BACTERIAL EXAMINATION OF WATER

The bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply of water for drinking, bathing, swimming and other domestic and industrial uses. The examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery.

Since human fecal pathogens vary in kind (viruses, bacteria, protozoa) and in number, it would be impossible to test each water sample for each pathogen. Instead, it is much easier to test for the presence of nonpathogenic intestinal organisms such as *E. coli*. *E. coli* is a normal inhabitant of the intestinal tract and is not normally found in fresh water. Therefore, if it is detected in water, it can be assumed that there has been fecal contamination of the water.

In order to determine whether water has been contaminated by fecal material, a series of tests are used to demonstrate the presence or absence of coliforms. The **coliform** group is comprised of Gram-negative, nonspore-forming, aerobic to facultatively anaerobic rods, which ferment lactose to acid and gas. Two organisms in this group include *E. coli* and *Enterobacter aerogenes*; however, the only true fecal coliform is *E. coli,* which is found only in fecal material from warm-blooded animals. The presence of this organism in a water supply is evidence of recent fecal contamination and is sufficient to order the water supply closed until tests no longer detect *E. coli.*

In this exercise, you will be testing water samples for the presence of coliforms. There will be three principal tests: the **presumptive**, **confirmed** and **completed** tests (see flow-chart).

STANDARD WATER ANALYSIS

The Presumptive Test

In the presumptive test, a series of lactose broth tubes are inoculated with measured amounts of the water sample to be tested. The series of tubes may consist of three or four groups of three, five or more tubes. The more tubes utilized, the more sensitive the test. Gas production in any one of the tubes is **presumptive** evidence of the presence of coliforms. The **most probable number** (MPN) of coliforms in 100 ml of the water sample can be estimated by the number of positive tubes (see MPN Table).

The Confirmed Test

If any of the tubes inoculated with the water sample produce gas, the water is presumed to be unsafe. However, it is possible that the formation of gas may not be due to the presence of coliforms. In order the **confirm** the presence of coliforms, it is necessary to inoculate EMB (eosin methylene blue) agar plates from a positive presumptive tube. The methylene blue in EMB agar inhibits Gram-positive organisms and allows the Gram-negative coliforms to grow. Coliforms produce colonies with dark centers. *E. coli* and *E. aerogenes* can be distinguished from one another by the size and color of the colonies. *E. coli* colonies are small and have a green metallic sheen, whereas *E. aerogenes* forms large pinkish colonies.

If only *E. coli* or if both *E. coli* and *E. aerogenes* appear on the EMB plate, the test is considered positive. If only *E. aerogenes* appears on the EMB plate, the test is considered negative. The reasons for these interpretations are that, as previously stated, *E. coli* is an indicator of fecal contamination, since it is not normally found in water or soil, whereas *E. aerogenes* is widely distributed in nature outside of the intestinal tract.

The Completed Test

The completed test is made using the organisms which grew on the **confirmed** test media. These organisms are used to inoculate a nutrient agar slant and a tube of lactose broth. After 24 hours at 37°C, the lactose broth is checked for the production of gas, and a Gram stain is made from organisms on the nutrient agar slant. If the organism is a Gram-negative, nonspore-forming rod and produces gas in the lactose tube, then it is positive that coliforms are present in the water sample.

FIRST PERIOD

Material:

1. Nine tubes of double-strength lactose broth
2. 10, 1.0 and 0.1 ml pipets
3. Water samples

Procedure: (work in groups of four)

Presumptive Test

1. Take a water sample (dilute as instructed in some cases) and inoculate three tubes of lactose broth with 10 ml, three tubes with 1.0 ml and three tubes with 0.1 ml.
2. Incubate all tubes at 37°C for **24 hours**.

SECOND PERIOD

Material:

1. EMB agar plates

Procedure:

Presumptive Test

1. Observe the number of tubes at each dilution that show gas production in 24 hrs. Record results.
2. Reincubate for an additional **24 hours** at 37°C.

Confirmed Test

1. Inoculate an EMB plate with material from a tube containing gas.
2. Invert and incubate the plate at 37°C for **24 hours**.

THIRD PERIOD

Material:

1. Lactose broth tubes
2. Nutrient agar slants

Procedure:

Presumptive Test

1. Observe the number of tubes at each dilution that show gas. Record results and determine the most probable number index.

Confirmed Test

1. Observe EMB agar plates. A positive confirmed test is indicated by small colonies with dark centers and a green metallic sheen (*E. coli*). Record results.

Completed Test

1. Inoculate a lactose broth tube and a nutrient agar slant with organisms from the EMB plate.
2. Incubate the broth tube and agar slant at 37°C for **24 hours**.

FOURTH PERIOD

Procedure:

Completed Test

1. Check for gas production in the lactose broth tube.
2. Make a Gram stain from the organisms on the nutrient agar slant.
3. Record results.

MPN DETERMINATION FROM MULTIPLE TUBE TEST

NUMBER OF TUBES GIVING POSITIVE REACTION OUT OF			MPN Index per 100 ml.	95 PERCENT CONFIDENCE LIMITS	
3 of 10 ml. each	3 of 1 ml. each	3 of 0.1 ml. each		Lower	Upper
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