Bio 3B Laboratory
Bacteriological Examination of Water

Objectives

- Carry out a presumptive test for the presence of coliform bacteria in a water sample
- Determine the most probable number (MPN) of bacteria in a positive presumptive sample
- Carry out a confirmed test to begin isolation of bacterial colonies
- Carry out a completed test using a Gram stain and morphology

Introduction

We consider fresh-water streams, lakes, ground water or coastal ocean water to be polluted when some condition makes the water unsafe for human recreation or consumption. We usually think of two forms of pollution: toxic chemicals or pathogenic microorganisms. Probably the largest single source of potentially pathogenic microbes is animal feces (including human), which contains billions of bacteria per gram. Although most intestinal microbes are non-pathogenic, some cause enteric disease. The organisms which cause typhoid fever (*Salmonella typhi*), cholera (*Vibrio cholera*), and bacterial dysentery (*Shigella flexneri*) are examples of enteric diseases caused by bacteria. In addition, some viral and protozoan pathogens are spread through water contaminated by feces. Testing for each organism separately would be extremely costly and time-consuming. Therefore, a simple rule is followed: if a water sample contains any microorganism common to animal intestines, it should not be consumed, because it may contain enteric pathogens.

Water testing for microbiological safety rests on the ability of microbiologists to detect coliform bacteria. The word "coliform" refers to any bacterium that is like *Escherichia coli* in the following characteristics: 1) it is a small, gram-negative rod; 2) it does not contain spores; 3) it ferments lactose with the production of acid and gas; 4) it produces a green metallic sheen on EMB agar.

E. coli, which is found in large numbers in the feces of all animals, lives longer in water than most intestinal pathogens do. Therefore, if no *E. coli* are present, there should be no intestinal pathogens present in the water sample. For this reason, testing for coliform organisms is performed daily by municipal water departments and waste-water (sewage) treatment plants. It is regularly tested for in coastal sea water samples, as well as runoff water.

The bacterial examination of water has been standardized into three tests. The first, or presumptive test, is a screening test to sample water for the presence of coliform organisms. A series of lactose fermentation tubes are inoculated with the water sample. If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe. If, however, any tube in the series shows acid and gas, the water is considered unsafe and the confirmed test is performed on the tube displaying a positive reaction. The presumptive test is also designed to estimate the concentration of coliform organisms, called the most probably number (MPN) in the water sample. The confirmed test is a second screening procedure in which a gram-negative selective medium is used (like EMB). This also allows for the differentiation of coliform (producing a green metallic sheen) from non-coliform colonies. The completed test is performed on a typical, well-isolated colony to reaffirm gas production in lactose, and to determine the morphology and gram reaction of the isolate from a nutrient agar slant.

Procedure 1
Presumptive Test and MPN

Each group will need:
3 triple strength lactose tubes with brom-thymol blue
6 regular strength lactose tubes with brom-thymol blue

Sterile water collection bottles for community sampling (or "doped" water samples)
10 ml and 1 ml sterile pipets

1. Collect approximately 50 ml of water to be tested (from pools, streams, ocean, etc.) or you may use the "doped" samples provided in class. Record the source and date of community samples or the sample number of the "doped" sample to be tested.
2. Vigorously shake the water sample to be tested by moving it 25 times through a 12- inch arch.
3. Transfer 10 ml of the sample into each of the three, triple strength lactose tubes. Always use aseptic technique in the water inoculations and label the tubes with the amount of water sample tested.
4. Transfer 1 ml of the sample into each of three regular strength lactose tubes. Using the same pipet, transfer 0.1 ml to each of the three remaining regular strength lactose tubes.
5. Incubate all tubes at 37^{o} C. until the next laboratory session.

Procedure 2
Confirmed Test

1. Examine the tubes from the presumptive test and determine if any has produced an acid/gas reaction. If so, this is a positive presumptive test. If no gas is present in any of the Durham tubes, this is a negative presumptive test.
2. Determine the number of tubes positive for acid/gas in each of the three volume categories. Determine the MPN of your water sample by comparing these numbers to the MPN Determination chart accompanying this exercise. Complete the table for the presumptive test as shown below and enter it into your laboratory notebook.

3. From any tube showing 10% gas production or more, streak one loopful of the broth onto an EMB plate using the isolation streaking technique. Incubate the plate at 37^{o} C. until the next laboratory session.

Procedure 3
Completed Test

1. Draw or describe your EMB plate in your notes. Carefully examine the plate, looking for well-isolated coliform colonies. Typically, E. coli colonies appear with a metallic green sheen on EMB. From one of these colonies, set up your completed test by inoculating a lactose fermentation tube and a NA slant. Incubate them at 37^{o} C. until the next laboratory session.
2. After this incubation, check the lactose tube for acid and gas production. If no gas is present, this is a negative completed test. Prepare a Gram stain from the NA slant. If the organism is a non-spore producing Gram negative rod and the lactose broth shows an acid/gas reaction, this is a positive completed test. Record your results in the table below in your laboratory notebook. Include a drawing of your Gram stain.

Results
Presumptive Test
Record the source of the water sample tested and the number of tubes in each category that produced acid/gas reactions. Determine MPN and record in the following table.

Sample Source	10 ml tubes	1.0 ml tubes	0.1 ml tubes	MPN

MPN Determination from Multiple Tube Test

Most Probable Numbers (MPN) Index for Various Combinations of Positive and Negative Results When Three 10-ml Portions, Three 1-ml Portions, and Three 0.1-ml Portions Are Used

Number of tubes giving positive reaction out of			MPN Index per 100 ml	95% Confidence Limits	
3 of 10 ml each	3 of 1 ml each	3 of 0.1 ml each		Lower	Upper
0	0	0	<3	<0.5	
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1,300
3	3	1	460	71	2,400
3	3	2	1,100	150	4,800
3	3	3	>2,400		

Sources:

Standard Methods for the Examination of Water and Wastewater, 13th Edition New York. American Public Health Association, 1971.

Standard Methods for the Examination of Water and Wastewater, 12th Edition New York. American Public Health Association, 1967, p. 608.