

SNS COLLEGE OF PHARMACY AND HEALTH SCIENCES

*Affiliated To The Tamil Nadu Dr. MGR Medical University, Chennai
Approved by Pharmacy Council of India, New Delhi.*

Coimbatore -641035



**COURSE NAME : PHARMACEUTICAL MICROBIOLOGY - BP303 T
B.PHARM II YEAR / III SEM**

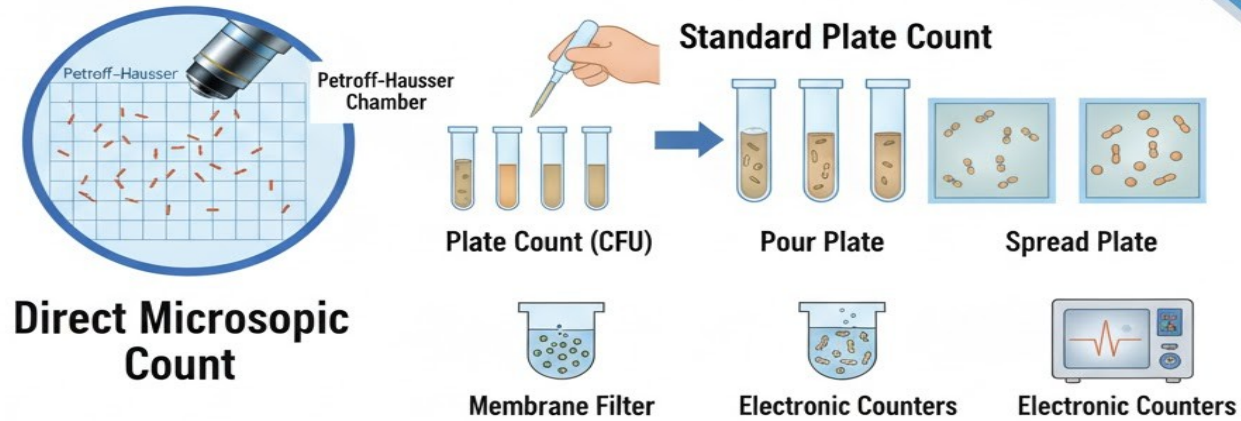
UNIT 1

SUB TOPIC : MEASUREMENTS OF MICROBIAL CELLS

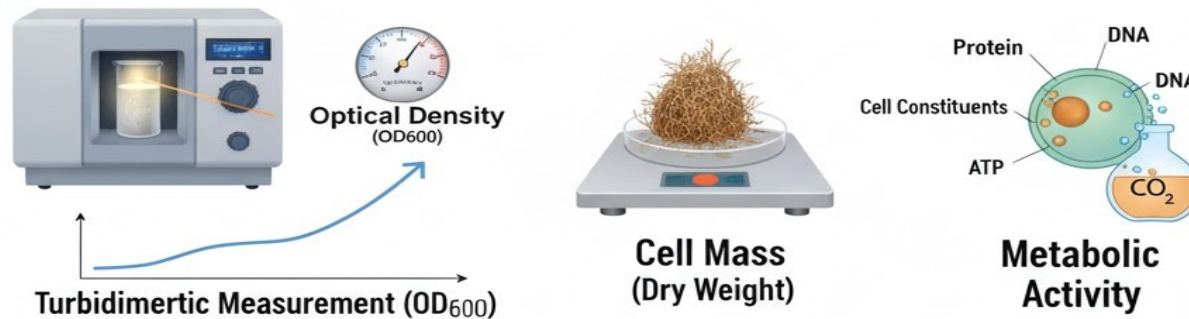
Quantitative Measurement of Bacterial Growth



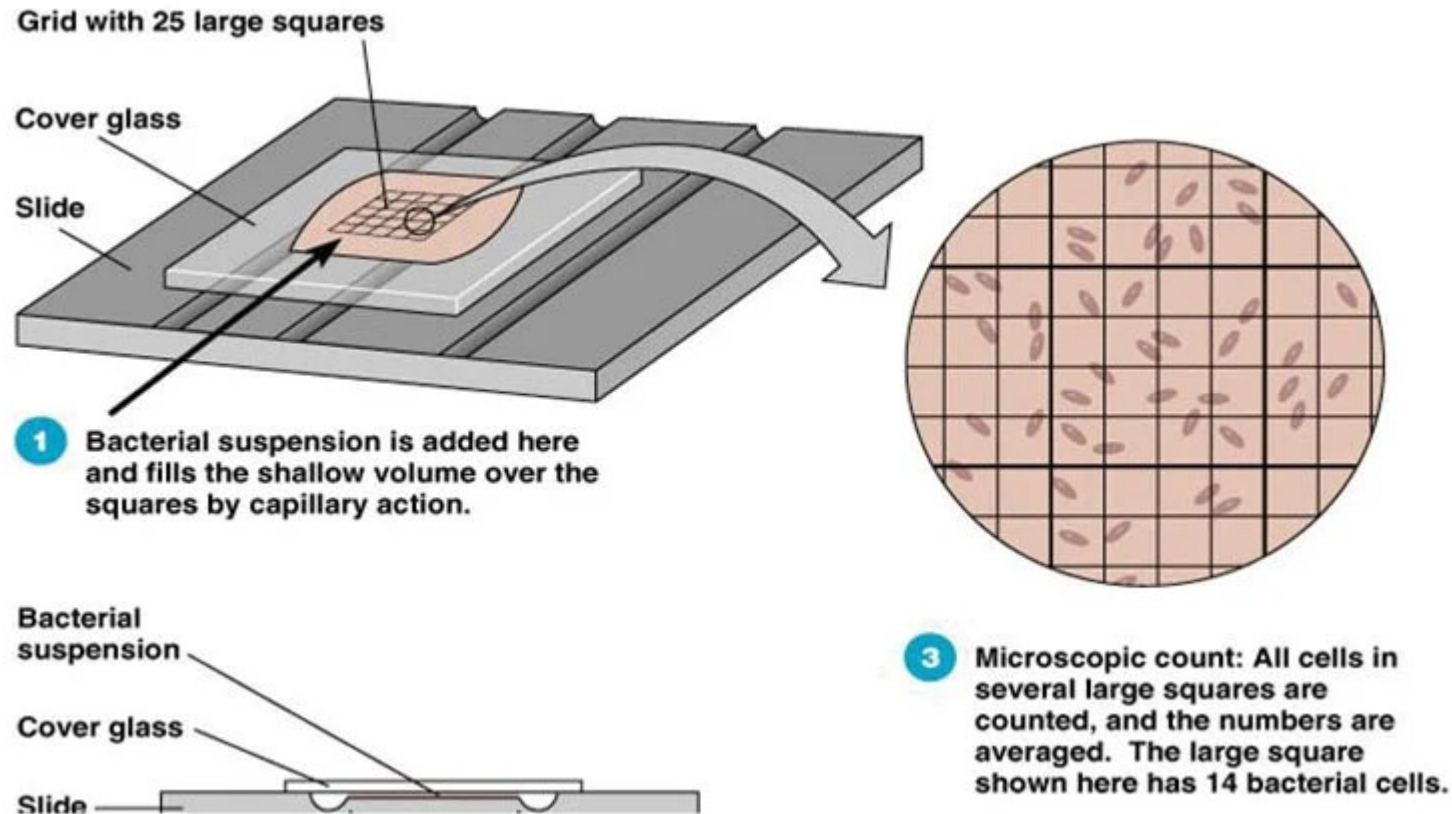
DIRECT METHODS (Measuring Cell Number)



INDIRECT METHODS (Measuring Cell Mass or Activity)

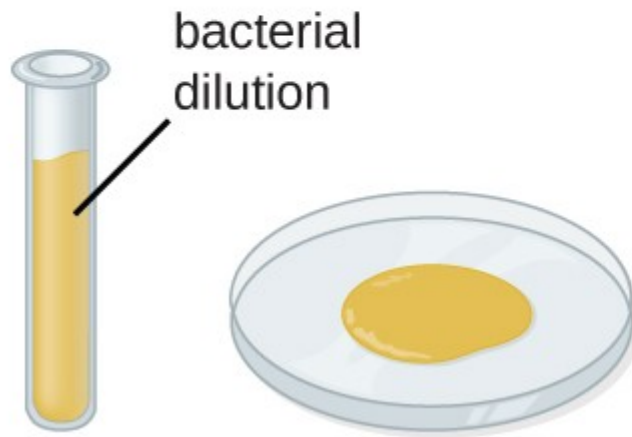


Direct microscopic count



Spread Plate Method

- 1 Sample (0.1 mL) poured onto solid medium



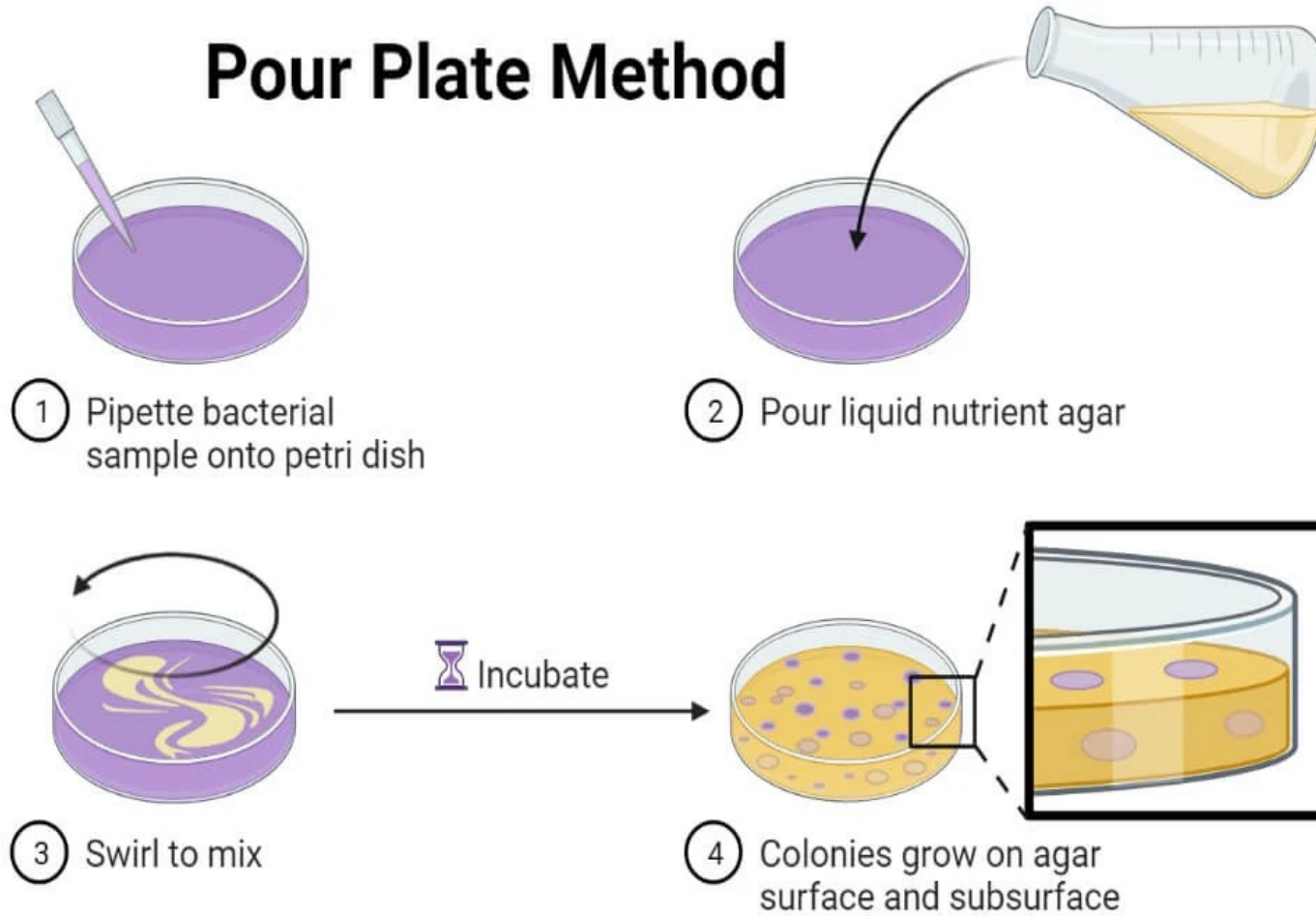
- 2 Spread sample evenly over the surface



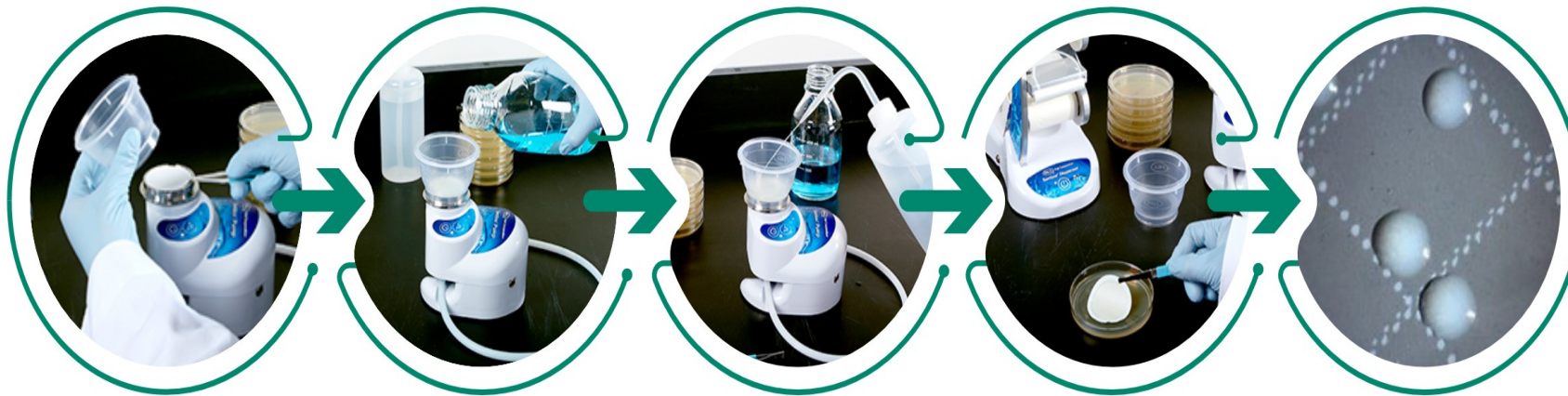
- 3 Plate incubated until bacterial colonies grow on the surface of the medium



Pour Plate Method



MEMBRANE FILTRATION METHOD



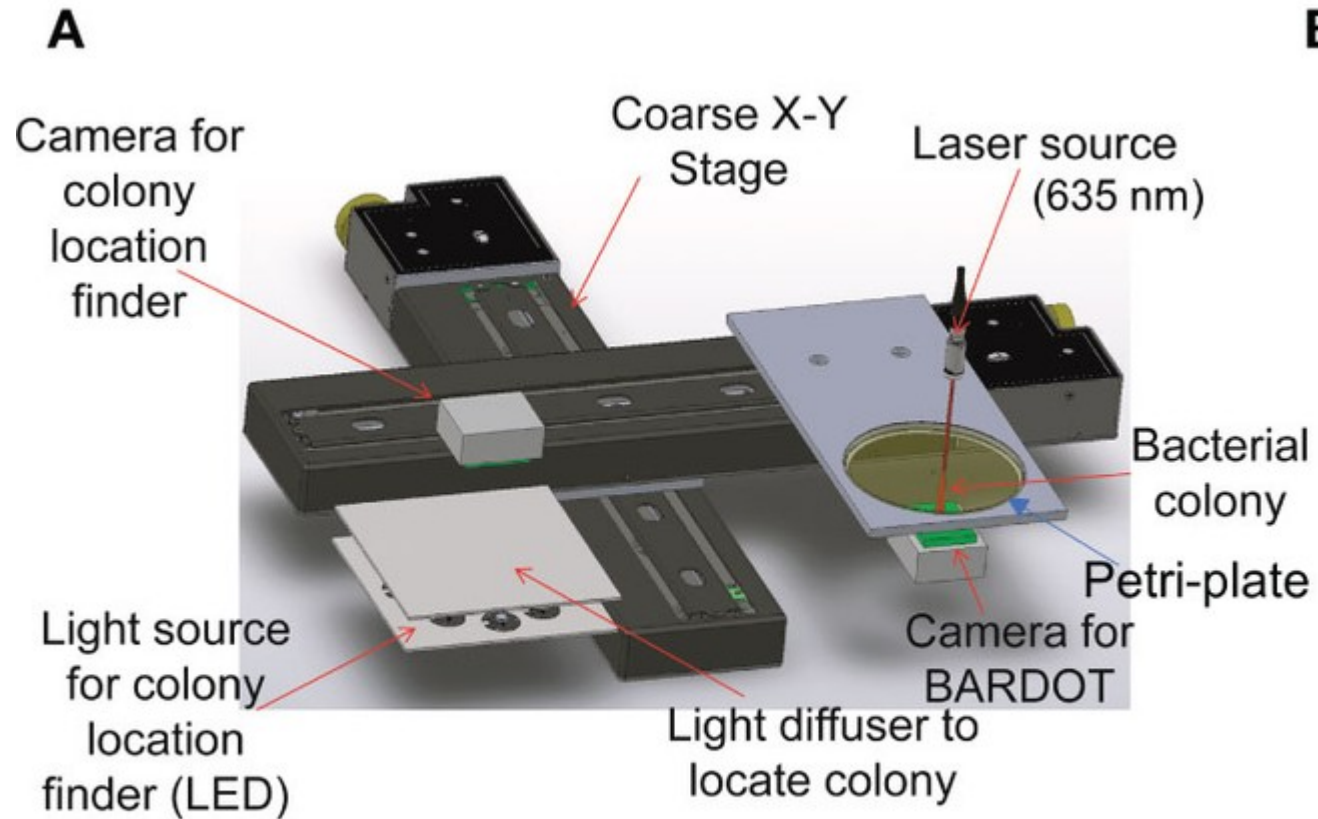
Membrane

**Filter
solution**

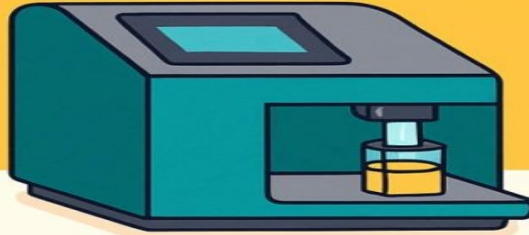
**Rinse
funnel**

**Plate and
incubate**

Count



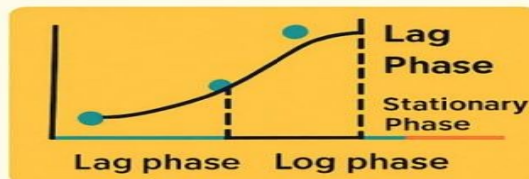
MEASUREMENT OF BACTERIAL GROWTH USING A SPECTROPHOTOMETER



Organism: E.coli
Medium: Luria-Bertani (LB) Broth
Wavelength: 600 nm (OD_{600})

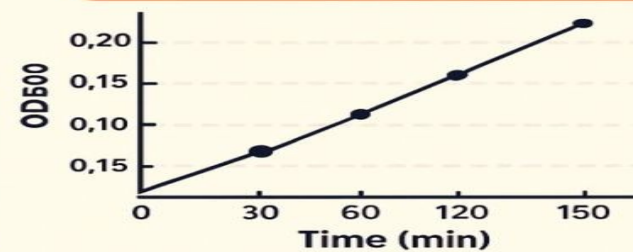
PROCEDURE

- 1 Inoculate E.coli in LB broth and incubate with shaking
- 2 Measure OD_{600} every 30 min
- 3 Plot OD_{600} vs. time



OBSERVATIONS	
Time (min)	OD ₆₀₀
0	0,145
30	0,150
60	0,155
90	0,160
120	0,162
150	0,192

GROWTH CURVE



DRY WEIGHT DETERMINATION: Microbial Biomass Measurement

1. PREPARATION



2. SAMPLE COLLECTION



6. WEIGHING



3. & 4. SEPARATION & WASHING



5. DRYING



7. CALCURATION

Dry Cell Weight (g/L) =
Dry Cell Weight (g/L)
 $[(W_2 - W_1) -$
Medium Residue Weight]

$W_2 = 0.253g = 0.253g$
Medium Residue = 1-1812g
Volume L

Biomass = 0.482 g/L

ADVANTGES

Accurate
Filamentous Organisms

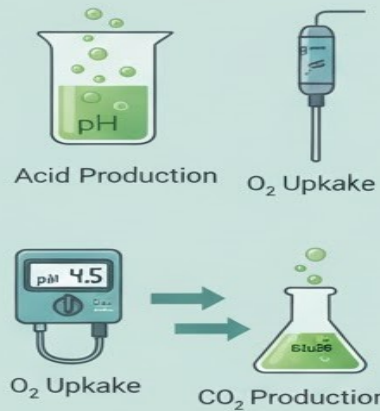
DISAVINTAGES

Time-Consusing
Counts Dead Cells

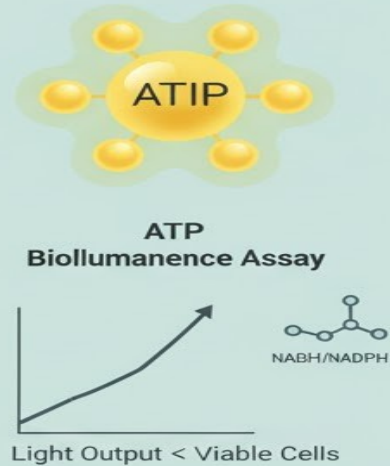
METABOLIC ACTIVITY MEASUREMENT MICROBIOLOGY

Principle: Quantify biochemical input/output (e.g., respiration, nutrient uptake, waste) to estimate the number of metabolically ACTIVE cells.

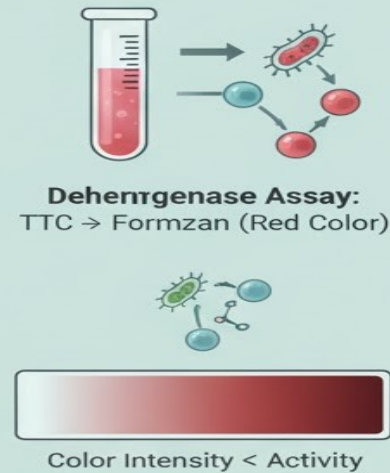
1. Substrate Consumption / Product Formation



2. Intracellular Components



3. Enzyme Activity Assays



ADVANTGES

- ✓ Measures Viable Cells
- ✓ Often Rapid Sensitive
- ✓ Complex Samples

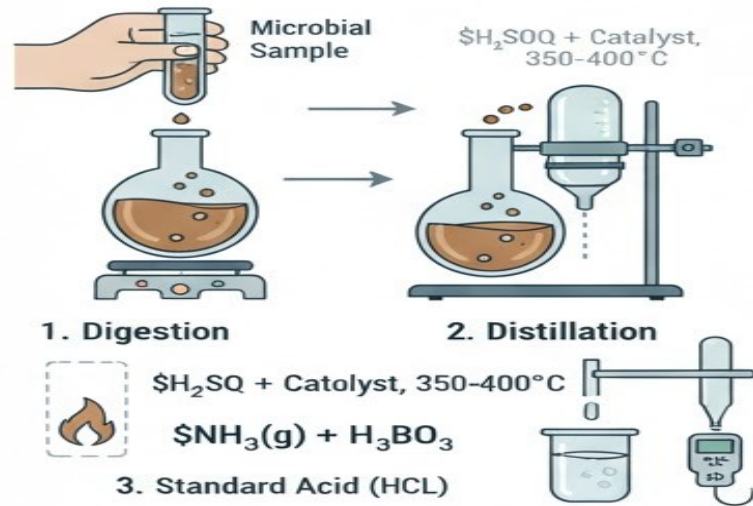
DISADVANTAGES

- ✓ Indirect Correlation ✗
- Affected by Physiology ✗
- Less Specific

CELL NITROGEN MEASUREMENT

Indirect Microbial Biomass Quantification

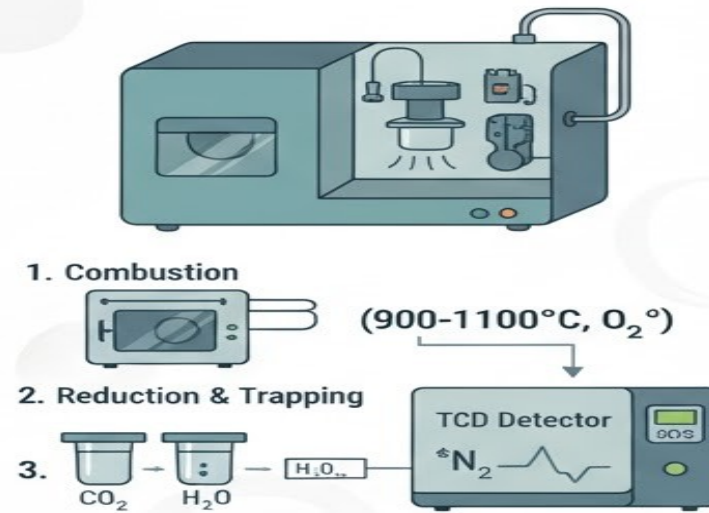
1. KJELLHALL METHOD (TKN)



Notes: Slow, Corrooive Chemicals, Fume Hood Required.



2. DUMAS METHOD (Combustion)



Notes: Fast, Automated, No Corrooives



APPLICATIONS IN MICRORCBOLOGY



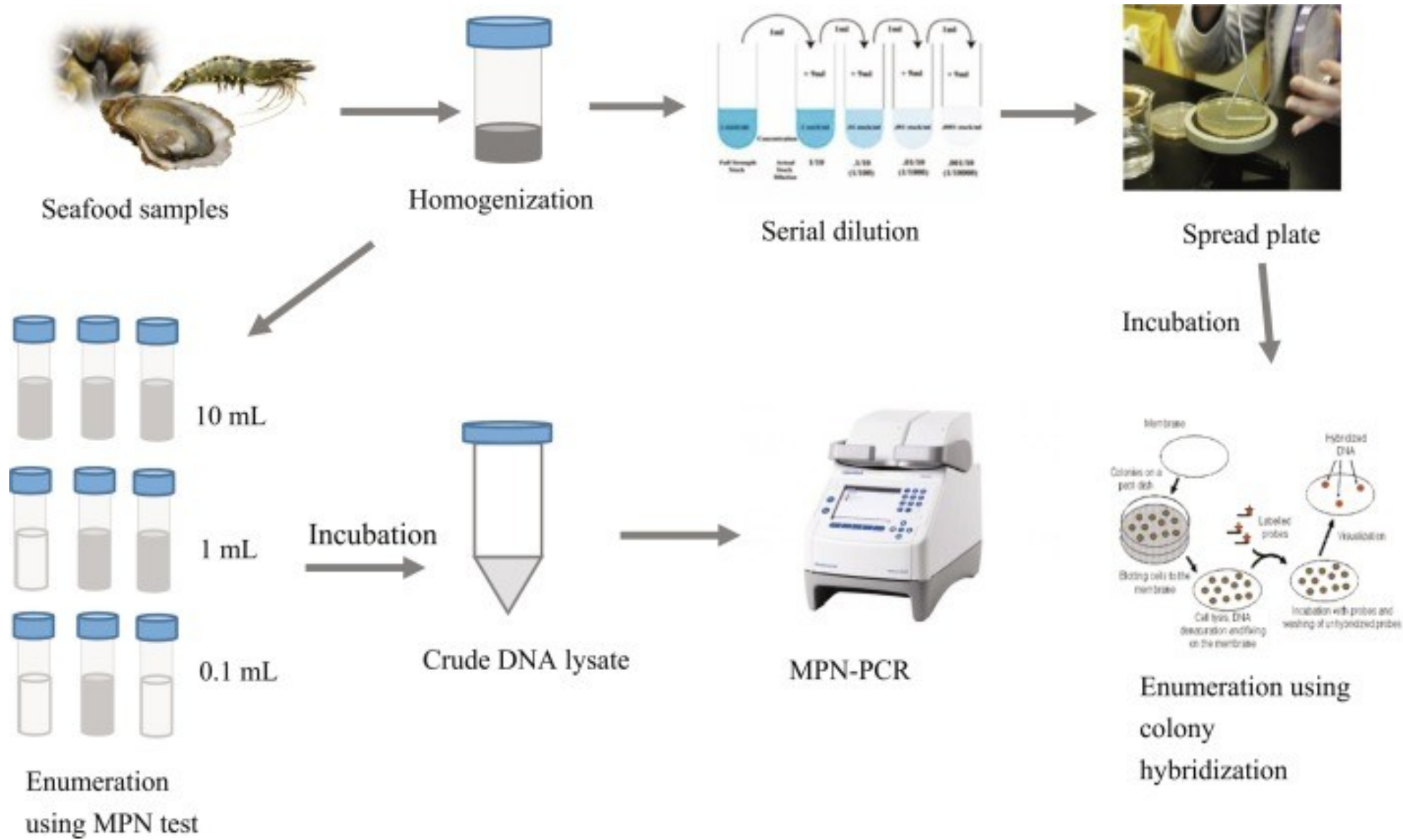
1. Monitor Biomass Growth

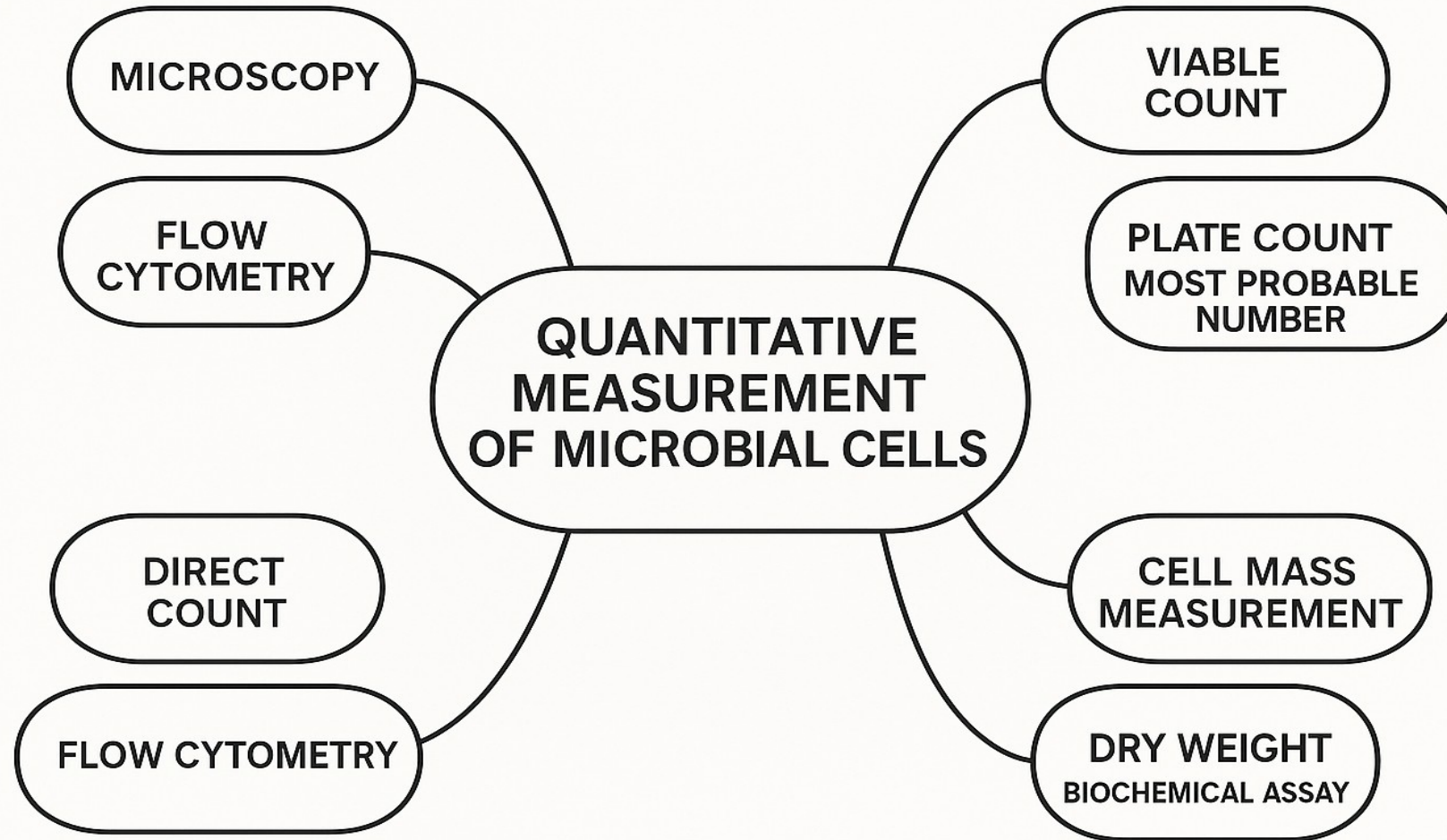


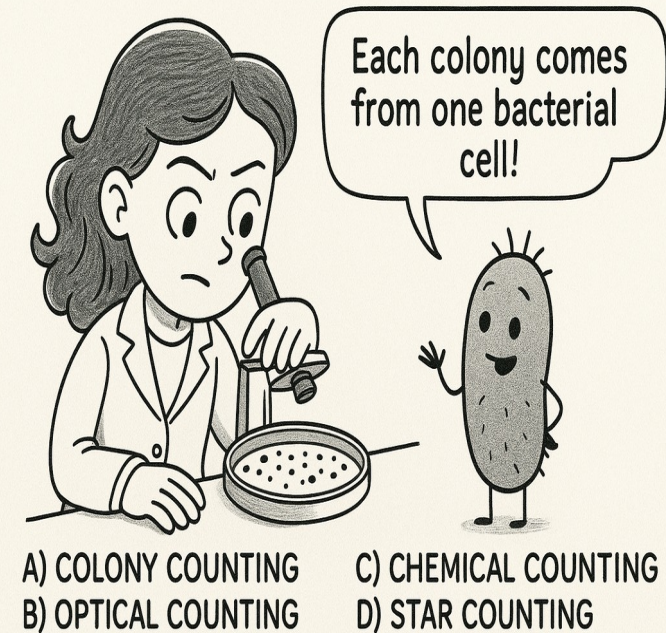
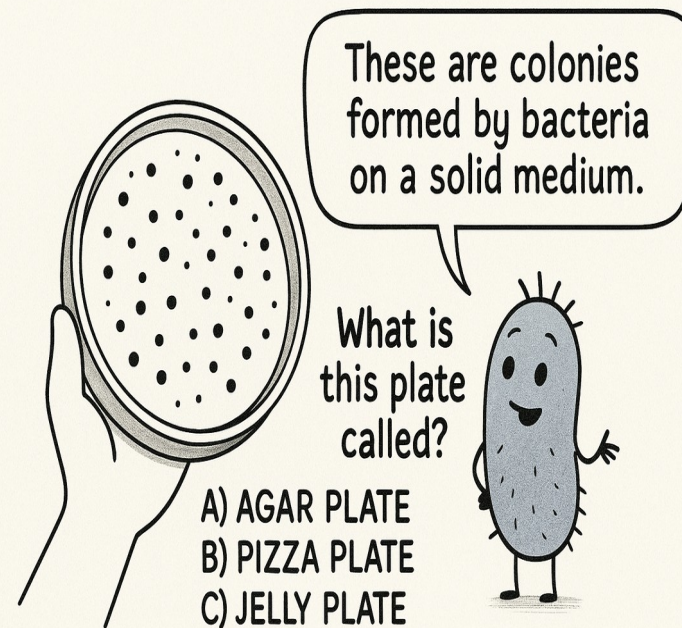
2. Crude Protein Estimation
($\text{N} \times 6.25$)

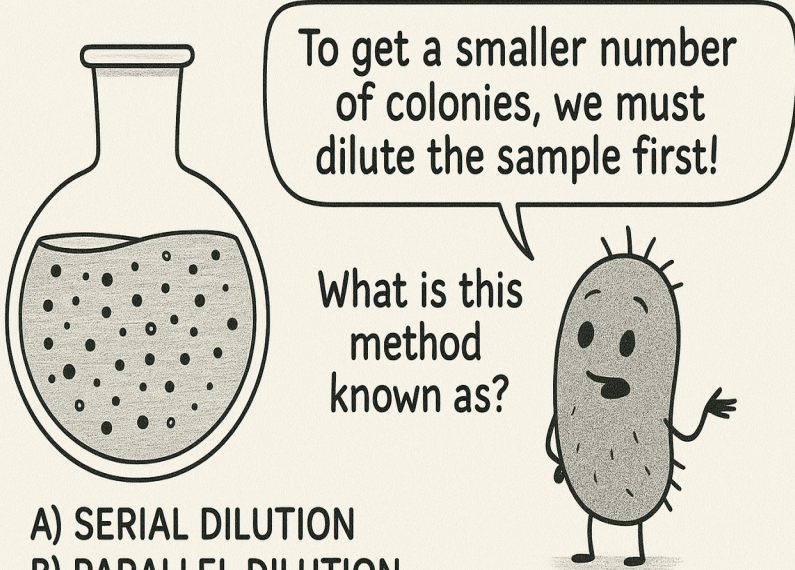


3. Nutrient Stochbetrity
(C:N:P Ratio)









To get a smaller number of colonies, we must dilute the sample first!

What is this method known as?

A) SERIAL DILUTION
B) PARALLEL DILUTION
C) FOOD COLORING
D) BUBBLE MIXING



It's shining light through the tube!

What is this method known as:

A) TURBIDITY MEASUREMENT
B) TRANSPARENCY TEST
C) BRIGHTNESS TEST
D) CLOUDY CHECK

REFERENCES

- 1.W.B. Hugo and A.D. Russel: Pharmaceutical Microbiology, Blackwell Scientific publications, Oxford London.
2. Prescott and Dunn., Industrial Microbiology, 4th edition, CBS Publishers & Distributors, Delhi.
3. Ananthanarayan : Text Book of Microbiology, Orient-Longman, Chennai

