Total Coliform and *E Coli*

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Date: 1-March-2016

SUMMARY

This procedure is used for measuring total coliform and *E Coli* in water samples using IDEXX Colilert and Quanti- Tray 2000 kits.

EQUIPMENT AND SUPPLIES

Isotemp forced air incubator at 37°C
pipette tips
Autoclaved ultrapure (Type 1) water
Colilert Kit and Quanti-Trays
100mL graduated cylinder (sterilized)
Autoclave Tape
Quanti-Tray sealer
UV Lamp
10mL pipette
Sample Log
New sample bottles
Bunsen burner
Gloves
Autoclave
1L Wheaton Bottles

NOTES

- All glassware used for samples, standards, or blanks needs to be autoclaved for 30 minutes at 121°C prior to use.
- Samples need to be analyzed as soon as possible (within 6 hours of receipt at the lab).
- Pipettes used in this procedure need to be calibrated annually and checked using a balance on a monthly basis or as problems arise.
- Incubator temperature should be confirmed using a glass thermometer in a monthly basis. If the temperature is off, use departmental incubators on the 2nd floor, and call for service.
- **Always use gloves during all steps of this process.**

REAGENTS

1. All chemicals used in this procedure are included in the Colilert/Quanti-Tray 2000 combo kit. Use care when handling so as not to contaminate them.

CONTROLS

1. Positive and negative controls are run whenever the lot number of the IDEXX kit changes. An IDEXX quality control kit (#UN3373-WQC-TCEC) containing *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* is used. Organisms are contained on a small disk and kept frozen. When ready to run controls, the disk is placed in 100mL of autoclaved water, shaken up, and colilert is added. This is then run through as a sample to test the effectiveness of the kit.
2. In addition to prepared controls, a negative control containing just autoclaved water and the colilert pack is run through at the end of aliquoting to ensure that contamination did not occur during the process.

SAMPLE PREPARATION AND STORAGE

Sample are collected in coolers containing ice packs to keep them as cool as possible in the field and should be stored at 4˚ C immediately upon arrival in the lab. Samples are to be analyzed within 6 hours of arrival at the lab.

SAMPLE ANALYSIS

Day Before Sampling:

1. Turn on incubator and set at 37°C.
2. Cover 100mL glass graduated cyinder with foil.
3. Fill autoclavable bottles with UV treated Ultrapure water found in the CAWS lab (267B).
4. Cap bottles and autoclave bottles and graduated cylinder using the liquid 6 setting (30 minutes at 121°C). Make sure that you mark some of the bottles with autoclave tape, so we know the cycle was successful.

5. Once bottles have cooled, set up Bunsen burner on clean bench space. Flame the tips of the graduated cylinder and the bottle of water that you are working with anytime the lid is replaced. **Never** sit the lid inside facing down on the counter. **Always** use gloves during all steps of this process.
6. Using the graduated cylinder, measure 90mL of autoclaved water into each bottle. Bottles used in this process should be either new or autoclaved. Cap immediately. Never sit caps on the countertop.
7. The last bottle you fill for the day should get 100mL of water instead of 90mL. This bottle serves as a negative control and needs to be run through steps 3 through 7 listed below in Day of Sampling.

Day Of Sampling:

1. Turn on Quanti-Tray Sealer to warm up.
2. Shake the sample bottle vigorously a minimum of 25 times.
3. Using a clean pipette tip, pipette 10mL of sample into a 90mL ultrapure aliquot bottle.
4. With the packet facing away from you, gently open Colilert pack and pour into bottle.
5. Shake gently to mix well.
6. Use one hand to hold open the Quanti-tray and squeeze gently. Pull the foil tab to open the tray. Do not touch the inside of the foil or the tray. Again, you should be wearing gloves!
7. Pour the mixture into the tray. Place the tray into the rubber insert with the well facing down, and tap gently a few times to release air bubbles.
8. Put tray through the sealer, and record the sample number and date on the back. Once you have 5-10 trays, place them in the incubator and record the samples numbers and time in the notebook.

Day After Sampling:

1. After 24 hours have passed (but before 27 hours), samples are ready to count. Two people must do the counts at all time to ensure accuracy. If at

any time both people do not agree on the counts, they must recount until they reach an agreed upon number. There is a color comparator in the fridge in 105 to settle disputes.

2. Yellow well are total coliform. Count the number of large and small wells for each sample and record the number.
3. Turn off lights and place trays under a UV light. Count the number of large and small wells that fluoresce, and record the number. These are *E Coli*.
4. Enter the values into the IDEXX MPN generator and record values.
5. Check values of blanks and controls. If any of the blanks are positive, record cooler that blank came out of and report it to the lab manager. If the positive control (if applicable) is not positive or the negative (if applicable) not negative, rerun the controls using the same lot number, and report this to the lab manager.

CLEAN UP

Turn off sealer. Throw used pipette tips and empty Colilert containers in the trash. Straighten bench space.