

LAB 9

ANALYSIS OF WATER FOR FECAL COLIFORMS

Introduction

Pathogenic microbes that can contaminate water include **bacteria** (such as *E. coli* and *Vibrio cholerae*), **protozoa** (such as *Balantidium coli* or *Entamoeba histolytica*), and **viruses**. The most common source of these microbes is the feces of animals – including humans.

Water quality can be determined by sampling for bacteria of fecal origin. These bacteria, including *E. coli*, are called **fecal coliforms**. Fecal coliforms are Gram-negative bacteria that ferment lactose and produce acid and gas within 48 hours at 37°C. These rod-shaped bacteria lack endospores. The EPA (Environmental Protection Agency) has set acceptable limits for fecal coliforms in water based upon the intended use of the water:

- drinking water cannot contain any fecal coliforms
- water for swimming may contain up to 400 fecal coliform colonies / 100 mL water

In this lab, a series of three tests will be conducted with water samples to test for fecal coliform contamination, and to estimate their numbers.

Part I: The Presumptive Test

The **Presumptive Test** is an initial test that tests only for lactose fermentation.

Materials and Methods:

- Water sample (35 mL or more)
- Sterile pipette tips and pipettors
- 6 single strength (SS) lactose tubes
- 3 double strength (DS) lactose tubes

Each group will collect a water sample in the sterile tube provided. Possible sources of water to test include water from drinking fountains, bottled water (opened or unopened), fish/turtle tank, and toilet.

If you sample from the fish tank or toilet, wear disposable gloves and discard them after use!

Your water sample origin: _____

Label the tubes according to how much water you add to each, as follows:

- 3 DS tubes: add **10 mL** water to each tube
- 3 SS tubes: add **1 mL** water to each tube
- 3 SS tubes: add **0.1 mL (100 µL)** water to each tube

Incubate the tubes for at least 48 hours at 37°C.

Part II: Most Probable Number Estimate and Gram Stain Confirmation

After incubation (next week), any yellow-colored tubes with gas in the Durham tube are positive for lactose fermentation. Record your results:

	DS (with 10 mL water)	SS (with 1 mL water)	SS (with 0.1 mL water)
Number of positive tubes (out of 3)			

Using the above data, refer to the **Most Probable Number (MPN)** Table on the next page to estimate how many bacteria were in your water sample.

MPN Estimate: _____ bacteria per 100 mL of water

A positive **Presumptive Test** result suggests that the water sample *might* contain fecal coliforms. However, it could be a different type of bacteria that ferments lactose, but is not a fecal coliform. Therefore, further testing is required to confirm fecal coliform contamination.

To test it further, select a positive lactose tube and use it to conduct a **Gram stain**. If your results are all negative, do not continue with the Gram stain. In that case, your water sample did not contain fecal coliforms.

Gram stain result: _____

Based on the Presumptive Test and Gram stain, were the bacteria in your water sample fecal coliforms?

Yes / No

MOST PROBABLE NUMBER (MPN) TABLE

Number of positive tubes			
Three 10 ml tubes	Three 1 ml tubes	Three 0.1 ml tubes	Probable number in 100 ml
0	0	0	<3
0	0	1	3
0	0	2	6
0	0	3	4
0	1	0	3
0	1	1	6
0	1	2	4
0	1	3	12
0	2	0	6
0	2	1	4
0	2	2	12
0	2	3	16
0	3	0	9
0	3	1	12
0	3	2	16
0	3	3	19
1	0	0	4
1	0	1	7
1	0	2	11
1	0	3	15
1	1	0	7
1	1	1	11
1	1	2	15
1	1	3	16
1	2	0	11
1	2	1	15
1	2	2	20
1	2	3	24
1	3	0	11
1	3	1	26
1	3	2	24
1	3	3	26

Number of positive tubes			
Three 10 ml tubes	Three 1 ml tubes	Three 0.1 ml tubes	Probable number in 100 ml
2	0	0	9
2	0	1	14
2	0	2	20
2	0	3	26
2	1	0	15
2	1	1	20
2	1	2	27
2	1	3	24
2	2	0	21
2	2	1	28
2	2	2	35
2	2	3	46
2	3	0	26
2	3	1	36
2	3	2	44
2	3	3	52
3	0	0	23
3	0	1	39
3	0	2	64
3	0	3	95
3	1	0	43
3	1	1	75
3	1	2	120
3	1	3	160
3	2	0	93
3	2	1	150
3	2	2	220
3	2	3	290
3	3	0	240
3	3	1	460
3	3	2	1100
3	3	3	>1100

Part III: MacConkey Agar Confirmation

Another way to test your sample for fecal coliforms is to use **MacConkey Agar**. MacConkey Agar is a **selective** medium that only allows Gram-negative bacteria to grow on it. It is also a **differential** medium, differentiating lactose-fermenting bacteria (including fecal coliforms) from bacteria that cannot ferment lactose.

When fecal coliform bacteria are grown on MacConkey Agar, the colonies will appear pink. Other Gram-negative bacteria that cannot ferment lactose (and thus are not fecal coliforms) will not appear pink.



A Petri plate of MacConkey Agar inoculated with non-coliforms (left) and fecal coliforms (right).

MacConkey Agar contains lactose, a pH indicator (which turns pink if acid is present), and chemicals that prevent Gram-positive bacteria from growing.

Pour a Petri plate of MacConkey Agar and let it solidify. Then streak a sample from a positive lactose tube (from the Presumptive Test) across the Petri plate and incubate it at 37°C. Next week, record your results.

MacConkey Agar results: _____

Based on the MacConkey Agar results, were the bacteria in your water sample fecal coliforms?

Yes / No