COLIFORM BACTERIA IN SURFACE WATERS

I. INTRODUCTION

Many organisms, including humans, have symbiotic bacteria in their guts that aid digestion. Symbiotic relationship is an intimate relationship between different organisms in which both the host organism, e.g. the human, and the symbiote, e.g. bacteria, benefit from each other. In this case, the bacterium gets a favorable environment and food source in the intestines of a human. In return, these bacteria improve the digestibility of food through a host of enzymatic processes.

A subset of this group of bacteria is known collectively as Coliform, which includes the well-known *E. coli*, some strains of which are necessary for human health, and some which are pathogenic and can make you very sick. Although the beneficial strains of this bacteria aid human digestion, finding these bacteria in waterways serves as a warning for the potential spread of disease because they can indicate human or animal feces. Therefore, coliform bacteria are used in water quality testing as indicators of other pathogenic bacteria that commonly are found associated with coliforms, e.g., *Salmonella*, *Shigella*, *Yersinia*, *Klebsiella* and certain strains of *E. coli*.

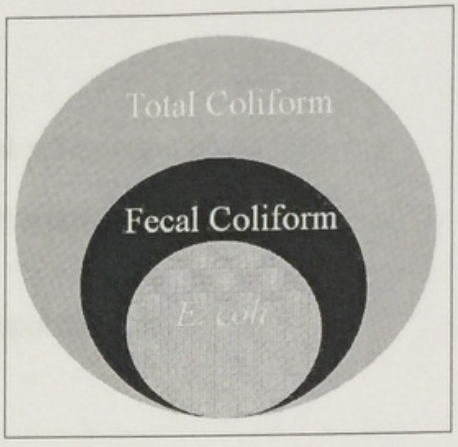


Figure 1. Breakdown of coliform into categories: total, fecal and *E. coli* (wqm.igsb.uiowa.edu)

How do Coliforms get there? What is required is any type of mammal or bird producing excrement. This could be as simple as a duck floating on a pond or a human flushing the toilet. These single events, however, will not produce enough bacteria to be detected. What is required is some concentrated amount of waste entering the environment.

For example, Ohio does not issue Clean Water Act permits specifically for Concentrated Animal feeding operations (CAFOS). Ohio is the number one egg producing state, and what happens with the excrement of 30 million chickens? For the most part, these wastes are applied to agricultural fields as fertilizer, which can be extremely beneficial. What is problematic is that the state has no local control over factory farm operations and allows these farms to dump manure offsite without revealing the location under the Ohio Trade Secrecy Act. Therefore, Ohio has no control over the timing of application, the concentration of application or the distance to any waterway. Furthermore, some Ohio politicians are currently attempting to provide greater leniency by changing the legal definition of a "stream" to exclude any primary or secondary drainage channels, which connect directly to larger waterways.

Livestock operations are not all to blame. In many urban areas, storm water sewers are combined with wastewater sewers in what is known as a _____. During large rain events, the amount of water in the Combined Sewer exceeds the capacity of the waste water channel and overflow directly into streams. This is unlawful under the Clean Water Act and municipalities (including Toledo) are now required to remove these types of systems. Even in low-density rural areas, faulty septic systems release coliform into waterways.

II. Lab Exercise: Coliform Bacteria in Surface Waters

Today you will test the water of the Ottawa River Watershed using Petrifilm media to test for total fecal bacteria, fecal coliforms and *E. coli.*

Supply List

1. Ottawa River water stored in 500 mL containers
2. (4) Dixie cups for samples, RO water, and waste
3. (1) 1 ml Transfer Pipettes
4. (1) Pipette Pump
5. (2) Petri Film Plates
6. Ziplock Bags
7. Sharpie

Procedure:

1. Water will be collected for you from Ottawa River Watershed at pre-selected points.
2. Using your sharpie, label a cup with the name of one site, label a cup with the name of a second site, for two cups total.
3. Pour approximately 10 mL of the source water into each corresponding cup.
4. Place the first transfer pipette in the pipette pump.
5. Label your first Petrifilm plate with the sample site, name and lab section.
6. Collect a 1 mL sample of sample site water, pull the cover sheet back and SLOWLY drop the water at the upper end of the Petrifilm.
7. Once the sample is on the film, SLOWLY drop the coversheet from top to bottom, to help spread the sample.
8. Rinse the pipette with Clean Water 5 times.
9. Repeat the procedure for the second sample site.
10. Give the samples to your instructor so that they may place them in a ziplock bag for incubation. They will be incubated at 34° C for 24 hours.
11. The following week, you will count the bubbles. Your instructor will show you how to count with a Power Point demonstration. Write the number of E. coli colonies, coliform colonies and total coliforms on the Petrifilm using a sharpie.
12. Record these numbers in the worksheet provided.
13. Share data among the groups.

Worksheet Exercise: Data Collection, Statistical Analysis, and Interpretation

NAME_____ DATE_____

Site	Team	E. coli - blue	Coliforms - red	Total Coliforms	Per 100 mL
River	Brid	15	500	15	51,500
Tap	Brid	0	0	0	0
River	***	18	391	409	40,900
Tap	***	0	0	0	0
River	us	9	623	632	63,200
Tap	us	0	0	0	0
River	penguins	15	45	465	46,500
Tap	penguins	0	0	0	0

Treatment Variable 1 Name (*1 point*): River water

Treatment Variable 2 Name (*1 point*): Tap water

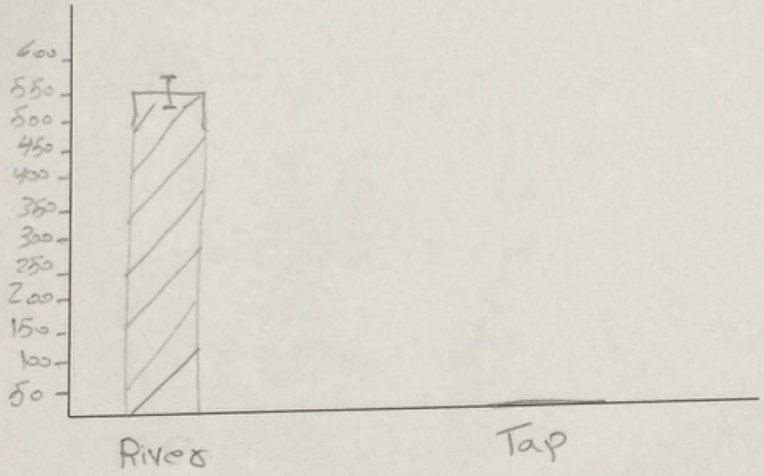
Response Variable You are Testing (*1 point*): Total coliforms

Your hypothesis *(2 points)*:

Calculate the mean, standard deviation and standard error at **http://www.graphpad.com/quickcalcs/ttest1.cfm**

Treatment 1 Name:	Rivers	Treatment 2 Name:	Tap
Treatment 1 Mean:	505.25	Treatment 2 Mean:	0
Treatment 1 Standard Deviation:	94.95	Treatment 2 Standard Deviation:	0
Treatment 1 Standard Error:	47.47	Treatment 2 Standard Error:	0

Draw a bar chart of the *means* and show *standard error* bars.
Include x and y axis labels, a title, and a scale. *(3 points).* How the sample site effects Total number of coliforms.



Is your hypothesis supported or unsupported? Why? (*2 points*)

What do you personally think about the result of this experiment? (*1 point*)

How can you reduce coliform inputs into our waterways? (*2 points*)