Bard Water Lab

IDEXX Colilert Protocol

Freshwater sample = 1:10 dilution
Effluent or otherwise highly contaminated = 1:100 dilution

TO BEGIN:
→TURN ON incubator at 41 degrees Celsius
→TURN ON the IDEXX Quanti-Tray sealer before starting so it will heat up.
→YOU MUST WEAR GLOVES and use sterile pipettes throughout.
→WARNING: DO NOT use the UV light without the viewing cover/box.

Preparing Sample:

1. Carefully crack open the Colilert media and empty into a sterile 120mL vessel (with sodium thiosulfate powder).
2. Gently shake the water sample you've collected and using a sterile pipette, place 10 ml of sample in the vessel, followed by 90 ml of autoclaved DI water (for a 1:10 dilution).
3. Close the vessel and invert gently until media is fully dissolved.
4. Carefully open a Quanti-Tray by pulling the tab gently outwards and gripping around the plastic side of the tray with one hand, pressing slightly to open it wider.
5. Pour the entire contents of the vessel into the tray. Pour slowly to avoid creating bubbles.
6. Gently tap out big bubbles before sealing.
7. Taking care not to spill contents, place the tray in the sealer mold (face down) and gently push into sealer (open end is up, last to go in). The sealer will "grab" the tray and roll it through at the proper speed. Push only enough to engage the tray.
8. Place a sticker label on the back of the tray and fill in the sample information.
9. Place tray in incubator set at **35 degrees Celsius**.

Evaluating MPN:

1. After the incubation period (24 hours), remove the tray from the incubator.
2. To measure total coliforms, count the number of yellow wells (as yellow or more yellow than the comparator), record number big and number small wells.
3. To measure E. coli, place the tray under the UV light box and count the number of large and small wells with a bright, blue fluorescence that are also yellow. Mark fluorescing wells with sharpie; this is a good idea in case you need to recount or get interrupted.
4. Use the MPN (most probable number) chart provided to determine the amount of enterococcus per 100mL sample. If the sample is diluted, you must multiple by the dilution factor (multiple by 10 for a 1:10 dilution).
5. Record data (number of positive wells is important information to gather) on the lab computer in the Water Lab Data spreadsheet under the 'Colilert' tab.

Quality Control Notes:

1. Blank: run one blank sample (using autoclaved DI water as "sample") during each sampling run.

2. Positive control: run a duplicate sample for Site #2 during each sampling run.