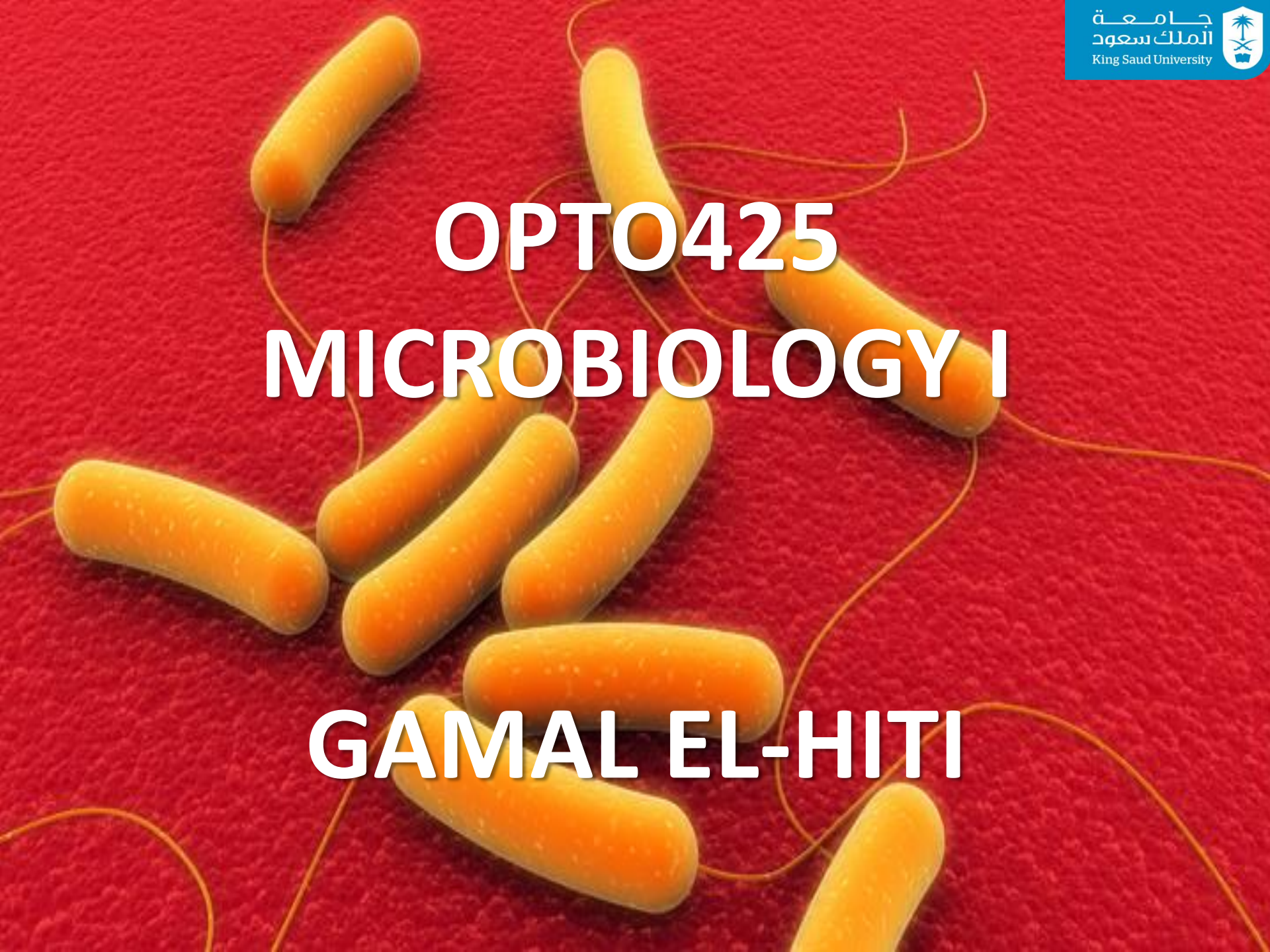
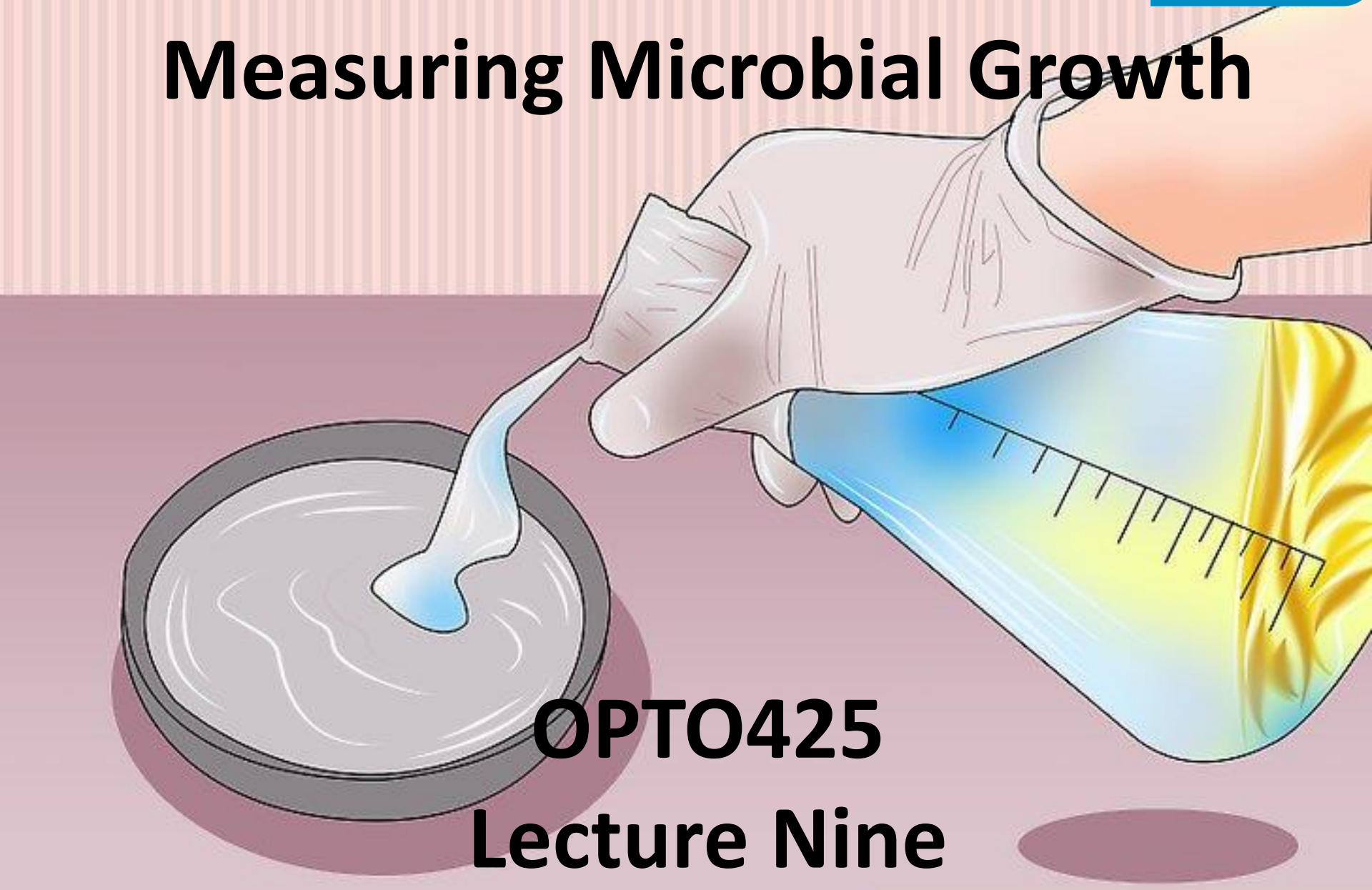


# OPTO425
# MICROBIOLOGY I

GAMAL EL-HITI

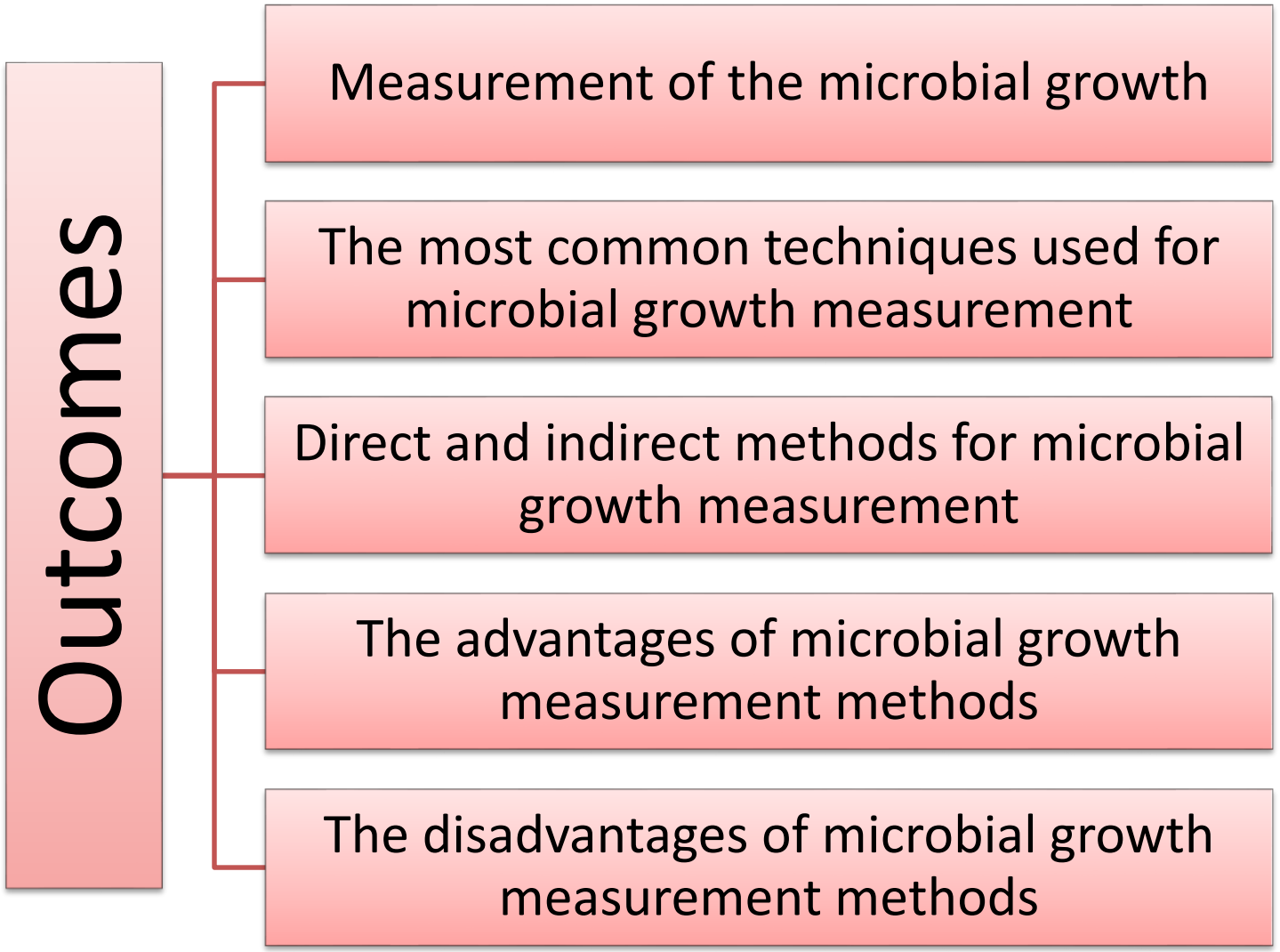
جامعة الملك سعود
King Saud University

# Measuring Microbial Growth




## OPTO425
## Lecture Nine

# Learning Outcomes



# Measuring Microbial Growth

- Direct Methods of Measurement

1) Plate Count Method

- Most frequently used method of measuring bacterial populations.
- Inoculate plate with a sample of bacteria and count number of colonies.
- Assumptions
- Each colony originates from a single bacterial cell and no cell aggregates.
- Original inoculum is homogeneous.

# Measuring Microbial Growth

- Advantages of plate count method
- Measures viable cells.
- Disadvantages of plate count method
- It takes 24 hours or more for visible colonies to appear.
- It can be only used to count between 25 and 250 colonies with high accuracy.
- It requires to perform serial dilutions to get the appropriate concentration to give desirable numbers of colonies per plate.

# Measuring Microbial Growth

- If the concentration of bacteria is too great, the colonies will grow into each other and the plate will be uncountable.
- Number of bacteria/mL = number of colonies on plate × reciprocal of dilution of sample.
- *e.g.*, if 32 colonies are on a plate of 1/10,000 dilution then what is the count?
- The count = 32 × 10,000 = 320,000/mL of sample.

# Measuring Microbial Growth

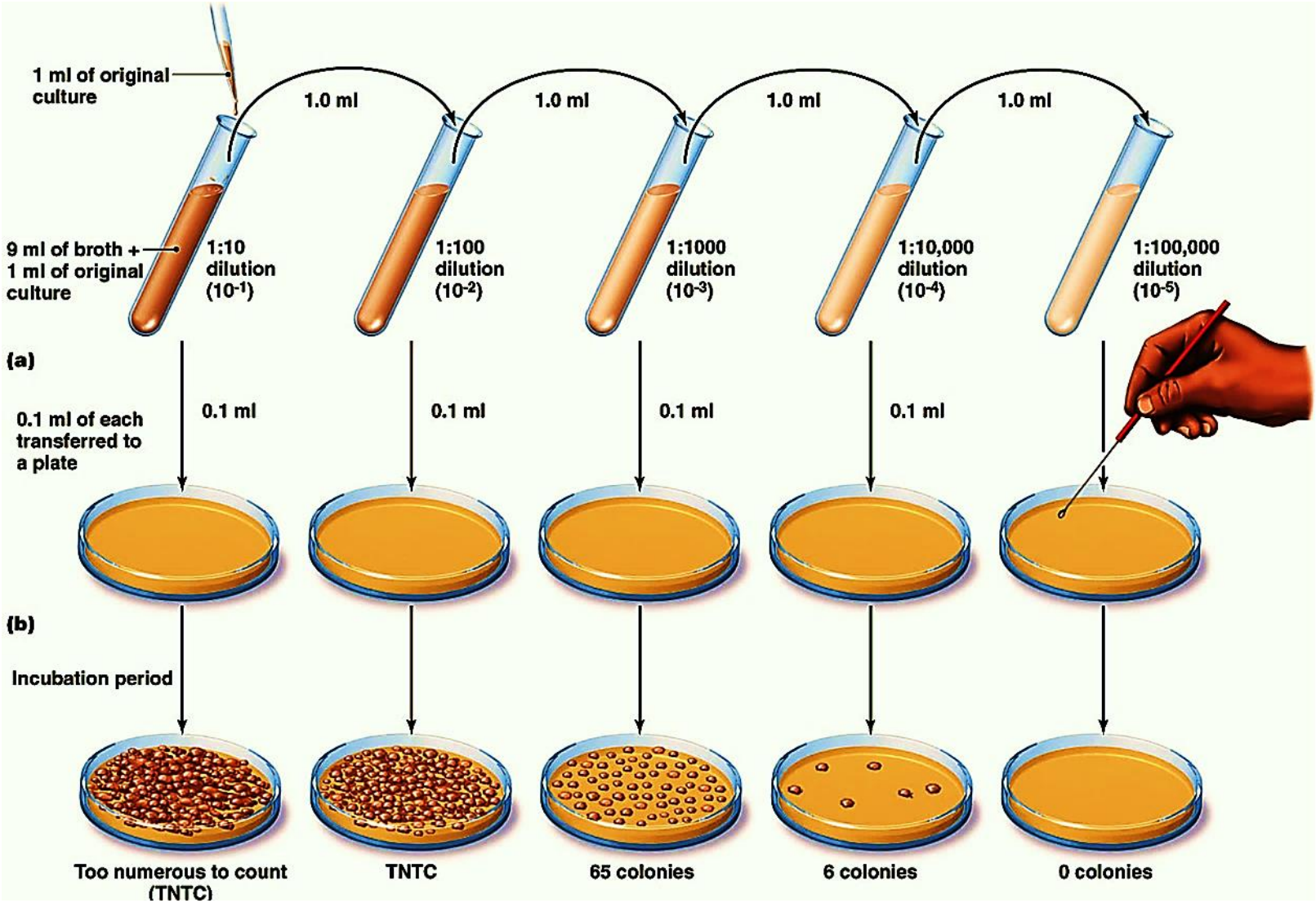



Plate Count Method

# Measuring Microbial Growth

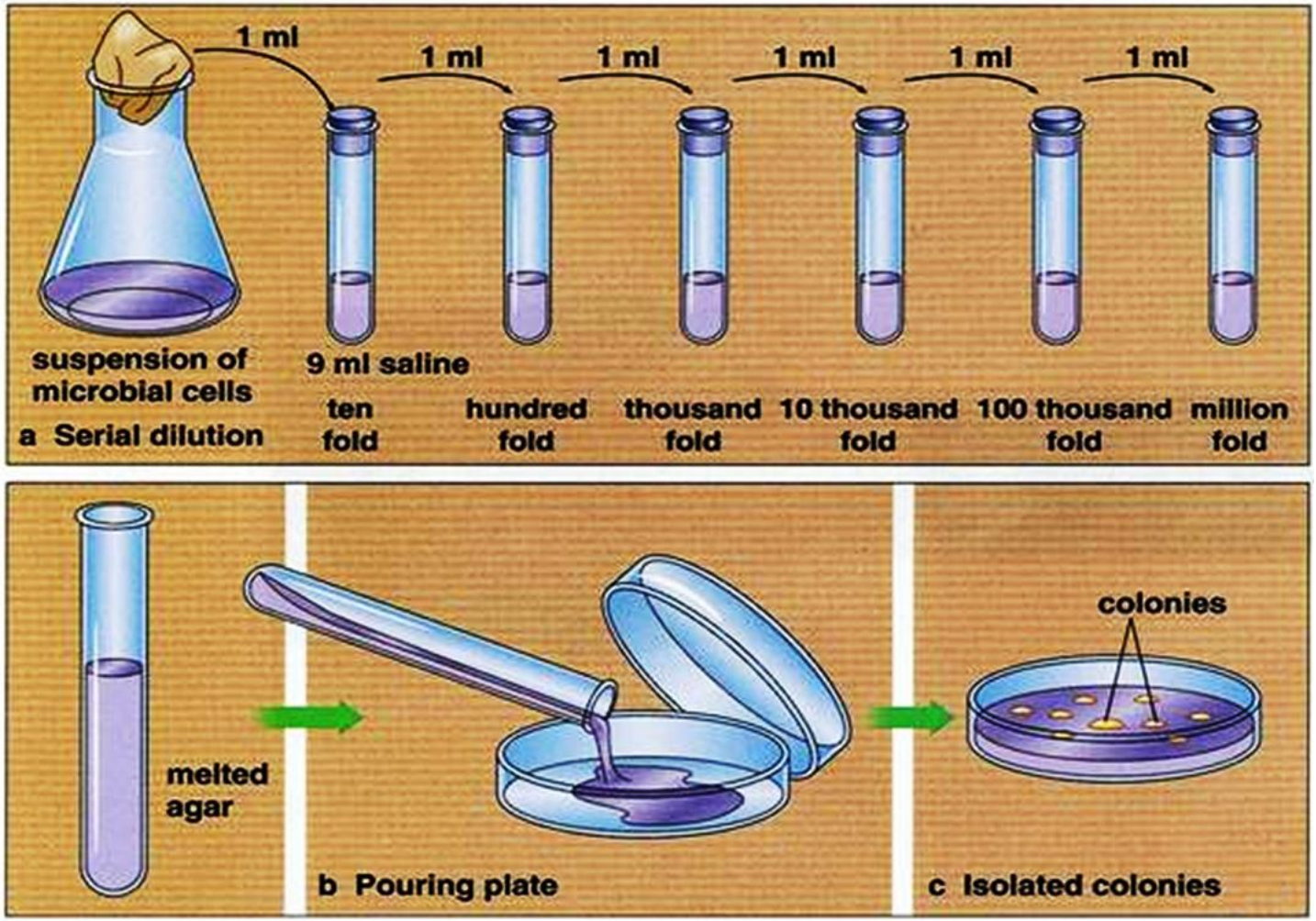
- Types of Plate Count Technique

A– Pour Plate Method

- Introduce a 1.0 or 0.1 mL inoculum into an empty Petri dish.
- Add liquid nutrient medium and kept at 50 °C.
- Gently mix, allow to solidify and incubate.
- Disadvantages of pour plate method
- Not useful for heat sensitive organisms.
- Colonies appear under agar surface.

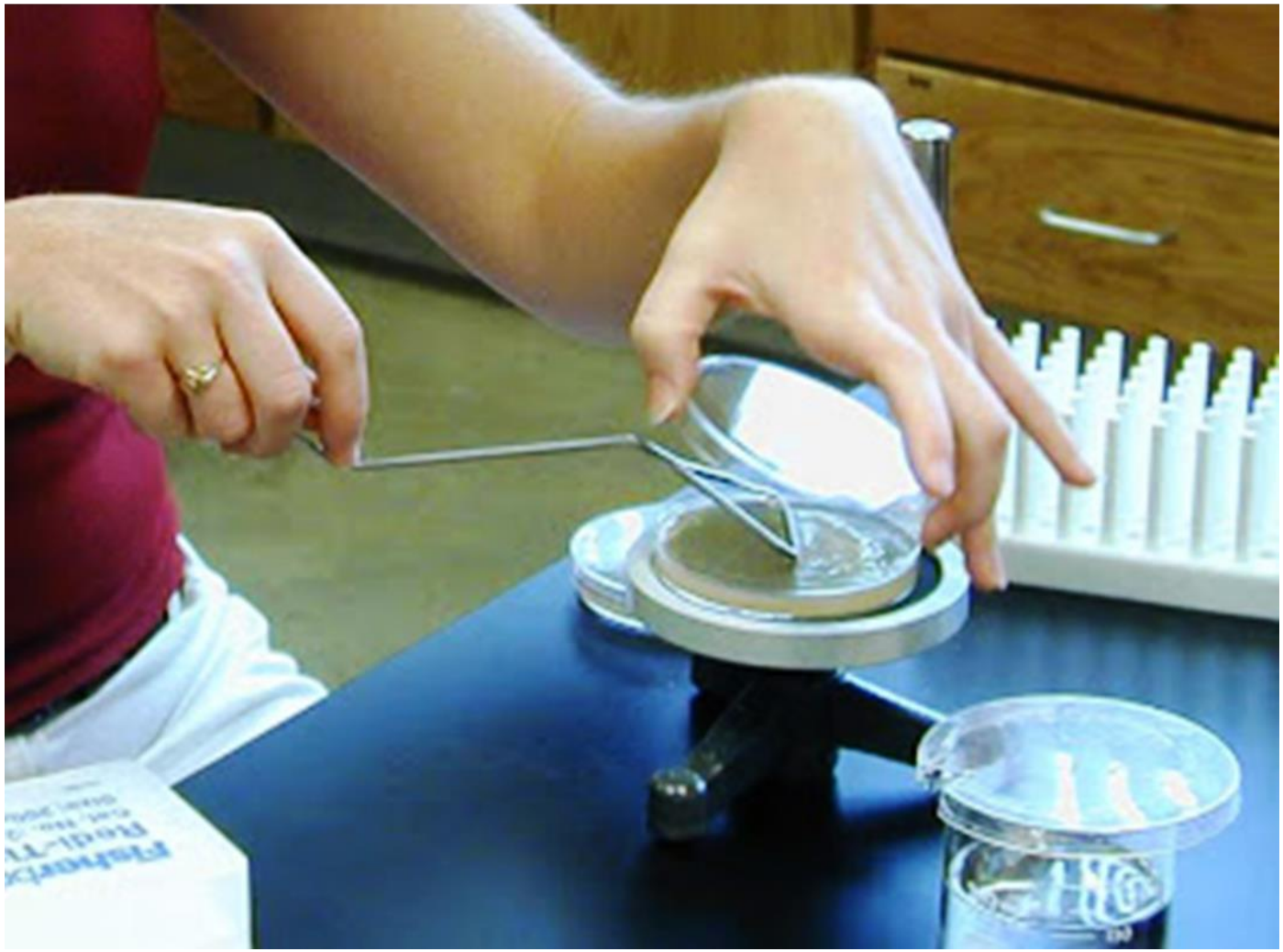
# Measuring Microbial Growth

## Pour Plate Method



# Measuring Microbial Growth

## Pour Plate Method



# Measuring Microbial Growth

## B– Spread Plate Method

- Introduce a 0.1 mL inoculum onto the surface of Petri dish.
- Spread with a sterile glass rod.
- Advantages of spread plate method
- Colonies will be on surface and not exposed to melted agar.

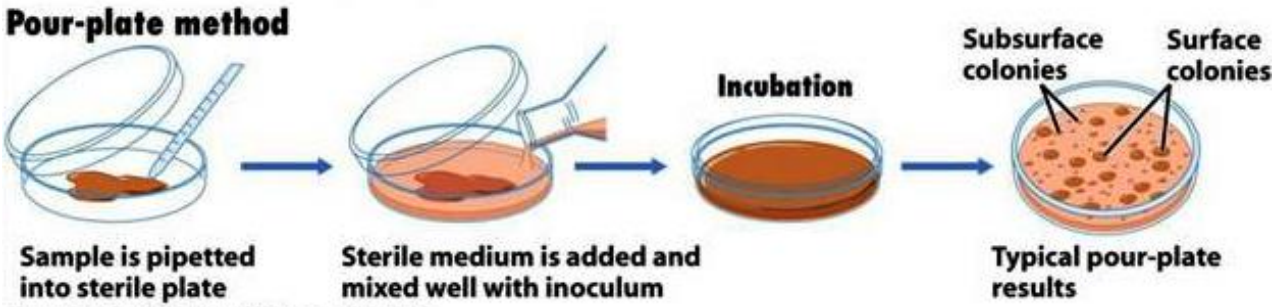



Figure 6-10 Brock Biology of Microorganisms 11/e
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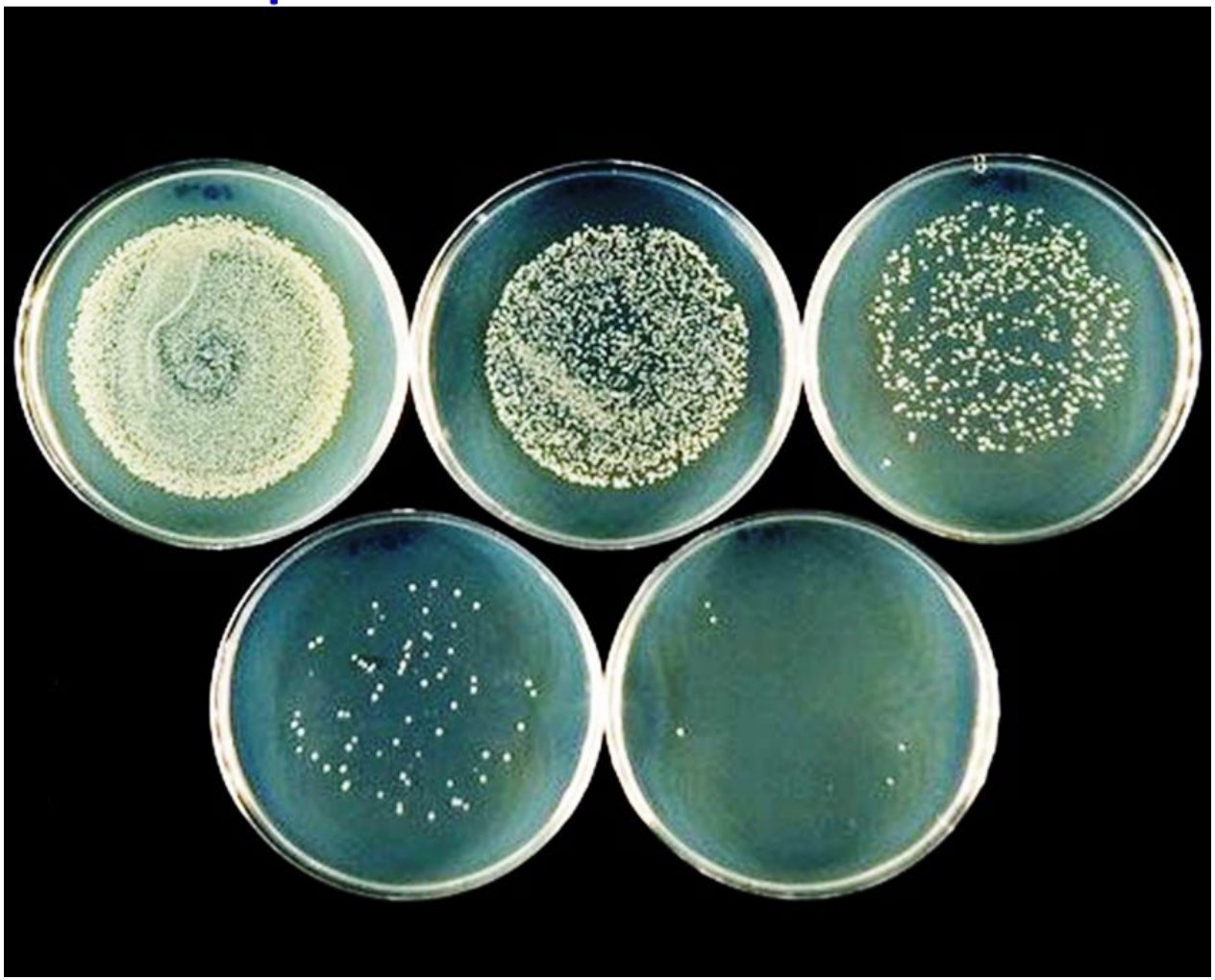
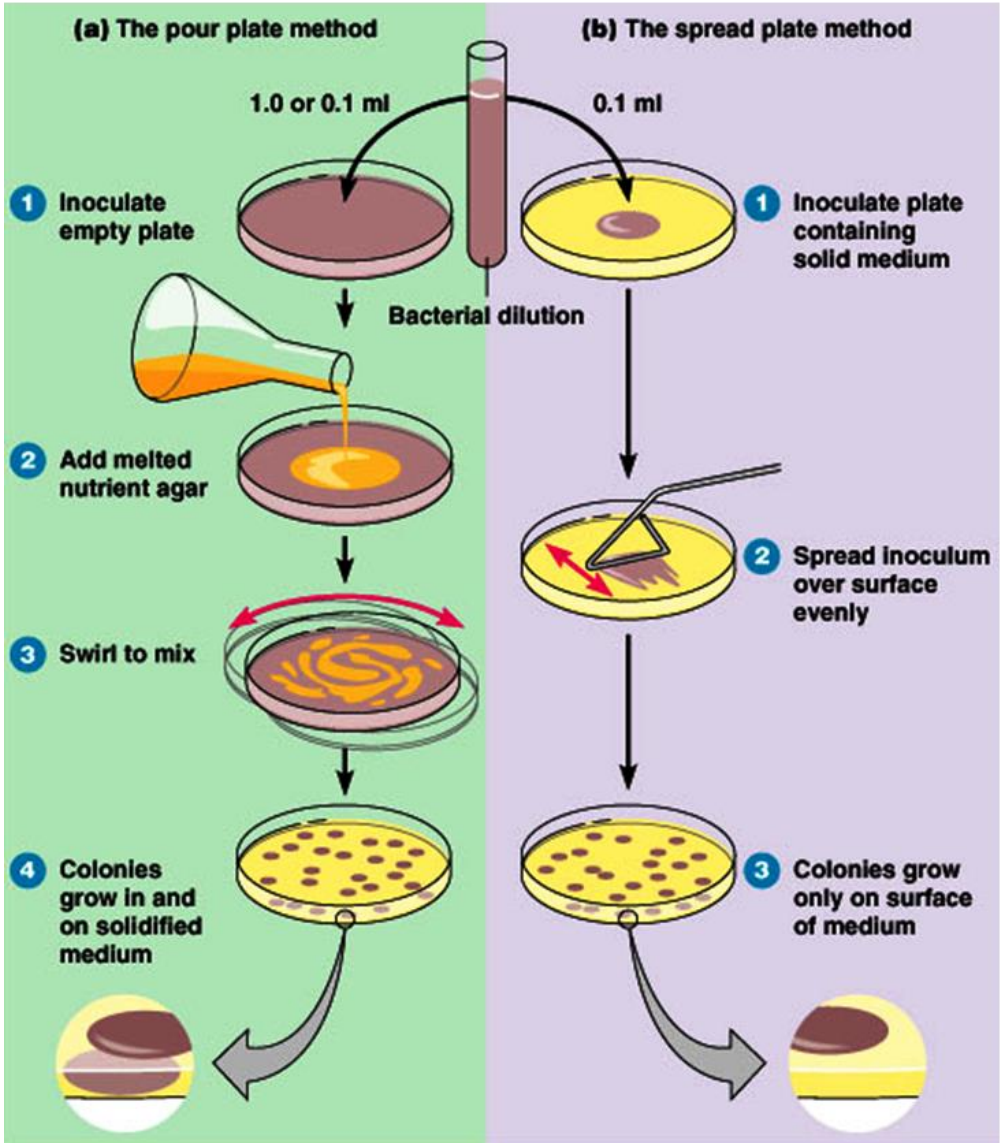
# Measuring Microbial Growth

## Spread Plate Method



# Measuring Microbial Growth




Pour Plates vs. Spread Plates

# Measuring Microbial Growth

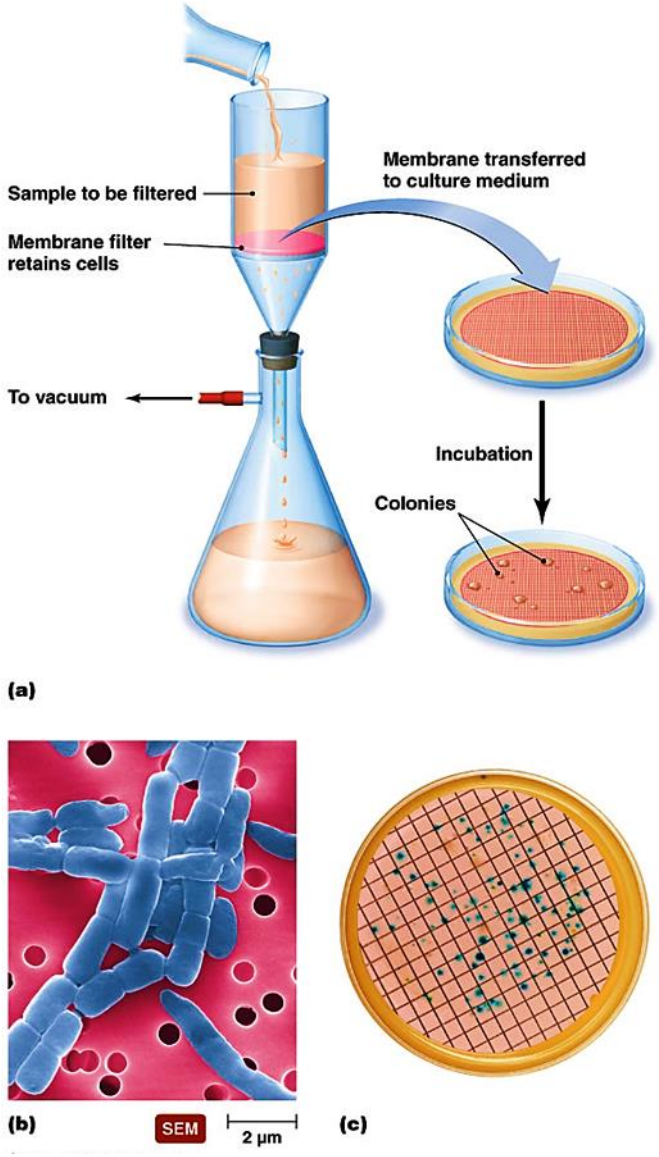
- Direct Methods of Measurement

## 2) Filtration

- It is used to measure small quantities of bacteria.
- *e.g*. Fecal bacteria in a lake water.
- A large sample (100 mL or more) is filtered to retain bacteria.

# Measuring Microbial Growth

- Bacteria are retained on the surface of a membrane filter.
- Bacteria was then transferred to a culture medium onto a Petri dish to grow.
- Incubate the bacteria under optimal conditions and count colonies.

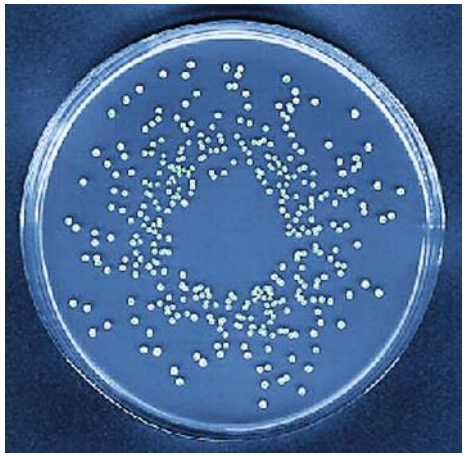


# Measuring Microbial Growth

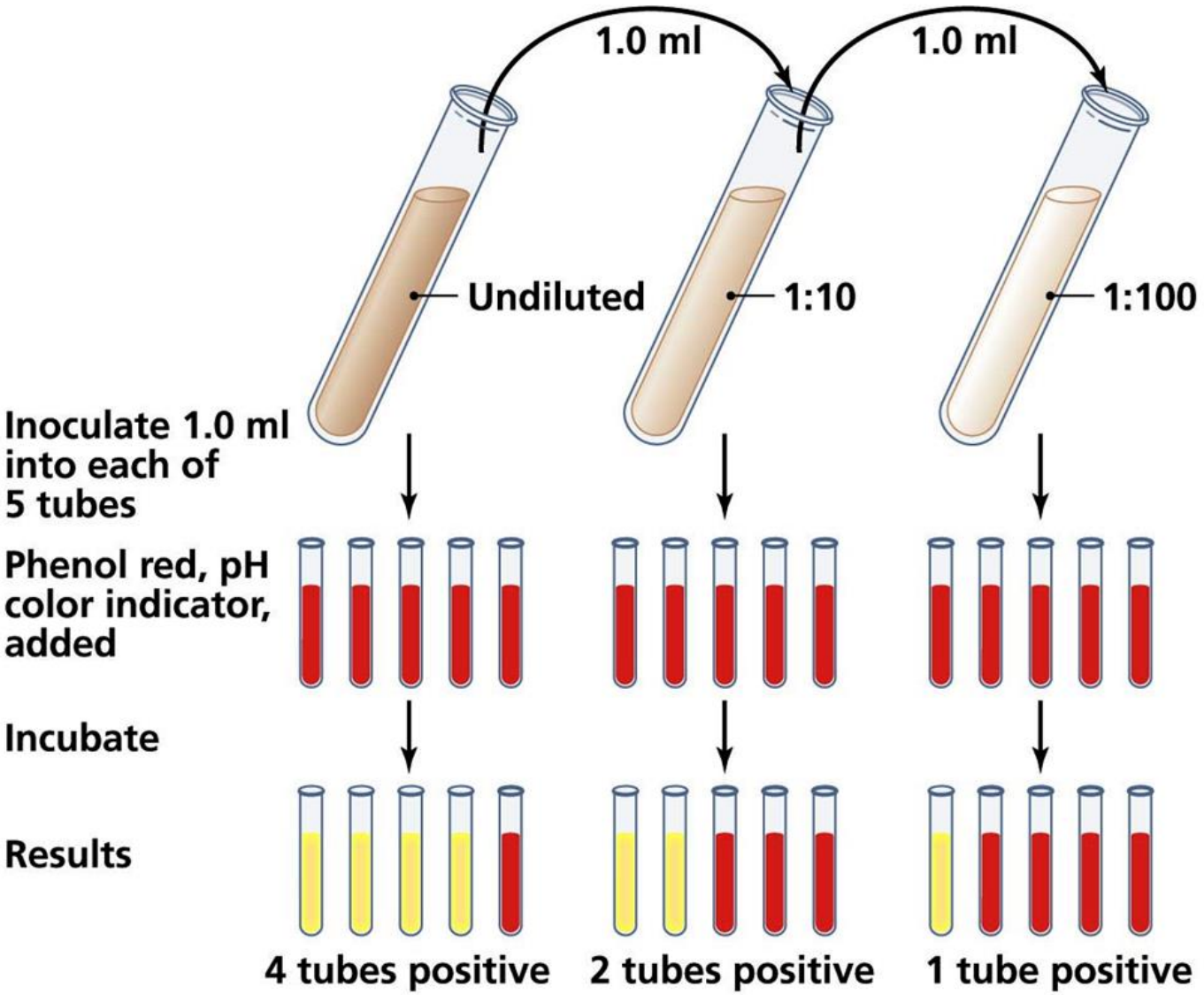
- Direct Methods of Measurement

3) Most Probable Number (MPN)

- It is used mainly to measure bacteria that will not grow on solid medium.
- Dilute a sample repeatedly and inoculate several broth tubes for each dilution point.
- Count the number of positive tubes along with negative ones in each set.



# Measuring Microbial Growth




Most Probable Number (MPN)

# Measuring Microbial Growth

- Statistical Method
- Determines 95% probability that a bacterial population falls within a certain range.
- Before the tubes are inoculated, the chance is at least 95% that the confidence interval associated with the eventual result will enclose the actual concentration.

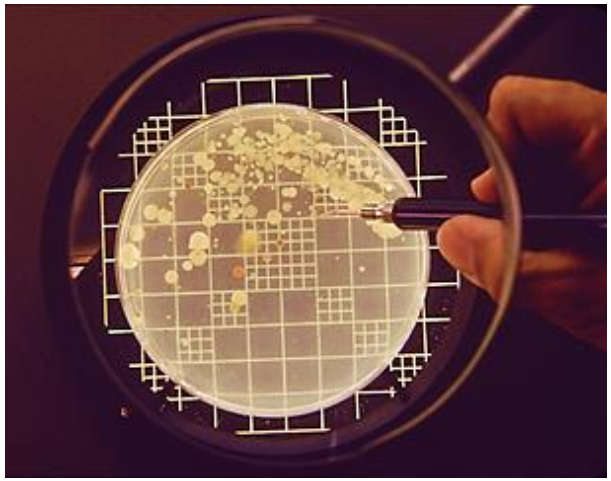
| Combination of Positives | MPN Index/ 100 ml | 95% Confidence Limits | |
|---|---|---|---|
| | | Lower | Upper |
| 4-2-0 | 22 | 9 | 56 |
| 4-2-1 | 26 | 12 | 65 |
| 4-3-0 | 27 | 12 | 67 |
| 4-3-1 | 33 | 15 | 77 |
| 4-4-0 | 34 | 16 | 80 |
| 5-0-0 | 23 | 9 | 86 |
| 5-0-1 | 30 | 10 | 110 |
| 5-0-2 | 40 | 20 | 140 |
| 5-1-0 | 30 | 10 | 120 |
| 5-1-1 | 50 | 20 | 150 |
| 5-1-2 | 60 | 30 | 180 |
| 5-2-0 | 50 | 20 | 170 |
| 5-2-1 | 70 | 30 | 210 |
| 5-2-2 | 90 | 40 | 250 |
| 5-3-0 | 80 | 30 | 250 |
| 5-3-1 | 110 | 40 | 300 |
| 5-3-2 | 140 | 60 | 360 |

# Measuring Microbial Growth

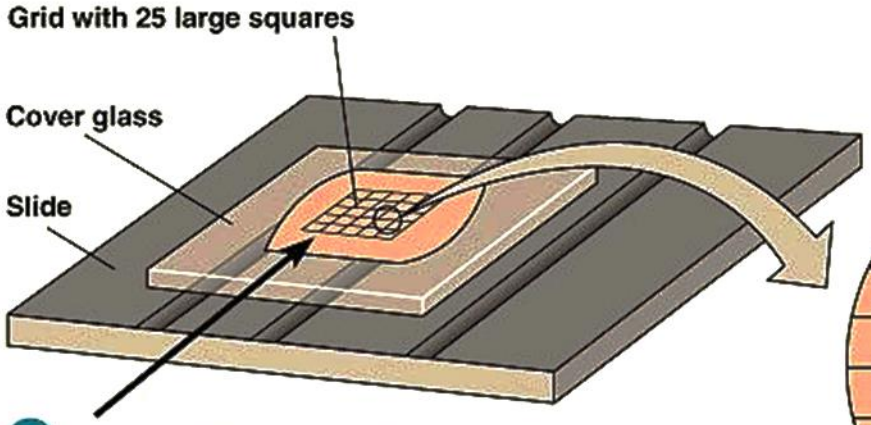
- Direct Methods of Measurement

## 4) Direct Microscopic Count

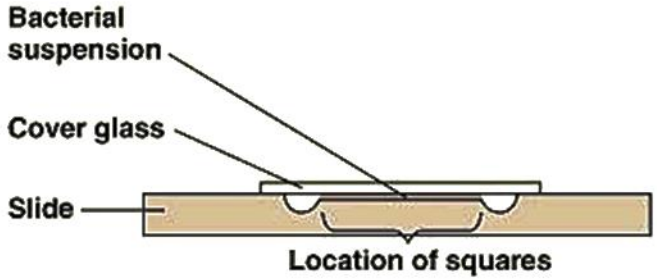
- A specific volume (0.01 mL) of a bacterial suspension is placed on a microscope slide with a special grid.
- Stain is added to visualize bacteria.
- Cells are counted and multiplied by a factor to obtain concentration.



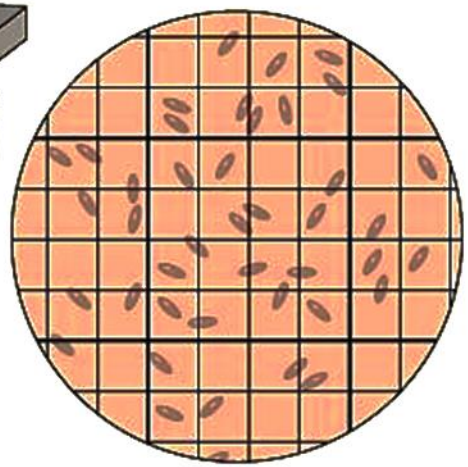
# Measuring Microbial Growth




1 Bacterial suspension is added here and fills the shallow volume over the squares by capillary action.




2 Cross section of a cell counter. The depth under the cover glass is known, and the area of the squares is known, so the volume of the bacterial suspension over the squares can be calculated (depth × area).



3 Microscopic count: All cells in several large squares are counted, and the numbers are averaged. The large square shown here has 14 bacterial cells.

4 The volume of fluid over the large square is 1/1,250,000 of a milliliter. If it contains 14 cells, as shown here, then there are 14 times 1,250,000 (17,500,000) cells in a milliliter.

Direct Microscopic Count

# Measuring Microbial Growth

- Advantages of direct microscopic count
- No incubation time is required.
- It is a rapid, simple and easy method requiring minimum equipment.

Disadvantages of direct microscopic count

- The method cannot always distinguish between live and dead bacteria.
- Motile bacteria are difficult to count.
- Requires a high concentration of bacteria (10 million/mL).

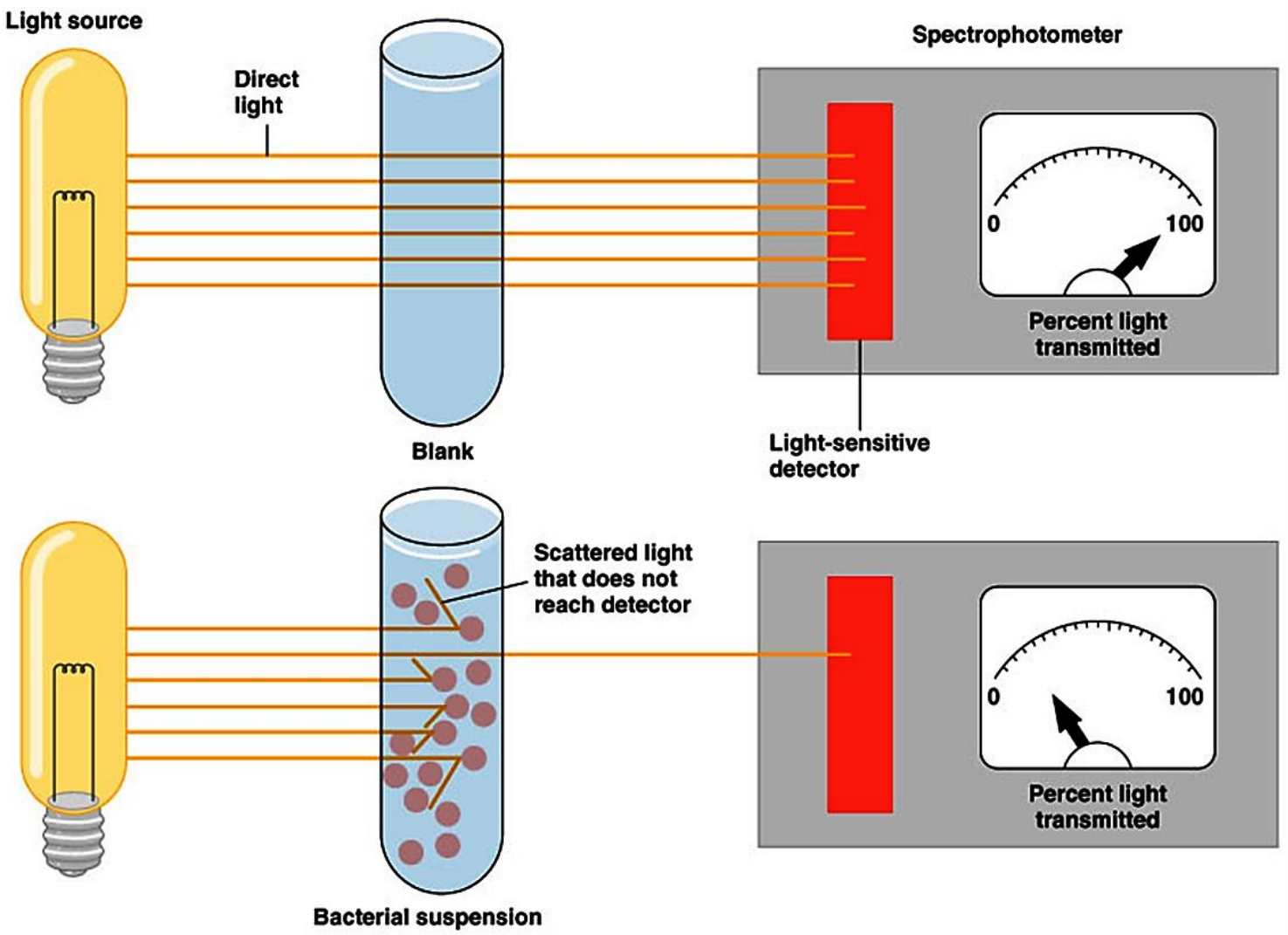
# Measuring Microbial Growth

- Indirect Methods of Measurement

1) Turbidity

- As bacteria multiply in media, it becomes turbid.
- A spectrophotometer is used to determine the transmission percentage or absorbance of the media.
- Multiply by a factor to determine the concentration of bacteria.
- More light is absorbed when more cells are present.

# Measuring Microbial Growth




Turbidity

# Measuring Microbial Growth

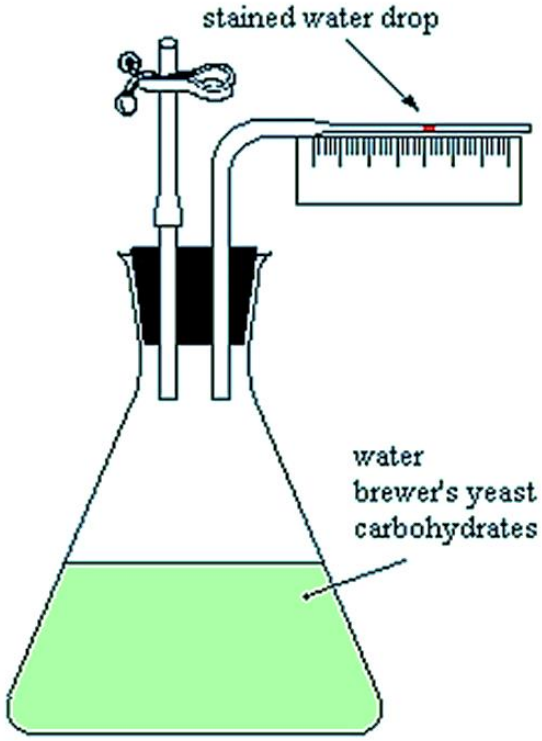
- Advantages of turbidity method
- No incubation time is required.
- Estimation of large numbers of bacteria in clear liquid media and broths.
- Disadvantages of turbidity method
- Cannot distinguish between live and dead bacterial cells.
- Requires a high concentration of bacteria not less than $10^7$cells per mL (10 to 100 million cells/mL).

# Measuring Microbial Growth

- Indirect Methods of Measurement

## 2) Metabolic Activity

- As bacteria multiply in media, they produce certain products, *e.g*. $CO_2$ and acids.
- The quantity of such metabolic products can be measured.
- The method is relatively expensive.



# Measuring Microbial Growth

- Indirect Methods of Measurement

3) Dry Weight

- Measurement of dry weight or wet weight of cells after centrifugation.
- Bacteria or fungi in liquid media are centrifuged.
- Resulting cell pellet is weighed.
- Measures total cell yield in cultures.
- Doesn't distinguish live and dead cells.
- An *E. coli* cell has a dry mass of about $7.0 \times 10^{-19}$ mg.

# Measuring Microbial Growth

- List of Reagents and Instruments for the Measuring Microbial growth
- Flasks, graduated cylinder and filtration unit with vacuum pump.
- Centrifuge, oven up to 100 °C, balance and spectrophotometer.
- Cell counting chamber, microscope.
- Petri dish, sterile pipets, sterile bottles.
- Flask of culture, nutrient agar and sterile water.