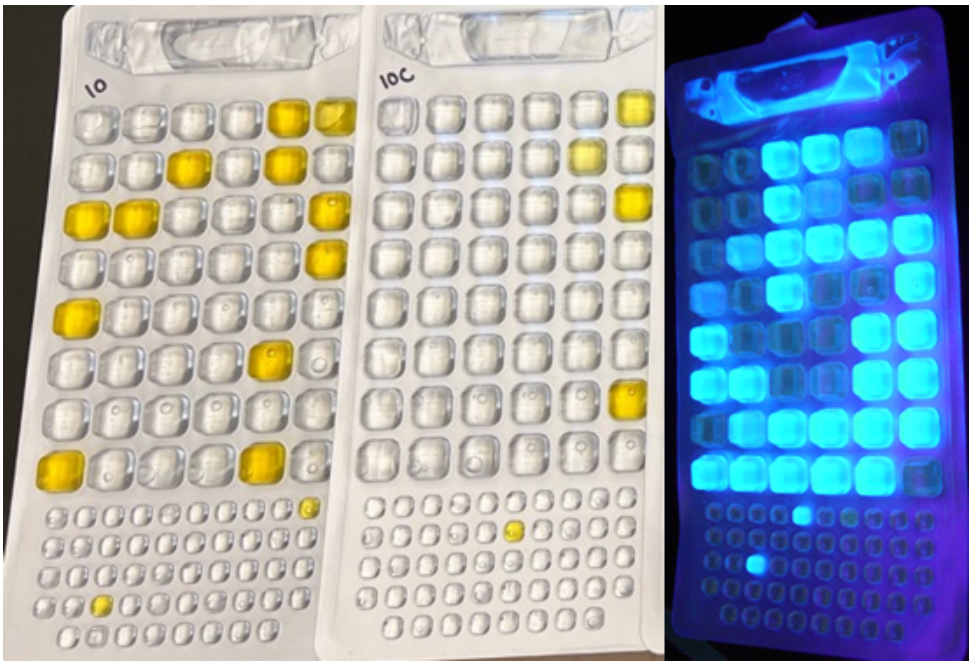
Note

Be sure to cover any exposed skin before working with the UV lamp, and wear UV-blocking glasses

22.2 **Colilert:** Count and record both yellow and fluorescing wells.

- Yellow wells indicate the presence of total coliforms. The wells must be as yellow as the Colilert Comparator (**See: Materials Quanti-Tray**)

COLORLESS	YELLOW	FLUORESCENT*
Negative for total coliforms and *E. coli*.	Positive for total coliforms.	Positive for *E coli*.
		*Use 365nm UV lamp



- Wells that are yellow **AND** fluoresce indicate the presence of *E. coli*.
- Do not count the wells that fluoresce but are not yellow.

22.3 **Enterolert:** Count and record the fluorescing wells.

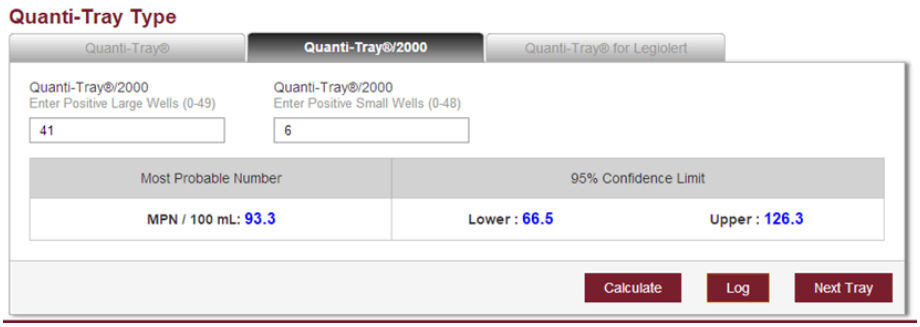
- Fluorescing wells indicate the presence of Enterococcus.
- Do not count the wells that fluoresce bright yellow.

22.4 Using the **Quanti-Tray/2000 MPN Table** software program (IDEXX MPN Generator) or the hardcopy that comes with the trays. Record the number of large and small wells to reach the **M**ost **P**robable **N**umber.

- With any dilution other than 10^0, you must account for the dilution in the final answer.
- Example: 41 Large Positive, 6 Small Positive tested at a 10^{-2} dilution

  $9.33X10^1 \rightarrow 9.33X10^3$

22.5 Example of MPN Generator



Protocol references

IDEXX Water Academy. 2020. "Testing for Coliform and E. coli" and "Testing for Enterococci." www.idexxwateracademy.com