PRACTICAL 10 MICROBIOLOGICAL STUDY OF WATER

Structure

10.1 INTRODUCTION

Water is the basic need of life. River, streams and underground water is the prime source for water. However, disposal of industrial, domestic, human and animal wastes in these water sources is of prime concern for human health. Entry of sewage and polluted water in these water bodies increases the amount of organic matter, which serves as an excellent medium for the growth of various microorganisms both pathogenic and non-pathogenic. Among these, the presence of pathogenic microoganisms is of importance. In this practical, we shall therefore focus on the microbiological study of water. What are the types of microorganisms likely to be present in the water? Which are these microorganisms which affect the quality of drinking water? How to know the potability of water by detecting the presence of indicator organisms? These are a few issues covered in this practical.

Objectives

After undertaking this practical, you will be able to:

- familiarize yourself with the types of microbial pathogen present in the water sample, and
- know the quality of drinking water or to know the potability of water by detecting the presence of indicator organisms, i.e. coliforms.

10.2 MICROBIOLOGICAL STUDY OF WATER

Water is a common carrier of infectious diseases. Even clean and clear water which looks pure may be contaminated with pathogenic microorganisms and may pose a serious health hazard. Many important human pathogens can survive in water and infect humans. These belong to fungi, protozoa, algae, bacteria, actinomycetes and viruses and are responsible for wide range of intestinal infections, like, bacillary dysentery, giardiasis, typhoid etc. Table 10.1 lists the common water borne infectious diseases.

Table 10.1: Some of the common water borne infectious diseases

1.	*Giardia lamblia*	:	Giardiasis
2.	*Entamoeba histolytica*	:	Dysentery
3.	Poliomyelitis virus	:	Poliomyelitis
4.	Hepatitis A	:	Infectious hepatitis
5.	*Salmonella typhi*	:	Typhoid
6.	*Vibrio cholera*	:	Cholera
7.	*V. parahaemolyticus*	:	Diarrhea on shell fish consumption
8.	*Yersinia enterocolitica*	:	Waterborne gastroenteritis
9.	*Pseudomonas aeruginosa*	:	Swimmer's ear & related infections
10.	*Campylobacter* sp.	:	Diarrhoea
11.	*Helicobacter pylori*	:	Type B gastritis, Peptic Ulcer
12.	Cryptosporidium	:	Acute enterocolitis
13.	Naegleria fowleri	:	Primary amoebic meningoencephalitis

You may recall studying about these microorganisms in the theory course in unit 5. Transmission of these waterborne diseases can be controlled by water purification and by monitoring the level of contamination. However, it is not possible to examine water routinely for each and every pathogen as it is time and labour consuming. Therefore, it is easy to detect the presence of *indicator organism(s)* as an index of possible water contamination by human pathogens. These indicator organisms are usually associated with the intestinal tract and their presence signals the faecal contamination of the water source.

Following criteria are suggested for an indicator organism:

i. It should be suitable for the analysis of all types of water, i.e., tap, river, ground, impounded, recreational, estuary etc.

ii. It should be present whenever enteric pathogens are present.

iii. It should survive longer than the hardiest enteric bacterium.

iv. It should not reproduce in the contaminated water and produce an inflated value.

v. Testing procedure should be easy, specific, have high sensitivity and can detect even low levels of indicator.

vi. It should be harmless to human.

vii. Level of indicator organism in contaminated water should have some direct relationship to the degree of faecal pollution.

So, then which is the ideal indicator organism? Researchers are still searching for an ideal indicator organisms though number of indicators have been suggested to be used in sanitary microbiology. These are:

(i) *Coliforms* – These are widely used indicators which belongs to the family *Enterobacteriaceae* and make up about 10% of the intestinal microflora of human and other animals. These are facultative anaerobes, gram negatives, non sporing rods that ferment lactose with gas formation within 48 hours at 35°C. These include *E.coli*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*. Absence of these in water sample indicates the absence of faecal contamination and thus makes the water suitable for human consumption.

(ii) *Faecal Coliforms* – The faecal coliforms include a wide range of bacteria, many of which are not primarily the intestinal bacteria. Therefore, focus is on faecal coliforms which are derived from the intestine of warm blooded animals and grow at more restrictive temperature of 44.5°C.

(iii) *E. coli* – It is always present in human intestine so its presence indicates the faecal pollution and possible presence of other human or animal intestinal pathogens.

Other indicator microorganisms include total coliforms, faecal streptococci, faecal enterococci etc. *Faecal enterococci* are increasingly being used as an indicator of faecal contamination in brackish and marine water because of slower death rate.

So now we know who is the culprit. But how do we detect the presence of these organisms in water? This is an important aspect which is covered in the next section.

10.3 METHODS FOR COLIFORM DETECTION IN WATER

Sanitary condition of drinking water can be determined by counting the coliform bacteria. It is a reliable and reproducible indicator of faecal contamination in all public water supplies. Both qualitative and quantitative methods can be used. Commonly used methods for coliform detection in water samples are:

i) Most probable number procedure, and

ii) Membrane filtration procedure

Other tests like presence-absence test, coli test, defined substrate test and molecular techniques can be used to detect coliforms in water and other environment. In a well regulated water supply system, coliform tests should be negative. Positive tests indicates a breakdown in purification or distribution system.

A discussion on most probable number procedure – a coliform detection method is presented next.

10.3.1 Most Probable Number Test (Multiple Tube Fermentation Test)

Presence of coliforms in the water sample can be detected by performing multiple tube fermentation test which include 3 basic tests – Presumptive, Confirmed and Completed tests – to be carried out sequentially on each sample under analysis. The complete process requires at least 4 days of incubation and transfers. Let us then understand these three basic tests.

a) *Presumptive Test:*

The Presumptive test is used to detect and estimate coliform population of a water sample. This test is carried out by inoculating a series of lactose broth medium with three different sample volumes (10 ml, 1 ml and 0.1 ml). The series consists of 3 groups, each composed of 5 tubes and each group is inoculated with the designated volume of the water sample. The greater the number of tubes per group, the greater the sensitivity of the test. Lactose medium contains the lactose, bile salt and pH indicator. Lactose and bile salts are selective for coliforms as lactose is used only by coliforms and not by other enteric bacteria while bile salts are depressent for enteric bacteria other than coliforms. A pH indicator such as *bromocresol purple* is added to the medium to detect acid fermentation from lactose. Production of acid changes the colour of the medium to yellow as shown in Figure 10.1. Gas production during fermentation is detected by inverted Durham tube in lactose medium. Positive test for coliforms is indicated by the production of acid and gas from lactose fermentation after 24-48 hours of incubation. Number of tubes showing positive test is counted and the result is expressed as most probable number (MPN) of coliforms by matching the results with those provided in a statistical table provided in Table 10.2.

Figure 10.1: Lactose broth tubes showing colour change

Table 10.2: The MPN Index per 100 ml for combination of positive and negative presumptive test results when five 10-ml, five 1-ml, and five 0.1-ml portions of sample are used.

Number of Positive Tubes/5 Tubes				Number of Positive Tubes/5 Tubes			
Five of 10 ml each	Five of 1 ml each	Five of 0.1 ml each	MPN index per 100 ml	Five of 10 ml each	Five of 1 ml each	Five of 0.1 ml each	MPN Index per 100 ml
0	0	0	<2	4	2	1	26
0	0	1	2	4	3	0	27
0	1	0	2	4	3	1	33
0	2	0	4	4	4	0	34
1	0	0	2	5	0	0	23
1	0	1	4	5	0	1	31
1	1	0	4	5	0	2	43
1	1	1	6	5	1	0	33
1	2	0	6	5	1	1	46
2	0	0	5	5	1	2	63
2	0	1	7	5	2	0	49
2	1	0	7	5	2	1	70
2	1	1	9	5	2	2	94
2	2	0	9	5	3	0	79
2	3	0	12	5	3	1	110
3	0	0	8	5	3	2	140
3	0	1	11	5	3	3	180
3	1	0	11	5	4	0	130
3	1	1	14	5	4	1	170
3	2	0	14	5	4	2	220
3	2	1	17	5	4	3	280
3	3	0	17	5	4	4	350
4	0	0	13	5	5	0	240
4	0	1	17	5	5	1	350
4	1	0	17	5	5	2	540
4	1	1	21	5	5	3	920
4	1	2	26	5	5	4	1600
4	2	0	22	5	5	5	>2400

Having counted the most probable number of coliform present in a sample, next we need to undertake confirmation tests. Let us get to know how?

b) *Confirmed Test:*

Confirmed test is carried out to rule out the possibility of any positive presumptive test because of the presence of noncoliform organisms which are not recognized as indicators of faecal pollution. The test is performed by streaking on selective and differential media, i.e., EMB agar and Endo agar from positive lactose broth tube in presumptive test. EMB contains lactose and dyes eosin and methylene blue. Methylene blue inhibits the growth of Gram positive bacteria. Lactose fermenters produce acid which results in the precipitation of dyes on to coliform colonies producing dark coloured center and a green metallic sheen. These results are characteristics of *E.coli*, the major indicator of faecal pollution. *Enterobacter aerogenes* produce pink and mucoidal colonies while nonlactose fermenters produce colourless, transparent colonies.

Endo agar is a nutrient medium containing dye fuchsin in decolourized state which produce a pink complex in presence of acid produced by coliforms resulting in pink colonies of *E. coli*.

Once we have confirmed the presence of indicator organism, we further examine it by completed test. Let us look at this test next.

c) *Completed test:*

Coliform colonies on EMB or Endo agar are further examined by completed test by inoculating lactose broth and nutrient agar slants from isolated colonies in confirmed test. Production of acid and gas in lactose broth and presence of gram negative rods in nutrient agar slants finally confirms the presence of *E.coli* in water sample and is considered to be a positive completed test.

Having gone through the discussion above, the 3 basic tests involved in detecting coliform in a water sample must be clearly understood by you. Make sure the procedure involved is clear, because we will try out these tests in the laboratory. Also a knowledge about the differential media, which are required for these tests is essential. So let us get to learn how to make these media.

The composition of differential media i.e., EMB agar, endo agar, lactose broth is presented next.

Composition of EMB (Eosin methylene blue) agar (pH 7.2)

Peptone	10.0 gm
Lactose	5.0 gm
Dipotassium Phosphate	2.0 gm
Agar	13.5 gm
Eosin Y	0.4 gm
Methylene blue	0.065 gm
Distilled water	1000 ml

Composition of Endo agar (pH 7.5)

Peptone	10.0 gm
Lactose	10.0 gm
Dipotassium phosphate	3.5 gm
Sodium sulphite	2.5 gm
Basic Fuchsin	0.4 gm
Agar	15.0 gm
Distilled water	1000 ml

Composition of lactose broth (pH 6.9)

Lactose	5.0 gm
Beef extract	3.0 gm
Peptone	5.0 gm
Distilled water	1000 ml

For 2 x broth use twice the concentration of the ingredients.

With this information we complete our study about the steps involved in coliform detection. Let us quickly review what we have learnt so far by answering the questions given in Review Questions herewith.

10.4 REVIEW QUESTIONS

1. Define Coliform and explain Coliform Test.

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2. Why is the coliform test used to access the purity of drinking water?

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3. What is an indicator organism?

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4. What properties an indicator organism should have?

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5. What is the significance of performing confirmed and complete test?

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6. Why EMB or endo agar is used to detect *E.coli* presence in the sample?

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7. How coliforms can be differentiated from faecal coliforms?

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8. Why *E.coli* is chosen as indicator of water potability?

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Geared with this knowledge, now get started with the exercises 1 to 3, which will give you good practice of the three basic tests involved in microbiological testing of water.

EXERCISE 1

TESTING QUALITY OF WATER USING PRESUMPTIVE TEST

Date : .....

Aim : To check the quality of a given water sample(s) by presumptive test.

Requirements

Sample : 3 water samples e.g. tap water, distilled water, sewage water

Media : Lactose broth fermentation tubes : 15 tubes (2x) and 30 tubes (1x) per student,

Equipments and Glassware : Bunsen burner, test tubes and test tube rack, sterile pipettes – 10 ml and 1 ml, glass marker.

Theory/Principle:

(Write the principle behind the presumptive coliform test in the space provided herewith).

Procedure:

Now carry out the exercise following the steps indicated herewith. The procedure is also graphically represented in Figure 10.2. This will help you carrying out the steps.

1. Make 3 series of lactose broth fermentation tubes (one each for tap water, distilled water, sewage water) each consisting of 15 test tubes, 5 tubes of double (2x) strength and 10 tubes of single (1x) strength.
2. Label 5 tubes of double strength in each series as 10, 5 tubes of single strength as 1 and other 5 tubes of single strength as 0.1, to indicate the sample volume to be inoculated. Also label the tubes for water source. One uninoculated tube each of the double and single strength is kept as a control.
3. Mix the sewage sample thoroughly and inoculate each tube in a series labeled for sewage water with 10 ml (5 tubes of 2x), 1 ml (5 tubes of 1x) and 0.1 ml (5 tubes of 1x) of the sample aseptically.
4. Repeat step 3 for tap water as well as for distilled water.
5. Incubate the tubes at 37^{o}C for 24 hours.
6. Observe all the lactose fermentation tubes for acid and gas production. If the test is negative, incubate the tubes further for next 24 hours. Production of acid and gas after 24 hours incubation indicates a positive presumptive test for coliforms. If it develops after 48 hours of incubation, presumptive test is doubtful. No acid and gas production after 48 hours shows negative presumptive test, i.e., absence of coliforms.

A positive or doubtful presumptive test suggests non-potability of water and should be tested further by confirmed test.

Precautions:

1. Handle the sewage water with care as it may contain enteric pathogens.
2. Sewage and pond water should be shaked before inoculating the fermentation tubes.
3. After sterilization Durham tubes in lactose fermentation broth should be free from air bubbles.

Figure 10.2: Presumptive, Confirmed and Completed test for detection of Coliform in water sample

Observations and Results:

Now record the observations here in the format provided.

MPN per 100 ml of the water sample is seen from the MPN table given in section 10.2 by applying the combination of the positive and negative test results observed in 15 inoculated lactose fermentation tubes. Write this value in the MPN/100 ml column.

Sample	Observations			Result MPN/100 ml
	Volume inoculated (ml)	Number of tubes showing		
		Colour change	Gas production	
Sewage sample	10.0			
	1.0			
	0.1			
Distilled water	10.0			
	1.0			
	0.1			
Tap water	10.0			
	1.0			
	0.1			

Inference / Conclusion

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Submit the exercise along with the Review Questions you answered earlier for evaluation.

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Counsellor Signature

EXERCISE 2

CONFIRMATION OF THE PRESENCE OF COLIFORM BACTERIA IN POSITIVE PRESUMPTIVE TEST

Date : .....

Aim : To confirm the presence of coliform bacteria in positive presumptive test sample.

Requirements

Culture : positive / doubtful presumptive lactose broth tube.

Media : Eosin methylene blue agar plates or endo agar plates.

Equipments and Glassware : Bunsen burner or spirit lamp, inoculating loop, incubator.

Theory/Principle:

(Write the principle behind the confirmatory coliform test in the space provided herewith).

Procedure:

This test procedure is in continuation to the presumptive test procedure followed on last experiment. Now carry out this exercise following the steps enumerated herewith. Look up the procedure also illustrated in Figure 10.2.

1. Label the EMB/Endo agar plates with the source of the water sample.
2. Streak the plates with the respective positive or doubtful presumptive broth culture using sterile inoculating loop.
3. Incubate all the culture plates in an inverted position for 24 hours at 37°C.
4. Observe the plates for the presence or absence of *E.coli* colonies. Appearance of typical colonies with dark centres and metallic sheen indicates positive confirmed test, i.e., water is non potable.

Precautions:

1. Streaking should be done aseptically.
2. Plates should be incubated in an inverted position.

Observations and Results:

Record your observations in the format given herewith:

Observations		Results (comment on the presence of *E. coli*)
Source of water sample	**Colour of water (pink/white/dark colour)**	
Tap water		
Distilled water		
Sewage sample		

Inference/Conclusion

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Submit the exercise for evaluation.

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Counsellor Signature

EXERCISE 3

PERFORMING THE COMPLETED COLIFORM TEST

Date : .....

Aim : To confirm the presence of coliform bacteria in a water sample by completed coliform test.

Requirements

Culture : 24 hours coliform positive EMB agar/Endo agar culture plate from confirmed test.

Media and Reagents : Lactose fermentation broth tubes and nutrient agar slants, Gram's iodine, crystal violet, 95% ethanol and safranin.

Equipments and Glassware : Bunsen burner, inoculating loop, glass slides, blotting sheet, permanent marker, microscope, immersion oil.

Theory/Principle:

(Write the principle behind the completed coliform test in the space provided herewith).

Procedure:

Completed test is the last step in the Coliform test procedure which is described herewith. Conduct the exercise following the steps enumerated herewith and also illustrated in Figure 10.2.

1. Label the lactose broth tubes and nutrient agar slants with the source of water sample.
2. Inoculate the lactose broth tubes and nutrient agar slants with the isolated coliform colonies on the EMB/Endo agar plates from each of the experimental water samples.
3. Incubate the inoculated broth tubes and slants at 37^{o}C for 24 hours.
4. Examine lactose broth fermentation tubes for production of acid and gas and nutrient slants for gram reaction and cell morphology.
5. Production of acid and gas in inoculated lactose broth and presence of gram negative rods in nutrient slants indicate a positive complete test and confirms the presence of coliforms in water sample.

Precautions:

1. Aseptic conditions should be adopted while performing the experiment.
2. Destaining is the critical step in grams reaction. It is to be performed till the colour comes out from the slide.

Observations and Results:

Record your observations in the format given herewith:

Observations			Results
Water Sample	**Acid and gas production on lactose broth (+/—)**	**Gram's reaction (+ or —)**	
Tap water			
Distilled water			
Sewage water			

Inference / Conclusion

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Submit the exercise for evaluation.

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Counsellor Signature