***Microbiology of water***

Polluted waters contain vast amounts of organic matter that serves as excellent nutritional sources for the growth and multiplication of microorganisms. The presence and number of coliform bacteria and other enteric organisms in water is indicative of fecal contamination and may suggest the presence of pathogens. These pathogens are responsible for intestinal infections such as bacillary dysentery, typhoid fever, cholera, and paratyphoid fever. Therefore, water is examined to detect *Escherichia coli*, the bacterium that indicates fecal pollution. Since *Escherichia coli* is always present in the human intestine, its presence in water alerts public health officials to the possible presence of other human or animal intestinal pathogens.

***1- Standard Qualitative Analysis of Water***

The three basic tests to detect coliform bacterial water are presumptive, confirmed, and completed. The tests are performed sequentially on each sample under analysis. They detect the presence of coliform bacteria (indicators of fecal contamination) the gram-negative, non sporeforming

bacilli that ferment lactose with the production of acid and gas that is detectable following a 24-hour incubation period at 37ºC.

**Coliform:** A group of bacteria which can be used as an indicator of pollution. A major portion of this group live in the intestinal tract of warm blooded animals, including human beings. They are easy to identify and count in the laboratory because of their ability to ferment lactose. Citrobacter, Klebsiella, Hafnia, Enterobacter and Fecal coliform.

***Presumtive coliform test***

***Purpose***

. 1-To determine the presence of coliform bacteria in a water sample

2-To obtain some index as to the possible number of organisms present in the sample under analysis.

***Principle***

The presumptive test is specific for detection of coliform bacteria. Measured aliquots of the water to be tested are added to a lactose fermentation broth containing an inverted gas vial( Durham tubes)

Because these bacteria are capable of using lactose as a carbon source , their detection is facilitated by use of this medium. Tubes of this lactose medium are inoculated with 10-ml, 1-ml, and 0.1-ml aliquots of the water sample. The series consists of at least three groups, each composed of five tubes of the specified medium. The tubes in each group are then inoculated with the designated volume of the water sample as described under "procedure". The greater the number of tubes per group, the greater the sensitivity of the test. Development of gas in any of the tubes is presumptive evidence of the presence of coliform bacteria in the sample.

The most probable number(MPN)of coliforms of water sample can be estimated by the number of positive tubes(see MPN table)

**The MPN** test is a statistics-based test which estimates the number of fecal coliforms in a water sample based on the degree of lactose fermentation by organisms in the sample

***Materials***

Cultures

Water samples from sewage plant and tap

Media

lactose broth

Equipment

Bunsen burner, sterile 10-ml pipettes, sterile 1-ml pipettes, mechanical pipetting device, and glassware marking pencil.

***Procedure***

1-Set up five separate series consisting of three groups, in a test-tube rack; for each tube, label the water source and volume of sample inoculated.

2-Mix sewage plant water sample by shaking thoroughly. Exercise care in handling sewage waste water sample because enteric pathogens may be present.

3-Flame bottle and then using a 10-ml pipette, transfer 10-ml aliquots to the three tubes.

4-Flame bottle and then using a 1-ml pipette, transfer 1 ml of water to the three tubes.

. 5-Repeat steps 2 through 5 for the tap water sample

. 6-Incubate all tubes for 48 hours at 37 degrees centigrade

7-Examine all tubes after 24 and 48 hours of incubation. Record your results in the

chart as:

a. Positive: 10 percent or more of gas appears in a tube in 24 hours.

b. Doubtful: Gas develops in a tube after 48 hours.

c. Negative: There is no gas in the tube in the series in 48 hours



***Confirmed coliform test***

**Purpose**

To confirm the presence of coliform bacteria in a water sample for which the presumptive test was positive.

***Principle***

The presence of a positive or doubtful presumptive test immediately suggests that the water sample is non-portable. Confirmation of these results is necessary, since positive presumptive tests may be the result of organisms of non-coliform origin that are not recognized as indicators of

fecal pollution. The confirmed test requires that selective and differential media such as eosinmethylene blue (EMB) or endo agar be streaked from a positive lactose broth tube obtained from the presumptive test. Eosin-methylene blue agar contains the dye methylene blue, which inhibits

the growth of gram-positive organisms. In the present of an acid environment, EMB forms a complex that precipitates out onto the coliform colonies, producing dark centers and a green metallic sheen.

***Materials***

Cultures

One 24-hour-old positive broth culture from each of the three series from the presumptive test

Media/ Eosin-methylene blue agar plates .

Equipment/ Bunsen burner, glassware marking pencil, and inoculating loop.

***Procedure***

1-Label the covers of the three EMB .with the source of the water sample (sewage and tap).

2-Using a positive 24-hour lactose broth culture from the sewage water series from the presumptive test, streak the surface of one EMB .

3-Repeat Step 2 using the positive lactose broth cultures from tap water series to inoculate the remaining plates.

4-Incubate all plate cultures in an inverted position for 24 hours at 37 degree centigrade.

***Completed coliform test***

**Purpose**

To confirm the presence of coliform bacteria in a water sample, or, if necessary, to confirm a suspicious but doubtful result of the previous test.

***Principle***

The completed test is the final analysis of the water sample. It is used to examine the coliform colonies that appeared on the EMB or endo agar plates used in the confirmed test. An isolated colony is picked from the confirmatory test plate and inoculated into a tube of lactose broth and

streak on a nutrient agar slant to perform a Gram stain. Following inoculation and incubation, tubes showing acid and gas in the lactose broth and the presence of gram-negative bacilli on microscopic examination are further confirmation of the presence E.coli, and indicative of a positive completed test.

***Materials***

Cultures

One 24-hour coliform-positive EMB or endo agar culture from each of the three series of the confirmed test.

Media/ Nutrient agar slants and lactose broth

Reagents/ Crystal violet, Gram's iodine, 95 percent ethyl alcohol, and safranin.

Equipment/ Bunsen burner, staining tray, inoculating loop, lens paper, blotting paper, microscope, glassware and marking pencil.

***Procedure***

. 1-Label each tube with the source of its water sample

2-Inoculate one lactose broth and one nutrient agar slant from the same isolated *E. coli* colony obtained from an EMB .

. 3-Incubate all tubes for 24 hours at 37 degrees centigrade

***2-Quantitative analysis of water***

***Purpose***

To determine the quality of water samples using the membrane filter method.

***Principle***

Bacteria-tight membrane filters capable of retaining microorganisms larger than 0.45 micrometer are frequently used for analysis of water. These filters offer several advantages over the conventional, multiple-tube method of water analysis:

 (1) Results are available in a shorter period of time.

(2) Larger volumes of sample can be processed.

 (3) Because of the high accuracy of this method, the results are readily reproducible.

 A disadvantage involves the processing of turbid specimens that contain large quantities of suspended materials; particulate matter clogs the pores and inhibits passage of the specific volume of water.

 A water sample is passed through a sterile membrane filter that is housed in a special filter apparatus contained in a suction flask.

Following filtration, the filter disc that contains the trapped microorganisms is aseptically transferred to a sterile petri dish containing an absorbent pad saturated with a selective, differential liquid medium. Following incubation, the number of colonies present on the filter is

counted with the aid of a microscope.



