F. Fecal Coliform

i. Why is This Test Important / What Does it Measure

Fecal coliform bacteria are found in the feces of humans and other warm-blooded animals. These bacteria can enter rivers directly or from agricultural and storm runoff carrying wastes from birds and mammals, and from human sewage discharged into the water. Fecal coliform by themselves are not all dangerous (pathogenic). Pathogenic organisms include bacteria, viruses, and parasites that cause diseases and illnesses. Fecal coliform bacteria naturally occur in the human digestive tract, and aid in the digestion of food. In infected individuals, pathogenic organisms are found along with fecal coliform bacteria.

If fecal coliform counts are high (over 200 colonies/100 ml of water sample) in the river, there is a greater chance that pathogenic organisms are also present. A person swimming in such waters has a greater chance of getting sick from swallowing disease-causing organisms, or from pathogens entering the body through cuts in the skin, the nose, mouth, or the ears. Diseases and illness such as typhoid fever, hepatitis, gastroenteritis, dysentery, and ear infections can be contracted in waters with high fecal coliform counts.
Pathogens are relatively scarce in water, making them difficult and time-consuming to monitor directly. Instead, fecal coliform levels are monitored, because of the correlation between fecal coliform counts and the probability of contracting a disease from the water.

Cities and suburbs sometimes contribute human wastes to local rivers through their sewer systems. A sewer system is a network of underground pipes that carry wastewater.

In a separate sewer system, sanitary wastes (from toilets, washers, and sinks) flow through sanitary sewers and are treated at the wastewater treatment plant. Storm sewers carry rain and snow melt from streets, and discharge untreated water directly into rivers. Heavy rains and melting snow wash wildlife, livestock and pet wastes from sidewalks and streets and may "flush out" fecal coliform from illegal sanitary sewer connections into the storm sewers.

In a combined sewer system, sanitary wastes and storm runoff are treated at a wastewater treatment plant. After a heavy rain, untreated or inadequately treated waste may be diverted into the river to avoid flooding the wastewater treatment plant. To avoid this problem, some cities have built retention basins to hold excess waste water and prevent untreated wastes from being discharged into rivers. Without retention basins, heavy rain conditions can result in high fecal coliform counts downstream from sewage discharge points. That is why it is important to note weather conditions on the days

before a fecal coliform measurement.

E. coli VS Fecal Coliform

Total coliform bacteria are a group of easily cultured organisms used to indicate water quality. The US Environmental Protection Agency considers any total coliform to be unacceptable in drinking water. Total coliform bacteria consist of environmental and fecal types. Coliforms are easy to isolate, present in larger numbers and usually survive longer in an aquatic environment than viruses, parasites and more serious types of bacteria. Most of the total coliforms are not considered pathogens under normal conditions.

E. coli is a species of coliform bacteria that is directly linked to fecal contamination by the wastes of warm-blooded animals, including humans. Some strains are pathogens in humans. E-coli produces a combination of sugars that are easily identified and are unique among Coliform bacteria because it can survive a higher incubation temperatures, up to 44.5ºC.

Non-coliform bacteria are mainly environmental organisms and in large numbers can compete with total coliform and make it difficult for coliform(s) to be detected. High levels of non-coliform bacteria indicate a reduction in water quality.

ii.Water Quality Standards/What is An Ideal Temperature?

Fecal and total coliform standards for water used for drinking, recreation, and treated sewage

Coliform Standards (in colonies/100 ml)

Drinking water	1 TC
Total body contact (swimming)	200 FC
Partial body contact (boating)	1000 FC
Treated sewage effluent	Not to exceed 200 FC

*Total coliform (TC) includes bacteria from cold-blooded animals and various soil organisms. According to recent literature, total coliform counts are normally about 10 times higher than fecal coliform (FC) counts.

iii. How to Conduct the Test

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 p-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 p-glucuronidase. Because E. coli produces both p-galactosidase and p-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.

Incbation temperature is extremely important! Due to the practical limitations of our method and equipment, it is important to note that at lower incubation temperatures (< 44.5ºC) coliform species of both fecal and non-fecal origin grow and will produce the p-galactosidase enzyme. However, only the E-coli bacterium will produce both enzymes and thus the purple color. Therfore, at incubation temperatures less than 44.5ºC, we can only ensure that the purple colonies are fecal coliforms and should be considered for counting purposes. As the incubation temperature rises (specifically at 44.5ºC), the non fecal coliforms are unable to establish growth. So if the means are availble, and you choose to incubate at 44.5ºC, it is appropiate to count both purple and pink colonies as fecal coliforms, but only at that specific temperature.

http://www.micrologylabs.com/page/93/Coliscan-Easygel
574-533-3351

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. In a sterile manner, 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
Environmental: River, lake, pond, stream, ditch	1.0 ~ 5.0mL
Drinking water: Well, municipal, bottled	5.0mL

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. Incubation (choose one method):
   a. Incubate plates at a constant room temperature for 48 hours.
   b. Incubate at a constant 35ºC for 24 hours.
   c. Incubate at a constant 44.5ºC for 24 hours.

6. Inspect the dishes.

a. **IF YOUR INCUBATION TEMP IS LESS THAN 44.5ºC:** Count **ONLY** the purple colonies on the Coliscan dish (disregard any pink, light blue, blue-green or white colonies), and report the results in terms of E. coli or Fecal Coliform per mL of water. At this temperature, only the E.coli can be ensured to be of fecal origin (other coliforms are of indeterminate origin at these temperatures)

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.**ONLY IF YOU USED AN INCUBATOR AT 44.5ºC:** 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.

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.

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.
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. 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 hours at elevated temperatures and 48 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 £. coif) 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. 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-fecal 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.

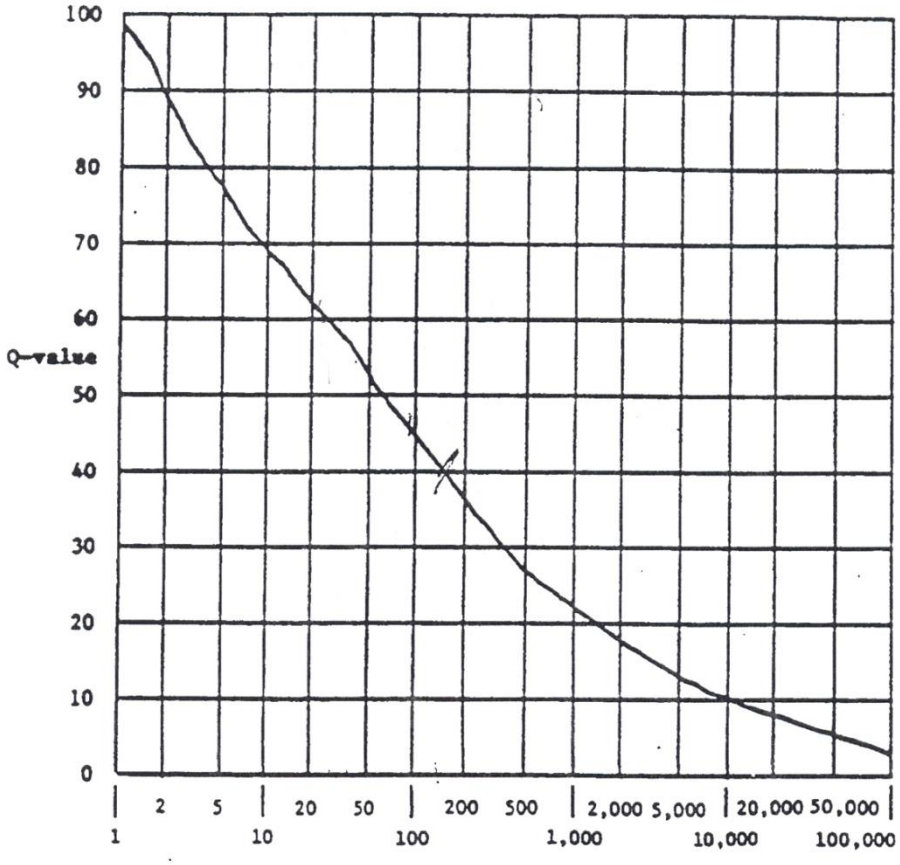
Interpretation of Results

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.

iv. Determining the Q-Value

Chart 2: Fecal Coliform (FC) Test Results



FC: /100ml

Note: if FC>10^5, Q=2.0

v.What to Watch Out For: Common Mistakes

-When transporting the petri dish from the testing site back to the school, make sure you keep it level, so the sample does not spill out

-Make sure you put the sample in the incubator when you get back to the school

-Make sure you check the sample in the 24-48 hour window

-Make sure your EasyGel bottle is thawed out but kept cool until it is time to do your test.

-Make sure you are using a treated petri dish, not a regular one.

vi.Consistency When Doing Multiple Tests

If you do multiple tests for fecal coliform, use the average (arithmetical mean) of the samples. If there is an extreme difference between the readings, contact your mentor or the FRWC for advice.

vii. How to Analyze Why The Results is Good or Bad

Any reading over 200 FC is cause for concern. You might want to consider re-testing the site. If the site continues to have high fecal coliform readings, this would be a good opportunity for students for try to determine potential sources of fecal contamination in the water.