

Detection of Waterborne *E. coli*, Total Coliforms, *Aeromonas* and *Salmonella* with ECA Check® (Plus) Easygel®

Instructions

1. Either collect your water sample in a sterile container and transport the water back to the test site, or take a measured water sample directly from the source and place directly into the bottle of ECA Check Easygel. Water samples kept longer than 1 hour prior to plating, or any ECA Check Easygel bottle that has had sample placed into it for transport longer than 10 minutes, should be kept on ice or in a refrigerator until plated.
2. Label the petri dishes with the appropriate sample information. A permanent marker or wax pencil will work.
3. Sterilely transfer water from the sample containers into the bottles of ECA Check Easygel (Consult the following table for rough guidelines for inoculum amount). Swirl the bottles to distribute the inoculum and then pour the medium/inoculum mixtures into the correctly labeled petri dishes. Place the lids back on to the petri dishes. Gently swirl the poured dish until the entire dish is covered with liquid (but be careful not to splash over the side or on the lid).

Inoculation of ECA Check (Plus) Easygel

Water Sources	Inoculum Amount
<u>Environmental:</u> River, lake, pond, stream, ditch	1.0 to 5.0 mL
<u>Drinking water:</u> Well, municipal, bottled	5.0 mL

4. The dishes may be placed right-side-up directly into a level incubator or warm level spot in the room while still liquid. Solidification will occur in approximately 45 minutes.
5. Incubate at 35° C (95° F) for 24 hours, or at room temperature for 48 hours.
6. Inspect the dishes.
 - a. *E. coli* colonies will appear in ambient visible light as dark blue circular dots.
 - b. *Salmonella* colonies will appear in ambient visible light as green circular dots.
 - c. Coliforms other than *E. coli* will appear as light pinkish purple circular dots.
 - d. *Aeromonas* species will appear as clear pink/red or uncolored circular dots and are oxidase positive (a simple spot test can be done by soaking a piece of filter paper with an aqueous solution of tetramethyl-P-phenylene diamine and picking a bacterial colony from a dish and smearing it onto the soaked filter paper. A purple spot will develop within seconds if positive. *E. coli*, *Salmonella* and Coliforms are all oxidase negative.

Total Coliforms are the sum of the *E. coli* (dark blue) and Coliform (pinkish-purple) colonies.

Additionally, you can verify or double check for the presence of *E. coli* by shining a long wave UV light on the back of the dishes (done in a dark room). If any of the colonies are *E. coli*, the area around the colonies will fluoresce a bright bluish color.

Note: To report in terms of colonies per 100 mL of water, first find the number to multiply by:

1. Divide 100 by the number of mL that you used for your sample.
 2. Multiply the count in your plate by the result obtained from #1.
- e.g. For a 3 mL sample $100 / 3 = 33.3$. So 4 *E. coli* colonies multiplied by 33.3 will be equal to 133.2 *E. coli* per 100 mL of water.

*Additionally, verification of *E. coli* is accomplished by shining a long wave (366 nanometer) UV light on the back of the dishes (do this in a dark room). If any of the colonies are *E. coli*, the area around the colonies will fluoresce a bright bluish color. This fluorescence can also be used as proof for the presence of *E. coli* in a sample, thus making the medium an effective P/A test for *E. coli* if quantitative results are not needed.

7. Do one of the following prior to disposal in normal trash:
 - a. Place dishes and Coliscan bottles in a pressure cooker and cook at 15 lbs. for 15 minutes. (This is the best method.)
 - b. Place dishes and Coliscan bottles in an oven-proof bag, seal it, and heat in an oven at 300° F for 45 minutes.
 - c. Place dishes and Coliscan bottles in a large pan, cover with water and boil for 45 minutes.
 - d. Place 5 mL (about 1 teaspoon) of straight bleach onto the surface of the medium of each plate. Allow to sit at least 5 minutes. Place in a water-tight bag and discard in trash.

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Micrology Laboratories LLC, Goshen IN 46526 USA www.micrologylabs.com P 574.533.3351 F 574.533.3370