

Detection of Waterborne Coliforms and Fecal Coliforms with Coliscan® Easygel®

Introduction

The Coliscan Easygel medium is a patented formulation for water testing. It contains a sugar linked to a dye which, when acted on by the enzyme β -galactosidase (produced by coliforms including *E. coli*), turns the colony a pink color. Similarly, there is a second sugar linked to a different dye which produces a blue-green color when acted on by the enzyme β -glucuronidase. Because *E. coli* produces both β -galactosidase and β -glucuronidase, *E. coli* colonies grow with a purple color (pink + blue). The combination of these two dyes makes possible the unique ability to use one test to differentiate and quantify coliforms and *E. coli*. (Because *E. coli* is a member of the coliform group, add the number of purple colonies to the number of pink colonies when counting total coliforms.)

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 Coliscan Easygel. Water samples kept longer than 1 hour prior to plating, or any Coliscan 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 Coliscan 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 Coliscan 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. (see Comments on incubation)

6. Inspect the dishes.
 - a. Count all the purple colonies on the Coliscan dish (disregard any light blue, blue-green or white colonies), and report the results in terms of *E. coli* or Fecal Coliform per mL of water.

Note: To report in terms of *E. coli* or Fecal Coliform 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.
 - b. Count all the pink and purple colonies on the Coliscan dish (disregard any light blue, blue-green or white colonies) and report the results in terms of coliforms per mL of water.

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.

Comments on Incubation

Micrology Laboratories, LLC. in-house studies indicate that **Coliscan** can effectively differentiate general coliforms from ***E. coli*** when incubated at either room temperatures or at elevated temperatures (such as 90-98° F). However, some further explanation may be helpful.

There is no one standard to define room temperature. Most would consider normal room temperature to vary from 68-74° F, but even within this range the growth of bacteria will be varied. Members of the bacterial family **Enterobacteriaceae** (which includes coliforms and ***E. coli****) are generally hardy growers that prefer higher than room temperatures, but which will grow at those temperatures. They tend to grow at a faster rate than most other bacterial types when conditions are favorable. It is therefore logical to try to place inoculated dishes in a "warm" place in a room for incubation if a controlled temperature incubator is not available. It is a very easy task to make an adequate incubator from a box with a 40-60 watt bulb in it to provide heat at an even rate. One can also use a heat tape such as is used to prevent the freezing of pipes in the winter as your heat source.

Our general instructions indicate that incubation times for coliforms (including ***E. coli***) are generally 24-48 hours at elevated temperatures (90-98° F) and 48 or more hours at room temperatures. At elevated temperatures, no counts should be made after 48 hours as any coliforms present will be quite evident by that time and if new colonies form after 48 hours they are most likely not coliforms, but some other type of slow growing organism that should not be included in your data. At room temperatures, the best procedure is to watch the plates by checking them at 10-12 hour intervals until you observe some pink or purple colonies starting to form and then allowing another 24-30 hours for the maturation of those colonies. Since the coliforms (including ***E. coli***) are generally the fastest growing organisms, these will be the first to grow and be counted. Colonies that may show up at a later time are likely to not be coliforms. As you can see, there are advantages to incubating your dishes at elevated temperatures. First, you can count the results earlier. At 95° F, it is often possible to do accurate counts at 18-20 hours of incubation. There is also less probability of variation from batch to batch when the incubation temperatures are kept at one uniform level. And a higher incubation temperature will tend to inhibit the growth of non-coliforms that may prefer lower temperatures.

****E. coli*** is the primary fecal coliform, however, ***Klebsiella*** is sometimes of fecal origin. Other general coliform genera include ***Enterobacter*** and ***Citrobacter***.

Interpretation of Results

This test method utilizes well established, widely accepted criteria for the recognition of coliforms and *E. coli* and proper application of the method will result in accurate results. Therefore, if you suspect that your water is dangerously contaminated based on the results you get using Coliscan Easygel, you should contact your local health department and ask for their help in performing an official assessment of the water.

Non-fecal coliforms are widely distributed in nature, being found both as naturally occurring soil organisms, and in the intestines of warm-blooded animals and humans. Fecal coliforms are coliforms found naturally only in the intestines of warm-blooded animals and humans. Fecal coliform contamination is therefore the result of some form of fecal contamination. Sources may be either animal or human.

General Notes on Differentiating Coliforms and *E. coli*

Generally, water containing *E. coli* (the fecal contamination indicator organism) should not be used for drinking water unless it is sanitized in some manner. Contact your local health department for guidelines regarding *E. coli* and coliforms in recreational waters. Inform them if you suspect that contamination may be occurring from a specific source.

Colonies which have the blue-green color are not exhibiting any β -galactosidase activity (which is evidenced by the pink color). Because of this, they are not considered to be either coliforms or *E. coli* and therefore should be ignored when counting your coliform or *E. coli* colonies. Similarly, colonies which are white are exhibiting neither color-causing enzyme, and should also be ignored.

Colonies on the surface of the plate are exposed to the medium on only the underside of the colony. This causes these colonies to appear with much less of the indicator color. *E. coli* colonies may only have a slight purple tinge to them, and it may appear only in the center of the colony with the remainder of the colony being white. Similarly, coliforms on the surface may be light pink or white with a pink center.

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Micrology Laboratories, LLC. PO Box 340 Goshen, Indiana 46527-0340 USA

Phone **574.533.3351** Fax **574.533.3370** E-mail **micrologylabs@juno.com**