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Standard Operating Procedure

Membrane Filtration Technique

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Membrane Filtration Technique for Total coliforms, Fecal coliforms and *Escherichia coli*	Approved by:	Shailaja Adhikari

1. Objectives and Scope

This document is Kathmandu Upatyaka Khanepani Limited (KUKL) Standard Operating Procedure (SOP) for the analysis of Total coliform, Fecal Coliform and *E. coli* in drinking water and Waste-water using Membrane Filtration Technique.

2. Applicability

This SOP includes the procedure for media preparation, quality control, sample collection, transportation and detection and enumeration of total, fecal coliform and *E. coli* by filtration using membrane filter and inoculation of filter paper on agar medium.

3. Definitions

Coliform: It is a group of aerobic and facultative anaerobic, gram negative, non-sporing bacilli that are present in the intestine of warm-blooded animals and can ferment lactose with the production of acid and gas within 48 hours at 37±1°C. It includes members of *Enterobacteriaceae* viz., the genera *Klebsiella, Escherichia, Enterobacter and Citrobacter*.

Fecal coliform: It is a subgroup of coliform which grows at higher temperature and is only associated with the fecal matter of warm-blooded animals.

Escherichia coli*: *E. coli conforms to the definitions of *Enterobacteriaceae*, coliforms, and fecal coliforms, but is further identified by an IMViC (indole, methyl red, Voges-Proskauer, citrate utilization) pattern of + + - - or - + - -. Its natural habitat is the intestines of vertebrate animals. Thus, the presence of *E. coli* in water indicates the possibility that fecal contamination has occurred and that other microorganisms of fecal origin, including pathogens, may be present. At present, *E. coli* is the best indicator of fecal contamination among the commonly used fecal-indicator organisms.

4. Responsibilities

Laboratory technicians, Microbiologists

5. Equipment, Reagents, and Supplies

- Sterile Sampling bottles
- Pipettes and graduated cylinders
- Containers for culture medium
- Culture dishes
- Sampling bottle labels and waterproof markers
- Forceps
- Ice box
- Filtration unit
- Autoclave
- Incubator
- Membrane filter
- Microscope
- Culture media (m-ENDO agar, EC broth, Biochemical media)
- Nutrient agar

6. Operation Procedure

I. Selection of sample size

Sample size is by expected bacterial density, degree of turbidity and, if applicable, regulatory requirements (Table 1).

Table 1: Suggested sample volume for Membrane filter total coliform test

Water source	Volume (X) to be filtered (mL)							
	100	50	10	1	0.1	0.01	0.001	0.0001
Drinking water	X							
Swimming pools	X							
Wells, springs	X	X	X					
Lakes, reservoirs	X	X	X					
Water supply intake			X	X	X			
River water				X	X	X	X	
Chlorinated sewage				X	X	X		
Raw sewage					X	X	X	X

An ideal sample volume will yield 20 to 80 total coliform colonies and less than/equal to 200 colonies of all types (typical, atypical, and non-coliform background colonies) on a membrane-filter surface.

Dilution: When filtering <10 mL of sample (diluted or undiluted), add approximately 10 mL sterile buffered dilution water to the funnel and then add sample followed by another 25 to 50 mL dilution water before filtration or pipet the sample volume into sterile dilution water and then filter the entire contents of dilution bottle (This increase in water volume helps disperse the bacterial suspension uniformly over the entire effective filtering surface).

II. Sampling, transportation, and storage:

- Turn the tap on full, and allow the water to run to waste for 1 minute.
- Hold the sterile bottle by the base in one hand.
- Use the other hand to remove the stopper and cover together.
- Do not touch the screw thread of the bottle neck or the inside of the cap.
- Replace the stopper and cover.
- Label the bottles properly.
- Transport the samples within 6 hours to the testing laboratory at room temperature. If delay is unavoidable, place the sample in icebox and transport to the laboratory at 4°C within 24 hours.
- *Neutralizing chlorine in water samples*: When the water to be examined is likely to contain chlorine or chloramine, add 0.1–0.2 mL sodium thiosulphate 30 g/l (3% w/v) to each bottle of 200 mL capacity before the bottle is sterilized to neutralize those substances.

III. Media preparation:

Media should be prepared in accordance with the manufacturer's instructions, as follows:

- Dissolve the stated amount of the dehydrated medium (m-ENDO agar/ EMB agar) in distilled water
- Boil to dissolve
- In case of m-ENDO agar, use 95% ethanol (20mL in 980mL media) for sterilization
- In case of EMB agar, sterilize in an autoclave. Use the autoclave tape for confirmation of sterilization with each batch of the items sterilized.

- Aseptically, pour the media into petri dishes (20-25mL in each plate)
- Let the petri dishes remain in room temperature for solidification.
- Store the medium at 2-8°C.
- Warm the medium to room temperature before use to ensure that all components have been dissolved.

IV. Quality control and assurance of culture media:

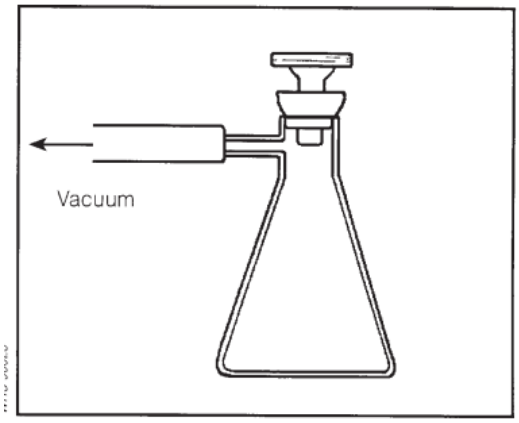
- For sterility control, a representative sample of each lot/batch of medium is incubated 37°C for 24 hours. As a general rule, for a lot of 100 or less units a 3-5% sample should be tested. For a larger lot, 10 random plates or tubes are taken. There should be no evidence of microbial growth after incubation. Discard all sterility samples when the tests have been completed.
- For utility control, inoculate *Escherichia coli* ATCC 00013 and incubate at 37°C for 24 hours.
- Stability test: Periodically perform the above procedures on stored prepared media in order to determine whether the storage conditions will give optimal results.

V. Sterilization of filtration units and Quality control

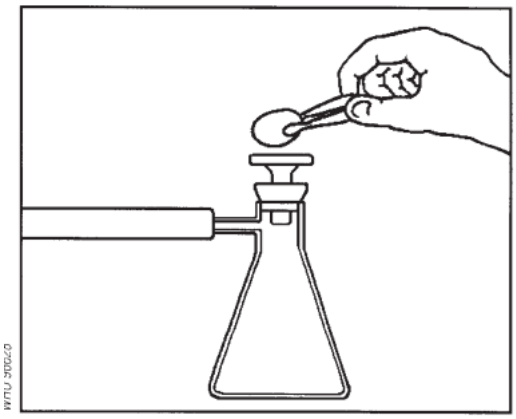
- Use sterile filtration units at the beginning of each filtration series
- Sterilize all units again if there is interruption of sample filtration for 30 minutes or longer

VI. Sample processing

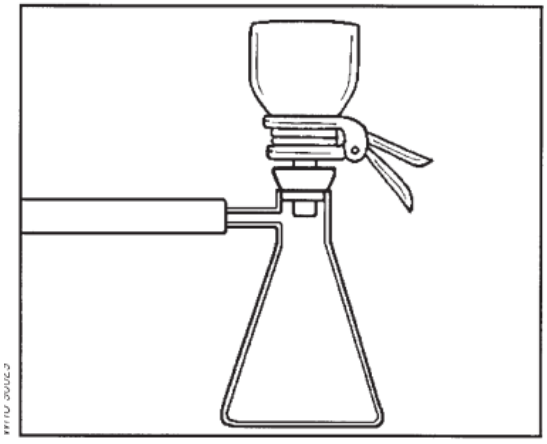
- Refer Table 1 for appropriate sample volume to be used
- Assemble the filtration apparatus aseptically



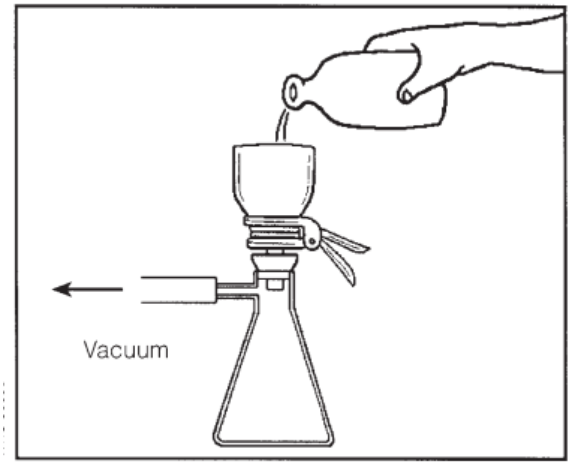
- Place the sterile membrane filter (grid side up) over the porous disc of the base, using sterile forceps



- Place the upper container in position and secure it. (The type of clamp used will depend on the type of equipment.)



- Pour the volume of sample chosen as optimal for the type of water (see Table 1) into the upper container and apply vacuum



- Invert the petri dish and incubate at 35 or 37°C (for total coliforms) and 44.5±0.2°C (fecal coliforms) for 18-24 hours

7. Results interpretation

- Typical colonies (pink to dark red color with a greenish metallic sheen) media represent coliform and atypical colonies (dark red/mucoid/nucleated without sheen) are represent non-coliforms
- Count both typical and atypical colonies promptly after incubation.
- For thermotolerant coliforms confirmation, transfer loopful of typical colonies to EC broth and incubate at 44.5±0.2°C for 24 hours. Gas production confirms thermotolerant coliforms
- For *E. coli* confirmation, inoculate typical colonies into EC-MUG medium and incubate at 44.5±0.2°C for 24 hours. Gas production and fluorescence under UV light confirms *E. coli*

8. Calculation of Coliform density

➢ Select membrane with acceptable number of colonies (Table 2) and ≤ 200 colony-forming units (CFU) per membrane, by following equation

$$\text{Total coliforms/100mL} = \frac{\text{coliform colonies counted} \times 100}{\text{volume (mL) of sample filtered}}$$

Table 2: Number of colonies in the ideal range for Quantitative determination

Test	**Colony Counting Range**	
	Minimum	**Maximum**
Total coliform	20	80
Fecal coliform	20	60
Fecal streptococci	20	100
Enterococci	20	60
E. coli	20	80

9. References

- APHA, 23rd Edition
- WHO guidelines, 4th Edition