Bacteriologic Water Quality: Multiple-Tube Test*

OBJECTIVES

After completing this exercise, you should be able to:

1. Define *coliform.*
2. Provide the rationale for determining the presence ofcoliforms.
3. List and explain each step in the multiple-tube technique.

BACKGROUND

Coliforms

Tests that determine the bacteriological quality of water have been developed to prevent transmission of waterborne diseases of fecal origin. However, it is not practical to look for pathogens in water supplies because pathogens occur in such small numbers that they might be missed by sampling. Moreover, when pathogens are detected, it is usually too late to prevent occurrence of the disease. Rather, the presence of **indicator organisms** is used to detect fecal contamination of water. An indicator organism must be present in human feces in large numbers and must be easy to detect. The most frequently used indicator organisms are the coliform bacteria. **Coliforms** are aerobic or facultatively anaerobic, gram-negative, non-endospore-forming, rod-shaped bacteria that ferment lactose with acid and gas formation within 48 hours at 35°C. Coliforms are not usually pathogenic, although they can cause opportunistic infections. The IMViC tests (Exercise 51) historically were used to identify coliforms, such as *Escherichia coli.*

Coliforms are not restricted to the human gastrointestinal tract and are found in other animals and in the soil. Tests that determine the presence of **fecal coliforms** (of human origin) have been developed. Appearance of coliform colonies on differential media incubated at 44.5°C is now considered positive for fecal coliforms. One of these differential media, MUG agar, will be used in this exercise and is discussed below.

Established public health standards specify the maximum number of fecal coliforms allowable in each 100 ml of water, depending on the intended use of the water (for example, water for drinking or water contact sports or treated wastewater for irrigation or for discharge into a bay or river).

MPN Test

The most accurate number of coliform bacteria is obtained by testing a large sample of water. Total coliforms can be detected and enumerated in the **multiple-tube technique** (Figure 1). In this method, coliforms are detected in two stages. In the **presumptive test,** dilutions from a water sample are added to lactose fermentation tubes. The lactose broth can be made selective for gram-negative bacteria by adding lauryl sulfate or brilliant green and bile. Fermentation of lactose to gas is a positive reaction.

Samples from the positive presumptive tube at the highest dilution are examined for coliforms by inoculating a differential medium in the **confirmed test.** A confirmed test can be done on **MUG agar.** Almost all strains of *E. coli* produce the enzyme β-glucuronidase (GUD). If *E. coli* is added to a nutrient medium containing 4-methylumbelliferone glucuronide (MUG), GUD converts MUG to a fluorescent compound that is visible with an ultraviolet lamp (Figure 2).

The number of coliforms is determined by a statistical estimation called the **most probable number** (MPN) method. In the presumptive test, tubes of lactose broth are inoculated with samples of the water being tested. A count of the number of tubes showing acid and gas is then taken, and then is compared to a statistical MPN table. The MPN number is *the most probable number* of coliforms per 100 ml of water.

MATERIALS per 3 students

FIRST PERIOD

Water sample, 50 ml (Bring your own from a pond or stream.)
9-ml, single-strength lactose fermentation tubes (6)
20-ml, 1.5-strength lactose fermentation tubes (3)
Sterile 10-ml pipette
Sterile 1-ml pipette

* Johnson, T. and C. Case. 2010. *Laboratory Experiments in Microbiology*. San Francisco: Benjamin Cummings.

SECOND PERIOD
Petri plate containing MUG agar

PROCEDURE First Period

Using Figure 3 as a reference, complete the following presumptive test:

1. Label three single-strength lactose broth tubes "0.I," label another three tubes "I," and label the three 1.5-strength broth tubes "10."
2. Inoculate each 0.1 tube with 0.1 ml of your water sample.
3. Inoculate each 1 tube with 1.0 ml of your water sample.
4. Inoculate each 10 tube with 10 ml of the water sample. Why is 1.5-strength lactose broth used for this step? _____
5. Incubate the tubes for 24 to 48 hours at 35°C.

PROCEDURE Second Period

1. Record the results of your presumptive test (Figure 1). Which tube has the highest dilution of the water sample? _____
2. Determine the number of coliforms per 100 ml of the original sample using the MPN table. If a tube has gas, streak the MUG agar with the positive broth. Incubate the plate, inverted, for 24 to 48 hours at 44.5°C.

PROCEDURE Third Period

1. Examine the plate using an ultraviolet lamp. **Do not look directly at the ultraviolet light, and do not leave your hand exposed to it.**
2. Record the results of your confirmed test (see Figure 1 and Figure 2). How can you tell whether coliform colonies are present? ___

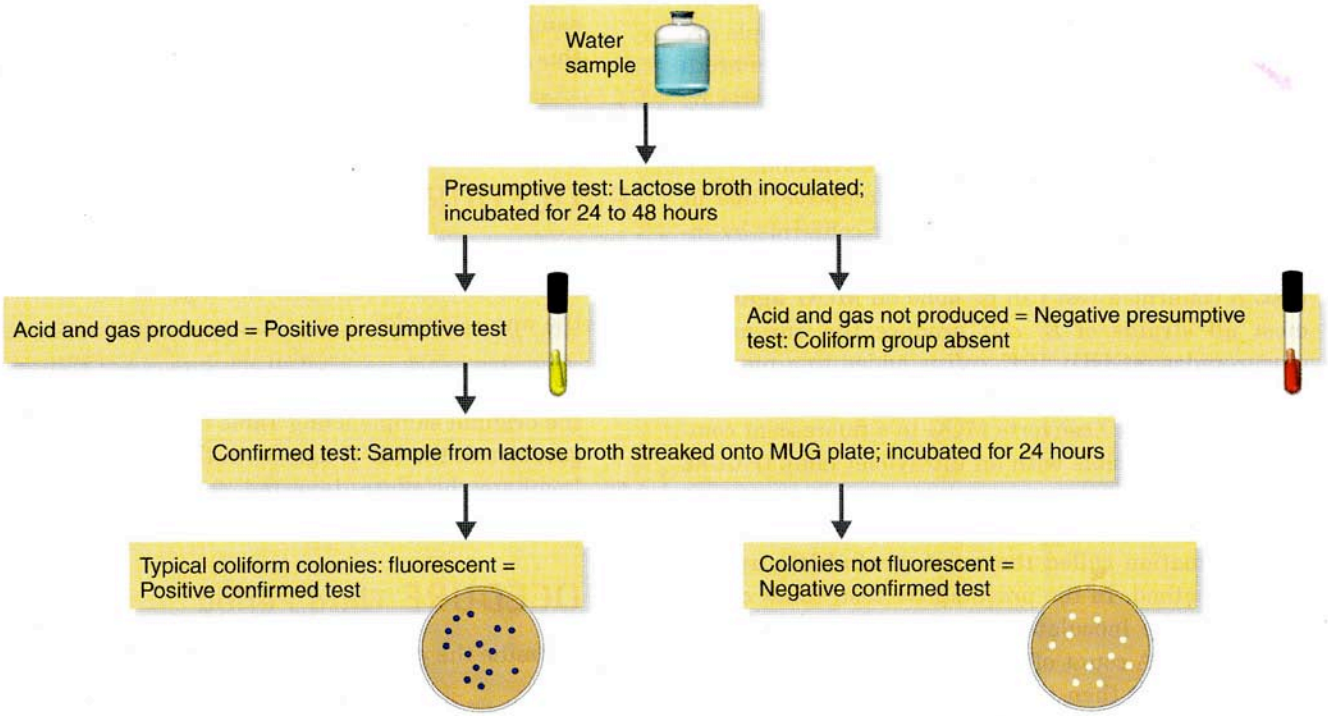

Figure 1. Analysis of drinking water for coliforms by the multiple-tube technique.

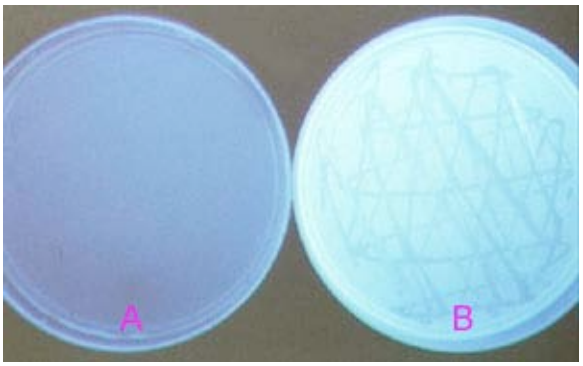

Figure 2. *E. coll.* A. Uninoculated plate. B. *E. coli* is identified by its ability to convert MUG to a fluorescent compound that is visible with an ultraviolet lamp.

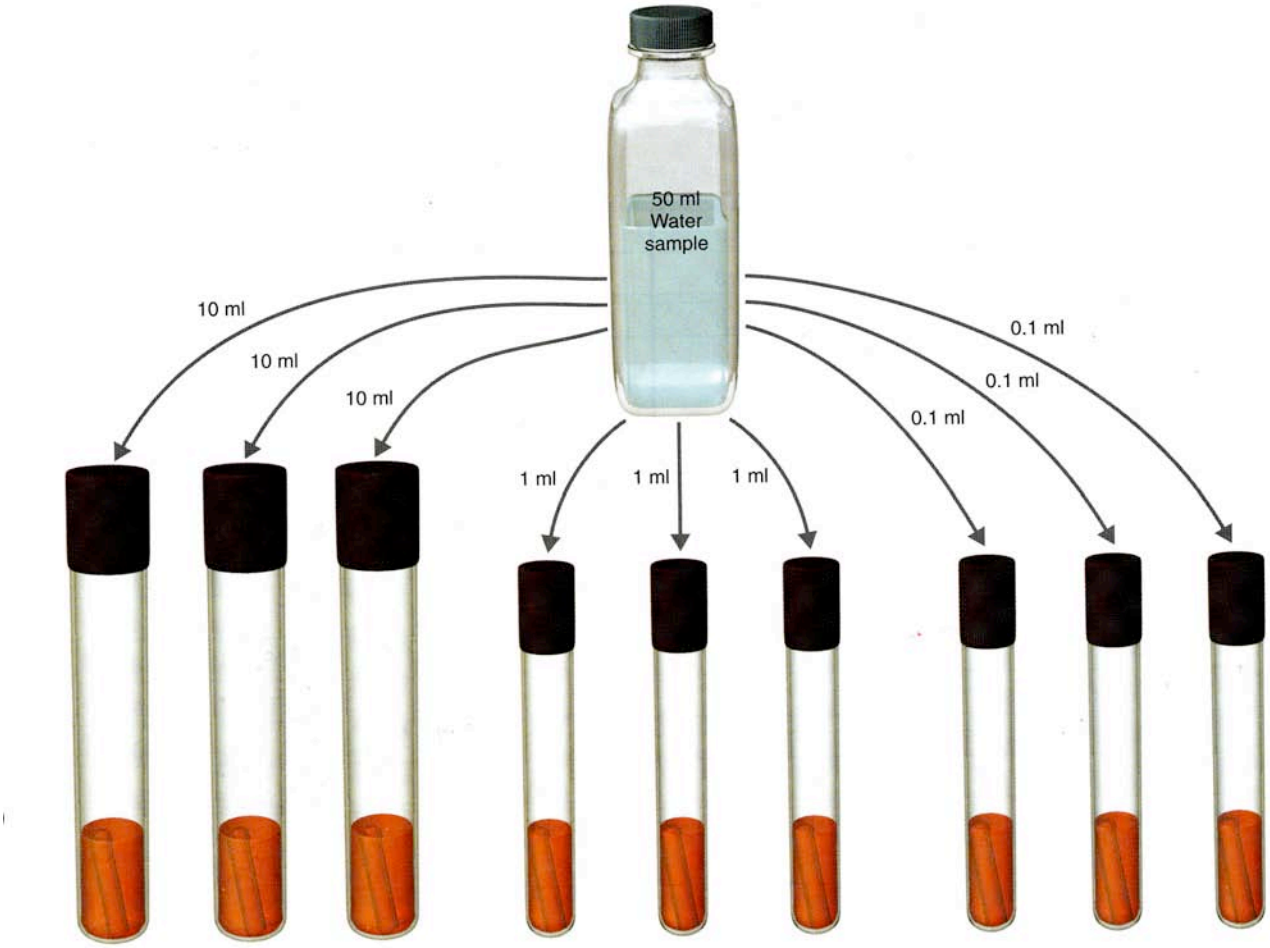

Figure 3. Most probable number (MPN) dilution series. There are three sets of tubes and three tubes in each set. Each tube in the first set receives 10 ml of the inoculum. Each tube in the second set of three tubes received 1 ml of the sample, and the third set, 0.1 ml each.

Bacteriologic Water Quality: Multiple-Tube Test

Name _____

Date _____

Purpose_____

_____

_____

Results

Water sample source: _____

Presumptive Test

	Number of Tubes with a Positive Results		
	Inoculum		
	10 ml	1.0 ml	0.1 ml
Growth			
Acid			
Gas			
Number of tubes positive for all 3			

Confirmed Test

Tube: _____

Appearance of colonies with white light: _____

Appearance of colonies with UV light: _____

Data from water samples tested by other students:

Sample	Coliforms?	MPN	Fecal Coliforms?

Conclusions

1. What is the MPN of your water sample? _____per _____ ml
2. Are fecal coliforms present? _____

Questions

1. Why are coliforms used as indicator organisms if they are not usually pathogens?

2. Why didn't we inoculate MUG agar directly and bypass lactose broth?