FE 204 Experiment 5
Temperature Lethal Effect and Membrane Filtration Method

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- Procedure

Temperature Lethal Effect

- Temperature is one of the most important factor influencing the activity of bacterial enzymes.
- Enzymes have minimum, optimum and maximum activity temperature.
- Microorganisms vary considerably in their temperature response.

Temperature Lethal Effect

Classification	Growth Temperature (°C)			Example
	Minimum	Optimum	Maximum	
Psychrophiles	-10	12-15	20	*Vibrio marinus*
Psychrotropic	-5	20	30	*Pseudomonas* spp.
Mesophiles	10	30-40	45	*E.coli*
Thermoduric	20	50-55	65	*Microbacterium*
Thermophiles	45	55-60	70	*B.stearothermophilus*

Temperature Lethal Effect

- At the optimum temperature, the enzymatic reaction progress at maximum rates.
- Below the minimum point, enzyme become inactive.
- Above the maximum point, enzyme destructing will be ocur.

Temperature Lethal Effect

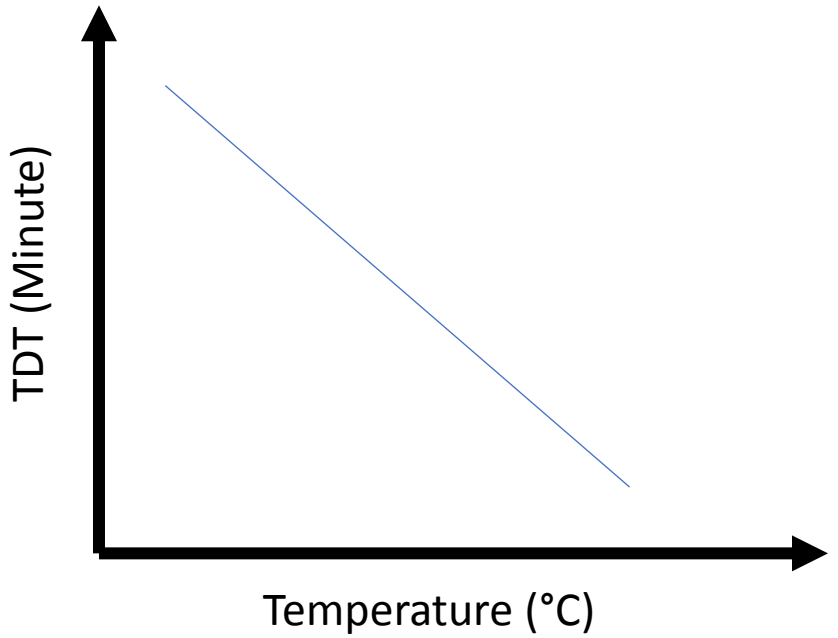
- In attempting to compare the susceptibility of different microorganisms to elevated temperatures, two methods are used:
  - **Thermal death point (TDP)**
  - **Thermal death time (TDT)**

Temperature Lethal Effect

- **Thermal death point (TDP):** TDP is the **temperature** at which all microorganism are killed in 10 min.
- İts related to **temperature**.

Temperature Lethal Effect

- **Thermal death time (TDT):** TDT is **time** reffered to kill all microorganism at a given temperature.
- İts related to **time**.



Material used in LAB

- Broth cultures of different microorganism
- Nutrient agar plate
- Nutrient broth tubes
- Wire loop
- Pipettes
- Glass spreaders
- Incubator

Procedure

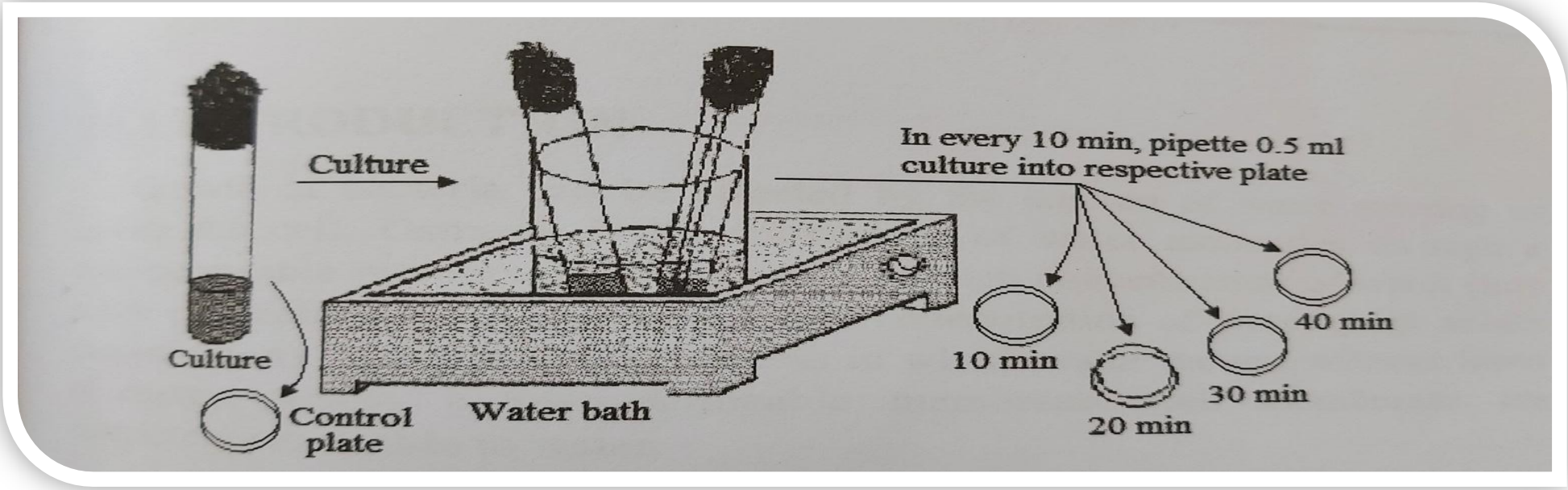
1) 24-hour nutrient broth cultures of *Escherichia coli* and *B.stearothermophilus* prepared.

2) Label plates with holding time (Control, 10, 20, 30 and 40 min) for the each temperature (40, 50, 60, 70, 80 °C).

Procedure

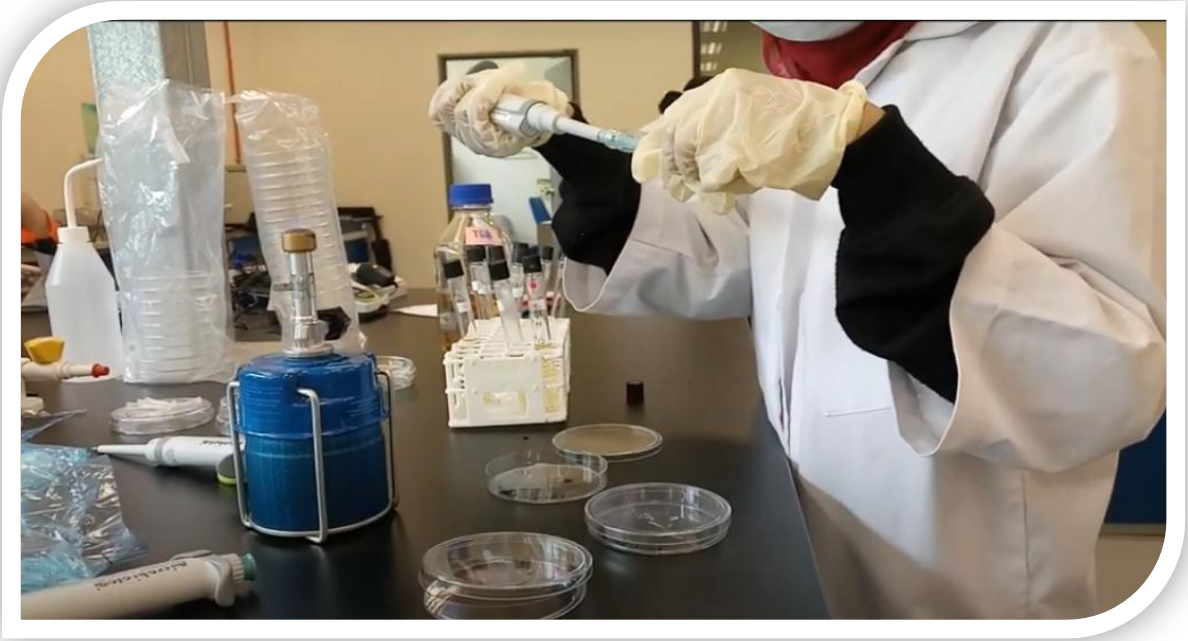
3) Inoculate to the control plate with 0.5 mL of culture before placing the tube in the water bath.

4) Place the culture and sterile nutrient broth into water bath.

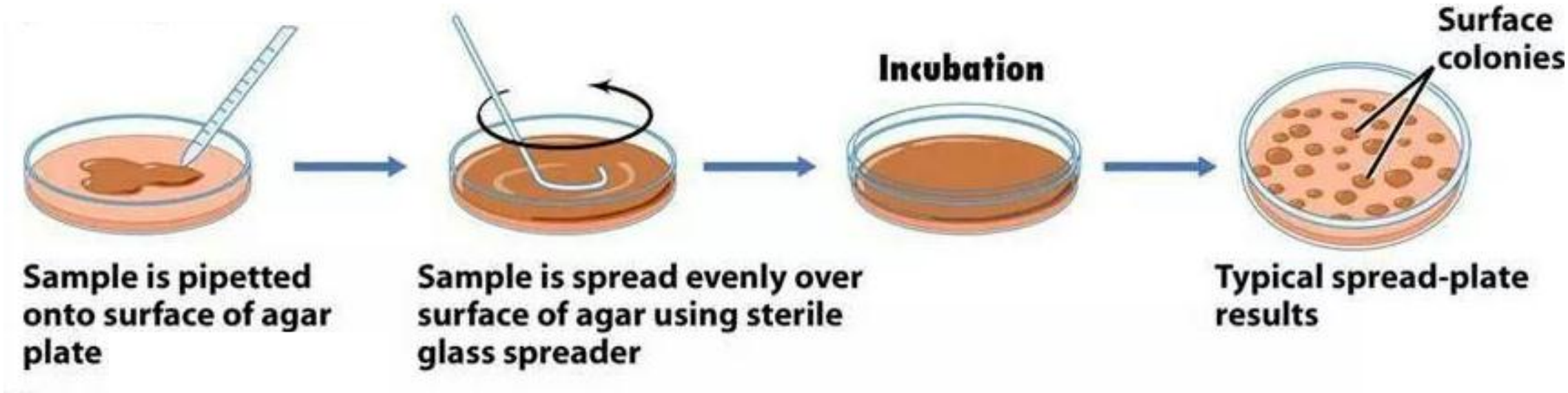


Procedure

5) After holding time has elapsed, spread plate 0.5 mL of culture.



Procedure


Incubation
Surface colonies
Sample is pipetted onto surface of agar plate
Sample is spread evenly over surface of agar using sterile glass spreader
Typical spread-plate results

Procedure

7) Incubate inverted plates at 35°C for 24 h. Examine plates and record the results.

Results

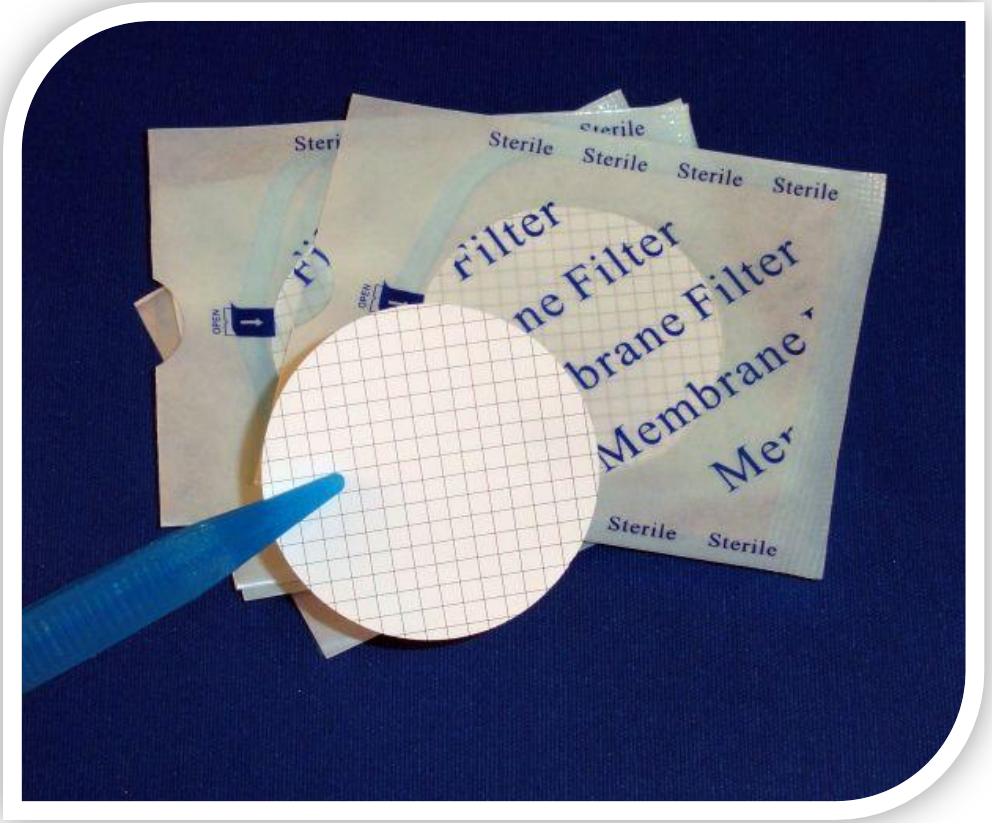
	Escherichia coli					*B.stearothermophilus*				
	40°C	**50°C**	**60°C**	**70°C**	**80°C**	**40°C**	**50°C**	**60°C**	**70°C**	**80°C**
Control	□	□	□	□	□	□	□	□	□	□
10'	□	□	□	×	×	□	□	□	□	□
20'	□	□	□	×	×	□	□	□	□	□
30'	□	□	×	×	×	□	□	□	□	×
40'	□	□	×	×	×	□	□	□	□	×

Results

	B.Suptilis				
	40°C	50°C	60°C	70°C	80°C
0'	□	□	□	□	□
10'	□	□	□	□	✕
20'	□	□	✕	✕	✕
30'	□	✕	✕	✕	✕
40'	□	✕	✕	✕	✕

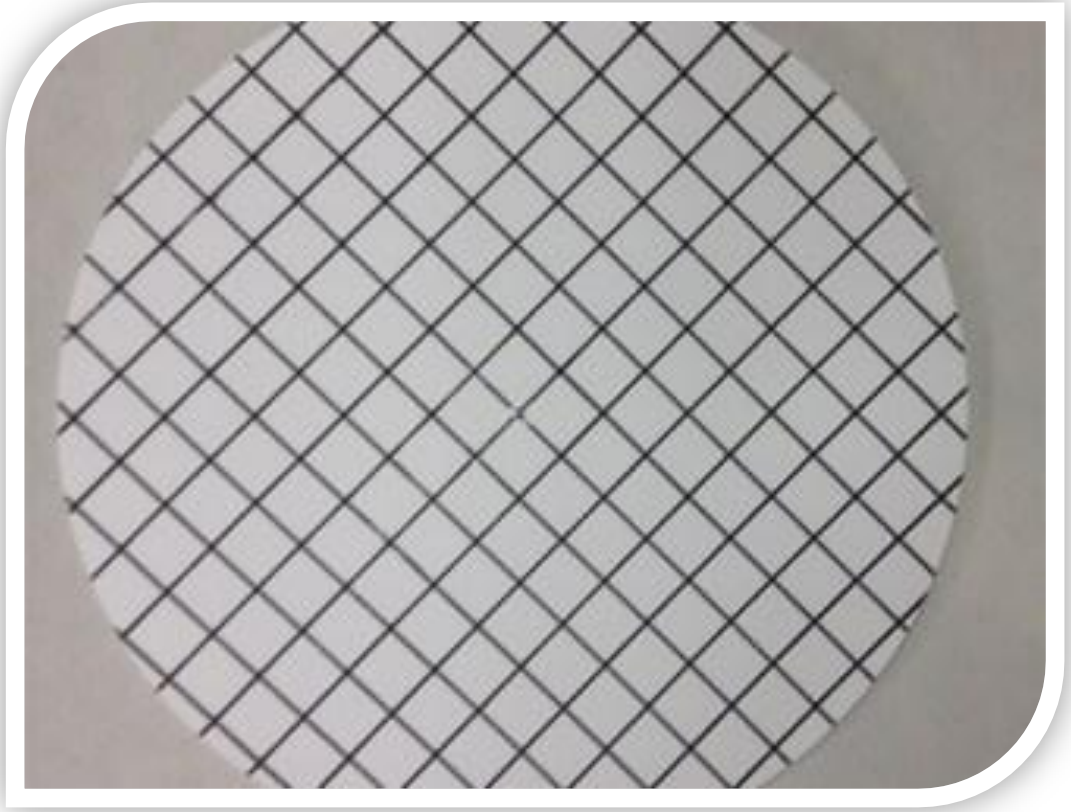
Membrane Filtration

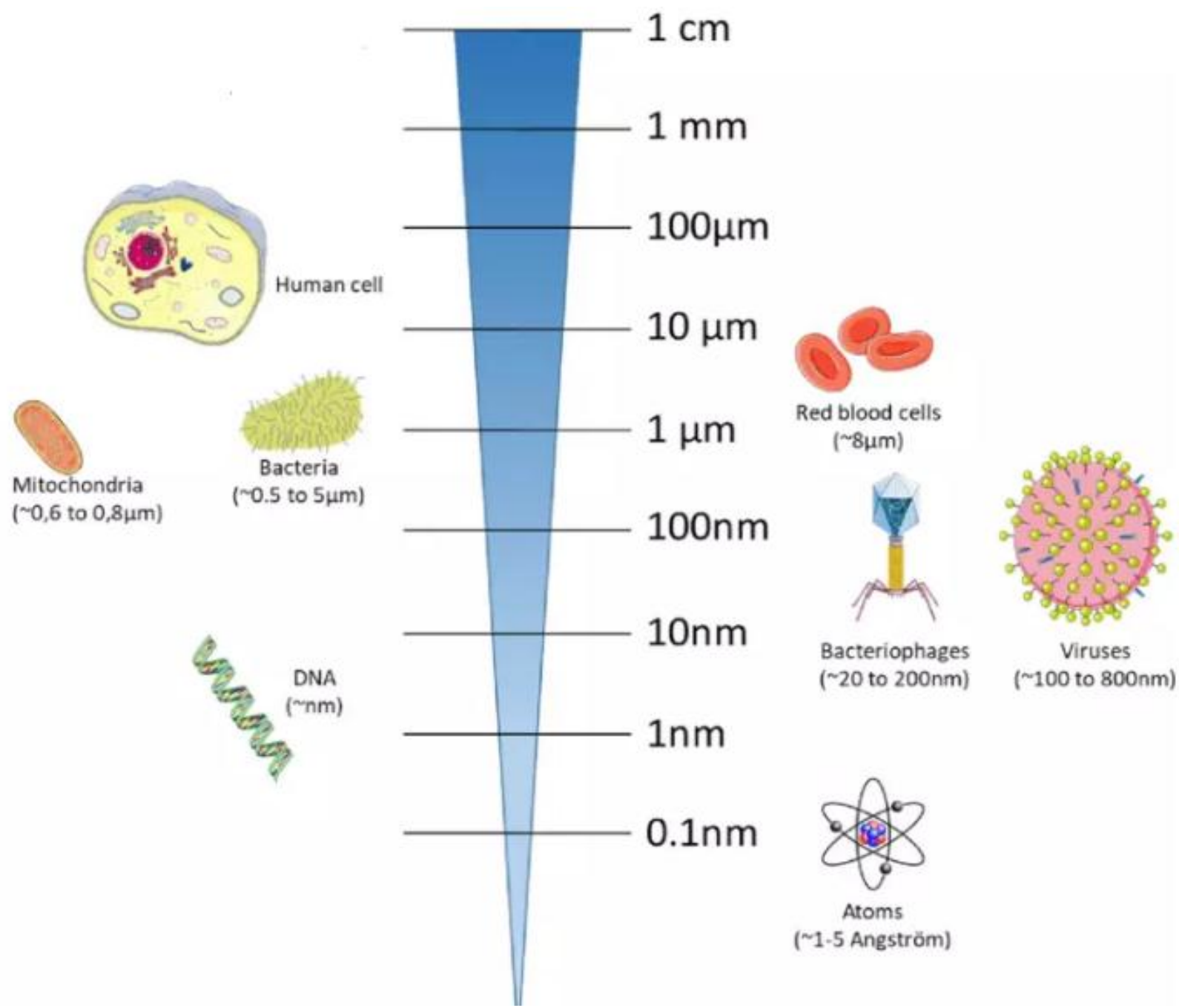
- Membrane filter disc consists of cellulose acetate, cellulose nitrate, or mixed cellulose esters.
- Pore size ranging from 10 nm to 8 µm.
- Bacteriological membrane pore size is 0.43- 0.47 µm.

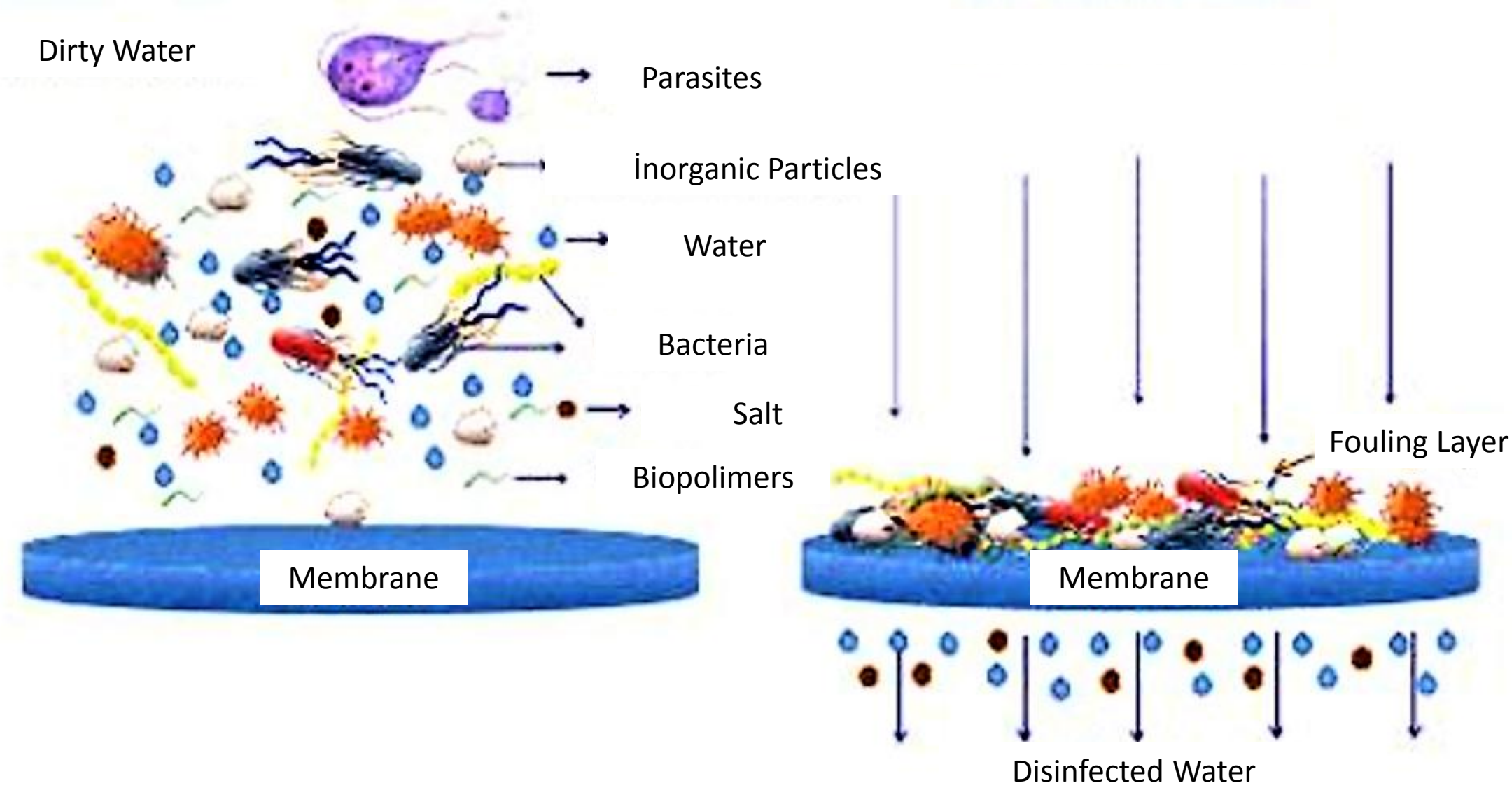


Membrane Filtration

- Pozitife or negative pressure can apply to membrane.
- The very small pore size prevents the passage of any bacteria present.
- The remaining bacteria on the membrane surface can be cultivated on a absorbent pad or liquid media.




1 cm
1 mm
100µm
10 µm
1 µm
100nm
10nm
1nm
0.1nm
Human cell
Mitochondria
(~0,6 to 0,8µm)
Bacteria
(~0.5 to 5µm)
DNA
(~nm)
Red blood cells
(~8µm)
Bacteriophages
(~20 to 200nm)
Viruses
(~100 to 800nm)
Atoms
(~1-5 Angström)


Dirty Water
Parasites
İnorganic Particles
Water
Bacteria
Salt
Biopolimers
Membrane
Fouling Layer
Membrane
Disinfected Water

Membrane Filtration

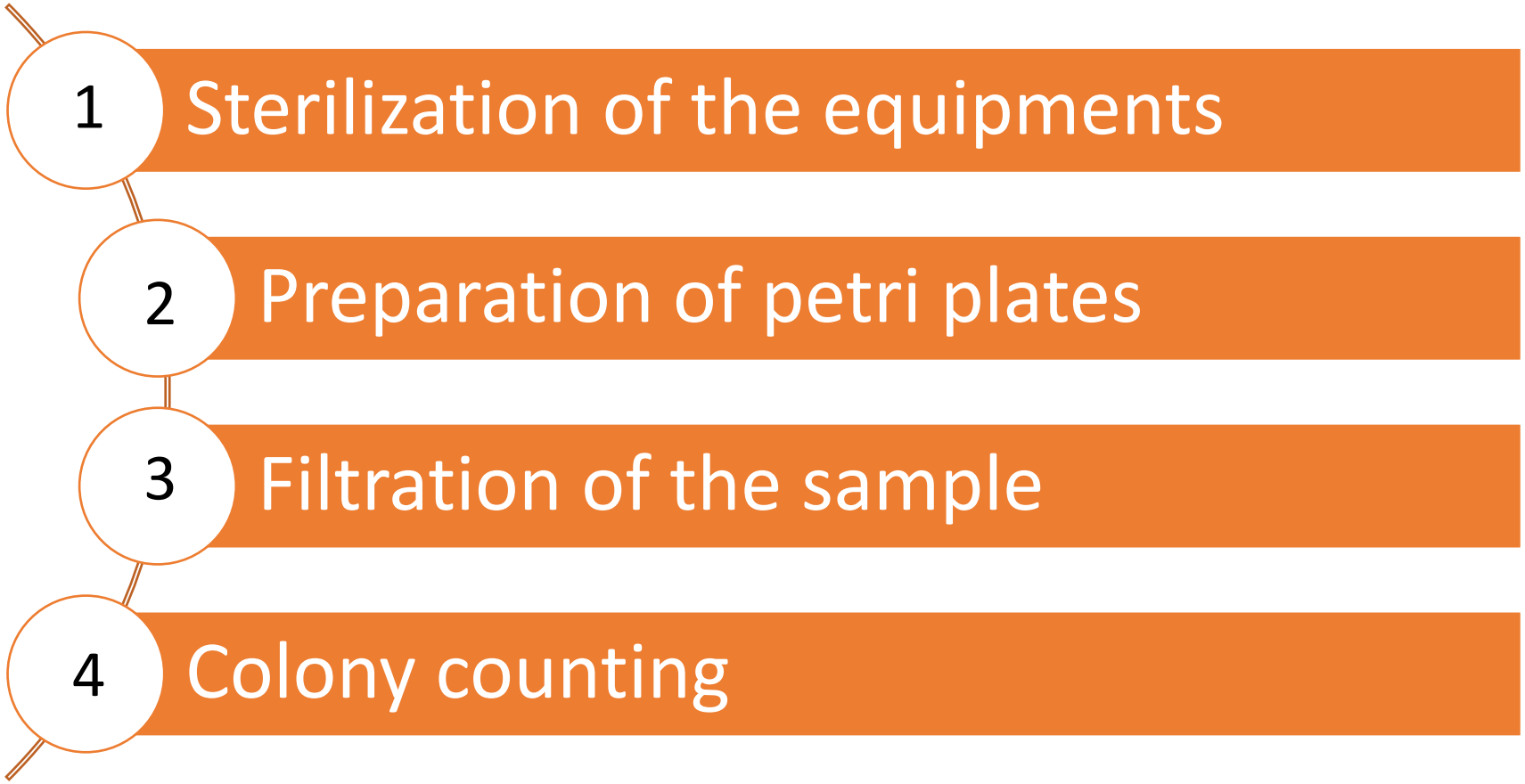
Through membrane filtration;

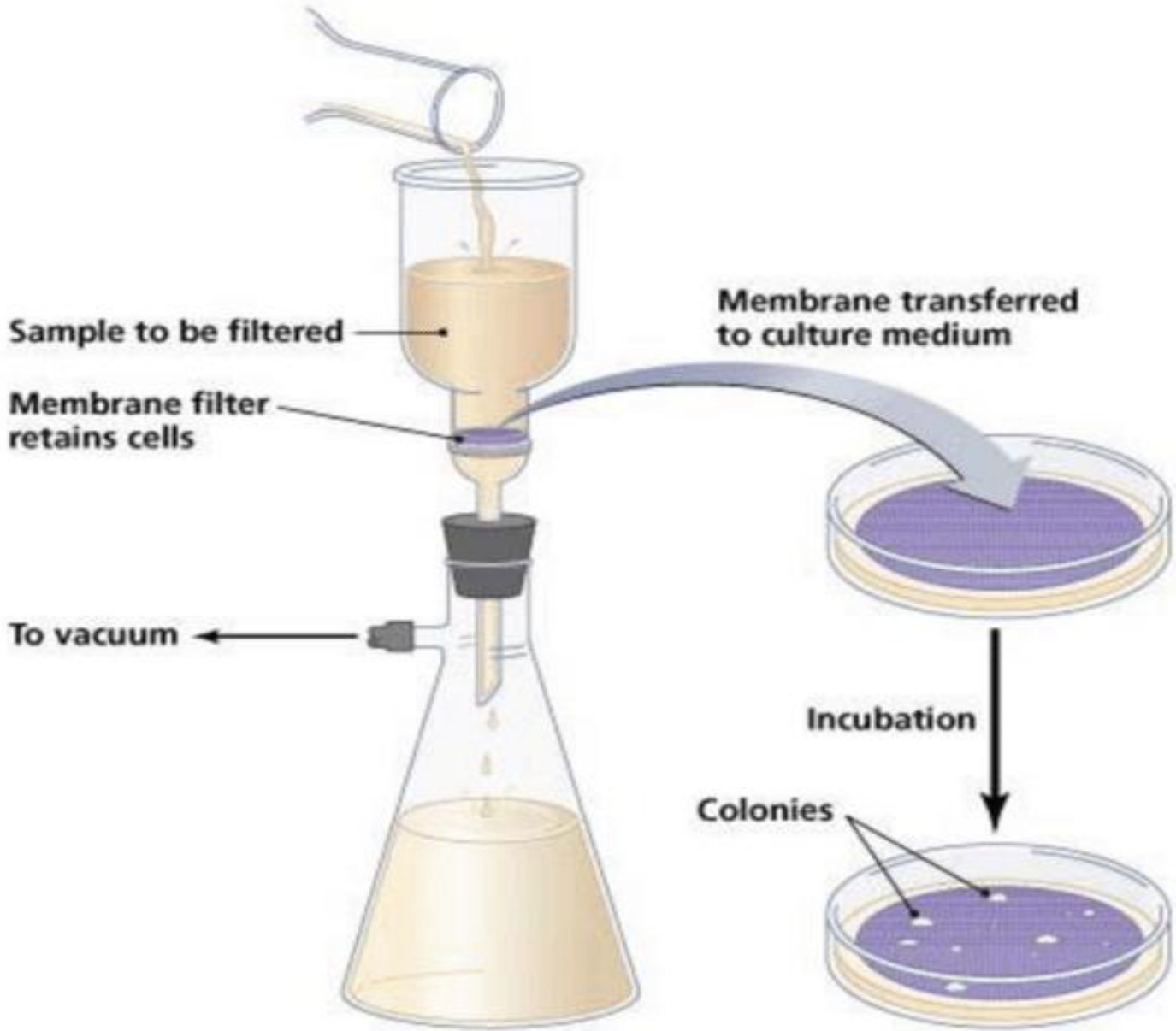
- Small numbers of organisms can be detected in large amounts of sample,
- Routine examination of water, air, sugar solution,
- Sterilization of fluid,
- Sterility testing of sterile liquids for clinical use.

Material used in LAB

- Membrane filtration apparatus
- Vacuum pump
- Plate Count Agar
- Sterile filter paper (0.45 µm)
- Forceps

Procedure


1
Sterilization of the equipments
2
Preparation of petri plates
3
Filtration of the sample
4
Colony counting


Sample to be filtered
Membrane filter retains cells
To vacuum
Membrane transferred to culture medium
Incubation
Colonies


JSR
Biological Safety Cabinet


MILLIPORE

Procedure

1- First sterilize the filtration apparatus and forceps by passing through flame or autoclave.

2- Place the coarse filter between flask and funnel and close the lid. Make sure the tap of funnel is closed.

Procedure

3- Take 50 mL of water sample and pour into funnel without filter paper to make a trial run to wet the surface of coarse filter. Run the vacuum pump and open the tap of funnel.

4- All of the sample should be passed through coarse filter.

5- Release the funnel lock and take a sterile filter paper.

6- Open the package of filter paper at aseptic conditions and take it by using sterile forceps.

7- Place the filter paper on the coarse (green side upper) filter.

8- Then place the funnel close the lock. Close the tap of funnel.

9- Add 100 mL of sample in the funnel at aseptic conditions and run the vacuum pump. Open the tap of funnel to pass the entire sample through the filter paper.

10- After all of the sample was passed, remove the funnel and remove the filter paper from the surface of coarse filter.

Procedure

11- Place the filter paper on PCA at aseptic conditions. And close the lid of petri dish.

12- Incubate the petri dishes in the 37°C Incubator.

13- Count formed colonies on the filter paper. Calculate number of microorganisms per ml of water sample.

Results

	Sample Volume	Colony number	Kob/ml or cfu/ml
Water 1	50 mL	35	35/50 = 0,70
	100 mL	38	0,38
	150 mL	57	0,38
Water 2	50 mL	34	0,68
	100 mL	44	0,44
	150 mL	72	0,48