Practical activity (10)

Microbial Examination of Water Samples Using the Membrane Filtration Technique

(Teacher's Guide)

I. Objectives of the experiment

1. To determine the microbiological quality of tap water and recreational water (swimming pool water) samples; and
2. To draw inferences about the suitability of the tested water for human consumption or for swimming.

II. Expected Learning Outcomes

Upon completion of the learning activities, students should be able to:

1. perform membrane filtration of water samples;
2. evaluate the microbiological quality of different water samples using the membrane filtration technique;
3. understand the relationship between the number of coliforms and water quality; and
4. recognise the safety standards of water for drinking and recreational uses.

III. Teaching notes:

1. Introduce the task by going through the "Background" and "Scenario" with students or have the students read through these parts and the "Guiding questions about the design of the experiment" as a pre-class activity.
2. Discuss the design of the experiment with the students using the "Guiding questions about the design of the experiment".
3. Plan the laboratory work with reference to the "Time allocation for the experimental activities" in section IV according to the lesson time of your school.
4. Always remind the students about the safety precautions of each part of the experiment.
5. Go through the "Results" and "Discussion" with the students.

IV. Time allocation for the experimental activities:

Practical work		Duration	
		Within class	**Out of class** (to be done by the laboratory technicians)
Lab session 1: Part (1) of the experiment (optional: may be prepared by the laboratory technicians)			
1	Collection of tap water sample	20 min	
2	Collection of water sample from the swimming pool	40 min (excluding travelling time)	
3	Storage of water samples at $4^{o}C$		Not more than 2 days
Lab session 2: Part (2) of the experiment			
1	Setup of a vacuum filtration unit	10 min	
2	Serial dilution of water samples	20 min	
3	Membrane filtration of water samples	50 min	
4	Incubation of membrane filters		1−3 days
Lab session 3: Part (3) of the experiment			
1	Counting colony-forming units and analysis of experimental results	30 min	
Total lesson time for the experimental activities		Lab session 1: 60 min Lab session 2: 1 hr 20 min Lab session 3: 30 min	

V. Equipment, materials, and preparatory work for the experiments

A. Part (1) of the experiment: Collection of water samples

a) Equipment (per group)

- Icebox × 1

b) Materials (per group)

- Sterile 200 ml sample bottle × 2
- Permanent marker × 1

c) Preparatory work

No specific preparatory work needed

B. Part (2) of the experiment: Membrane filtration of water samples and incubation of microbes on the membrane filters

a) Equipment (per group)

- Bunsen burner × 1
- Spark lighter × 1
- Complete membrane filtration apparatus × 1

b) Materials (per group)

- Sample collected in Part (1) × 2
- Dilution bottle with 99 ml sterile buffered solution × 3
- Sterilised 250 ml measuring cylinder × 7
- Sterilised 20 ml measuring cylinder × 3
- Sterile membrane filter × 7
- Forceps × 1
- LB agar plate × 7
- Sterilised distilled water (100 ml) × 1
- *E. coli* solution (100 ml) × 1

- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Sterilised distilled water in wash bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

c) Preparatory work

Preparation of LB agar plates and *E. coli* solution **(To be done by teacher/laboratory technicians one day before lab session)**

1. Dissolve LB agar powder in distilled water according to the manufacturer's instructions. Autoclave the LB agar solution. Pour it into Petri dishes (15–20 ml per dish). As soon as the agar is solidified, wrap the dishes with parafilm and store them properly at 4°C until use. Prepare 7 LB agar plates per group. If there is not enough time, purchase ready-to-use LB agar plates from biotechnology companies.

2. Grow a tube of *E. coli* culture (Sigma-Aldrich or Promega) in an appropriate broth medium (e.g. LB broth) for about 16 hours. Make a 1:100 dilution of the overnight culture of *E. coli* cells. Aliquot 100 ml *E. coli* solution into sterile bottles. Prepare 1 bottle per group.

Sigma-Aldrich distributor in Hong Kong: Tin Hang Technology; Tel: 2817 2121
Website: www.tinhangtech.com/home/

Promega distributor in Hong Kong: Bio-Gene Technology Ltd; Tel: 2646 6101
Website: www.bio-gene.com.hk

Preparation of sterile buffered solution and other related solutions **(To be done by teacher/laboratory technicians one day before lab session)**

Phosphate buffer solution

1. Dissolve 34.0 g potassium dihydrogen phosphate, KH_2PO_4, in 500 ml distilled water.
2. Using a pH meter, adjust this solution to pH 7.2 with 1N NaOH. This can be purchased from biotechnology companies.
3. Dilute to 1 litre with sterile distilled water, using a measuring cylinder.

Magnesium chloride solution

1. Dissolve 81.1 g magnesium chloride hexahydrate, $MgCl_2$ $6H_2O$, in distilled water. Dilute to 1 litre.

Preparation of sterile buffered solution

1. In a 1 L bottle, add 1.25 ml of stock phosphate buffer solution.
2. Add 5 ml of magnesium chloride solution.
3. Fill to a final volume of 1 litre with sterile distilled water.
4. Autoclave the solution.

Aliquot of the buffered solution and distilled water **(To be done by teacher/laboratory technicians one day before lab session)**

1. Aliquot 99 ml autoclaved buffered solution into sterile dilution bottles. Prepare 3 bottles per group.

2. Aliquot 100 ml distilled water into sterile dilution bottles. Prepare 1 bottle per group.

Sterilisation and disposal

After the experiment, some equipment and materials to discard (solid or liquid) should be sterilised by steam under pressure (autoclave) at 121°C, 15 psi pressure for at least 30 minutes or immersed in 10% chlorine bleach for at least 2 hours.

C. Part (3) of the experiment: Analysis of experimental results

a) Equipment (per group)

No equipment needed

b) Materials (per group)

- LB agar plate from Part (2) × 7
- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

c) Preparatory work

No specific preparatory work needed

d) Sterilisation and disposal

After the experiment, some equipment and materials to discard (solid or liquid) should be sterilised by steam under pressure (autoclave) at 121°C, 15 psi pressure for at least 30 minutes or immersed in 10% chlorine bleach for at least 2 hours.

VI. Suggested answers to "guiding questions about the design of the experiment"

1. Why do we need to test the microbial quality of water samples?

   Testing of microbiological quality of water can protect the public from disease and illness caused by the consumption of water that may contain disease-causing bacteria, thus preventing waterborne disease outbreaks.

2. What does the presence of coliforms in the water sample indicate?

   The presence of coliforms in water samples shows that the water is not safe for human consumption and if consumed, it may cause health problems.

3. What is the acceptable level of coliforms for tap water?

   Ideally, the coliform count in tap water should be 0 CFU/100 ml

   Reference link:

   https://www.wsd.gov.hk/en/core-businesses/water-quality/my-drinking-water-quality/hong-kong-drinking-water-standards/index.html

4. What is the acceptable total bacterial count for swimming pool water?

   Ideally, the total bacterial count for swimming pool water should not exceed 200 bacteria/ml of pool water sample.

   Reference link:

   https://www.lcsd.gov.hk/en/beach/atten-general/swim-watqal.html

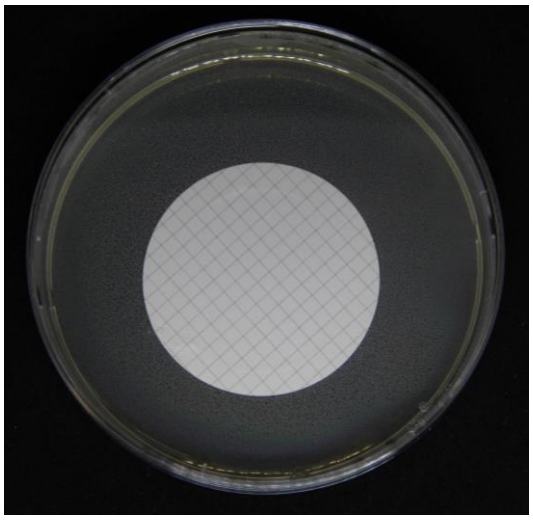
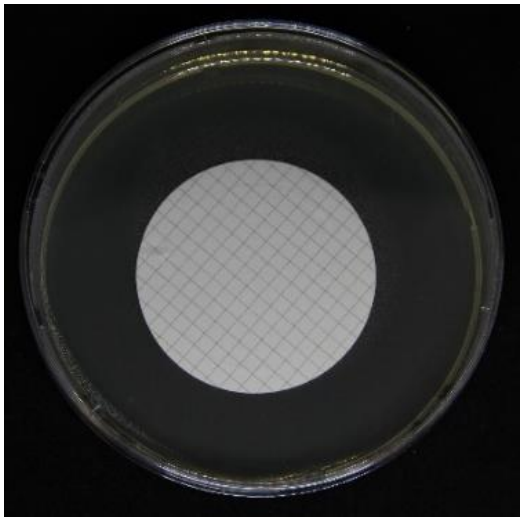
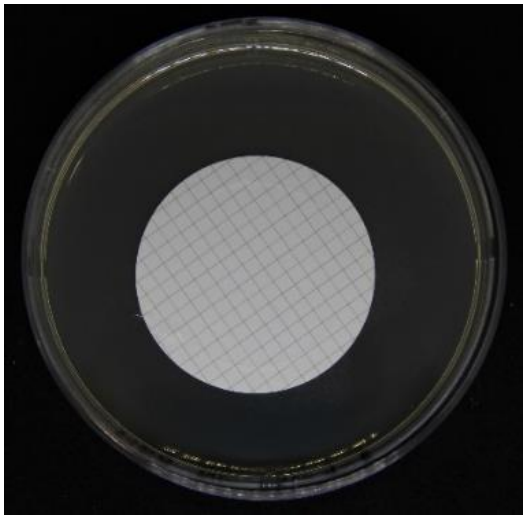
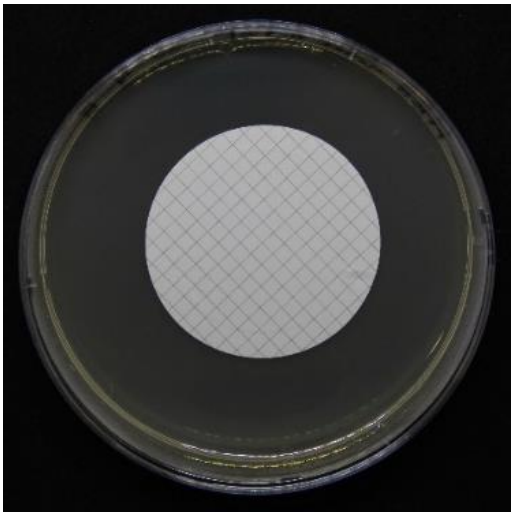
   (Note: The coliform count may be higher in each source if

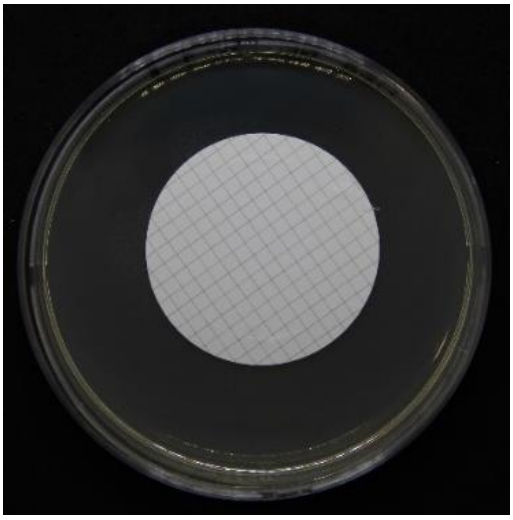
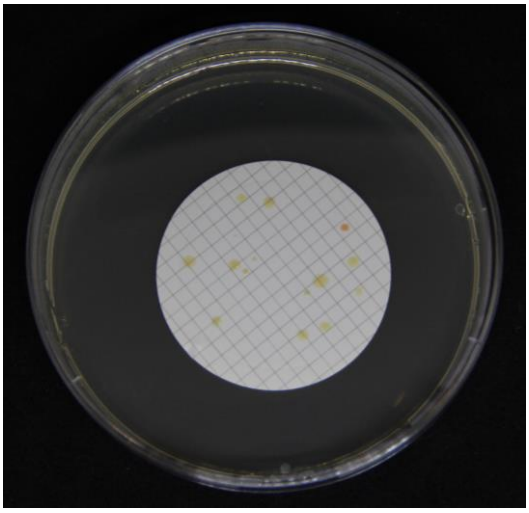
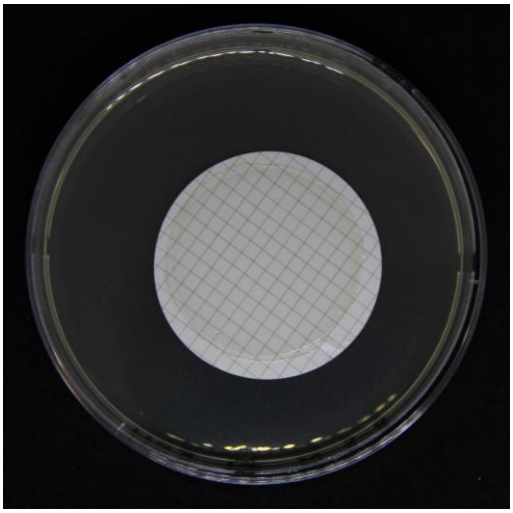
   - *there is contamination of a water source by human activity*
   - *there is leakage in water supply lines*
   - *the water is not effectively disinfected*
   - *there is poor swimming hygiene)*

VII. Results

1. Photo or drawing of the membrane filters of the samples (after incubation on the LB agar plates).

   [For reference only]

(1) Sterilised distilled water sample 	**(2) Tap water sample** 
(3) Swimming pool sample (1:1000) 	**(4) Swimming pool sample (**1:100) 

(5) Swimming pool sample (1:10) 	**(6) Undiluted swimming pool sample** 
(7) *E.coli* solution 	

2. Complete the table below.

Table 2

Agar plate	1	2	3	4	5	6	7
Label of sample	H_2O	Tap	SP; 1:1000	SP; 1:100	SP; 1:10	SP	*E. coli*
Sample	Sterilised distilled water (negative control)	Tap water	Swimming pool water; 1:1000	Swimming pool water; 1:100	Swimming pool water; 1:10	Swimming pool water; undiluted	*E. coli* solution (positive control)
CFU	*nil*	*nil*	*nil*	*nil*	*nil*	*15*	*TNTC*
CFU / 100 ml	*nil*	*nil*	*nil*	*nil*	*nil*	*17 (=15/90 x100)*	*TNTC*

VIII. Discussion

1. Compare the results of your water samples with plate 1 (negative control). Are the results close to what you expected?

   The results are close to my expectation. Although the number of bacterial colonies found in most water samples is the same as that of plate 1 (nil) as expected, the undiluted swimming pool water sample shows a detectable level of bacteria. Such a result indicates that the swimming pool water is not fit for human consumption, but it is suitable for recreational use.

   Note: If any colonies are found in the negative control, there's contamination. Such contamination may be derived from other environmental/external sources if the sample was not handled properly. Based on the same principle, the number of CFU in a given sample may be higher than expected if contamination occurs. Thus, a further confirmation experiment is needed.

2. Why is a positive control needed for this experiment?

   The high count of CFU in plate 7 should indicate proper execution of the membrane filtration and microbial incubation procedures, because the E. coli solution should give rise to an observable microbial growth. If a high count of CFU is not obtained in plate 7, then errors existed in the experiments. The underestimation of bacterial count in the water samples may cause a serious problem.

3. What factors in sample collection and filtration processes might have influenced your results?

   - Contamination of sample bottles and/or membrane filters
   - Pipetting errors in experiments
   - Improper handling of water samples

4. Why is sample dilution required for some sample types?

   If the bacterial level in the source of the water sample is presumed to be very high, there is a possibility there will be more than 250 CFU on the membrane filter, which is out of the countable range.

   If the turbidity of the water sample is very high, based on the mentioned principle, there is a high possibility of having more than 250 CFU on the membrane filter, which is out of the countable range. Therefore, dilution is required in this situation as well.

5. Is the tested water suitable for human consumption / recreational use? Give a brief explanation on the suitability of each water sample.

   The microbiological quality of tap water is good, as no bacterial colony is obtained, even from the undiluted sample. Thus, the tap water sample is suitable for human consumption.

   The microbiological quality of swimming pool water is not so good, as bacterial colonies are detected from the undiluted sample. The CFU count is 17 per 100 ml. However, such a result is still within the acceptable limit according to the total bacterial count standard given by the LCSD. Thus, the swimming pool water is suitable for human recreational use.

   Website: www.lcsd.gov.hk/en/beach/atten-general/swim-watqal.html.

IX. References

Bartram, J., & Ballance, R. (1996). *Water quality monitoring–A practical guide to the design and implementation of freshwater quality studies and monitoring programmes*. Boca Raton, FL: CRC Press.

Forster, B., & Arango Pinedo, C. (Eds.). (2015). Bacteriological examination of waters: membrane filtration protocol. *American Society for Microbiology* (ASM).

National Environmental Methods Index. EPA-approved method for analysis of E. coli in ambient water. *Standard Methods*, 20th ed., 9213D.

Pan American Health Organization. *Manual for bacteriological analysis of natural water supply sources in disaster situations*.

Pelczar, M. J., Chan, E. C. S., & Krieg, N. R. (2007). *Microbiology*. 5th edn. New Delhi: Tata McGraw Hill-Education.

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