

Blue Thumb Coliform Data Sheet

Site Name: _____ WBID #: _____

Legal/County: _____ Date (MM/DD/YY): _____

Lat/Long: _____ Site Time (Military): _____

Samplers: _____

Reading Date/Time: _____

Volume of Sample Water
<u> </u> mL

	<i>E. coli</i> count (violet)	Total Coliforms count (violet + pink)
Petri dish 1		
Petri dish 2		
Petri dish 3		
Sum		
Average		

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 *Escherichia 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. Store the Coliscan Easygel bottles in the freezer. Coliscan Easygel can be refrozen if it has been thawed and not used.
2. The night before monitoring, take three bottles of Coliscan Easygel for each site from the freezer and thaw in the refrigerator.
3. When heading to the site, take the three bottles, a sterile pipette (1 mL), and a small ice chest with ice.
4. Just before leaving the site, take a measured water sample (1 – 5 mL) from the stream with the sterile pipette and place it directly into each of the three bottles of Coliscan Easygel using the same amount of stream water for each bottle. If you are unsure how much water to use, start with 5 mL. Your own experience will tell you if you need to use less next month. Put the Coliscan Easygel bottles on ice until plating the sample. **Note the volume of sample water on this sheet.**
5. Plug in the incubator to begin preheating.

6. Label three Petri dishes with the site name, the date, and the time you pour the sample into the dish. (The larger diameter is the lid.) You must use the dishes we provide!
7. Swirl the Coliscan Easygel bottles to distribute the inoculum and then pour the medium/inoculum mixtures into the Petri dishes. Place the lids back on 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.)
8. While the contents of the dish are still liquid, place the dish with the lid side up in a warm level spot. The liquid will solidify in about 30 – 45 minutes.
9. When the medium has solidified in 45 minutes, turn the dish upside down (with the lid side down.) Incubate the Petri dishes 24-48 hours in a preheated incubator at 35°C.
10. Colonies will begin to appear after 24 hours. **The best time to count colonies is after 30 hours of incubation.** Colonies should not be counted after 72 hours. Count the colonies with the dish upside down. Colonies may appear inside the medium. Count all colonies growing on the surface **and** within the medium.
11. To determine the results as ***E. coli*** or Fecal Coliform, count the **purple** (and dark blue) colonies. Disregard any light-blue, blue-green or white colonies.
12. To determine the result as **Total Coliforms** (*E. coli* + non-fecal coliforms) count the number of **pink and purple** colonies. Disregard any light blue, blue-green or white colonies.
13. If there are more than 300 CFU (colonies) of one color, the result should be recorded as Too Numerous To Count (TNTC). If the *E. coli* purple colony count is less than 300, even though there are more than 300 total colonies of all colors and types, the purple colonies can be counted and a result given for *E. coli*. Record TNTC for coliforms or non-coliforms that are present at greater than 300 CFU.
14. Count all three Petri dishes and average the results. Be sure to record your results on the data sheet and send it in.
15. Any materials containing living or viable microbes should be disinfected before being discarded. Before disposal in normal trash, treat the Petri dishes by pouring one tablespoon of undiluted household bleach on the dish. Wait 10 minutes. Place the dish in a waterproof bag and discard in the trash.

Interpretation

- 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, such as *E. coli*, are coliforms found naturally only in the intestines of warm-blooded animals and humans. The presence of fecal coliforms is therefore the result of some form of fecal contamination from either animal or human.
- Be aware of animals, like ducks or geese, which may be upstream from where the sample was taken because their feces will increase the *E. coli* and coliform count temporarily, therefore, the results will not reflect the true nature of the water quality.
- If your results are TNTC or appear to indicate dangerously contaminated water, please call us so we can help you with a dilution and/or have a laboratory test the water.
- A smaller sample size should be used for samples with large *E. coli* concentrations to bring the number of colonies into a practical range. The target range should be between 20 and 300 colonies. Below 20 colonies the results are not significant. Colonies greater than 300 are too numerous to count (TNTC). (A total coliform number TNTC is fine. We are concerned mostly with *E. coli*.)

For help or information call:
Kim Shaw Cheryl Cheadle
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(701) 659-0008

Enter your data using the Adobe Fillable
Form or mail your data sheet to:
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