5. SAMPLING AND MICROBIOLOGICAL EXAMINATION OF WATER

SCOPE:

To examine water for microbiological parameters such as SPC and coliform.

DILUENT, CULTURE MEDIA AND REAGENTS:

- Plate Count Agar (PCA)
- MacConkey Broth

APPARATUS, INSTRUMENTS AND GLASSWARES:

- Laminar airflow chamber
- Autoclave
- Hot air oven
- Sterile petri plates
- Colony counter
- Weighing balance
- Dilution bottles
- Water bath
- Test tubes
- Sterile spatula
- Micropipette
- Sterile tips

PROCEDURE:

SAMPLING:

- Sterilize the plastic bottles used for collecting the water samples in autoclave at 121^{o}C ± 0.5 ^{o}C for 15 lbs for 15 min. (If the water to be sampled contains or likely to contain chlorine, sodium thiosulphate can be added in the sample to give a final concentration of 100mg/litre. This can be done by adding 0.5ml of 5% sodium thiosulphate solution to a 250ml bottle before sterilization).
- Spray 70% isopropanol on the outside of the tap nozzle and allow it to dry.
- Open the tap fully and allow water to run for 2 to 3 minutes.
- Restrict the flow to permit filling the bottle without splashing.
- Held the sterile plastic bottle close to the tap, remove the lid, fill approximately 200ml

of water without rinsing and immediately replace the lid.

- Mark the bottles with sampling point, section, time, shift & date of collection and keep it refrigerated until analysis.

STANDARD PLATE COUNT:

- Transfer 1 ml of water sample into sterile Petri plates with duplicates.
- Pour about 15 to 20 ml of sterile plate count agar (previously melted and cooled to 45°C) in each plate.
- Mix the content well and allow to solidify on a levelled surface.
- Incubate the plates at 37°C for 24 hours.
- After incubation, observe the colonies, count the numbers and report the average of the plates as the number of cfu/ml of water sample.

COLIFORM:

- Transfer 50 ml portion of the sample into a bottle containing 50 ml double strength MacConkey broth and also transfer 10 ml portions of the sample into 5 tubes of double strength MacConkey broth.
- Incubate at 37°C for 24 - 48 hrs. Examine the bottle and each tube at the end of 24 hours and at the end of 48 hours for acid and gas production. Record the presence or absence of acid and gas at each examination of the tubes regardless of the amount.
- Formation of the acid and gas within 48 ± 3 hrs in any amount, in the Durham's tubes constitutes a positive test. The absence of acid and gas formation at the end of 48 ± 3 hrs of incubation constitutes a negative test.
- Report the result as Coliform MPN/100 ml based on the number of positive tubes with reference to MPN table.

MPN TABLE

Most probable number (MPN) of organisms per 100 ml of sample using 1 tube of 50 ml and 5 tubes of 10 ml

Number of Positive Tubes		MPN/100 ml	Limits within which MPN/100 ml can lie	
50 ml Tubes	10 ml Tubes		Lower Limit	Upper Limit
0	1	1	<0.5	4
0	2	2	<0.5	6
0	3	4	<0.5	11
0	4	5	1	13
1	0	2	<0.5	6
1	1	3	<0.5	9
1	2	6	1	15
1	3	9	2	21
1	4	16	4	40

REFERENCE:

IS 1622 - 1981 Reaff 2009, Methods of Sampling and Microbiological Examination of Water