Fecal Coliform (Bacti): IDEXX Quanti-Tray Method

Test Station: Benchtop on far right of lab
Notebook: "Fecal Coliforms"
Estimated Time: 15 minutes
Overview: The product utilizes a nutrient indicator (ONPG) that produces a yellow color when metabolized by fecal coliforms at 44.5 ± 0.2 °C. When the reagent is added to the sample and incubated, it can detect fecal coliforms at 1 CFU/ml at 18 and up to 22 hours.

Materials needed	**Location**
Hype-Wipe with bleach	Dispenser on bench above station
1 Quanti-Tray (200, or 2000)	Cabinet below workspace
1 packet of Colilert-18 media	Cabinet below workspace
Equipment needed	**Location**
IDEXX Quanti-Tray Sealer	Benchtop at station
Quanti-Tray rubber insert	Atop Quanti-Tray sealer
1000 mL flask with arm	At station
Bunsen burner	At station
Lighter for Bunsen burner	Top corner drawer under dissecting scope
Gas	Blue knob on left side of work station
IDEXX MPN tables (for Q-tray 200 & 2000)	In file rack on table
Samples needed	**Location**
100 mL Dechlorinated effluent in a 120mL vessel with Sodium Thiosulfate	Brought in by operators in the morning (clean fridge)

Preparation

1. Test Bacti sample within 6 hours of collection.
2. In the bacti notebook, write down the sample information. Use the previous entry as a reference for formatting.
3. Determine if you will be using the 51-well tray (normal Q-tray) which can detect up to 200 coliforms, or the Q-tray 2000 (with big and small wells) which can detect up to 2,419 coliforms. If you expect to see a lot of coliforms, are testing a new/different water source, or completing a PT or QC test, then plan to use the Q-tray 2000.
    a. Clues to the coliform concentration in dechlor can be: the residual chlorine level, recent trends, and current plant processes.
    b. Dilutions may also be made, as long as the final solution in the Q-tray is 100mL
4. Sterilize and clean work counter, flasks, and Bunsen burner with a Hype-Wipe.
5. Turn on Bunsen burner
    - Hold the lit lighter over the burner → turn the blue gas nozzle on.
    - Keep work in a close radius to the flame to keep the area sterile.
6. Retrieve a Q-tray (TRAYS ARE ALL OPEN; be careful to Hype-wipe the outside of the bag of trays and stay close to flame to keep sterile). Do NOT use the Hype-Wipe on the open edge of the Q-Tray, you could inadvertently allow particles inside the tray and contaminate sample.

7. Also retrieve one snap-packet of Colilert-18 dry media and your dechlor eff sample.
8. Use the Hype-Wipe to sterilize the sample bottle.
9. Turn on the Quanti-Tray Sealer via the rocker switch on the back of the machine. The red bar indicates its level of preheating. Once the green light turns on, the machine is up to temperature and is ready for use.
10. Place the appropriate-sized rubber insert (blue for Q-tray 200, black for Q-tray 2000) right-side up on the conveyer table to the right side of the sealer.

Filling the Tray

1. Hold the sample bottle close to the flame. In a clean beaker, pour out the excess sample. YOU NEED 100mL. More than 100 will overfill the Q-tray and empty into the rubber insert and/or into the sealer machine. Too little sample won't fill the tray accurately.
    a. Try to keep the sample covered with the cap and under the burner as much as possible to prevent contamination.
    b. Keep in mind that some samples containing humic material may have an innate color. If your sample is not clear but contains a tint of color, then a control blank of the same water sample may be required for comparison to the inoculated sample.
2. Carefully separate one snap pack from the strip taking care not to accidentally open the adjacent pack. Tap the pack so the powder is in the bottom. Hold the pack face down (paper-side-up) above the open sample and bend it along the score-line to open, such that the powder falls into the open sample.
3. Mix sample by inversion. Do not vigorously shake. It will take about 5 minutes for the powder to completely dissolve. If foamy, you may let the sample sit under the burner for a few min and allow foam to dissipate. (If especially foamy, look into using the anti-foam reagent). Once mixed, you may unscrew the cap, but leave it covered loosely.
4. Now open the Q-tray:
    a. DO NOT try to open the tray by pulling the tab!!! It will tear.
    b. Take the tray in one hand (holding near flame) with the paper side out, gently squeeze the Q-tray inward with your hand. With your other hand, make a fist and press it into the paper side of the tray. Allow the tray to bend at the indents in the large well.
    c. While bent inward, you may carefully pull the tab outward. The tray opening will widen.
    d. DO NOT TOUCH the inside of the tray.
5. While holding the open Q-tray upright near the burner, uncap and gently pour the sample/colilert-18 solution into the open tray. Relax your grip on the tray to allow the opening to close a bit.
    a. Note: bubbles in the wells are fine, as long as all the wells end up filled.
6. Carefully place the filled tray into the appropriate rubber insert on the right side of the sealer. (it will not spill out as long as the open tray is laid at an angle)
7. Ensuring that the sealer is up to temp, you may push the rubber insert into the sealer. About 1/3 of the way in, the sealer will automatically grab the insert and push it through

the machine itself. The sealed tray will exit the machine on the left side. Again, the sealer will automatically push it out to a point, then you have to pull the rest of it out.

8. With a broad-tipped permanent marker (fine tips may puncture the tray), write the sample information on the paper-side of the tray. (Writing this before sealing the tray will stain the roller inside the machine and will wipe off the writing on the tray).
9. Place the sealed tray either in an air incubator or water bath that is set at 44.5°C. If in water bath, you will need to weigh it down using a weighted ring, careful not to puncture the tray. Record the time you put it in, in the bacti book. Leave the tray to incubate for at least 18 hours and up to 22 hours.

Reading Results

1. You may read results at 18 hours, however, affected wells are often easier to distinguish if incubated 20-22 hours.
2. A positive well will turn yellow. If it is difficult to distinguish a positive well, you may:
    a. Compare the well to the IDEXX prepared comparator (located in labelled blue bag in cabinet beneath work station). If your sample's wells are a darker yellow or just as yellow as the comparator, the well is positive for fecal coliforms. If it is less yellow, it is negative. (Keep in mind any coloration of your sample before adding the Colilert-18 reagent).
    b. Put it back into the incubator/water bath to finish the 22 hours incubation period. Often, lightly yellowed wells will darken to a brighter, more distinguishable yellow.
3. To keep track of yellow wells, it is helpful to mark a positive well with a slash mark using a permanent marker.
    a. Although it is not required, you may also count the number of wells that fluoresce (fluorescent wells indicate E.Coli specifically, which we are not permitted on). The UV lamp is located either above work station or under inside a cabinet. While under light, mark fluorescing wells with another slash to make an X. (only yellow wells will fluoresce).
4. Record the number of yellow wells in the bacti book. Use the number of yellow wells to infer the MPN (Most Probable Number)/100mL of fecal coliforms--- according to the MPN table provided by IDEXX for the Q-tray you used (MPN tables differ between Q-tray 200 and 2000). Record the MPN and respective 95% confidence range in the bacti book.

Quanti-Tray Disposal

Store used trays in the fridge until ready to discard. When about 6-8 trays pile up, double-bag them all in large autoclaveable bags. Seal the bags as tight as you can. TRAYS WILL LEAK a bit. Place bag of trays on a solid tray (not a wire tray or the ones with holes). The tray will catch the remaining leakage from the Q-trays. Sterilize for 15 min using the 't-shirt' icon on autoclave. After cycle, throw in trash and rinse the autoclave tray in sink using distilled water.