Methods to Detect Potability of Water Sample

METHODS TO TEST WATER QUALITY

- STANDARD PLATE COUNT
- COLIFORM TEST
- MEMBRANE FILTER TECHNIQUE

- **PRESENCE/ ABSENT TEST**

CHARACTERISTICS OF

* Enterobacteriaceae members
* Facultative anaerobes,
* gram negative,
* non-spore forming,
* rod shaped bacteria
* ferment lactose to produce gas and acid within 48hrs @ 35ºc

COLIFORM

1. COLIFORMS:

* **Total Coliforms:** include bacteria that are found in the soil, in water and in human or animal waste. (Enterobacter, Klebsiella, citrobacter, escherichia,Hafnia).
* **Fecal coliforms:** Found in the gut and feces of warm-blooded animals.
* Ex: E. coli (not found growing and reproducing in the environment).
* Best indicator of fecal pollution than the total coliforms.

2. STREPTOCOCCI:

* Fecal streptococci is found in stomachs and intestines of humans and animals.
* Their presence indicates the presence of fecal pathogens in water. Ex: Enterococci

SIGNIFICANCE OF TESTING COLIFORMS

* There are Numerous water born pathogens
* Individual pathogen numbers may be too low to detect in a reasonable sized water sample.
* Isolation and detection of some pathogens can take several days, weeks, or months.
* Absence of one particular pathogen does not rule out the presence of another
* Coliforms come from the same sources as pathogenic organisms.
* Coliforms are relatively easy to identify, are usually present in larger numbers than more dangerous pathogens.

As a result, presence of coliform bacteria in water sample is an indication of presence of pathogenic bacteria.

STANDARD PLATE COUNT

Total Plate Count (TPC) / Total Viable Count (TVC)

A popular method for the routine determination of cell number in food, medical, aquatic and research laboratories.

STEPS:

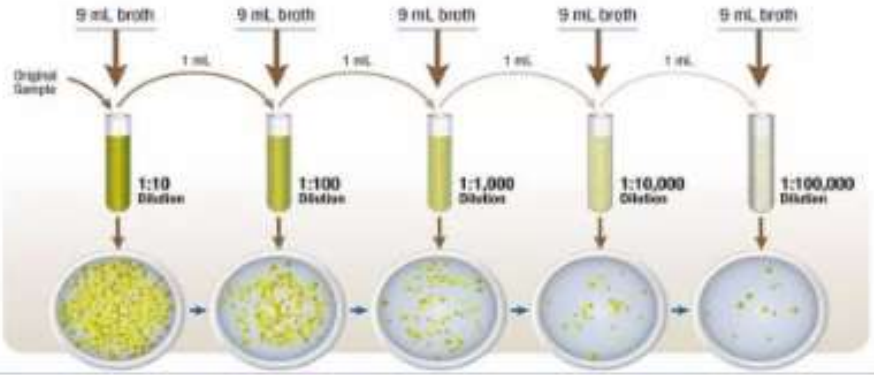
1. **Serial dilution**
2. **Sample Inoculation** (Spread/Pour Plate Technique)
3. **Colony counting**
4. **CFU Calculation**

SERIAL DILUTION

* **Serial dilution** is the stepwise dilution of sample in solution (diluent).
* The diluent is sterile water/broth/saline
* At each step, 1ml of the previous dilution is added to 9ml of distilled water.
* Each step results in 10-fold decrease in the concentration from the previous concentration.

Significance:

* The number of bacteria in a given sample is often too many to be counted directly.
* To obtain the appropriate colony number, the sample to be counted must always be diluted.
* Single isolated bacteria form visible isolated colonies.



*10-fold dilution

SPREAD-PLATE TECHNIQUE

Procedure:

* Pipette 0.1 ml of sample onto the center of an agar medium plate.
* Dip a glass spreader into a beaker of ethanol.
* Briefly flame the ethanol soaked spreader and allow it to cool.
* Spread the sample evenly over the agar surface with the sterilized spreader.
* Incubate the plates at 37°C for 24-48hrs

Results

* Results in visible and isolated colonies of bacteria appear

Advantages:

* Evenly distributed colonies in the plate are countable.

POUR PLATE TECHNIQUE

Procedure:

* 1ml of diluted sample mixed with liquid agar that has been cooled to about 45°C and poured immediately into sterile culture dishes.
* The plates are incubated at 37°C for 24-48hrs

Results:

* The surface colonies are circular (similar in appearance as those on a streak plate).
* subsurface colonies are small & confluent.

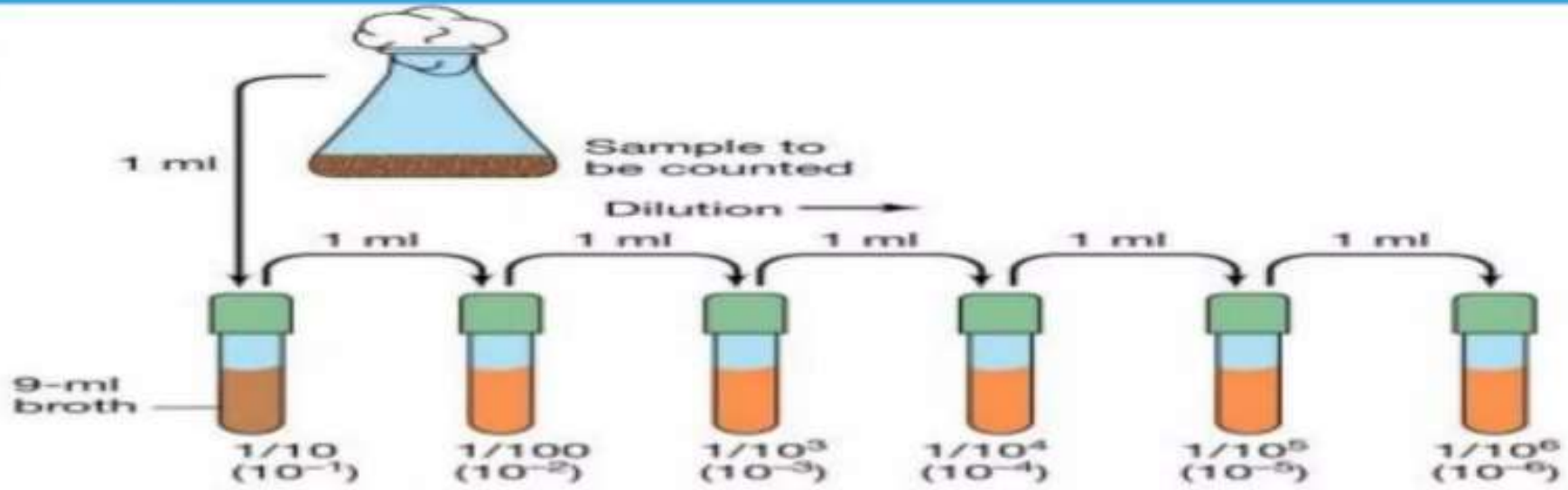
Advantage

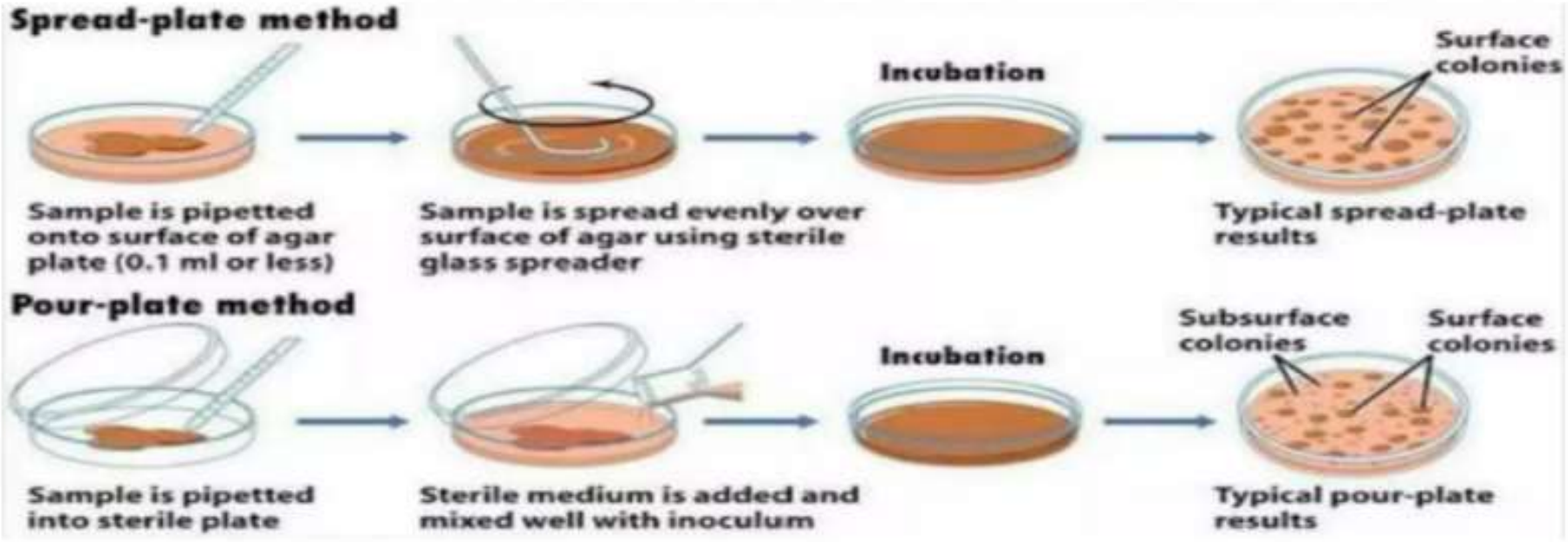
* Microorganisms will grow both on the surface and within the medium.
* Do not require previously prepared plates.
* Counting bacteria is more precise than other method

Disadvantage

* Gives lower count as heat sensitive microorganisms may die when they come contact with hot, molten agar medium.

Flow Chart For Standard Plate Count



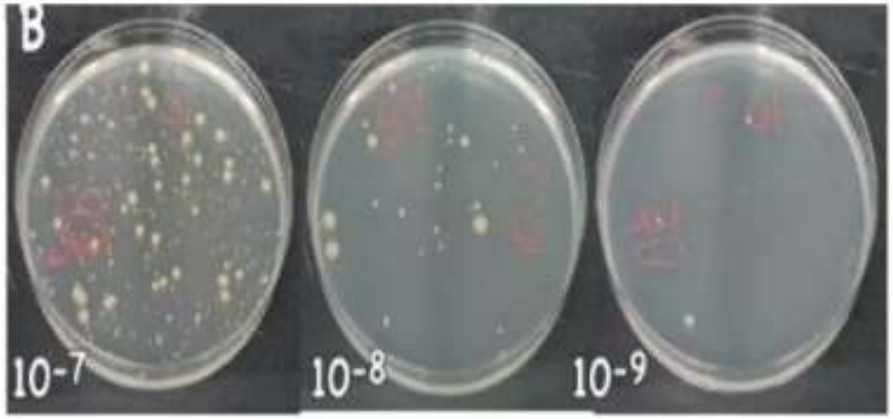


INTERPRETATION OF RESULTS

Colony Counting : QUBEC Colony Counter

* Plates containing between 30 and 300 colonies are counted using magnifying colony counter.
* the number of colonies equal the number of viable organisms in the sample

https://www.indiamart.com/proddetail/digital-colony-counters-6228427497.html



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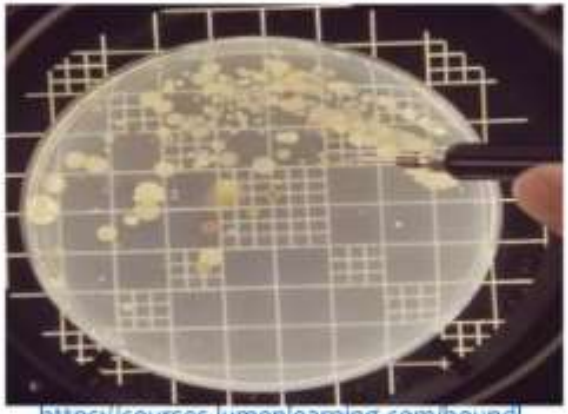


https://courses.lumenlearning.com/boundless-microbiology/chapter/counting-bacteria/

Colony Forming Units (CFU)

* The number of microorganisms that can form colonies when cultured using spread plates or pour plates

CFU/ml =

$$\frac{\text{No.of colonies on plate X reciprocal of dilution of sample}}{\text{volume plated}}$$

* For example, suppose the plate of the 10-6 dilution yielded a count of 130 colonies.
* Then, the number of bacteria in 1 ml of the original sample can be calculated as follows:

$$\text{cfu/ml} = \frac{(130) \times (10^6) = 1.3 \times 10^9 \text{ or } 13,00,000,000}{0.1\text{ml}}$$

Advantages and Disadvantages

Advantages

* The technique is sensitive and counts living bacteria,
* Any concentration of microorganism can be easily counted, if the appropriate dilution is plated.
* The equipment necessary for performing viable plate counts is cheap & available in lab.
* By using a selective medium it is possible to determine the number of bacteria of a certain class, even in mixed populations.

Disadvantages

* One colony does not equal one cell (*Staphylococcus*).
* Great care must also be taking during dilution and plating to avoid errors.
* The rate at which bacteria give rise to an observable colony can also vary.
* The temperature of incubation and medium conditions must also be optimized
* one day to several weeks might be necessary to determine the number of CFUs

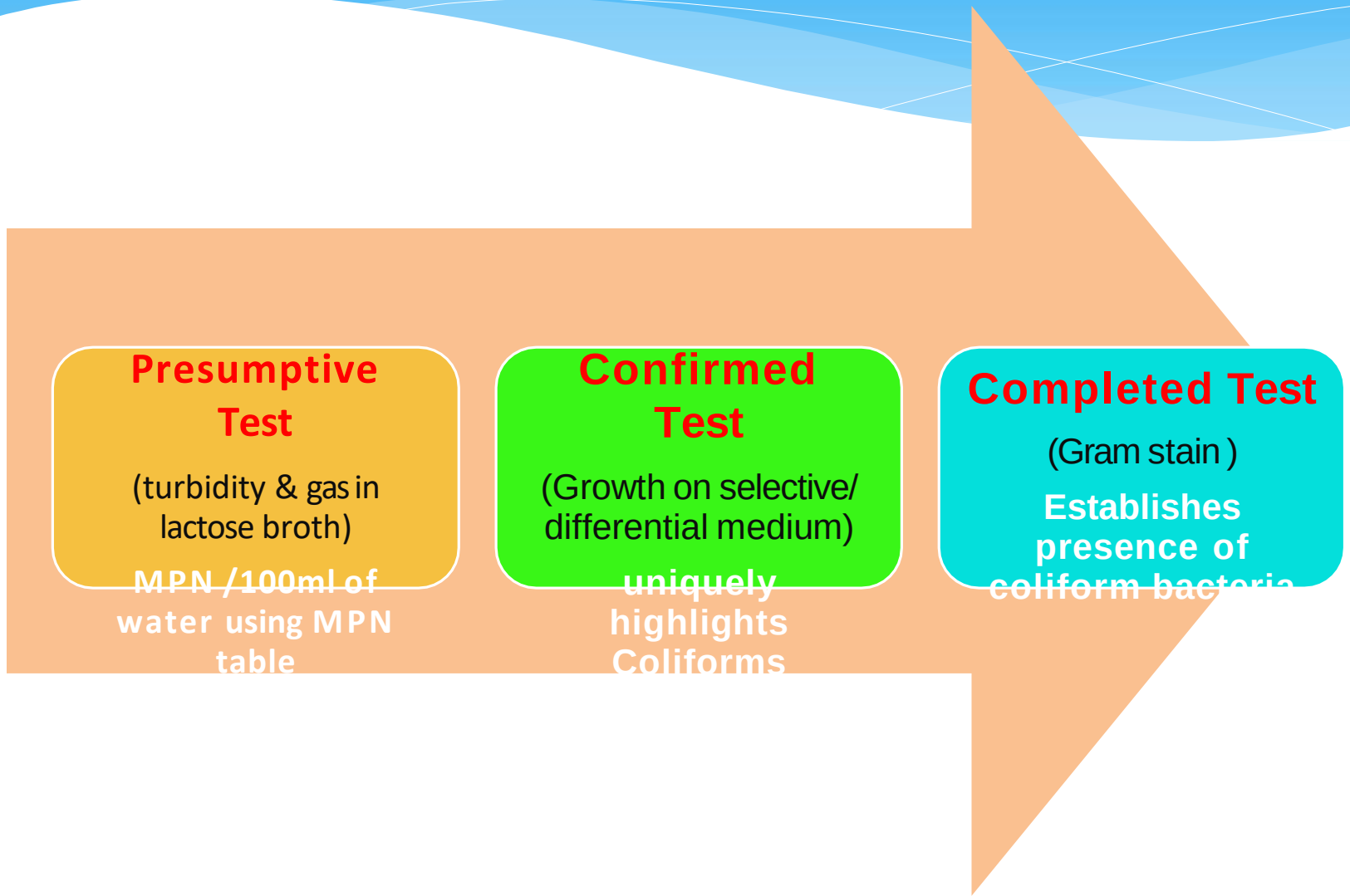
COLIFORMS TEST

- Most Probable Number Test
- Presence-Absence Coliform Test
- Colilert (Minimal Media ONPG-MUG Test)

Most Probable Number/Multiple tube Fermentation Test

* The Most Probable Number method is used to check potability (if water is safe enough to be drinking water) of water.
* The MPN method looks for the presence of potential pathogenic bacteria that may be in the water due to contamination of the water supply.
* MPN method enumerates the enteric bacteria called coliforms, specifically fecal coliforms (E. coli)

MPN test includes 3 levels of testing:



COLIFORM TEST

PRESUMPTIVE TEST

Inoculation of lactose broth

Gas produced = presumptive evidence of coliforms
Examination continued

No gas = coliform not present
Examination stop here

CONFIRMED TEST

Transfer made from lactose broth tubes with gas to:

Brilliant green lactose bile broth (BGLB)
This medium inhibits the growth of lactose fermenters
Other than coliforms; thus gas formation in BGLB medium
Constitutes a confirmed test; i.e. coliform present.

Eosin methylene blue agar (EMB)
Coli-aerogenes organisms produce
characteristic colonies; small, dark almost dark
Centers with greenish metallic sheen

COMPLETED TEST

The most typical colonies are selected from EMB plate

Lactose broth (coliform produce gas)

Gram staining
(Coliform are gram negative, non sporulating bacilli)

PRESUMPTIVE TEST

Principle:

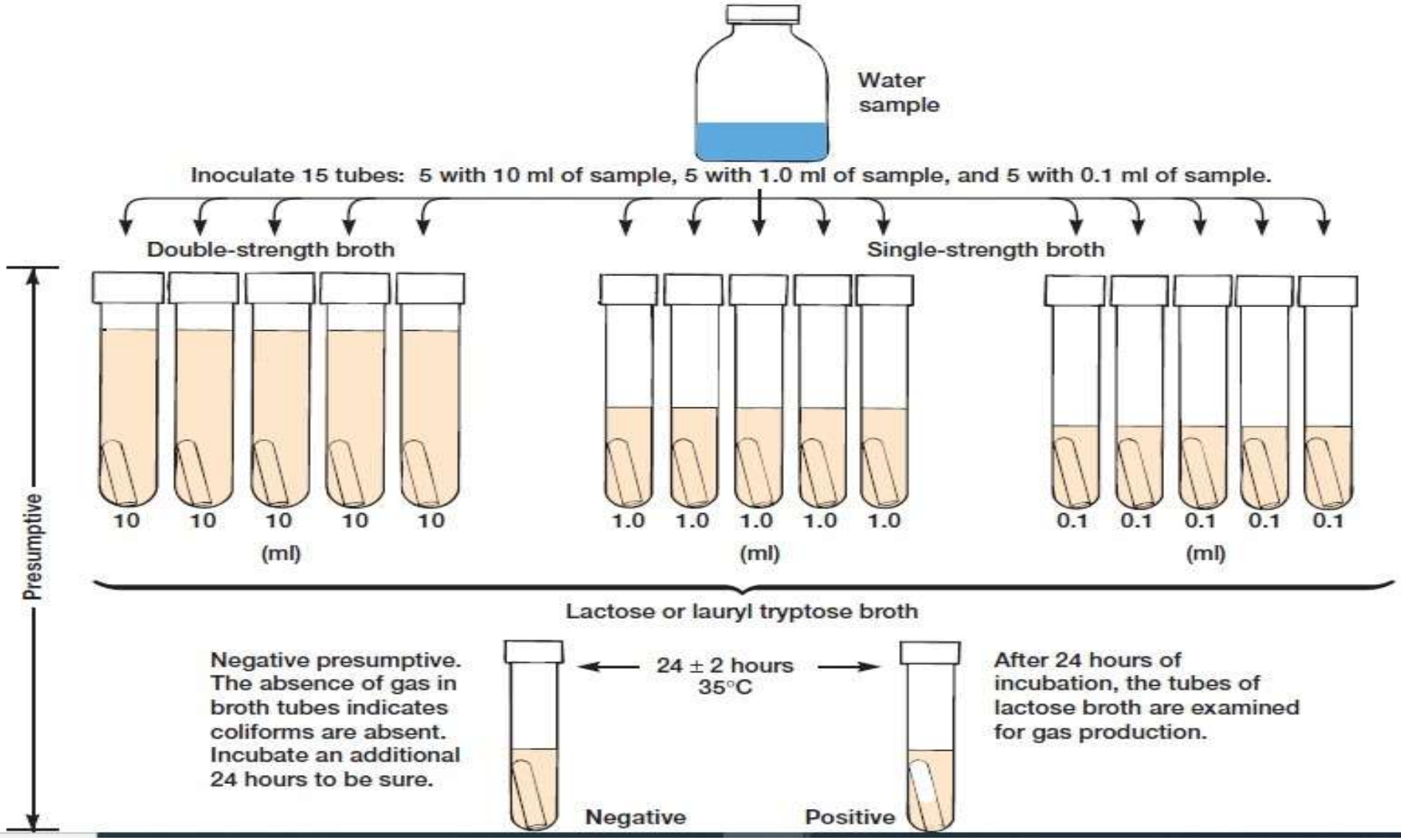
* The presumptive test looks for presence of fecal coliforms in the water sample by inoculating **lactose broth** containing **Durhams tubes** with the water sample

Procedure:

Three sets of lactose broths are inoculated with varying dilutions of the sample:

* first set of 5 tubes inoculated with 10ml of sample;
* second set of 5 tubes inoculated with 1ml of sample;
* third set of 5 tubes inoculated with 0.1ml of sample
* Incubate all tubes at 35°C for 24-48 hours

Flow chart of Presumptive



Source: Prescott–Harley–Klein: Microbiology, Fifth Edition, © The McGraw–HillCompanies, 2002

Interpretation of Results:

* **Positive Test:** Those tubes that show turbidity with gas are marked as +ve and those with no turbidity/gas as -ve.
* +ve marked tubes are counted from all 3 sets.
* The combination of positives in the 3 sets is used to find out the MPN /100ml of water using the MPN table

Result

s: MPN Index: 5 5 3

* No.of Coliforms: 920/100ml water sample

Table A5.3 MPN values per 100 ml of sample and 95% confidence limits for various combinations of positive and negative results (when five 10-ml, five 1-ml and five 0.1 ml test portions are used)

No. of tubes giving a positive reaction :			MPN (per 100 ml)	95% confidence limits	
5 of 10 ml	5 of 1 ml	5 of 0.1 ml		Lower	Upper
0	0	0	<2	<1	7
0	1	0	2	<1	7
0	2	0	4	<1	11
1	0	0	2	<1	7
1	0	1	4	<1	11
1	1	0	4	<1	11
1	1	1	6	<1	15
2	0	0	5	<1	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	79
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	93
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	110
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	150
5	2	0	49	17	130
5	2	1	70	23	170
5	2	2	94	28	220
5	3	0	79	25	190
5	3	1	110	31	250
5	3	2	140	37	340
5	3	3	180	44	500
5	4	0	130	35	300
5	4	1	170	43	490
5	4	2	220	57	700
5	4	3	280	90	850
5	4	4	350	120	1000
5	5	0	240	68	750
5	5	1	350	120	1000
5	5	2	540	180	1400
5	5	3	920	300	3200
5	5	4	1600	640	5800
5	5	5	>1800	—	—

Source: Philippe Et Al, Clinical & Biomedical Sciences Of Tropical Diseases , Practical Notes, Bacteriological Examination Of Water (2009) Pg 12

False Positive Presumptive

* A positive, presumptive test does not necessarily mean that members of the colon group are present.
* In most cases it is true, but there are exceptions.

False, positive, presumptive tests are caused by

1. the presence of other organisms capable of fermenting lactose with the production of acid and gas and
2. bacterial associations or synergism: Joint action of two organisms on a carbohydrate resulting in the production of gas that is not formed by either organism when grown separately.(G+ve & G-ve)

Elimination of False, Presumptive Tests: Addition of triphenyl methane dye in the lactose-broth medium inhibits G+ve bacteria.

CLASSES OF WATER

Based of British Ministry of Health

* CLASS I – regarded as highly satisfactory and contains less than 1coliform/100mL
* CLASS II – regarded as satisfactory and contains 1-2 coliform/100mL
* CLASS III – regarded as suspicious and contains 3-10 coliforms/100mL
* CLASS IV – regarded as unsatisfactory and contains more than 10 coliforms/100mL

Guidelines for Enumerating coliforms

Drinking Water

* EPA guidelines for coliforms in drinking water are < 1CFU/100 ml.
* Action limit of 4 coliform per 100 ml.

Action limit: means water provider must take immediate action against coliform

Recreational water

◦ 200 fecal coliforms /100 ml

CONFIRMED

* Done to determine the origin of coliforms (fecal or not) and they are E. coli or not.

Media:

* Brilliant Green lactose bile broth/EMB agar/Endo's Agar

Procedure:

* **Eosin methylene blue agar (EMB):** prepared by adding the two dyes eosin and methylene blue to melted lactose agar
* A loopful of the culture from the lactose broth with gas is streaked over the surface of EMB agar plates.
* The plates are incubated at 37°C for 24 or 48 hr
* Coliforms produce characteristic colonies on EMB agar
* **E.coli :** small colonies ,dark ,almost black centers with greenish metallic sheen
* **Enterobacter :** large pinkish colonies ,dark centers ,rarely show metallic sheen

INTERPRETATION OF RESULTS

CONFIRMED TEST ON EMB Agar

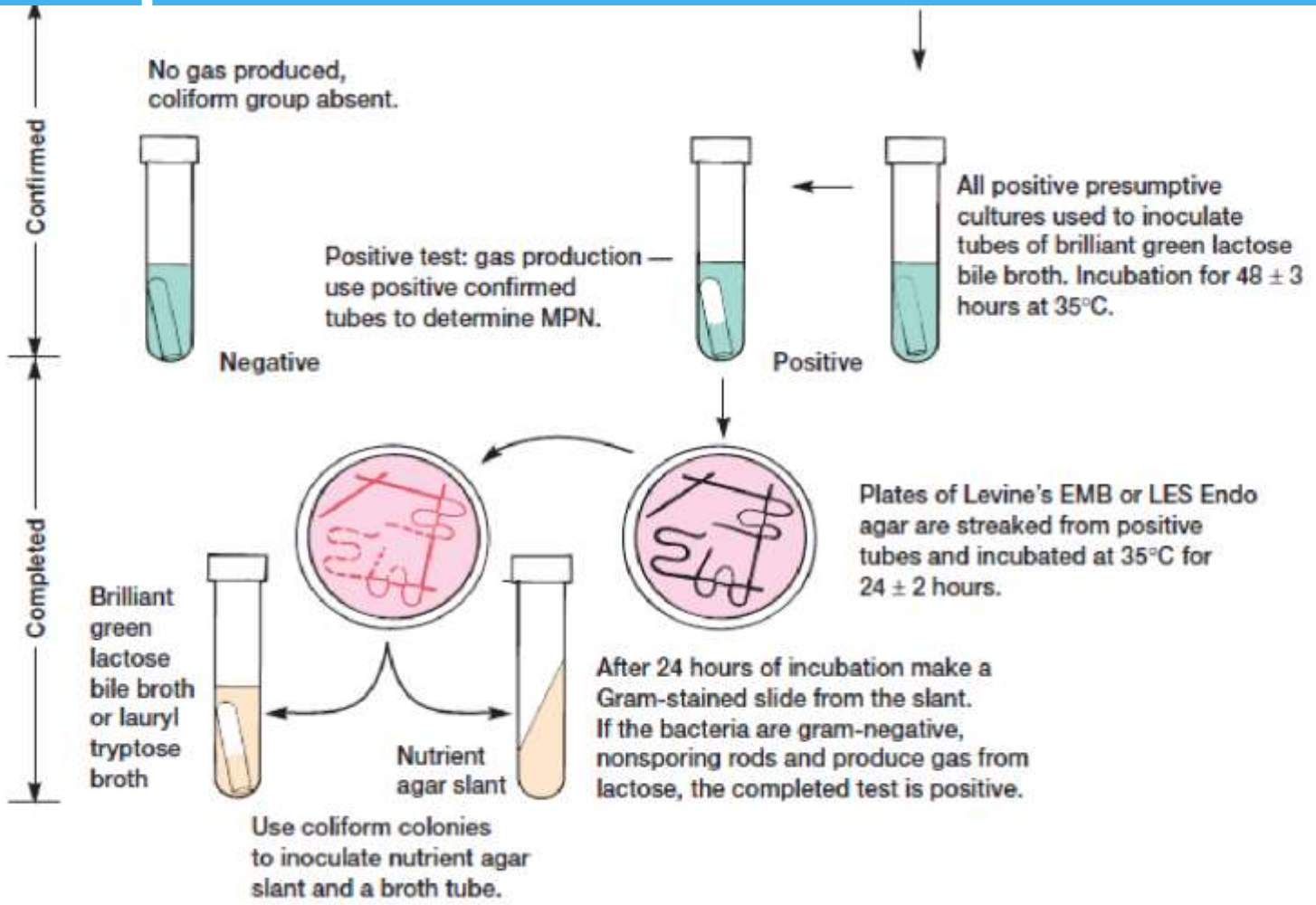
Typical colonies: Bluish green metallic sheen (E.coli)

Atypical colonies: Pink mucoid (Enterobacter)

Positive test:

* Appearance of at least one typical colony or,
* if no typical colonies are present,
* at least two atypical colonies

Flow chart of Confirmed & Completed Test



Source: Prescott–Harley–Klein: Microbiology, Fifth Edition, © The McGraw–HillCompanies, 2002

COMPLETED TEST

The purpose of the completed test is to determine:

* (1) if the colonies appearing on E.M.B. agar plate are again capable of fermenting lactose with the production of acid and gas and
* (2) if the organisms transferred to an agar slant show the morphological properties of coliforms.

Procedure:

* The typical/atypical colonies from EMB plate are inoculated into Lactose broth and Agar slant and incubated at 37℃ for 24-48hrs

INTERPRETATION OF RESULTS

Nutrient Agar slant

Grams staining

gram -ve non-spore forming bacilli (+ve test)

Formation of gas in Durhams tube (+ve test)

Result: confirms presence of coliforms

Advantages and

Advantages:

* **Correlated to the presence of pathogens**
* **coliform population is large enough to isolate in small water samples (100 mL)**
* **Rapid**
* **Inexpensive**
* **Safety, not culturing pathogens**

Disadvantages:

* labor intensive ,Large amount of glassware is required
* Its lack of precision, large errors
* still requires survival and culture of organisms in lab

PRESENCE-ABSENCE TEST

Principle:

* Presence-Absence (P-A) test is a presumptive detection for coliforms in water.
* The test is a simple modification of the multiple-tube test.
* This test is based on the principle that coliforms and other indicator organisms should not be present in a 100 mL water sample.

Procedure:

* 100 mL test sample incubated in a culture bottle with a triple-strength broth containing lactose broth, lauryl tryptose broth
* Bromcresol Purple is used as an indicator dye

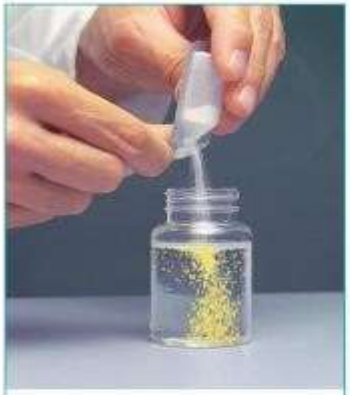
lactose-fermenting organisms turn the medium from purple to yellow with or without gas production

Colilert (Minimal Media ONPG-MUG Test)

Colilert uses the patented Defined Substrate Technology® (DST®) to simultaneously detect total coliforms and *E. coli*.

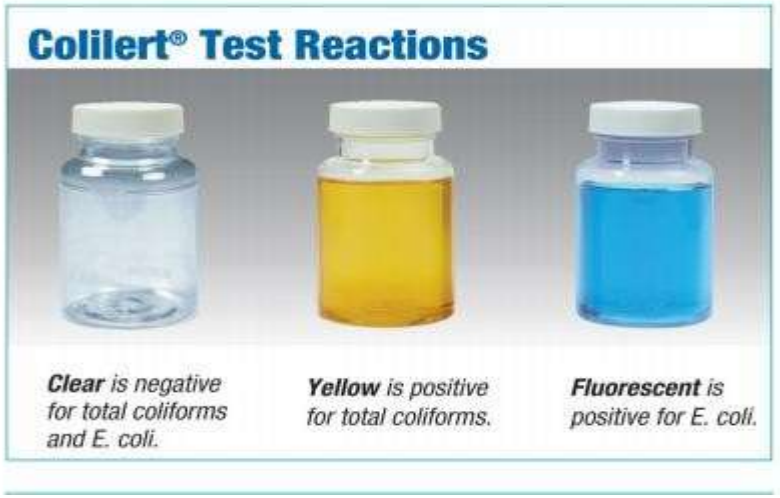
Principle:

* Total coliforms release β -d- galactosidase which hydrolyses, ortho- nitrophenyl- -d- galactopyranoside (ONPG) - change it from colorless to yellow
* *E. coli* uses enzyme *β*-glucuronidase to metabolize 4-methylumbelliferyl- - glucuronide (MUG) - Fluoresces when hydrolyzed



Add reagent to sample.

Incubate at 35°C. for 24 hours, 18 hours for Colilert-18 (P/A).

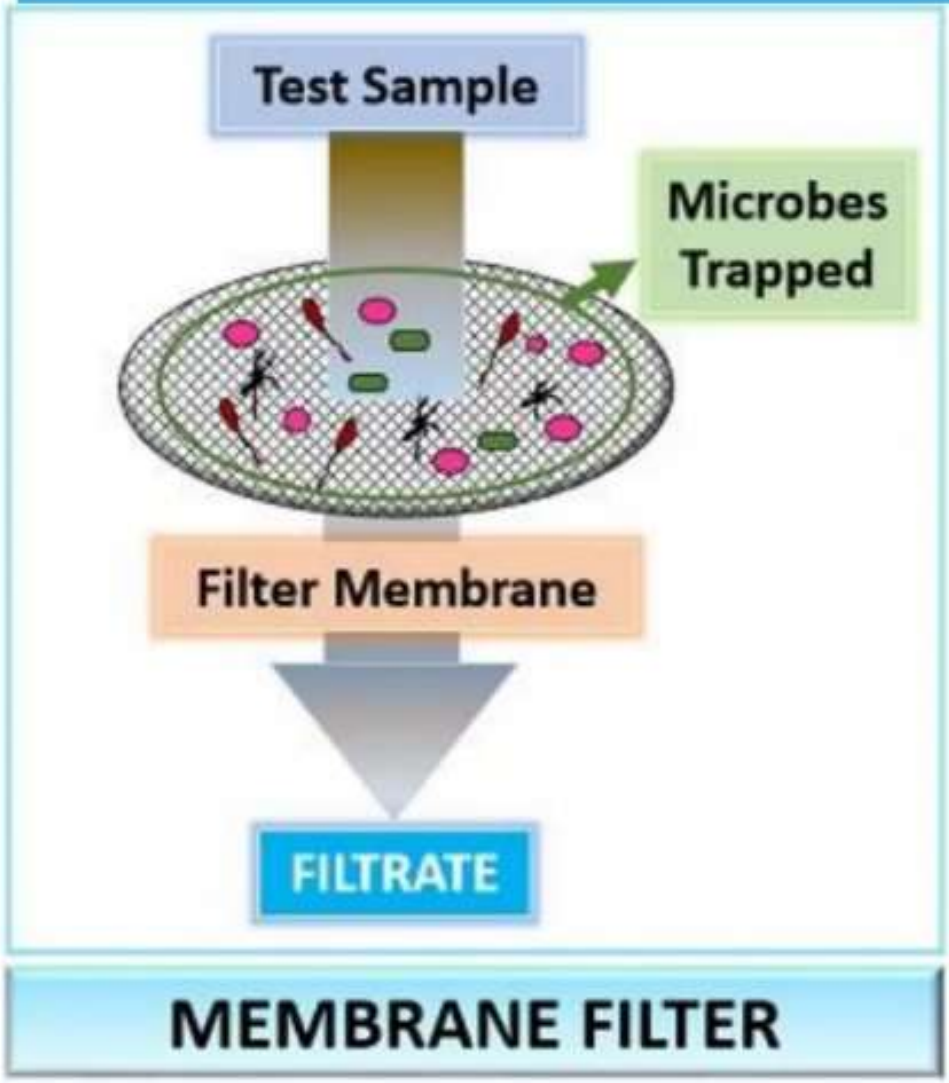


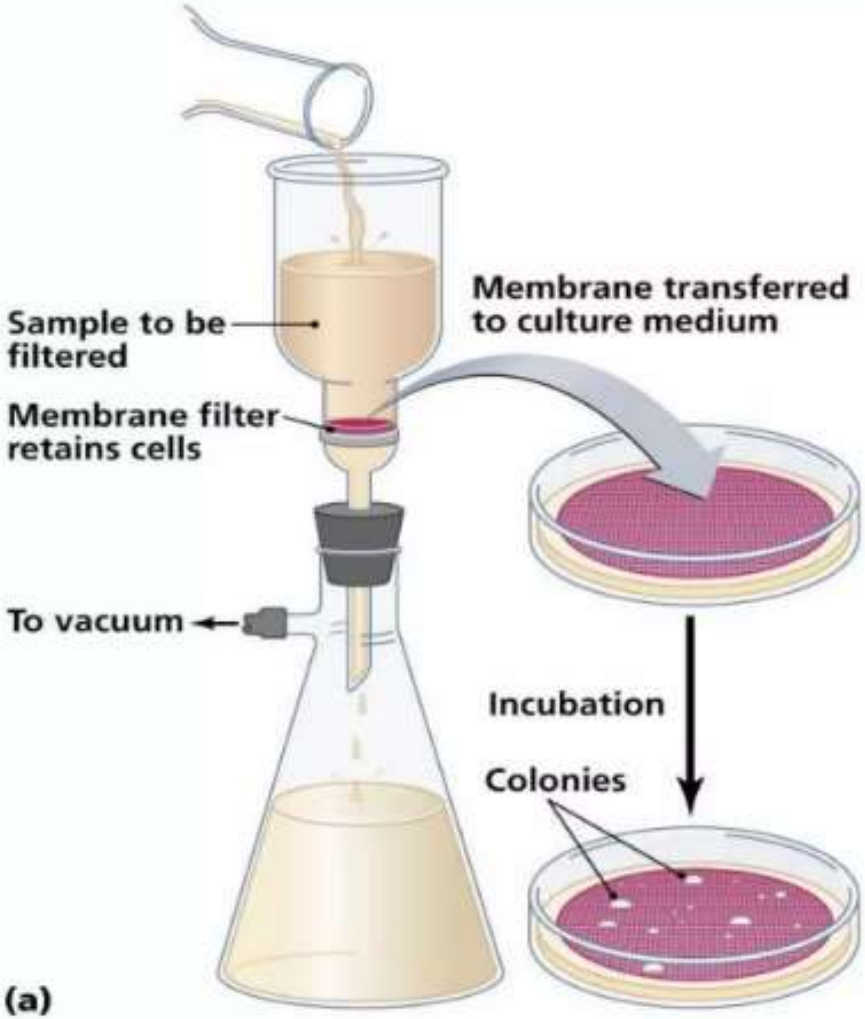
Source: weberscientific.com

MEMBRANE FILTER TECHNIQUE

PROCEDURE

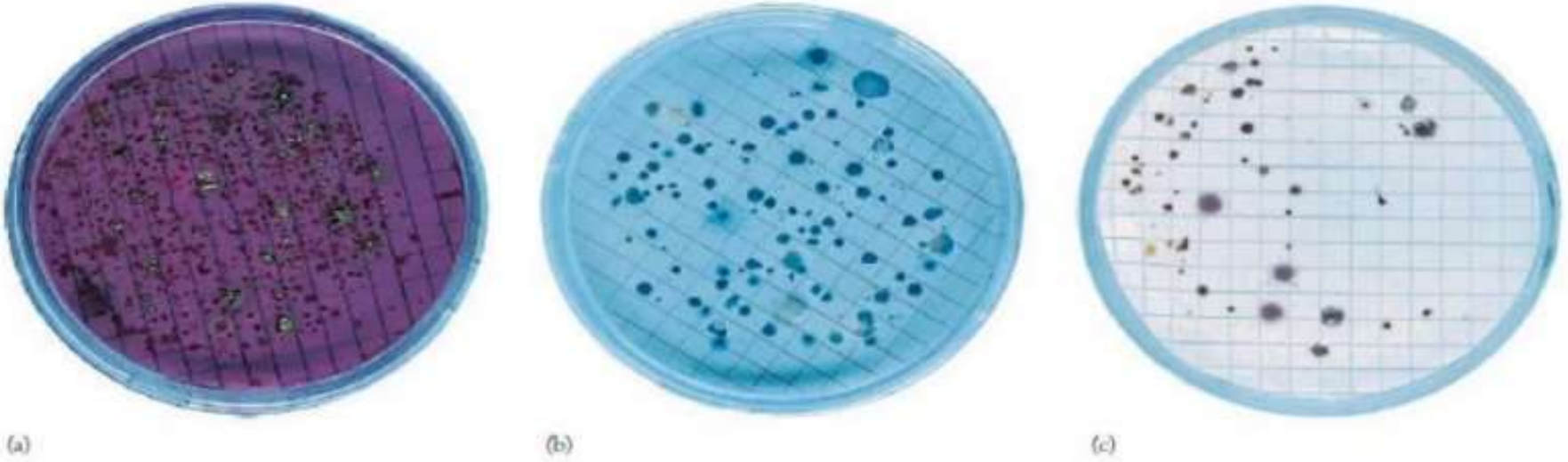
* This technique involves filtering 100 ml for drinking water through a special sterile filter.
* These filters are made of nitrocellulose acetate or polycarbonate, are 150 µ thick, and have 0.45 µ diameter pores.
* When the water sample is filtered, bacteria (larger than 0.45 µ) in the sample are trapped on the surface of the filter.
* The filter is then carefully removed, placed in a sterile Petri plate on a pad saturated with a liquid or agar-based medium,
* Incubated for 20 to 22 hours at 35°C.
* it is assumed that each bacterium trapped on the filter will then grow into a separate colony.
* By counting the colonies one can directly determine the number of bacteria in the water sample that was filtered.





INTERPRETATION OF RESULTS

* Use of the proper medium allows the rapid detection of total coliforms, fecal coliforms, or fecal streptococci by the presence of their characteristic colonies
* A grid pattern is typically printed on these filter disks in order to facilitate colony counting.



Coliform and Enterococcal Colonies

(a) Coliform reactions on an Endo medium.
(b) Fecal coliform growth on a bile salt medium (m-FC agar) containing aniline blue dye.
(c) Fecal enterococci growing on an azide-containing medium (KF agar) with TTC, an artificial electron acceptor, added to allow better detection of colonies.

Advantages and Disadvantages

Advantages

* Good reproducibility
* Single-step results often possible
* Filters can be transferred between different media
* Large volumes can be processed to increase assay sensitivity
* Time savings are considerable
* Ability to complete filtrations on site
* Lower total cost in comparison with MPN procedure

Disadvantages

* High-turbidity waters limit volumes sampled
* High populations of background bacteria cause overgrowth
* Metals and phenols can adsorb to filters and inhibit growth

References

* Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes Edited by Jamie Bartram and Richard Balance, Published on behalf of United Nations Environment Programme and the World Health Organization © 1996 UNEP/WHO ISBN 0 419 22320 7 (Hbk) 0 419 21730 4 (Pbk)
* Fundamental Principles of Bacteriology BY A. J. SALLE, B.S., M.S., PH.D. Associate Professor of Bacteriology University of California Los Angeles Second edition sixth impression. Mcgraw-hill Book Company, INC. New York And London 1943
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THANK YOU