CULTURE MEDIA COMPOSITION

- Culture media supply the nutritional needs of microorganisms (C ,N, Phosphorus, trace elements, etc)
  - **Defined medium** : precise amounts of highly purified chemicals
  - **Complex medium** (or undefined) : highly nutritious substances.
- In clinical microbiology,
  - **Selective** : contains compounds that selectively inhibit
  - **Differential**: contains indicator
  - terms that describe media used for the isolation of particular species or for comparative studies of microorganisms.

TYPES OF MEDIA

- Media can be classified on three primary levels
    1. Physical State
    2. Chemical Composition
    3. Functional Type

PHYSICAL STATES OF MEDIA

- Liquid Media
- Semisolid
- Solid (Can be converted into a liquid)
- Solid (Cannot be converted into a liquid)

LIQUID MEDIA

- Water-based solutions
- Do not solidify at temperatures above freezing / tend to be free flowing
- Includes broths, milks, and infusions
- Measure turbidity
- Example: Nutrient Broth, Methylene Blue Milk, Thioglycollate Broth

SEMI-SOLID MEDIA

- Exhibits a clot-like consistency at ordinary room temperature
- Determines motility
- Used to localize a reaction at a specific site.
- Example: Sulfide Indole Motility (SIM) for hydrogen sulfide production and indole reaction and motility test.

SOLID MEDIA

- Firm surface for discrete colony growth
- Advantageous for isolating and culturing
- Two Types
    1. Liquefiable (Reversible)
    2. Non-liquefiable
- Examples: Gelatin and Agar (Liquefiable)
  Cooked Meat Media, Potato Slices (Non-liquefiable)

CHEMICAL COMPOSITION OF CULTURE MEDIA

1. Synthetic Media
   - Chemically defined
   - Contain pure organic and inorganic compounds
   - Exact formula (little variation)
2. Complex or Non-synthetic Media
   - Contains at least one ingredient that is not chemically definable (extracts from plants and animals)
   - No exact formula / tend to be general and grow a wide variety of organisms

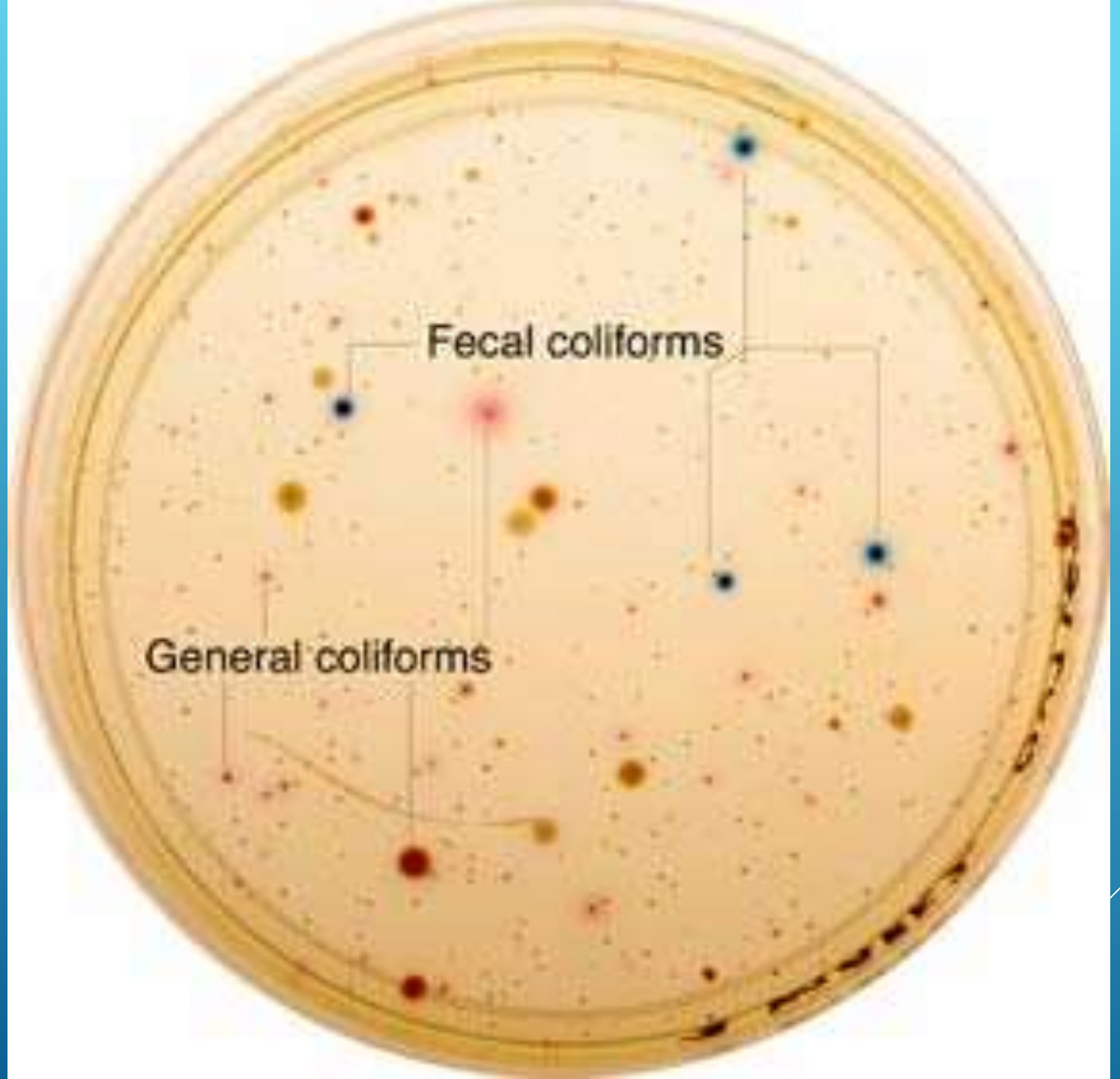
SELECTIVE MEDIA

- Contains one or more agents that **inhibit** the growth of a certain microbe and thereby encourages, or **selects**, a specific microbe.
- Example: Mannitol Salt Agar [MSA] encourages the growth of *S. aureus*. MSA contain 7.5% NaCl which inhibit the growth of other Gram + bacteria

Growth of *Staphylococcus aureus* on Mannitol Salt Agar results in a color change in the media from pink to yellow.

DIFFERENTIAL MEDIA

- Differential shows up as **visible changes** or variations in colony size or color, in media color changes, or in the formation of gas bubbles and precipitates.
- Example: Spirit Blue Agar to detect the digestion of fats by lipase enzyme. Positive digestion (hydrolysis) is indicated by the dark blue color that develops in the colonies. Blood agar for hemolysis (a,β,and γ hemolysis), EMB, MacConkey Agar, …etc.


Fecal coliforms
General coliforms

ENRICHMENT MEDIA

- Is used to encourage the growth of a particular microorganism in a mixed culture.
- Ex. Manitol Salt Agar for *S. aureus*
- Blood agar , chocolate agar, Slenite F broth

BACTERIAL COLONIES ON SOLID MEDIA

P. aeruginosa (TSA)

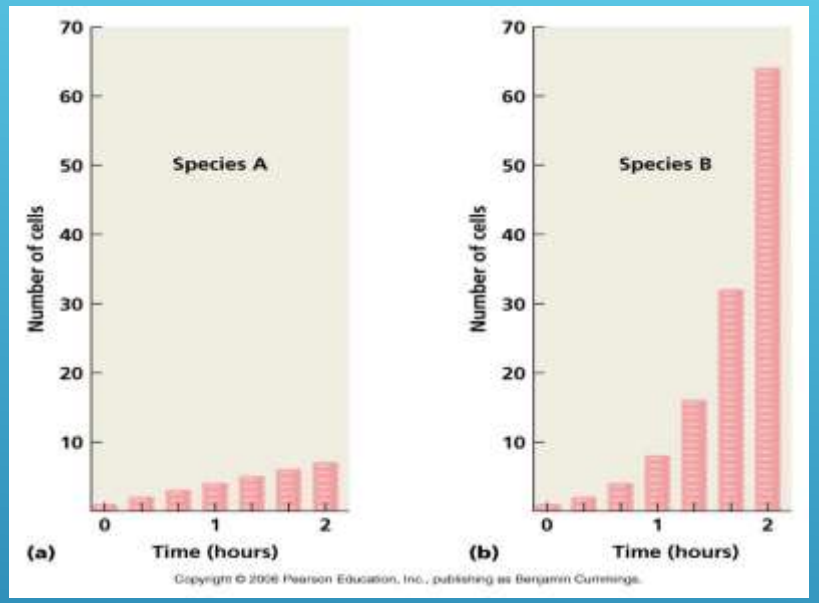
S. Flexneri (Mac)

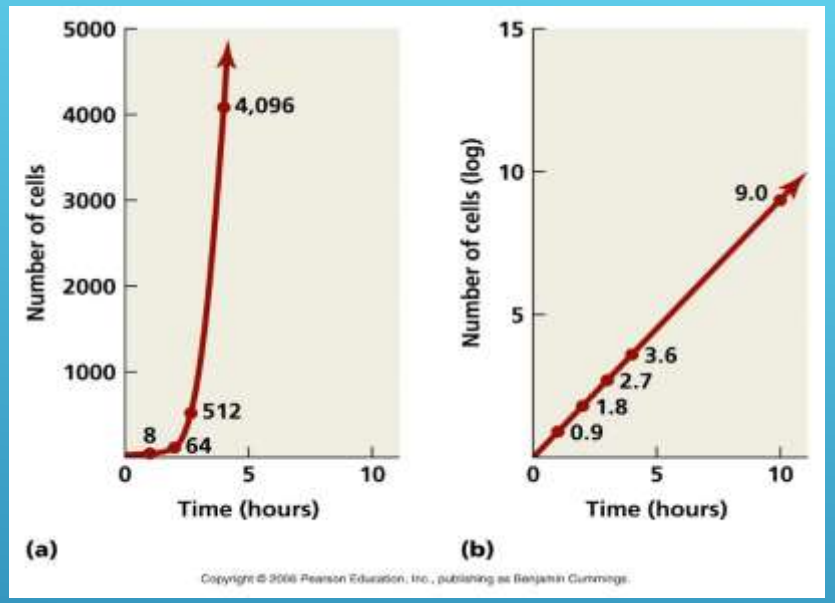
S. Marcescens (Mac)

LABORATORY CULTURE OF MICROORGANISMS

- Microorganisms can be grown in the laboratory in culture media containing the nutrients they require.
- Successful cultivation and maintenance of **pure cultures** of microorganisms can be done only if **aseptic technique** is practiced to prevent contamination by other microorganisms.

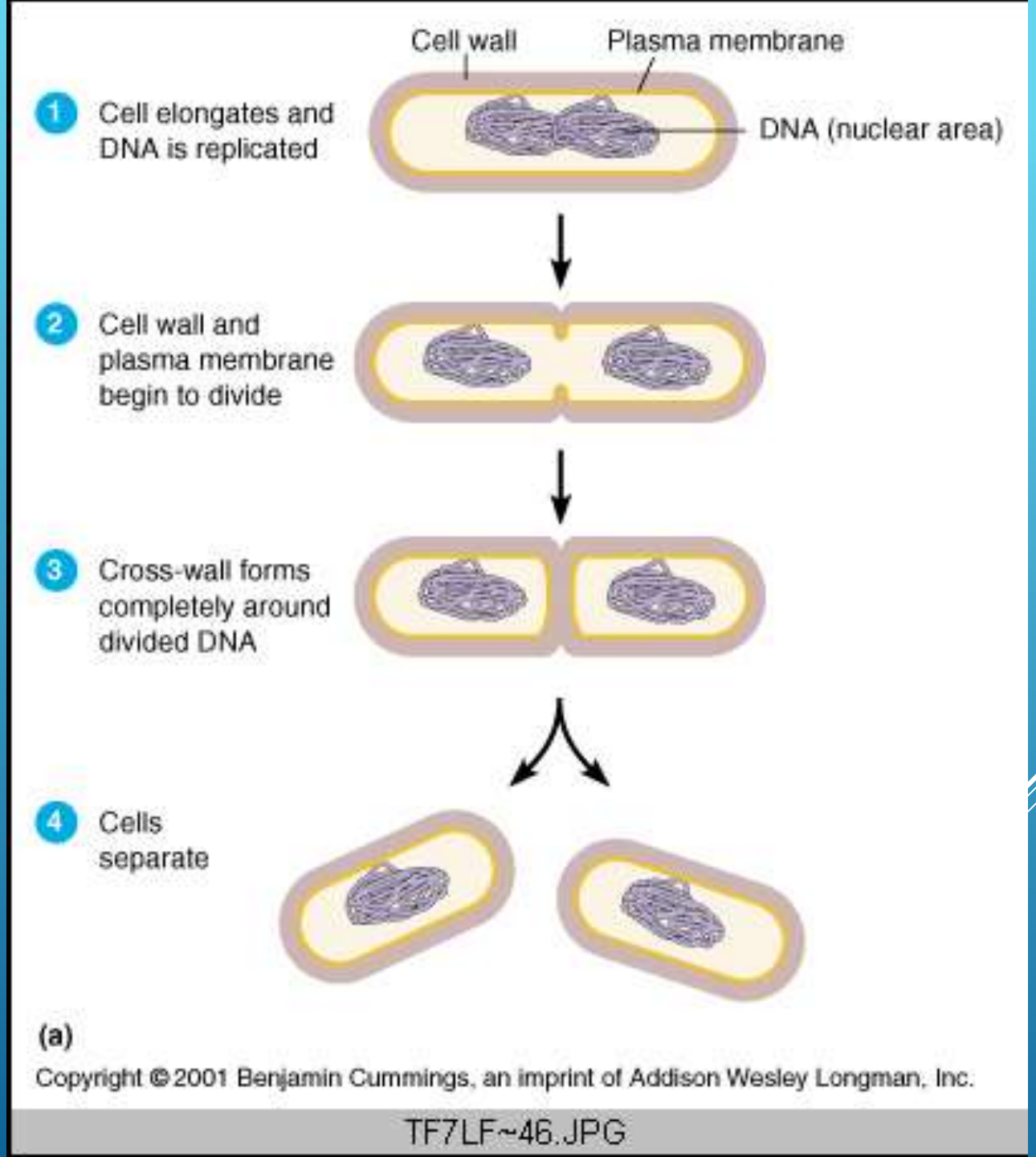
MICROBIAL GROWTH





- Microbes grow via binary fission, resulting in exponential increases in numbers
- The number of cell arising from a single cell is 2^n after n generations
- Generation time is the time it takes for a single cell to grow and divide

Binary Fission



Rapid Growth of Bacterial Population

Arithmetic Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

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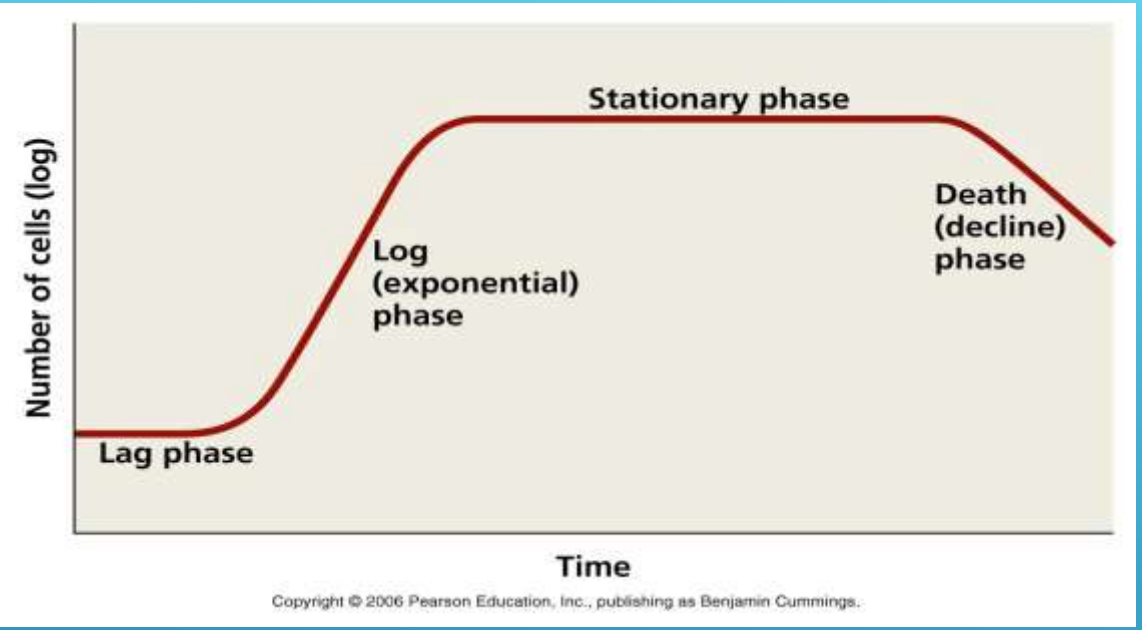
GROWTH CURVE



- During lag phase, cells are recovering from a period of no growth and are making macromolecules in preparation for growth
- During log phase cultures are growing maximally
- Stationary phase occurs when nutrients are depleted and wastes accumulate (Growth rate = death rate)
- During death phase death rate is greater than growth rate

METHODS USED TO MEASURE MICROBIAL GROWTH

Plate Count Method:

This method involves spreading a known volume of a microbial sample onto the surface of a solid agar medium.
After incubation, colonies formed by individual viable cells are counted.
Results are expressed as colony-forming units (CFUs) per unit volume.

Turbidity Measurement (Spectrophotometry):

This method measures the cloudiness of a liquid culture, which is directly proportional to the microbial cell density.
A spectrophotometer is used to measure the absorbance of light passing through the culture at a specific wavelength.
Turbidity measurements are expressed in optical density units (OD) or absorbance units.

Impedance Microbial Growth Analyzer:

This method measures the electrical impedance of a culture medium, which changes as microorganisms grow and metabolize nutrients.

Flow Cytometry:

This technique uses lasers and detectors to analyze individual cells in a liquid sample.
Cells are tagged with fluorescent dyes, and the resulting signals are used to determine cell size, complexity, and viability.

Biomass Determination:

This method involves direct measurement of the microbial biomass.
Dry weight, protein content, or other cellular components can be used to estimate the biomass concentration.
It requires harvesting and drying the cells before measurement.

Viable Cell Count:

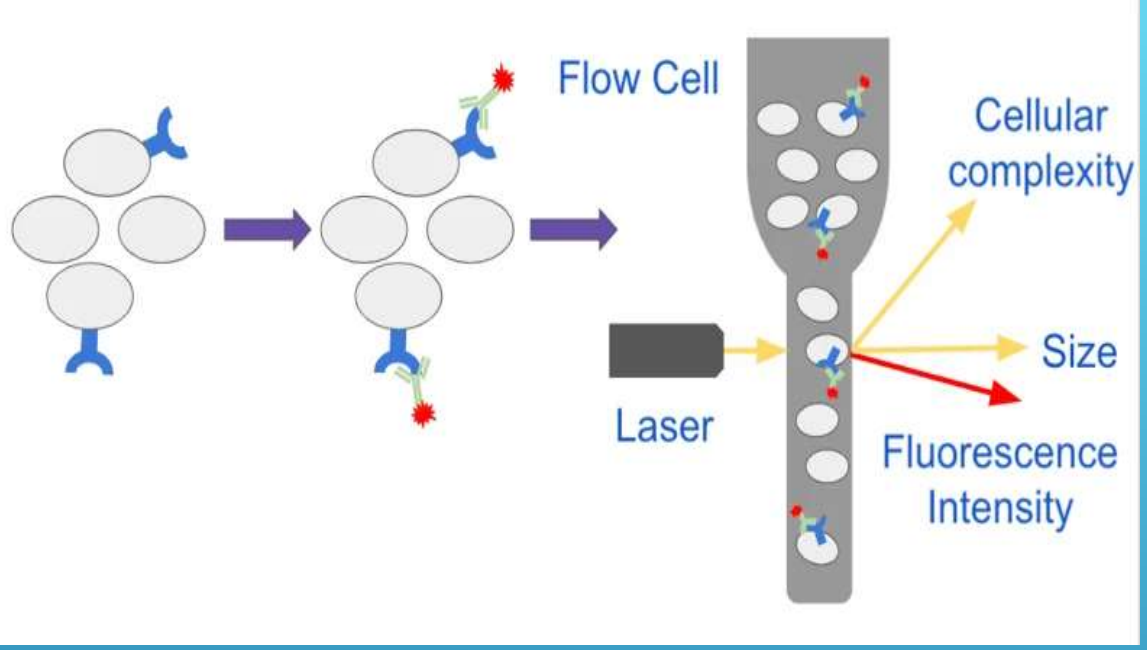
In addition to plate counting, other methods like the Most Probable Number (MPN) method or the use of fluorescence-based dyes (e.g., acridine orange) can be used to count viable cells.
The MPN method involves statistical estimation based on the presence or absence of growth in multiple tubes or wells.

Real-time PCR (Polymerase Chain Reaction):

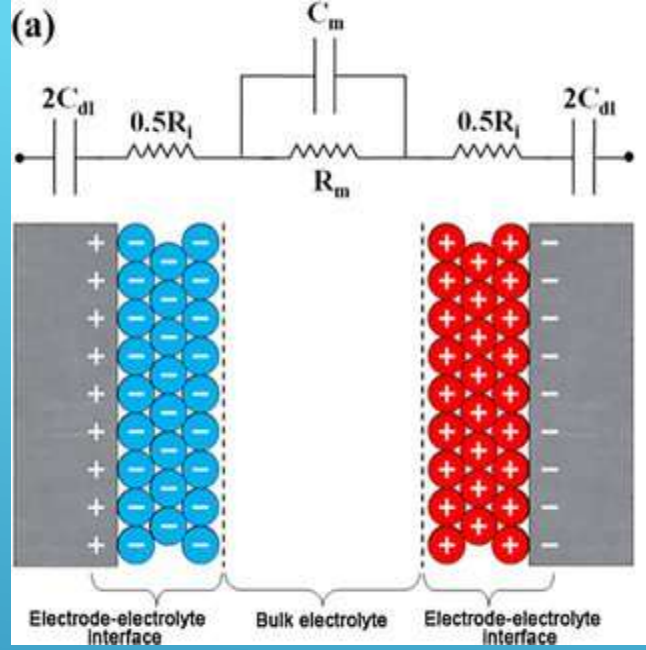
Quantitative PCR can be used to measure the amount of DNA in a sample, providing an indirect measure of microbial growth.

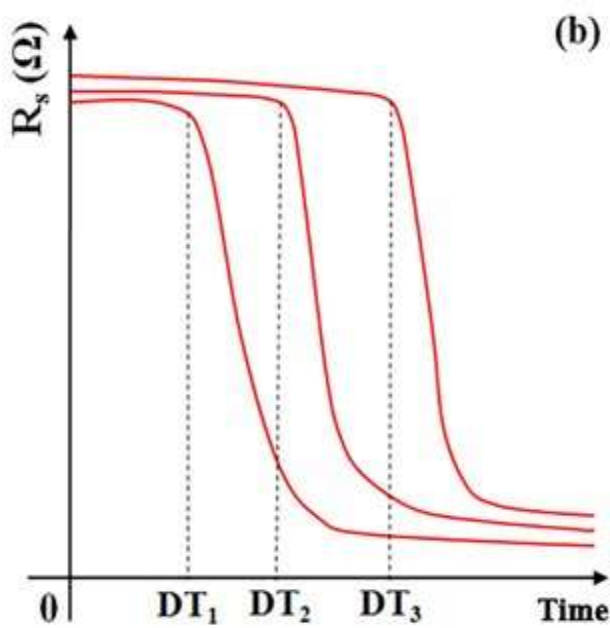
Metabolic Activity Measurement:

Monitoring the metabolic activity of microorganisms, such as the production of carbon dioxide, can provide an indirect measure of microbial growth.
Resazurin reduction assays are an example of a metabolic activity measurement.

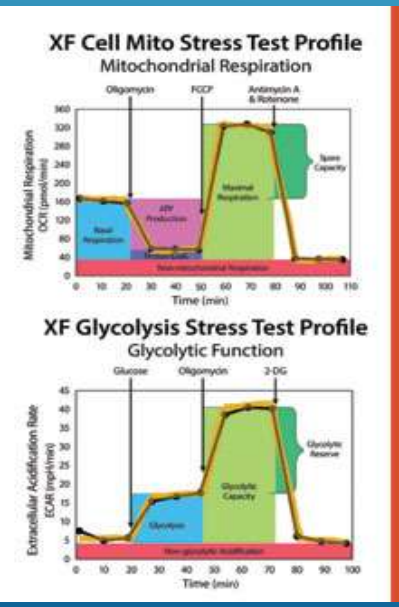


Flow Cytometry

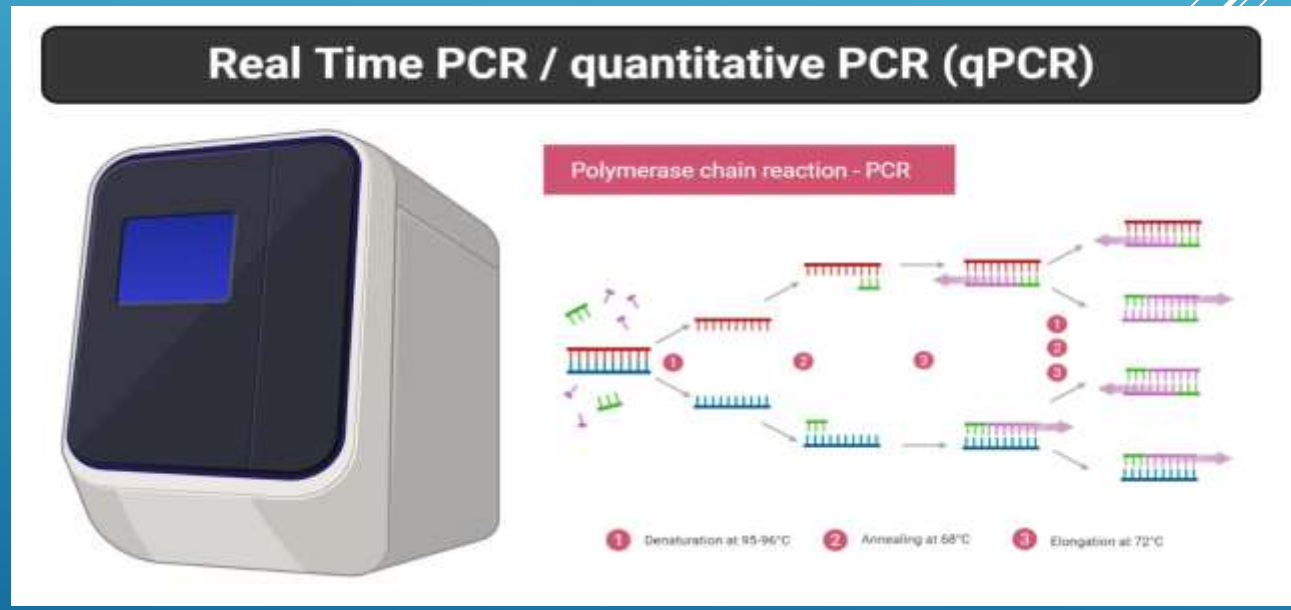




Impedance Microbial Growth Analyzer

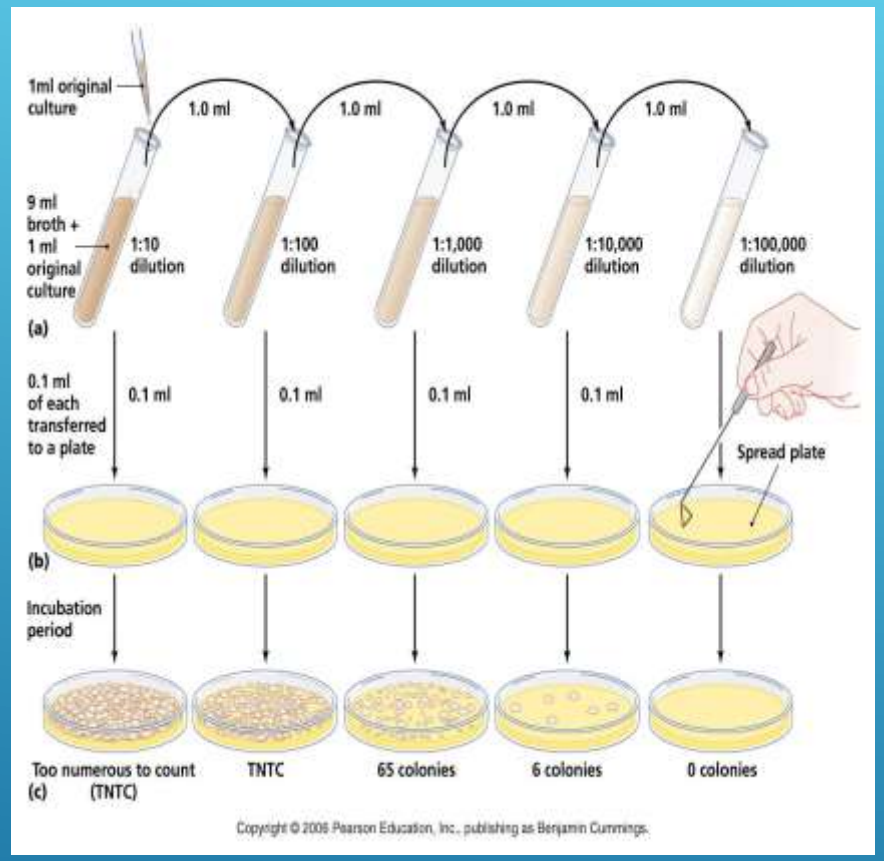


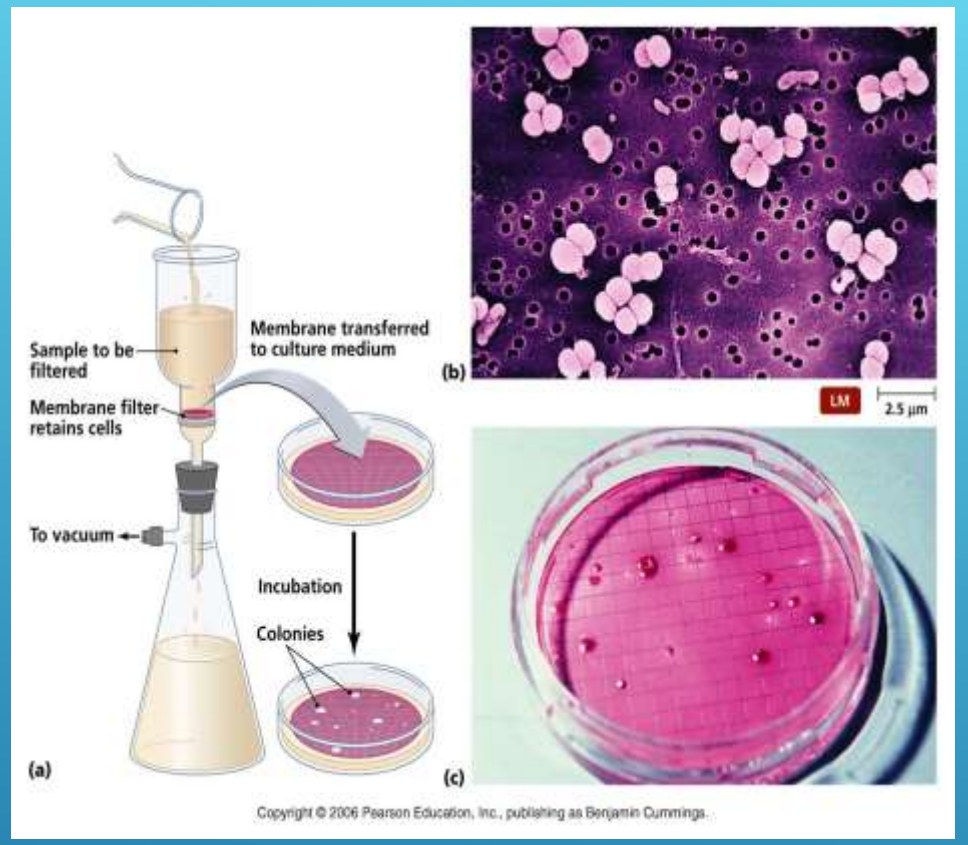
Metabolic Activity Measurement:



Real-time PCR

VIABLE COUNTS

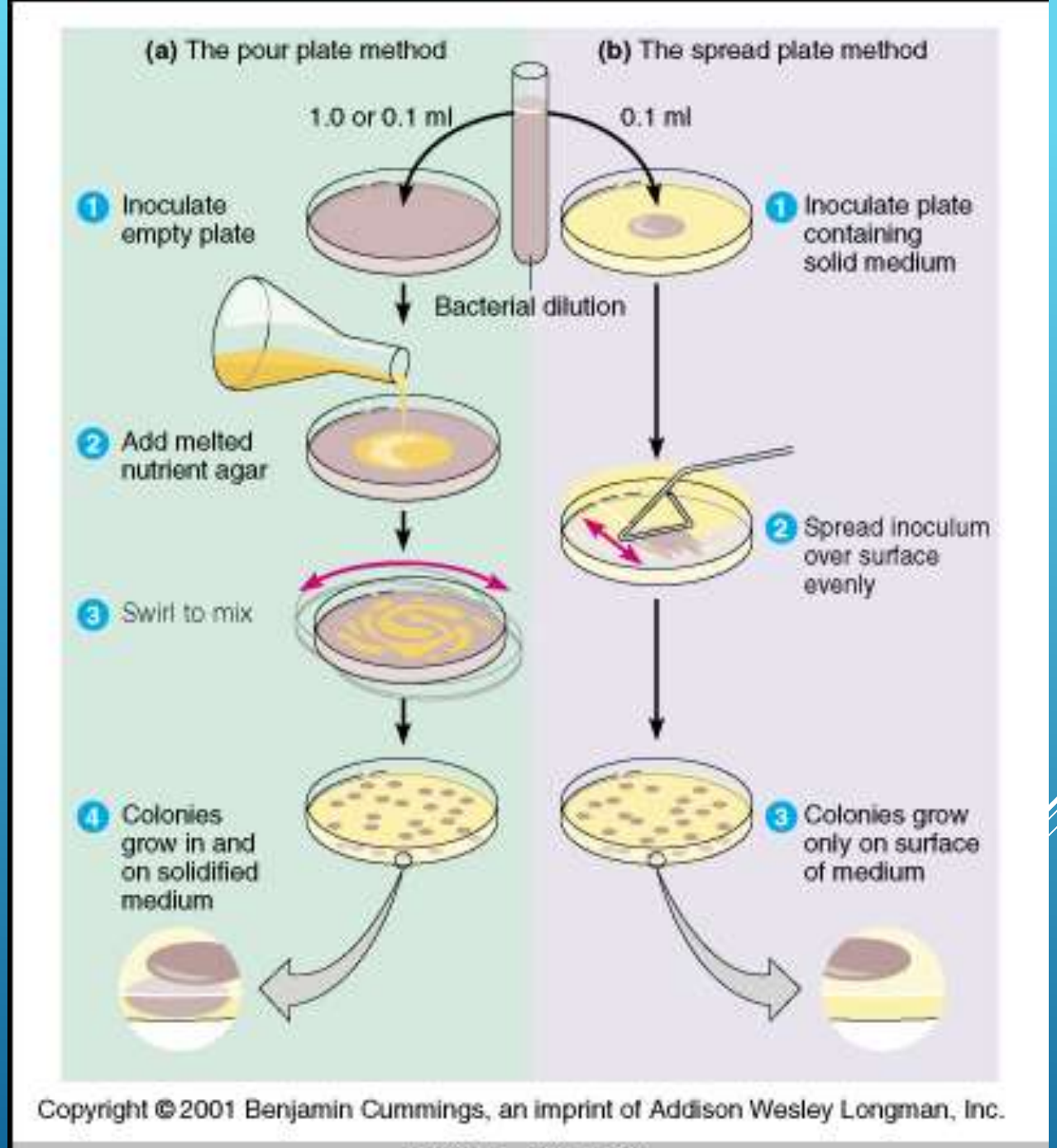


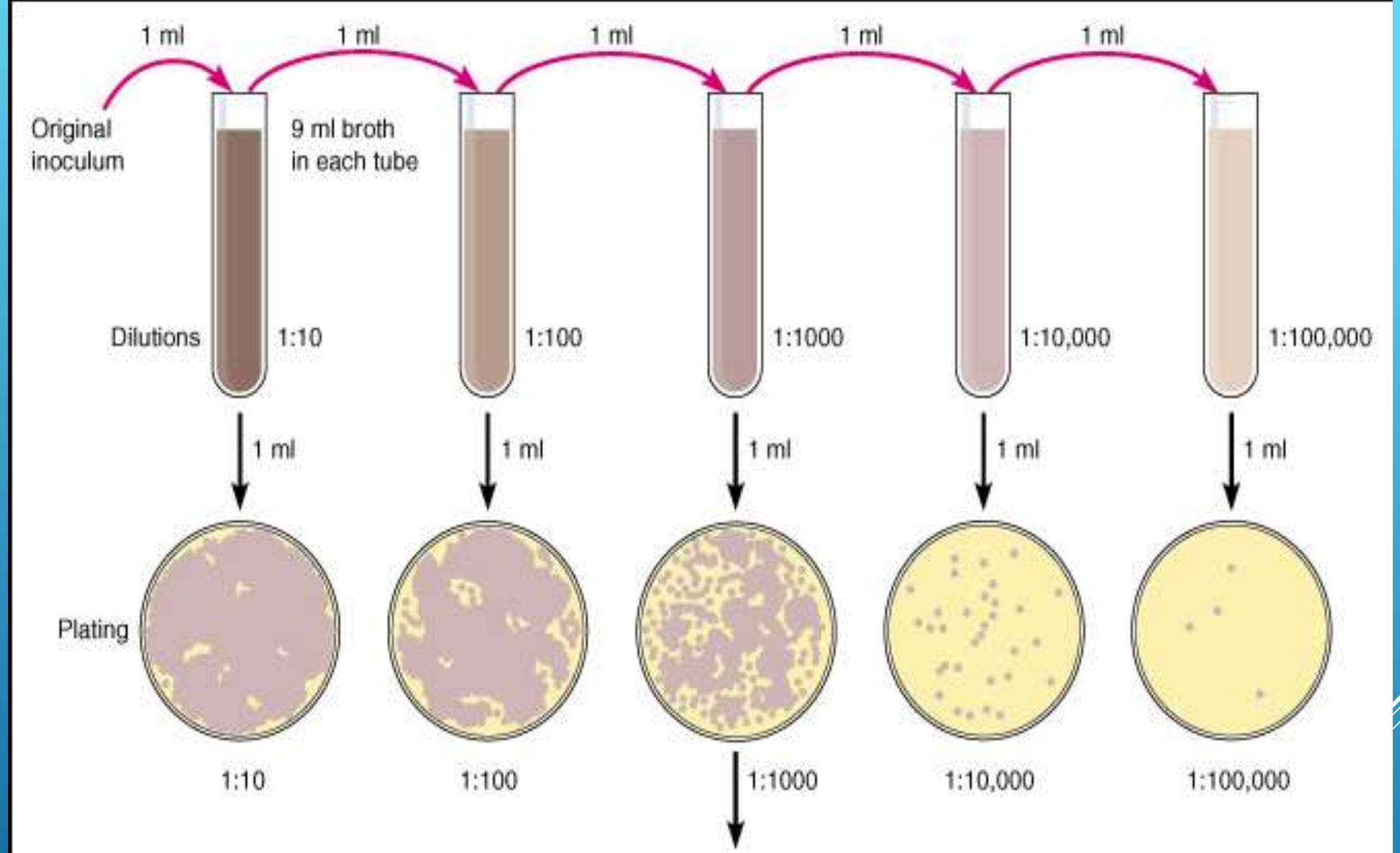


- Each colony on plate or filter arises from single live cell
- Only counting live cells

Direct Count

Pour Plate





Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000/ml in sample.)

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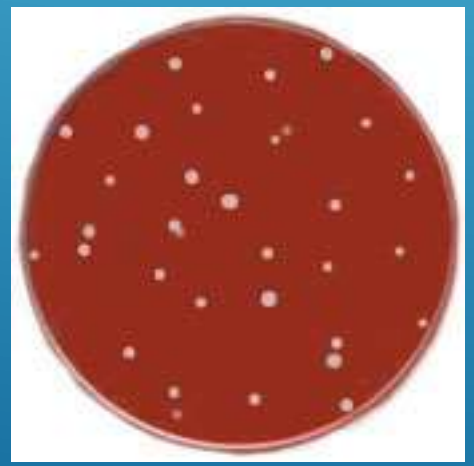
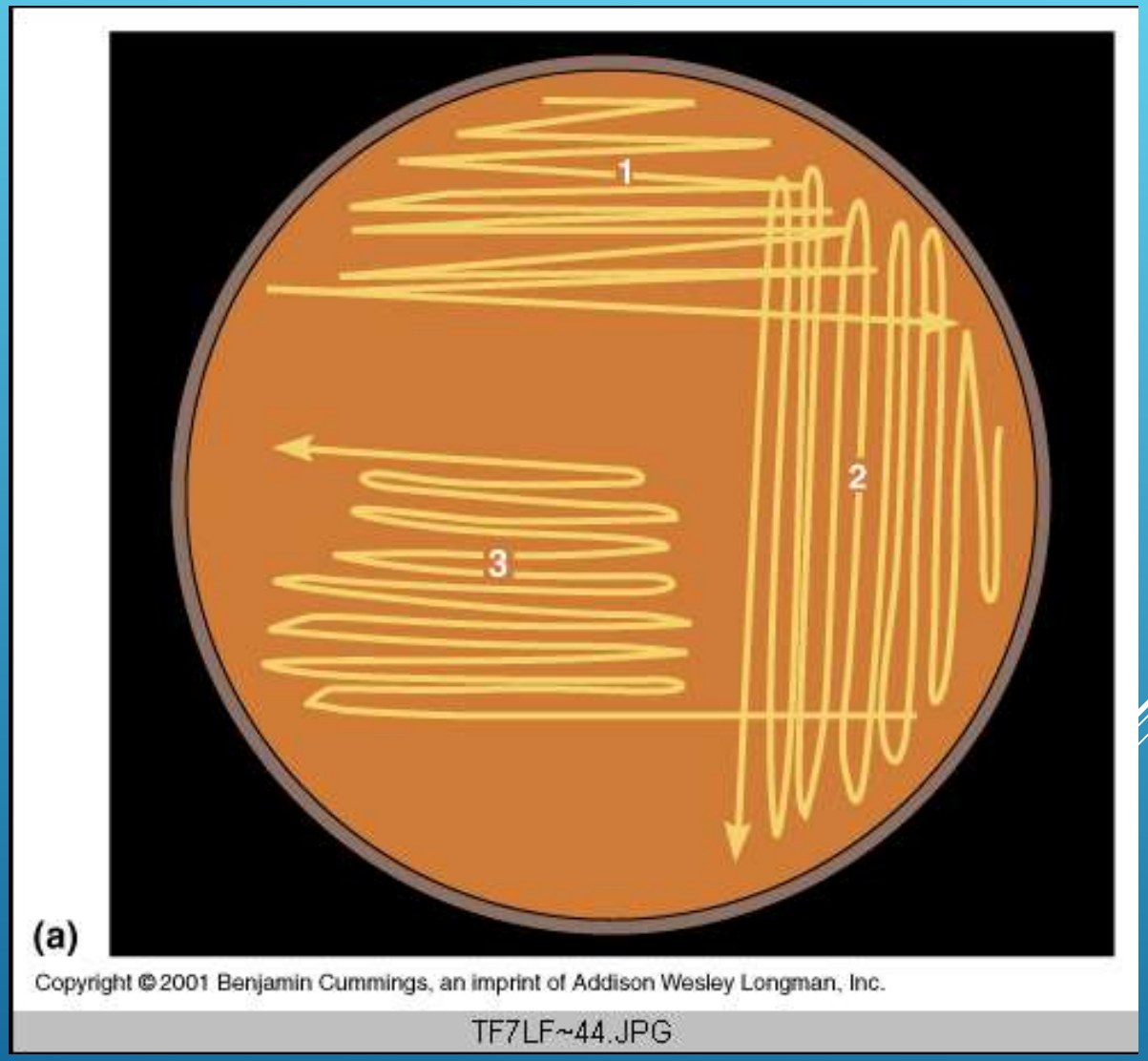
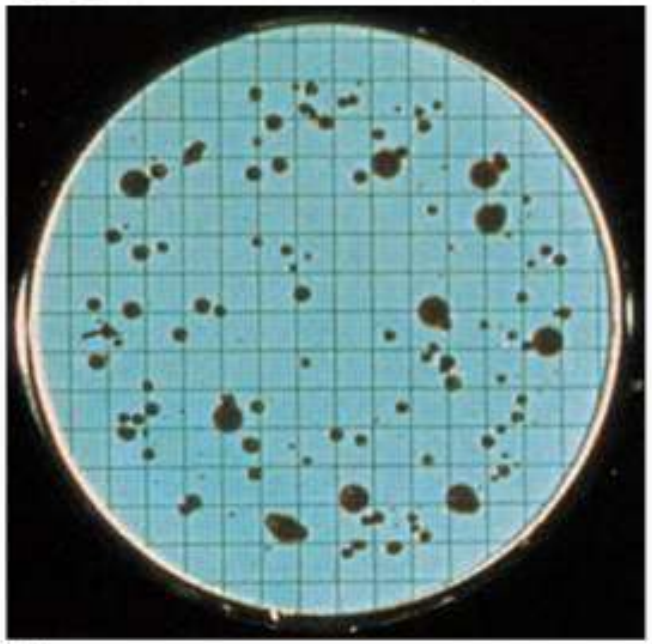
Direct Count

Spread or Streak Plate







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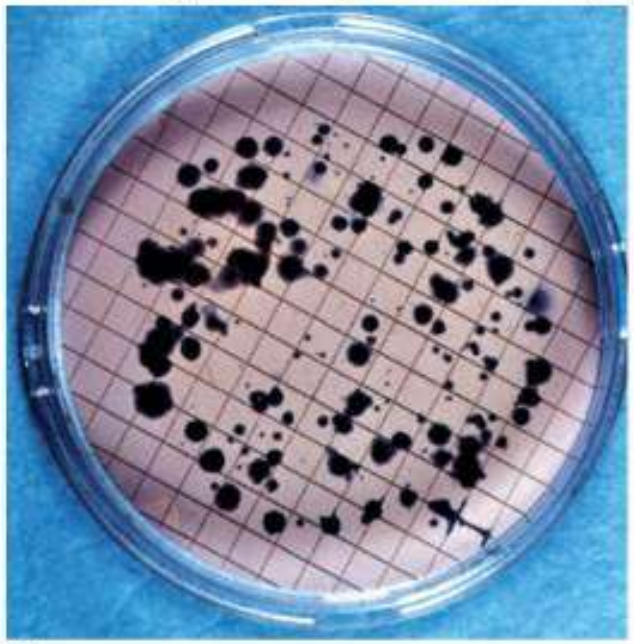
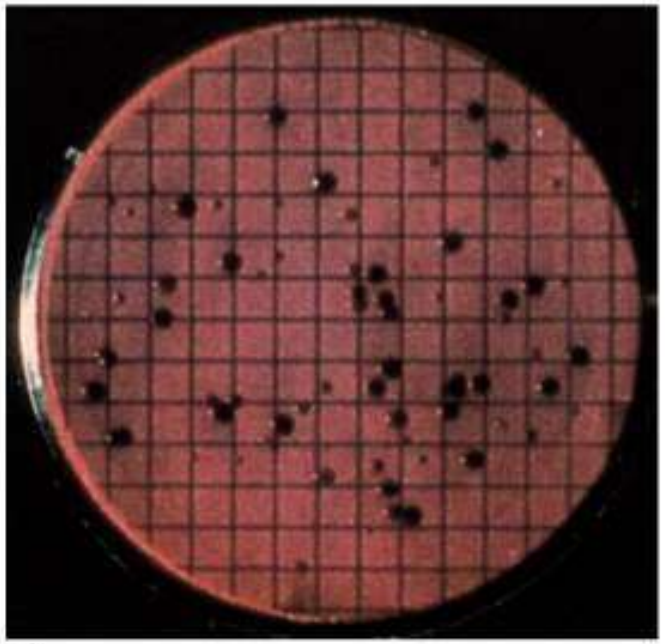
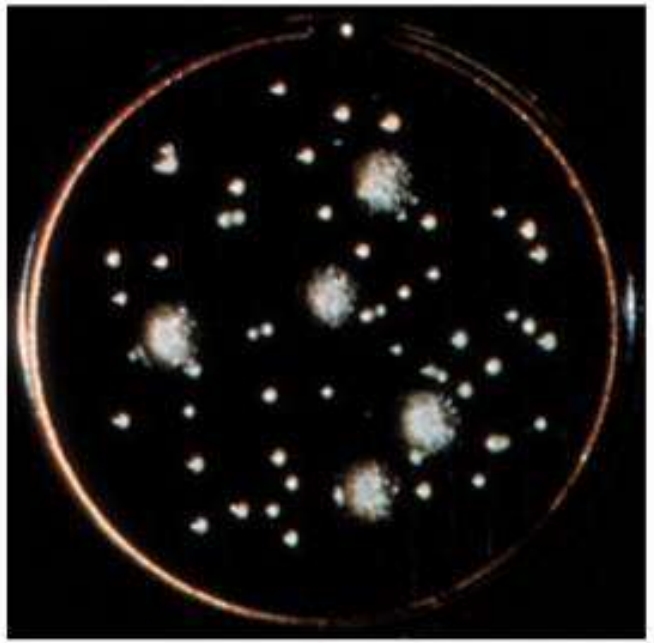


(b)

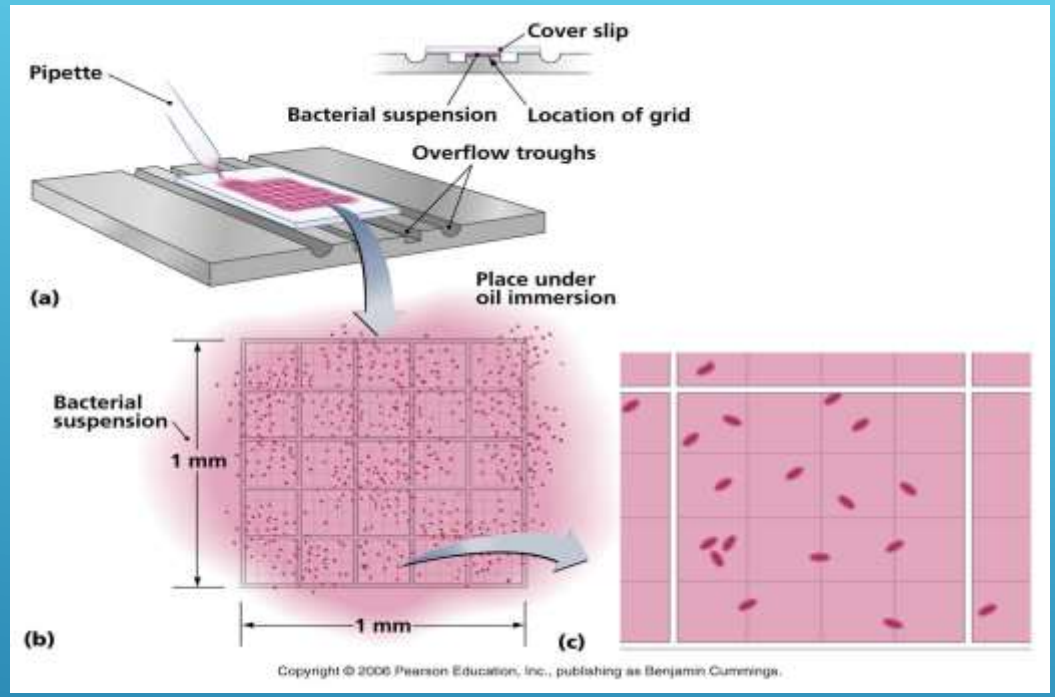


(c)



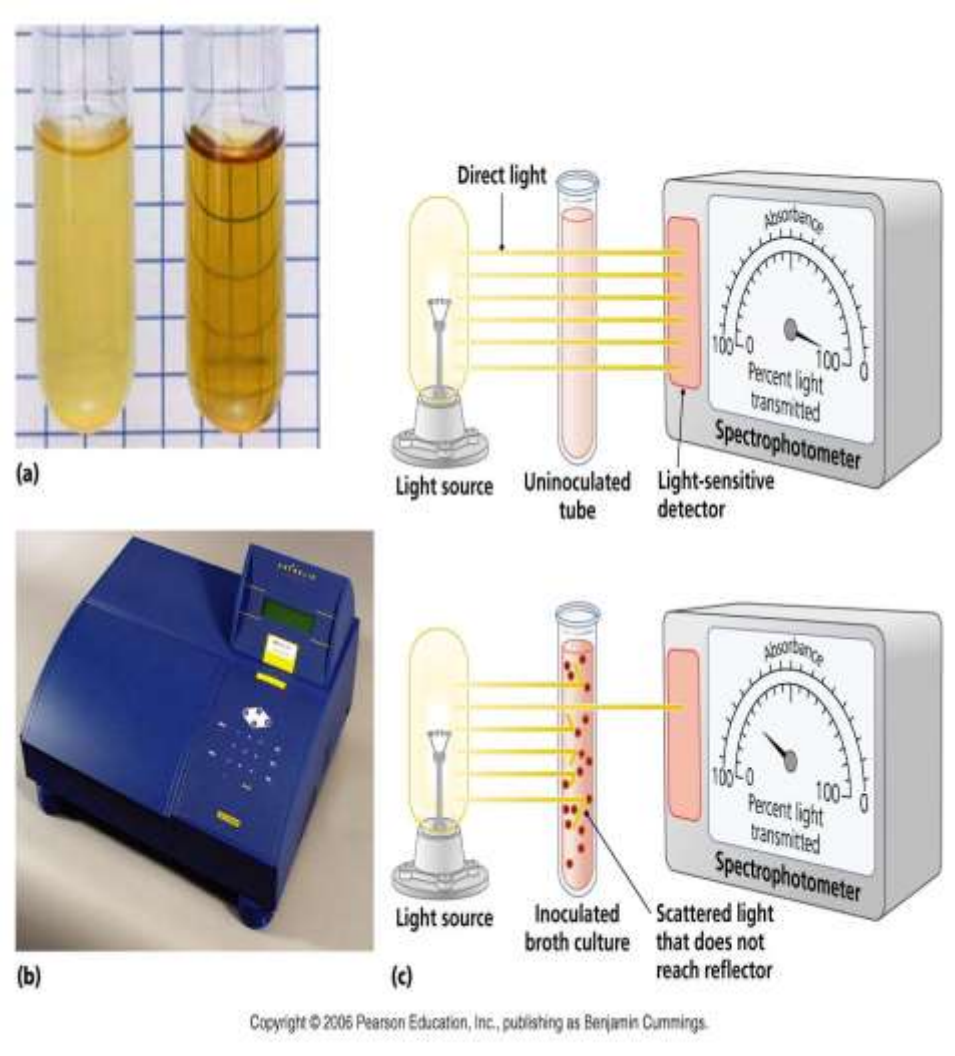
(d)

MICROSCOPIC COUNTS



- Need a microscope, special slides, high power objective lens
- Typically only counting total microbe numbers, but differential counts can also be done

TURBITITY



- Cells act like large particles that scatter visible light
- A spectrophotometer sends a beam of visible light through a culture and measures how much light is scattered
- Scales read in either absorbance or % transmission
- Measures both live and dead cells

INOCULATION

- Sample is placed on sterile medium providing microbes with the appropriate nutrients to sustain growth.
- Selection of the proper medium and sterility of all tools and media is important.
- Some microbes may require a live organism or living tissue as the inoculation medium.

INCUBATION

- An incubator can be used to adjust the proper growth conditions of a sample.
- Need to adjust for optimum temperature and gas content.
- Incubation produces a culture – the visible growth of the microbe on or in the media

ISOLATION

- The end result of inoculation and incubation is isolation.
- On solid media we may see separate colonies, and in broth growth may be indicated by turbidity.
- Sub-culturing for further isolation may be required.

INSPECTION

- Macroscopically observe cultures to note color, texture, size of colonies, etc.
- Microscopically observe stained slides of the culture to assess cell shape, size, and motility.

IDENTIFICATION

- Utilize biochemical tests to differentiate the microbe from similar species and to determine metabolic activities specific to the microbe.