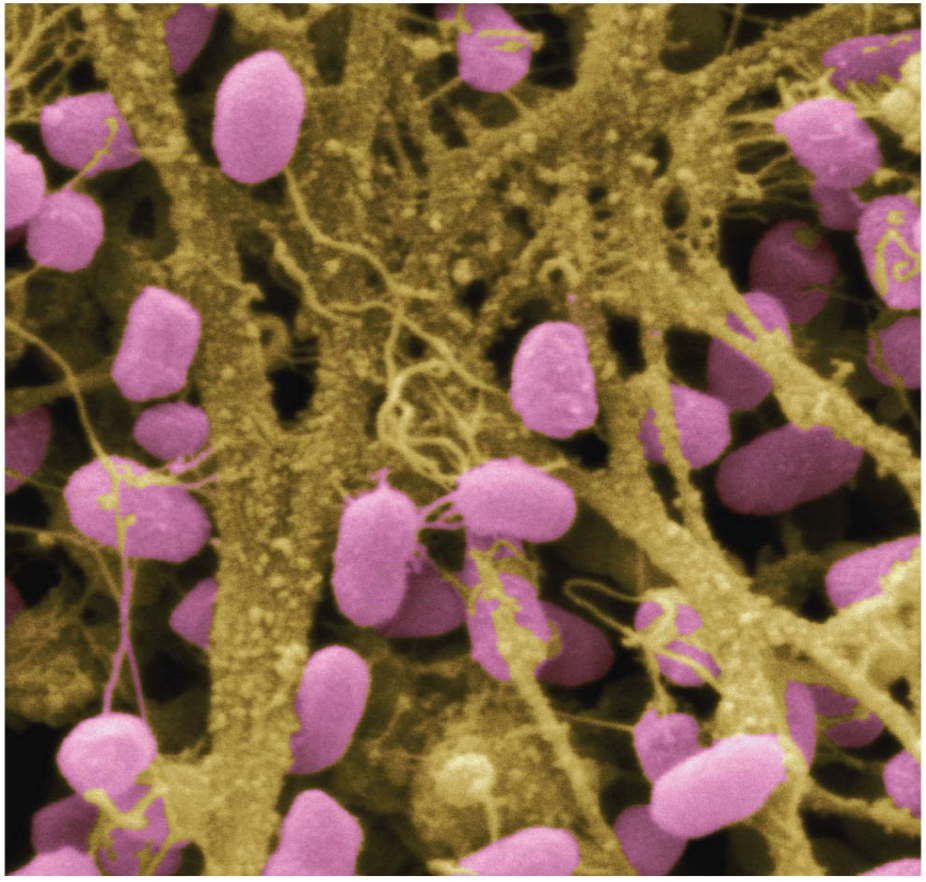
Chapter 6

Microbial Growth



Microbial Growth

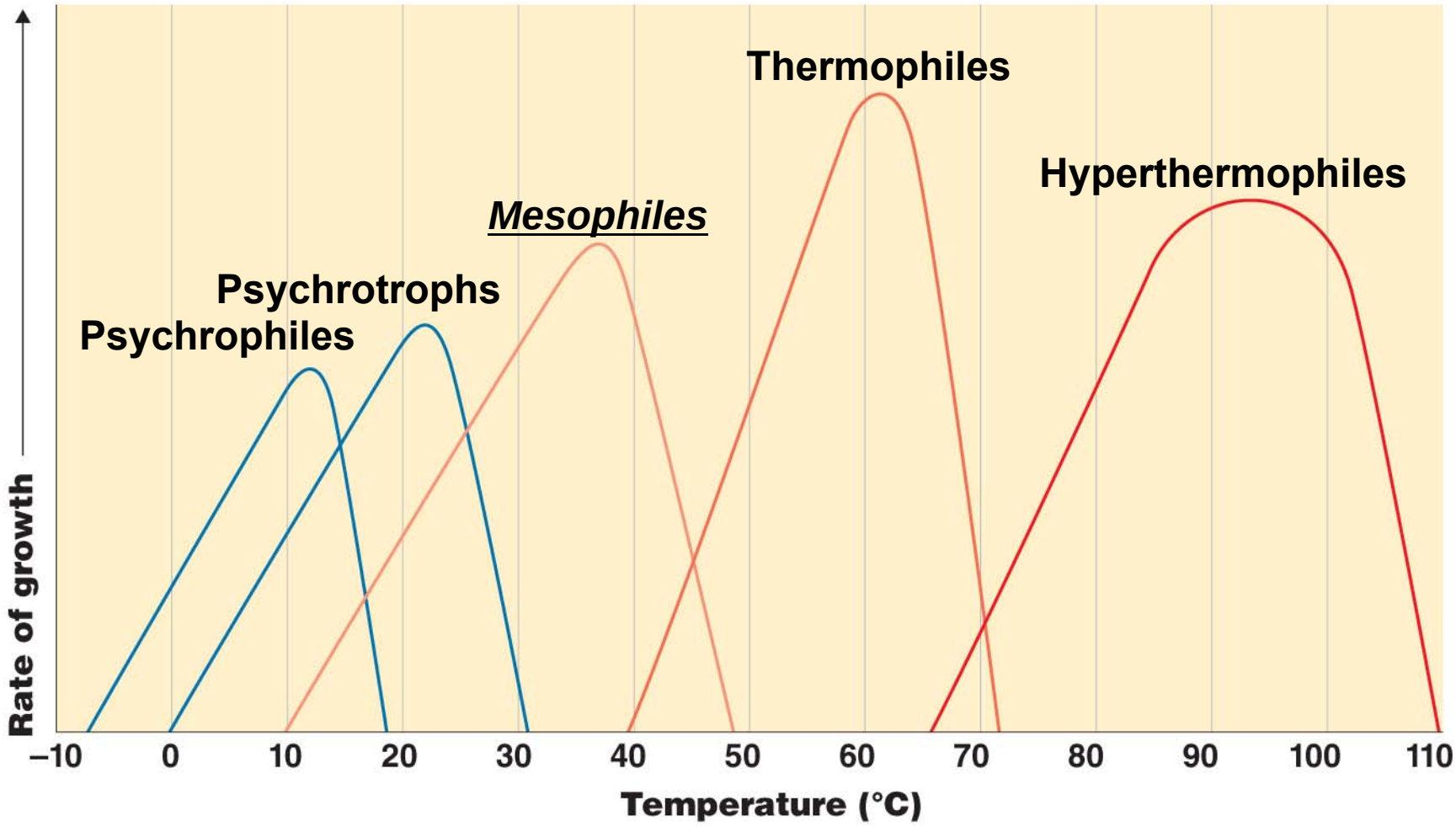
- Growth = measuring the increase in the number of cells (not their size)
- Bacteria however, do typically double their size in a life cycle
- Colonies of bacteria // cell number increases so growth now visible with naked eye
  - May indicate 100,000 to million to billion of cells

Important Terms

- Facultative = means "optional" or "discretionary" (i.e. an antonym to obligate), used mainly in biology in phrases such as:
  - **Facultative** anaerobe - an organism that prefers no oxygen but can tolerate oxygen.
- Obligate = something that you must do because of a law, rule, promise, etc. // limited only to that condition
  - obligate parasite (e.g. virus) – only can grow inside another cell.

The Requirements for Growth

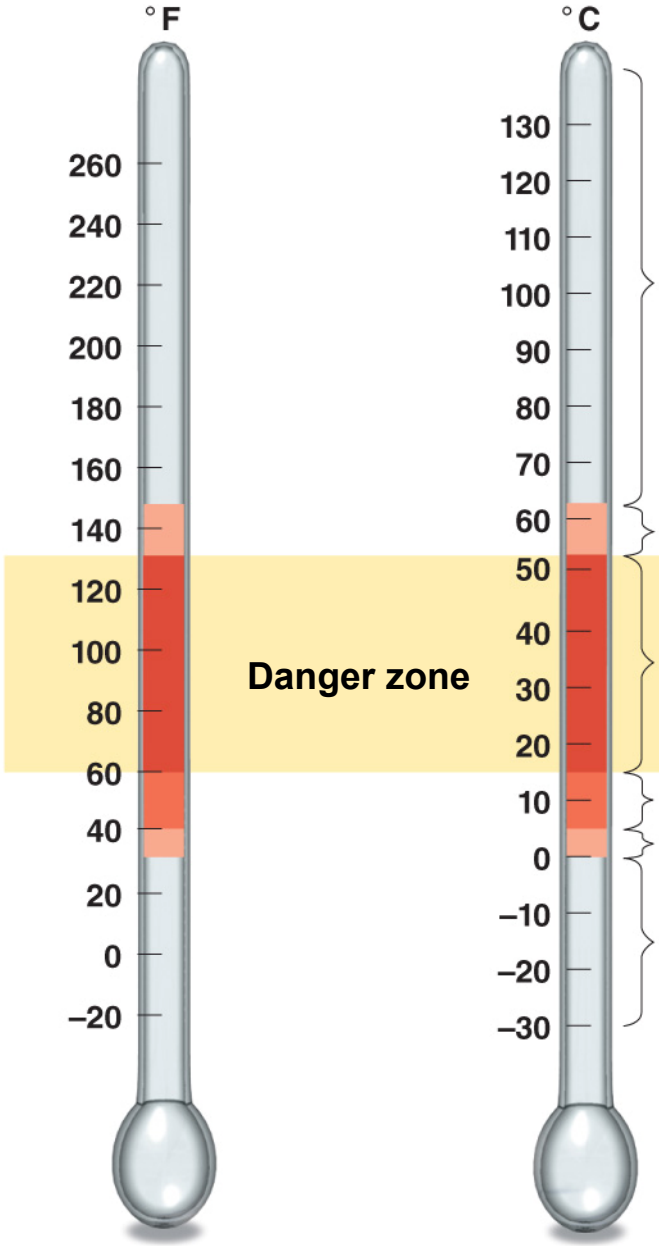
- Physical requirements
  - Temperature
  - pH
  - Osmotic pressure
- Chemical requirements
  - Carbon
  - Nitrogen, sulfur, and phosphorous
  - Trace elements
  - Oxygen
  - Organic growth factor



Typical growth rates for different types of microorganisms in response to temperature.

Minimum / Optimum / Maximum Growth Temperature

Food preservation temperatures.



Temperatures in this range destroy most microbes, although lower temperatures take more time.

Very slow bacterial growth.

Rapid growth of bacteria; some may produce toxins.

Many bacteria survive; some may grow.

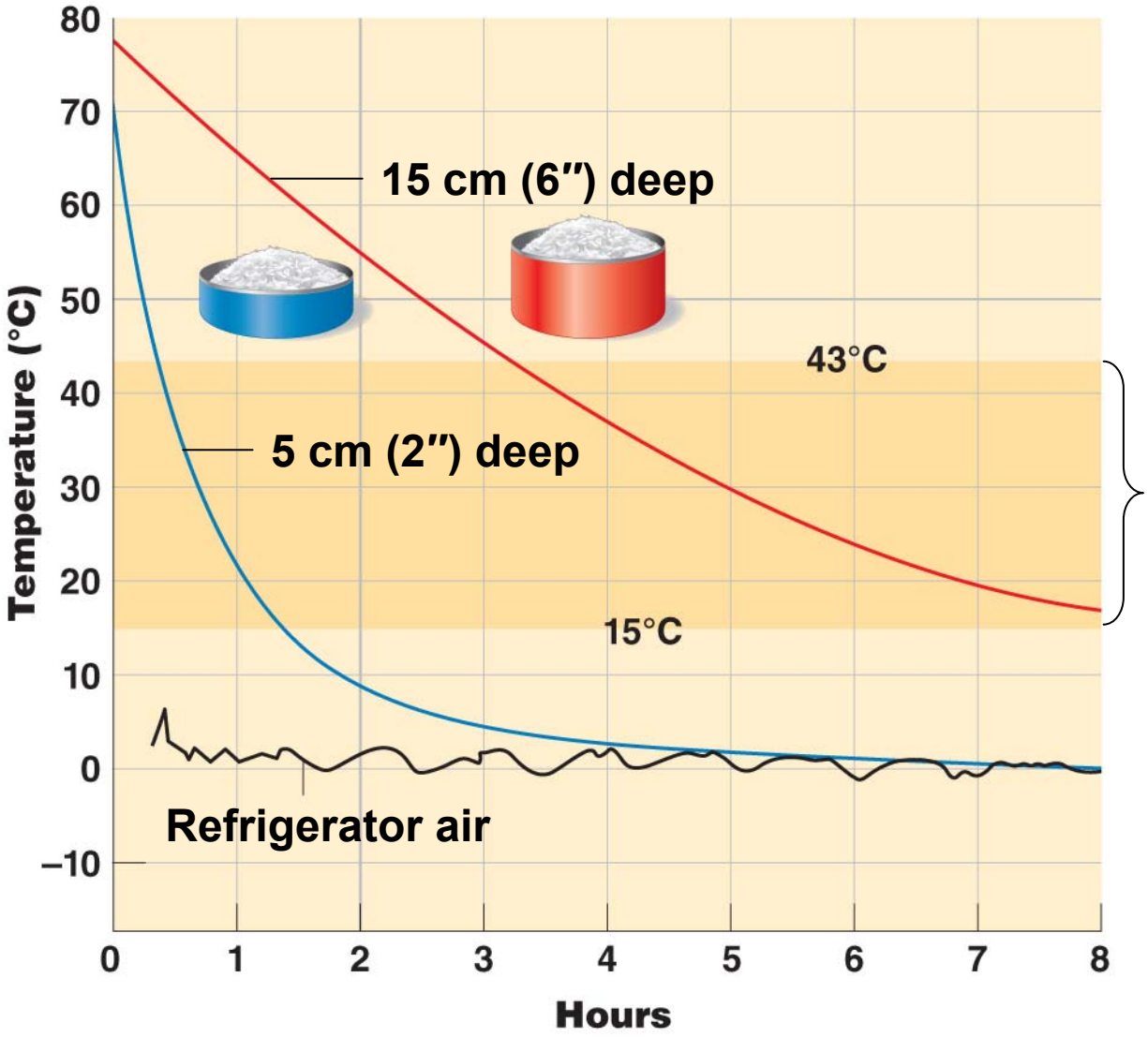
Refrigerator temperatures; may allow slow growth of spoilage bacteria, very few pathogens.

No significant growth below freezing.

Psychrotrophs grow between 0°C and 30°C // Cause food spoilage

A white microbial biofilm is visible on this deep-sea hydrothermal vent. Water is being emitted through the ocean floor at temperatures above 100°C.

The effect of the amount of food on its cooling rate in a refrigerator and its chance of spoilage.



Approximate temperature range at which *Bacillus cereus* multiplies in rice

Note: refrigeration is the most common method of preserving household food.

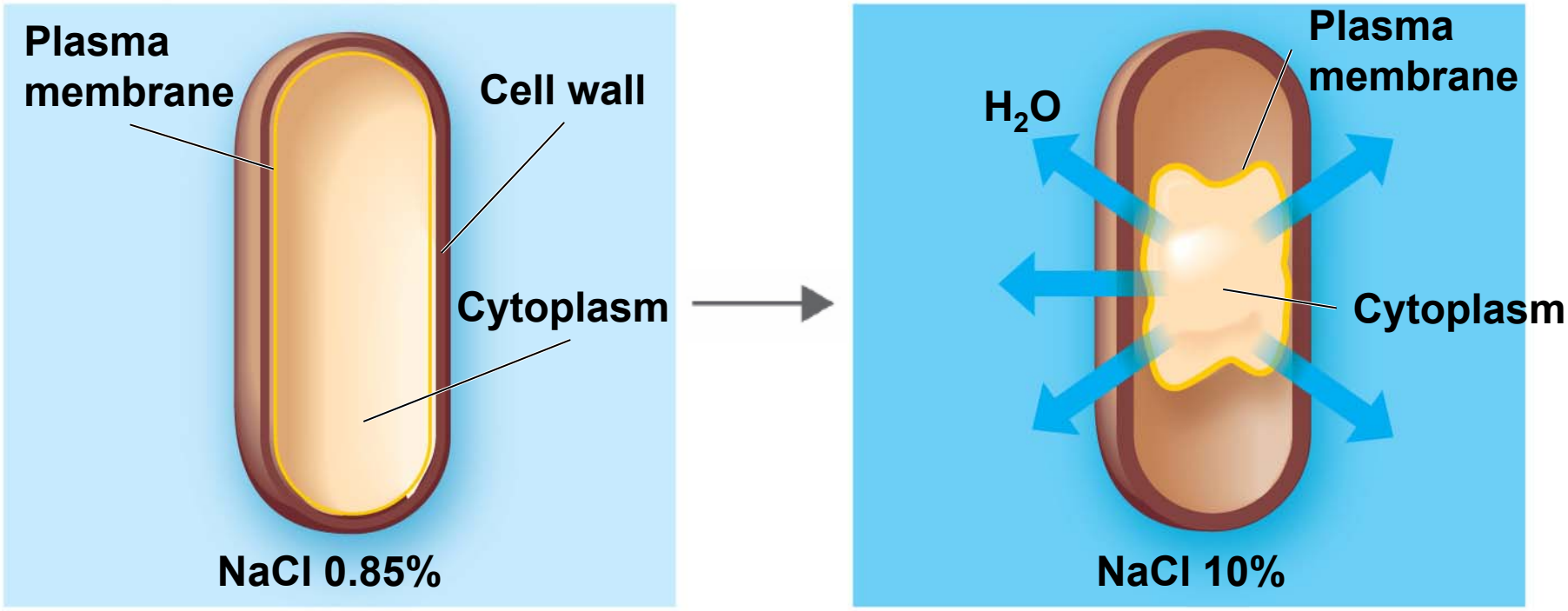
pH

- Most bacteria grow between pH 6.5 and 7.5 (best test answer would be pH7)
- Molds and yeasts grow between pH 5 and 6 // just on the acidic side of pH scale
- **Acidophiles** grow in acidic environments

Osmotic Pressure

- Hypertonic environments, or an increase in salt or sugar, cause **plasmolysis**
- **Extreme** or **obligate** **halophiles** require high osmotic pressure
- **Facultative** **halophiles** tolerate high osmotic pressure

Plasmolysis.



(a) Cell in isotonic solution.

(b) Plasmolyzed cell in hypertonic solution.

Note: explains why sugar and salt may be used as a food preservative in foods like condensed milk, honey, and fish.

Chemical Requirements - Carbon

- Carbon – all organisms need a carbon source to build their macromolecules
  - Heterotrophs use an organic carbon sources
  - Autotrophs use CO_2 from “air” // able to “fix” carbon dioxide from the atmosphere into organic molecules
    - carbon dioxide is “reduced”
    - meaning hydrogen atoms removed from another molecule (e.g. water or sulfate = H2S) and attached to the carbon dioxide
    - this reduction process used to make chemical energy (ATP) which then used to make organic molecule (e.g. glucose).

Chemical Requirements - Nitrogen

- Nitrogen
  - In amino acids and proteins
  - Radioactive nitrogen in growth media will incorporate into proteins and nucleic acids
  - Most bacteria decompose proteins
  - Some bacteria use NH_4^+ (i.e. for their hydrogen to reduce CO2 or NO_3^- as in respiration to accept electrons)
  - A few bacteria use N_2 gas /// this is called nitrogen fixation
    - Bacteria associated with plant roots fix nitrogen so plants can incorporate nitrogen into macromolecules (i.e. proteins and nucleic acids)

Chemical Requirements - Sulfur

- Sulfur
  - Required for amino acids, thiamine, and biotin
  - Most bacteria decompose proteins
  - Some bacteria use SO_4^{2-} or H_2S
- Phosphorus
  - Required for DNA, RNA, ATP, and membranes
  - PO_4^{3-} is a source of phosphorus

Chemical Requirements

- Trace elements
  - Inorganic elements required in small amounts
  - Organic / Usually as enzyme cofactors

Oxygen – Friend or Foe

- Earth in its earliest days was free of oxygen and life itself
- This environment allowed for the formation of oceans with a high concentration of highly reduced molecules
- Abiogenisis preceded biologic life
- First photosynthetic organisms did not produce oxygen // helped to preserve balance which favored highly reduced molecules
- Second form of photosynthesis developed later which used sun energy to remove hydrogen atoms from water to produce chemical energy (ATP). In the process free oxygen was released into the atmosphere
- Free oxygen “oxidizes” reduced molecules // in effect oxygen destroys the structure of highly reduced molecules.
- This oxygen produced the first massive extinction of most obligate anaerobes. (the oxygen catastrophe)

Photosynthesis / Two "Types"

- Oxygenic:

$$6\ CO_2 + 12\ H_2O + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\ H_2O + 6\ O_2$$

- Anoxygenic:

$$6\ CO_2 + 12\ H_2S + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\ H_2O + 12\ S$$

The Effect of Oxygen on the Growth of Various Types of Bacteria

TABLE 6.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

Oxygen – As a Weapon of Destruction

- **Singlet oxygen**: $^1O_2^-$ boosted to a higher-energy state // very unstable // very reactive with other molecules
- **Superoxide free radicals**: $O_{2\bullet}^-$

$$O_2^{\bullet-} + O_2^{\bullet-} + 2\,H^+ \xrightarrow{\text{Super-oxide dismutase}} H_2O_2 + O_2$$

- **Peroxide anion**: O_2^{2-}

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

$$H_2O_2 + 2\,H^+ \xrightarrow{\text{Peroxidase}} 2\,H_2O$$

- **Hydroxyl radical** (OH•)

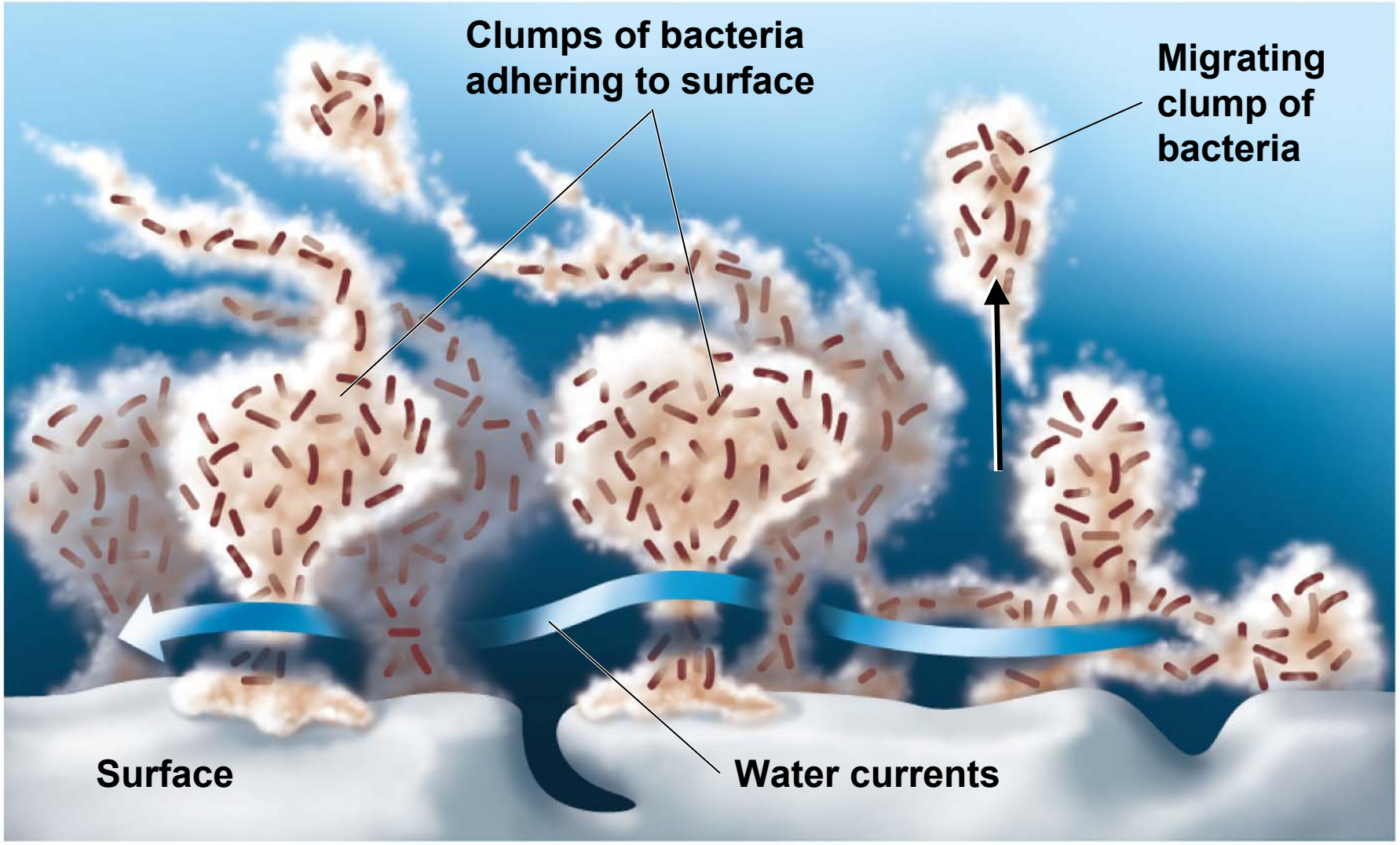
Organic Growth Factors

- Organic compounds obtained from the environment
- Vitamins, amino acids, purines, and pyrimidines

Biofilms

- Microbial “mixed” communities
- Formation aided by fibria and capsules
- Form slime or hydrogels
- Bacteria attracted by chemicals via quorum sensing // ability to communicate between members of the colony
- Share nutrients
- Sheltered from harmful factors
- Reduce metabolic rate of mixed colonies // become more resistant to antibiotics (100X plus)
- Dental plaque is an example of a biofilm
- Biofim also grow on surfaces of many types of surgical implants

Biofilms

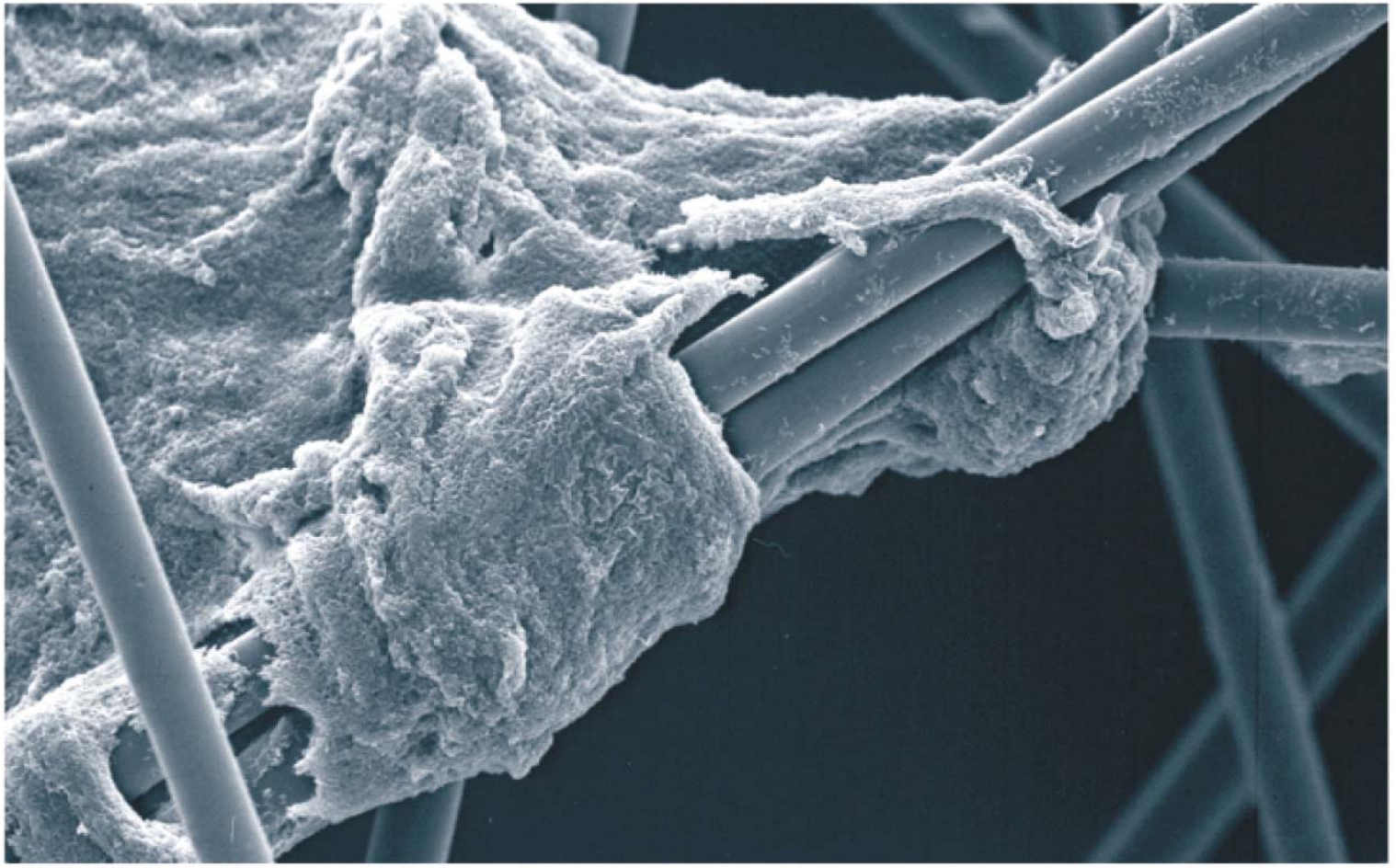


- Note: antibiotics affect bacteria most during the log growth phase – when actively growing!

Biofilms Grow Slowly

- Patients with indwelling catheters may received contaminated heparin
- Bacterial numbers in contaminated heparin were too low to cause symptoms of an infection
- 84 – 421 days after exposure, patients developed infections
- Note: watch video features posted on Web site

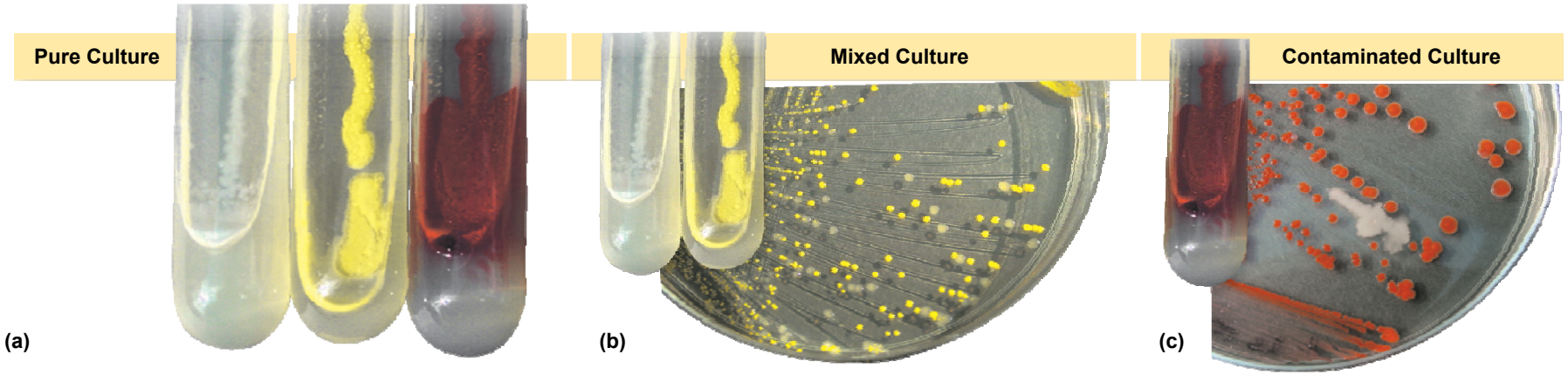
Pseudomonas aeruginosa biofilm.



Culture Media

- **Culture medium**: nutrients prepared to support microbial growth
- **Sterile medium**: no living microbes are present
- **Inoculums**: introduction of microbe(s) into medium
- **Culture**: microbes that are growing “in or on” culture medium

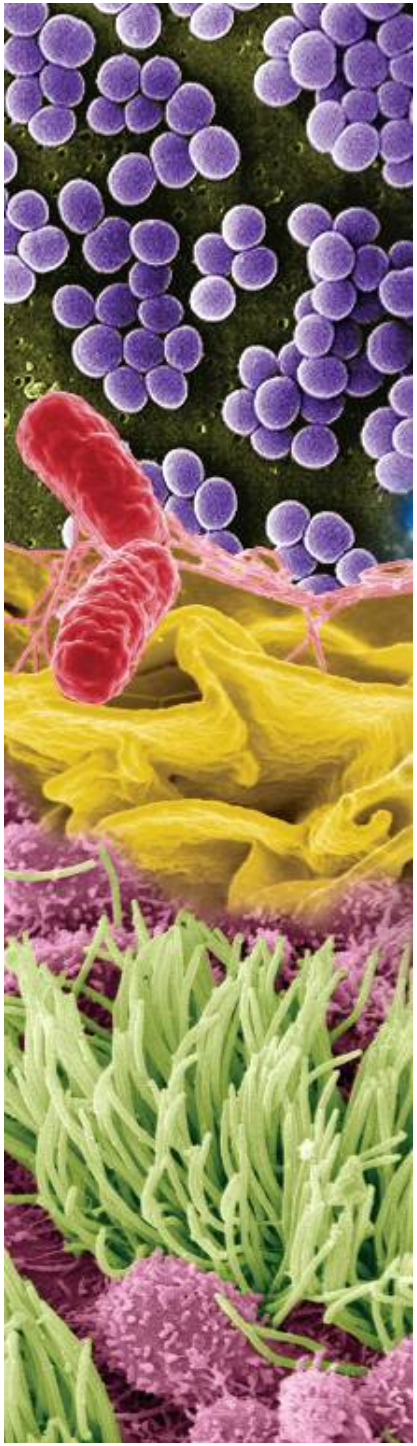
Various Conditions of Cultures



Various conditions of cultures. (a) Three tubes containing pure cultures of Escherichia coli (white), Micrococcus luteus (yellow), and Serratia marcescens (red). A pure culture is a container of medium that grows only a single known species or type of microorganism. This type of culture is most frequently used for laboratory study, because it allows the systematic examination and control of one microorganism by itself.

(b) A mixed culture is a container that holds two or more identified, easily differentiated species of microorganisms, not unlike a garden plot containing both carrots and onions. Pictured here is a mixed culture of M. luteus (bright yellow colonies) and E. coli (faint white colonies).

(c) A contaminated culture was once pure or mixed (and thus a known entity) but has since had contaminants (unwanted microbes of uncertain identity) introduced into it, like weeds into a garden. Contaminants get into cultures when the lids of tubes or Petri dishes are left off for too long, allowing airborne microbes to settle into the medium. They can also enter on an incompletely sterilized inoculating loop or on an instrument that you have inadvertently reused or touched to the table or your skin. This plate of S. marcescens was overexposed to room air, and it has developed a large, white colony. Because this intruder is not desirable and not identified, the culture is now contaminated.



The Media

(Different physical states of the media)

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liquid

semisolid

solid (some maybe converted to liquid with heat)

solid (cannot be liquefied)

The Media
Food for Microbes in the Laboratory

Agar - complex polysaccharide from the alga *Gellidium*

Used as solidifying agent for culture media in Petri plates, slants, and deeps

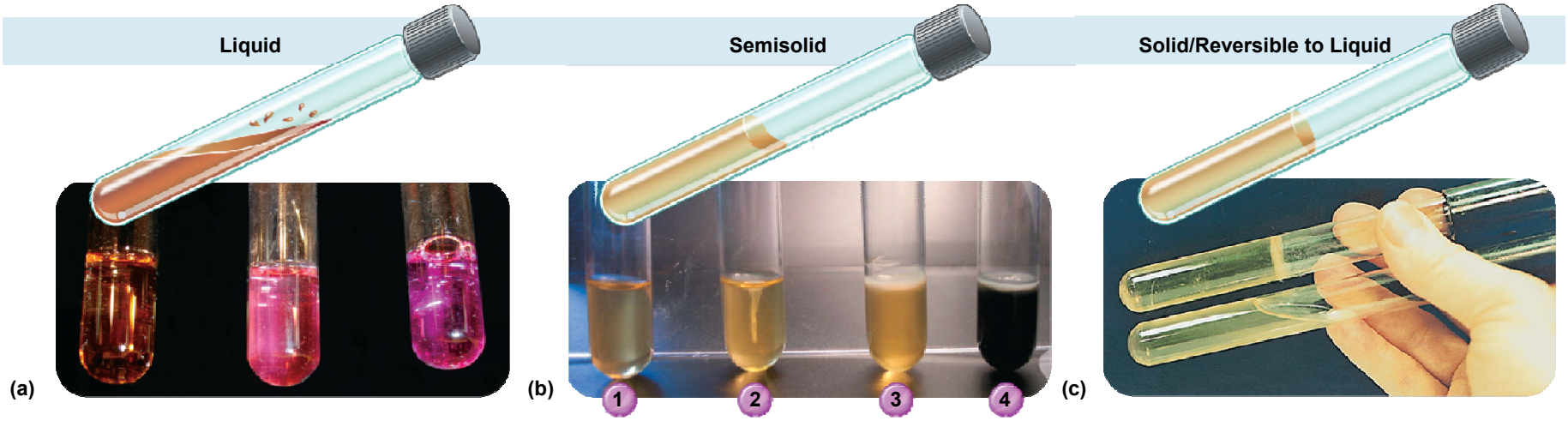
liquefies at 100°C and solidifies at 42°C

can be poured in liquid form that will not harm the microbe or the handler

flexible and moldable; can hold moisture and nutrients

Generally not a digestible nutrient for most microorganisms

Media in Different Physical Forms



Media in different physical forms. (a) Liquid media are water-based solutions that do not solidify at temperatures above freezing and that tend to flow freely when the container is tilted. Growth occurs throughout the container and can then present a dispersed, cloudy, or particulate appearance. Urea broth is used to show a biochemical reaction in which the enzyme urease digests urea and releases ammonium. This raises the pH of the solution and causes the dye to become increasingly pink. Left: uninoculated broth, pH 7; middle: weak positive, pH 7.5; right: strong positive, pH 8.0.

(b) Semisolid media have more body than liquid media but less body than solid media. They do not flow freely and have a soft, clotlike consistency at room temperature. Semisolid media are used to determine the motility of bacteria and to localize a reaction at a specific site. Here, sulfur indole motility medium (SIM) is pictured. The (1) medium is stabbed with an inoculum and incubated. Location of growth indicates nonmotility (2) or motility (3). If H_2S gas is released, a black precipitate forms (4).

(c) Media containing 1%–5% agar are solid enough to remain in place when containers are tilted or inverted. They are reversibly solid and can be liquefied with heat, poured into a different container, and resolidified. Solid media provide a firm surface on which cells can form discrete colonies. Nutrient gelatin contains enough gelatin (12%) to take on a solid consistency. The top tube shows it as a solid. The bottom tube indicates what happens when it is warmed or when microbial enzymes digest the gelatin and liquefy it.

Culture Media

- **Chemically defined media**: exact chemical composition is known
- **Complex media**: extracts made from digests of yeasts, meat, or plants added to agar

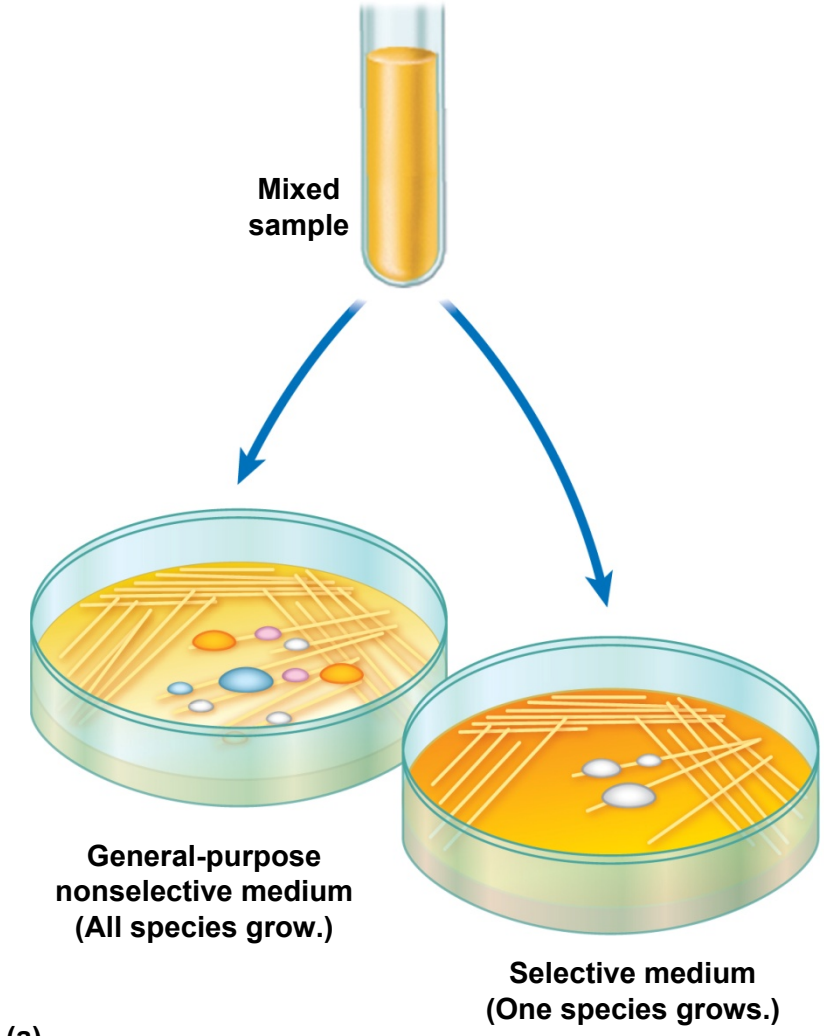
Selective Media

- Suppress unwanted microbes while encourages desired microbes

Differential Media

- Make it easy to distinguish colonies of different microbes

Comparison of Selective and Differential Media



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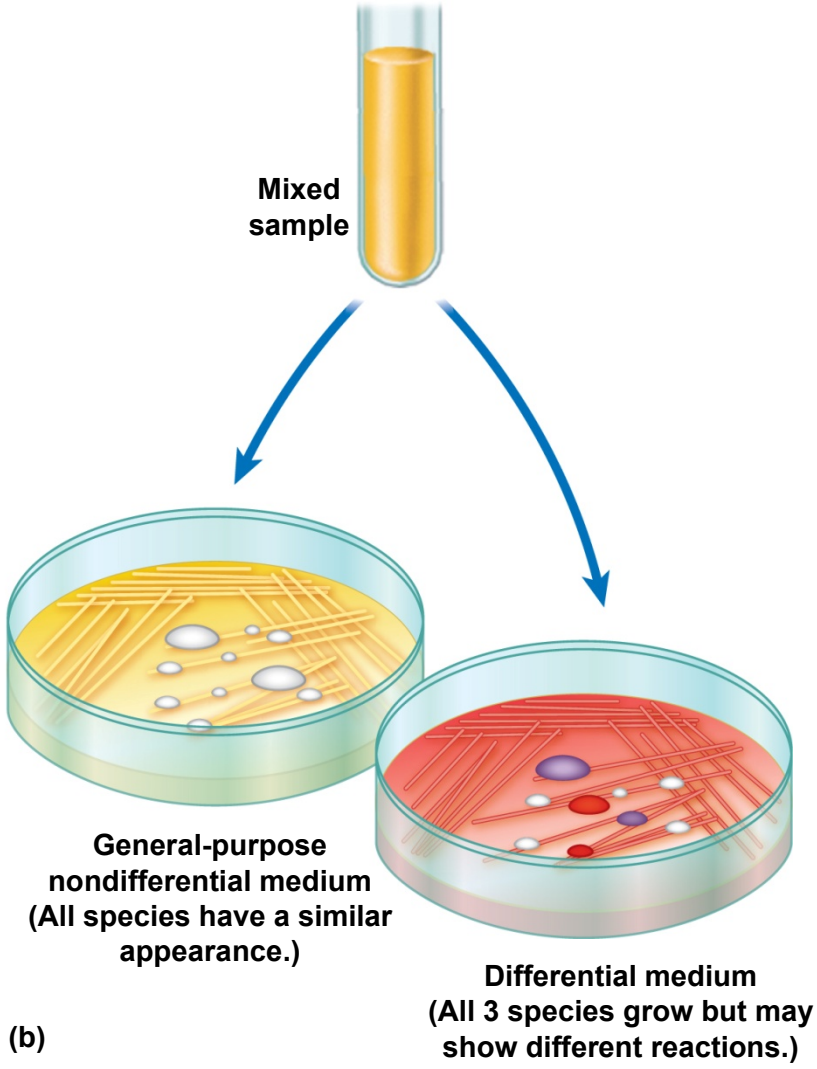


(b)

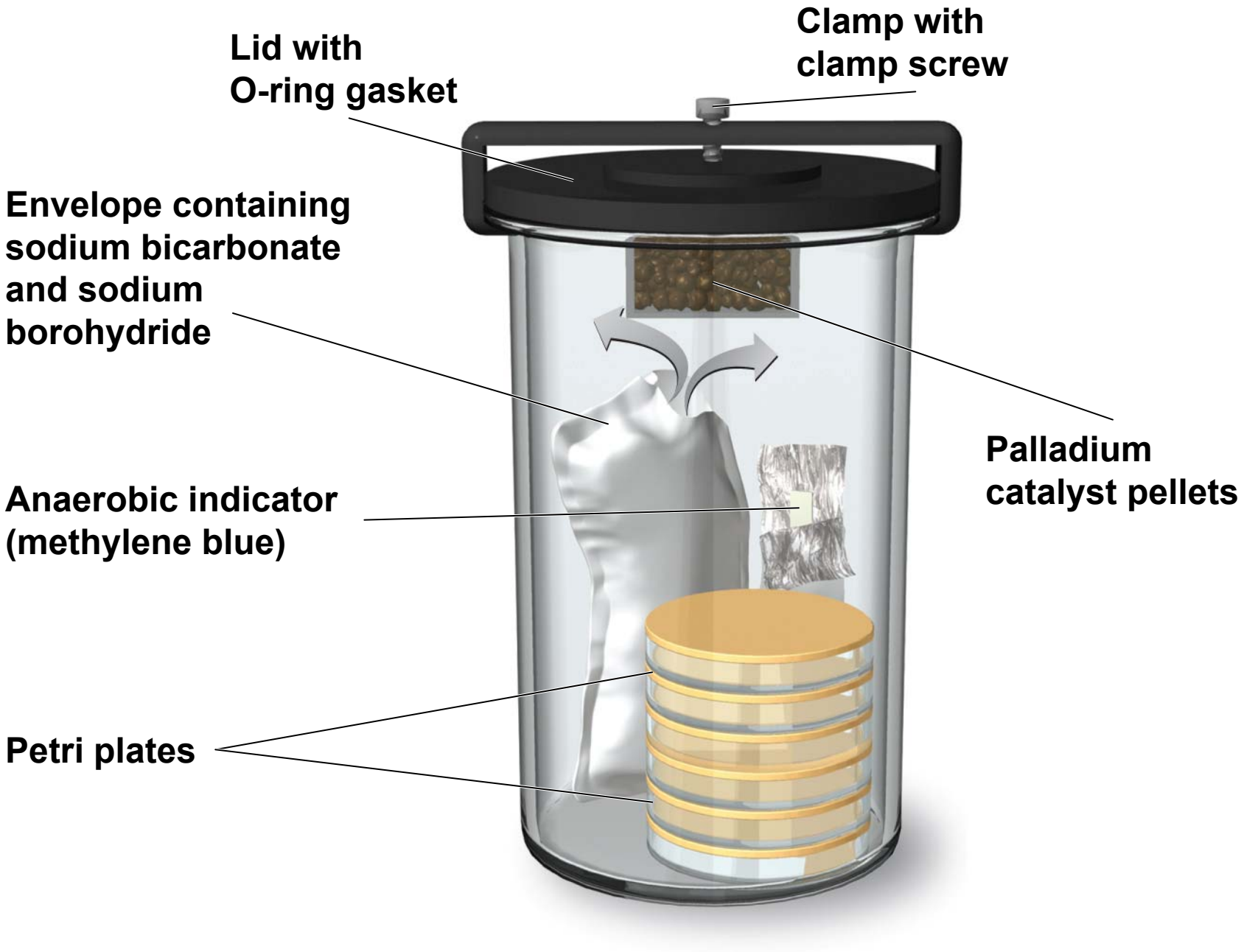
Enrichment Culture

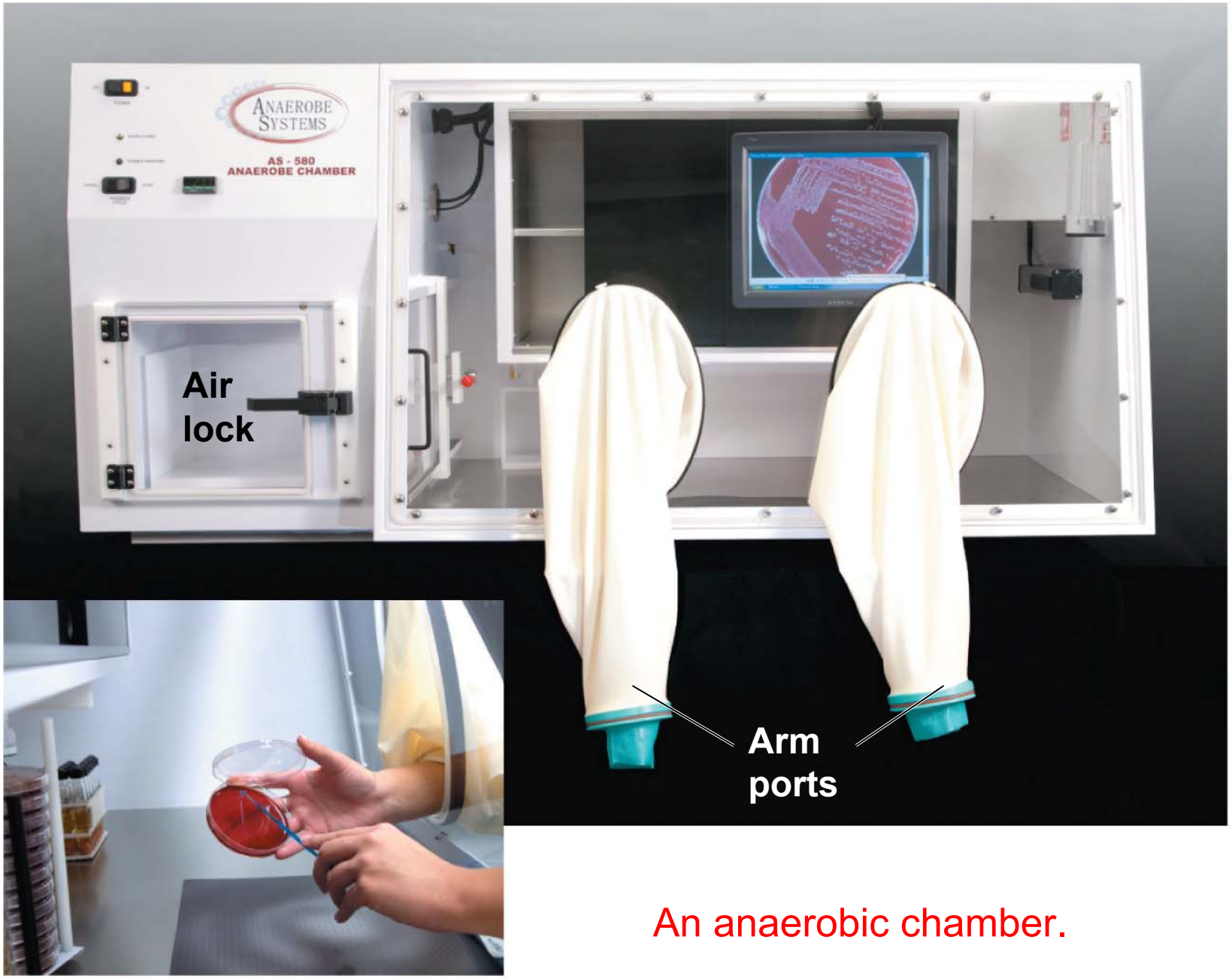
- Encourages growth of a particular desired microbe
- Assume a soil sample contains a few phenol-degrading bacteria and thousands of other bacteria
  - Inoculate phenol-containing culture medium with the soil, and incubate
  - Transfer 1 ml to another flask of the phenol medium, and incubate
  - Transfer 1 ml to another flask of the phenol medium, and incubate
  - Only phenol-metabolizing bacteria will be growing

Anaerobic Culture Methods

- **Reducing media**
  - Contain chemicals (thioglycolate or oxyrase) that combine O_2
    - this removes free oxygen // creates anaerobic environment
  - Used to grow anaerobic microbes
  - Also heated to drive off O_2

A jar for cultivating anaerobic bacteria on Petri plates.





An anaerobic chamber.

A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 . 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

TABLE 6.3 Defined Culture Medium for *Leuconostoc mesenteroides*

Carbon and Energy
Glucose, 25 g
Salts
NH_4Cl, 3.0 g K_2HPO_4*, 0.6 g KH_2PO_4*, 0.6 g $MgSO_4$, 0.1 g
Amino Acids, 100–200 µg each
Alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine
Purines and Pyrimidines, 10 mg of each
Adenine, guanine, uracil, xanthine
Vitamins, 0.01–1 mg each
Biotin, folate, nicotinic acid, pyridoxal, pyridoxamine, pyridoxine, riboflavin, thiamine, pantothenate, *p*-aminobenzoic acid
Trace Elements, 2–10 µg each
Fe, Co, Mn, Zn, Cu, Ni, Mo
Buffer, pH 7
Sodium acetate, 25 g
Distilled Water, 1,000 ml
*Also serves as buffer.

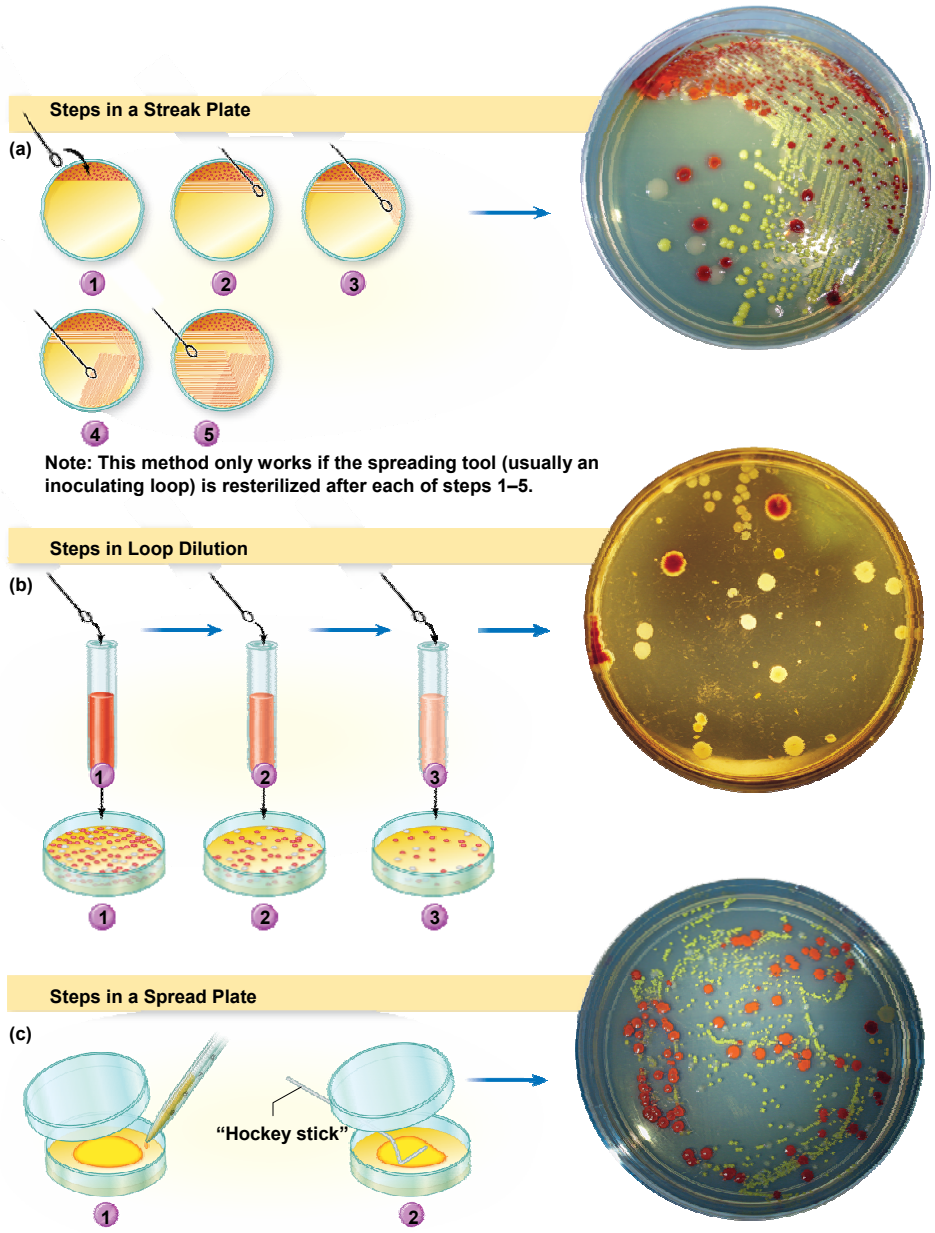
Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

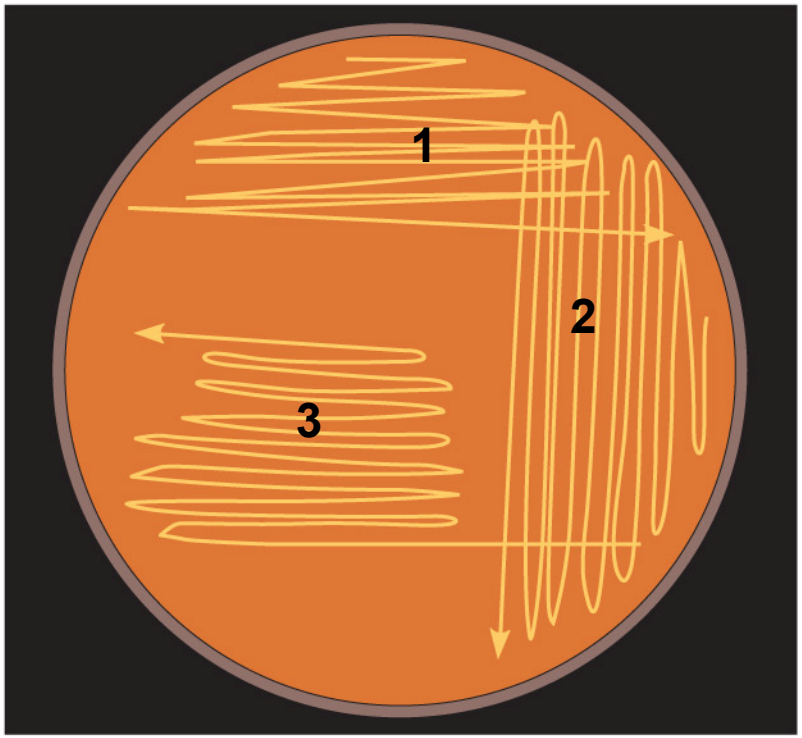
Obtaining Pure Cultures

- A pure culture contains only one species or strain
- A **colony** is a population of cells arising from a single cell or spore or from a group of attached cells
- A colony is often called a **colony-forming unit** (CFU)
- The **streak plate method** is used to isolate pure cultures

Methods for Isolating Bacteria



The streak plate method for isolating pure bacterial cultures.



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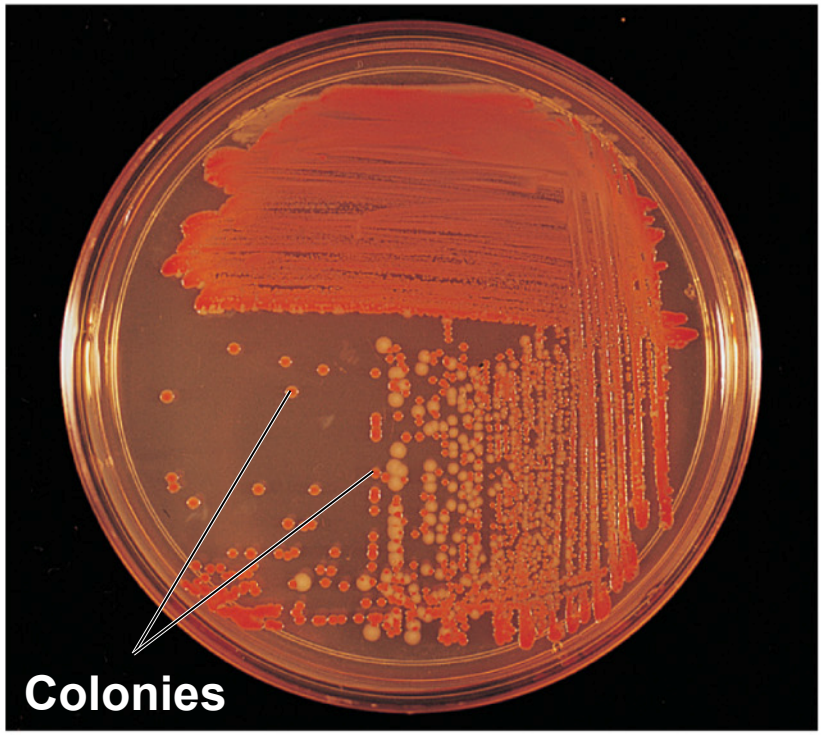
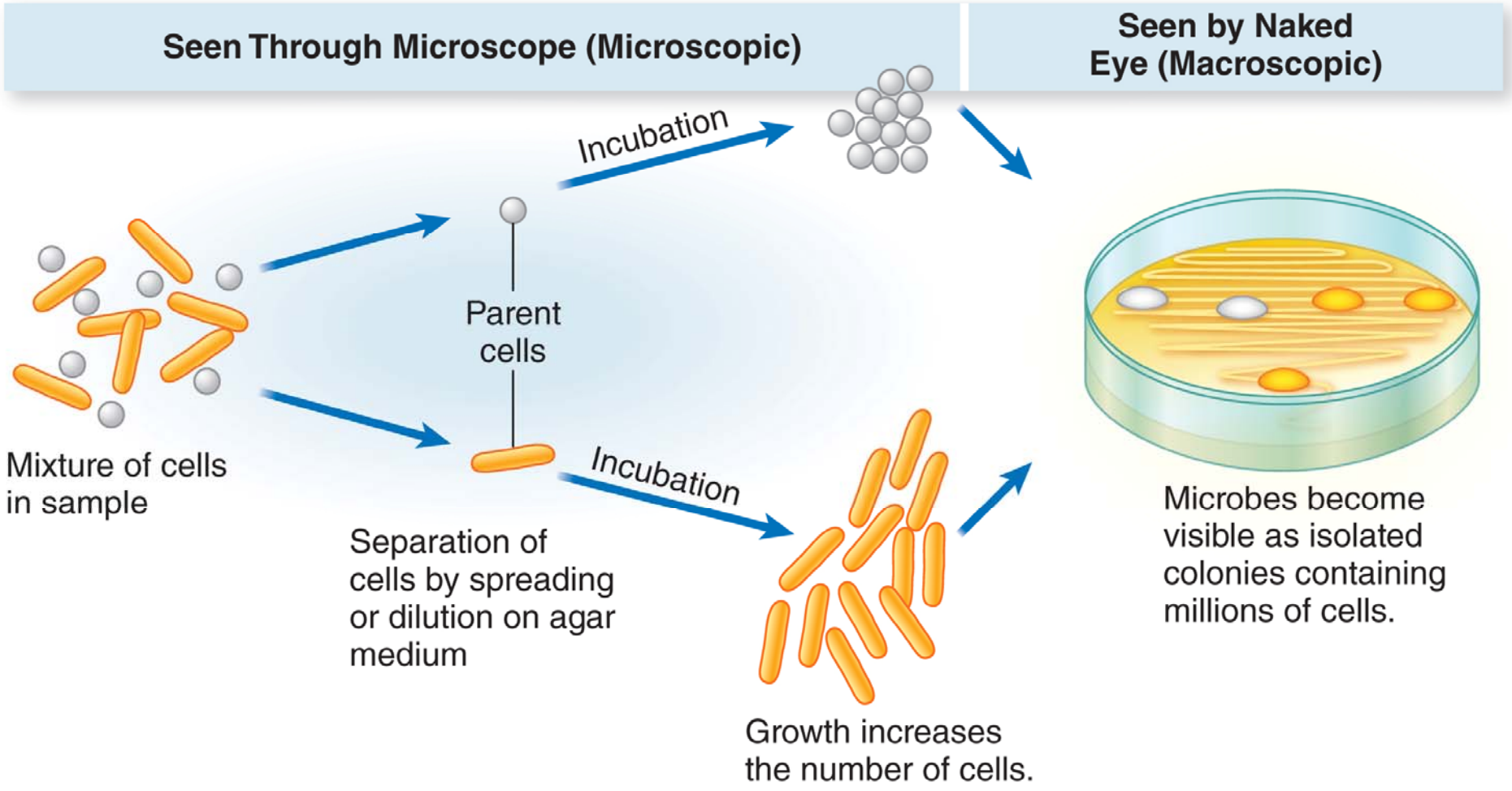


(b)



Preserving Bacterial Cultures

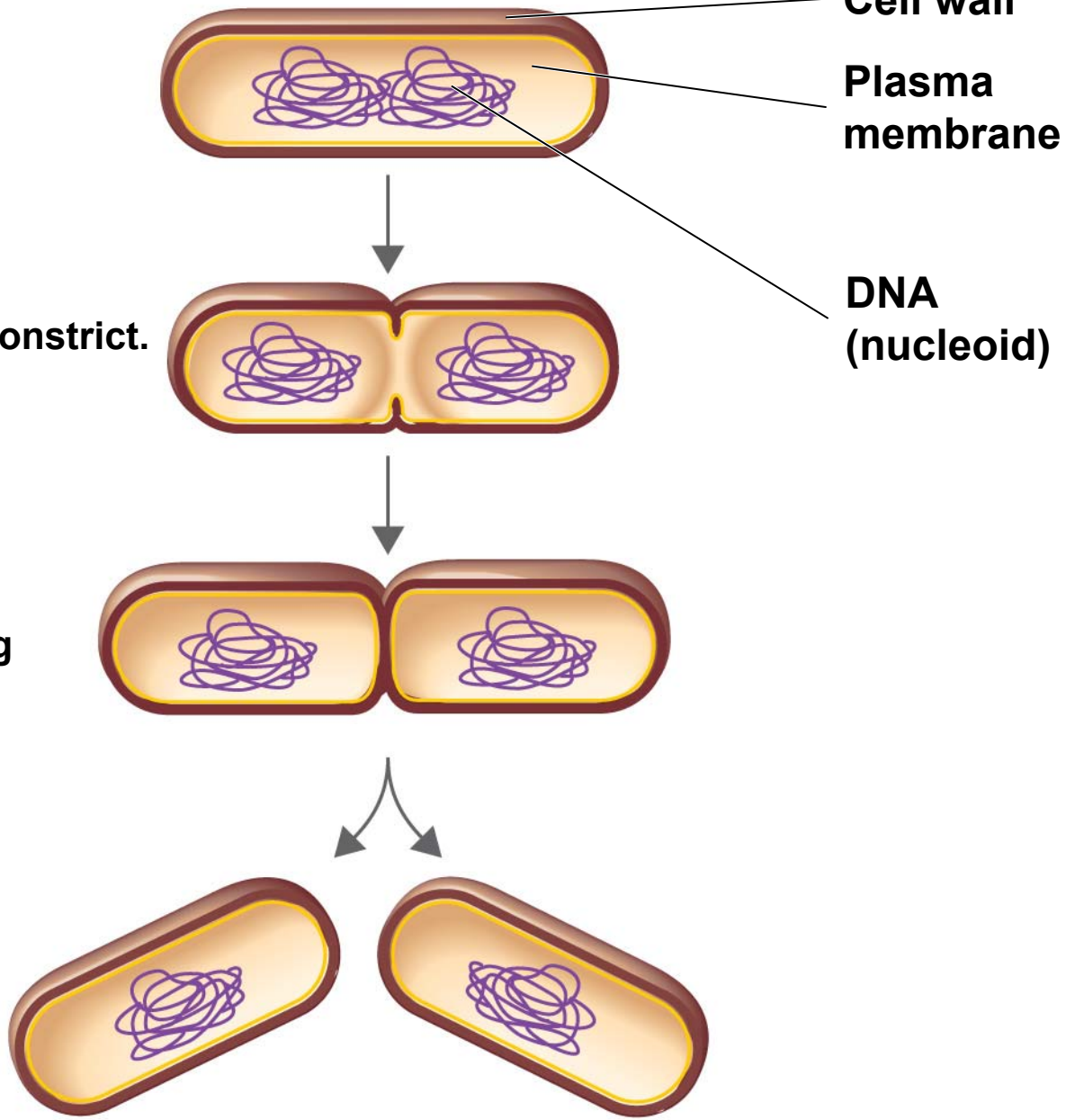
- **Deep-freezing**: –50° to –95°C
- **Lyophilization (freeze-drying)**:
  - frozen (–54° to –72°C)
  - then dehydrated in a vacuum

Methods Used by Prokaryotes to Reproduce

- Binary fission // most bacteria use this method
- Budding // yeast use budding
- Conidiospores (actinomycetes) // molds produce spores which are “reproductive” – don’t confuse with bacterial “endospores” (different structures)
- Fragmentation of filaments // hyphae of molds

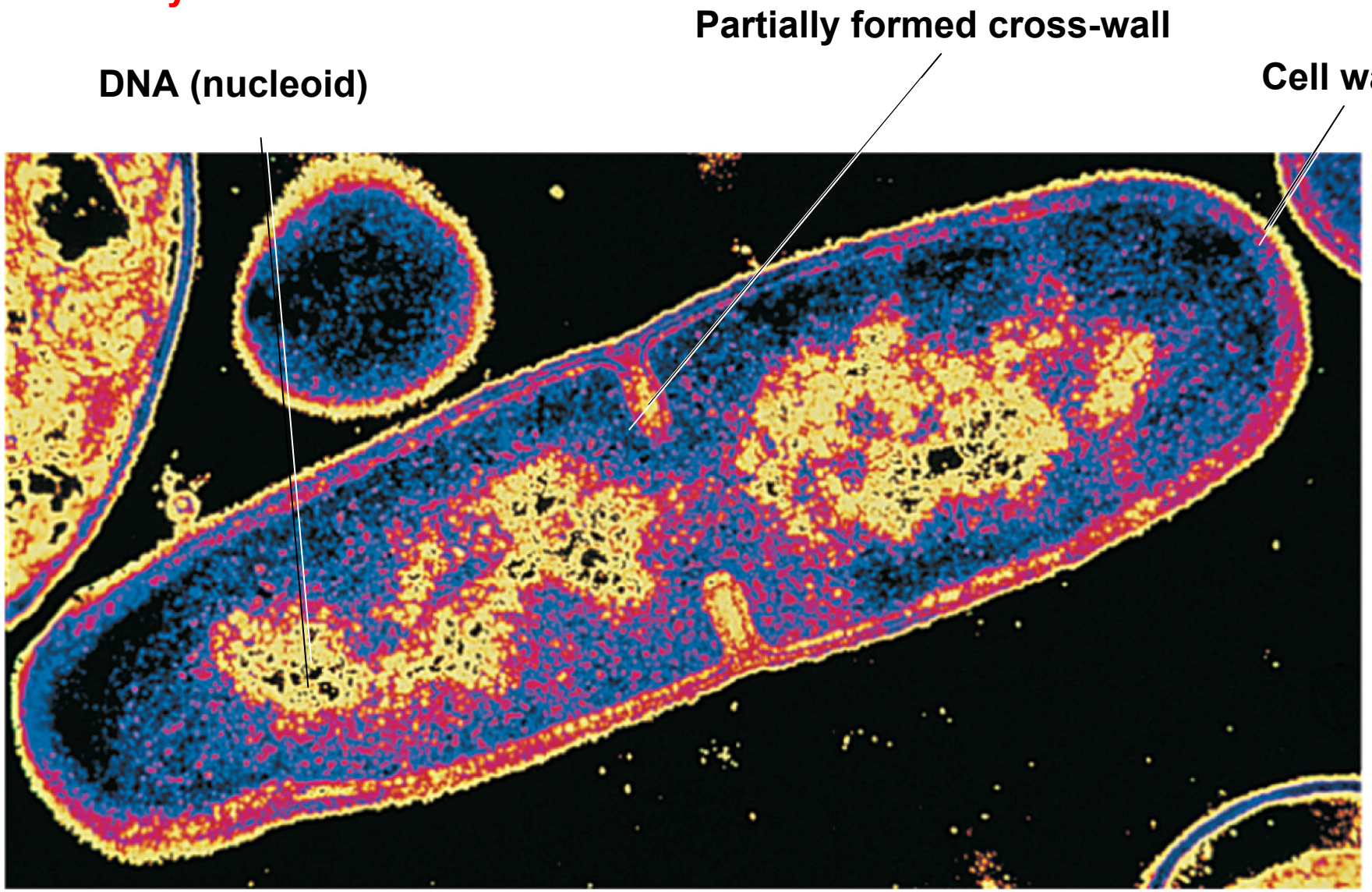
Binary fission in bacteria.

1. Cell elongates and DNA is replicated.
2. Cell wall and plasma membrane begin to constrict.
3. Cross-wall forms, completely separating the two DNA copies.
4. Cells separate.



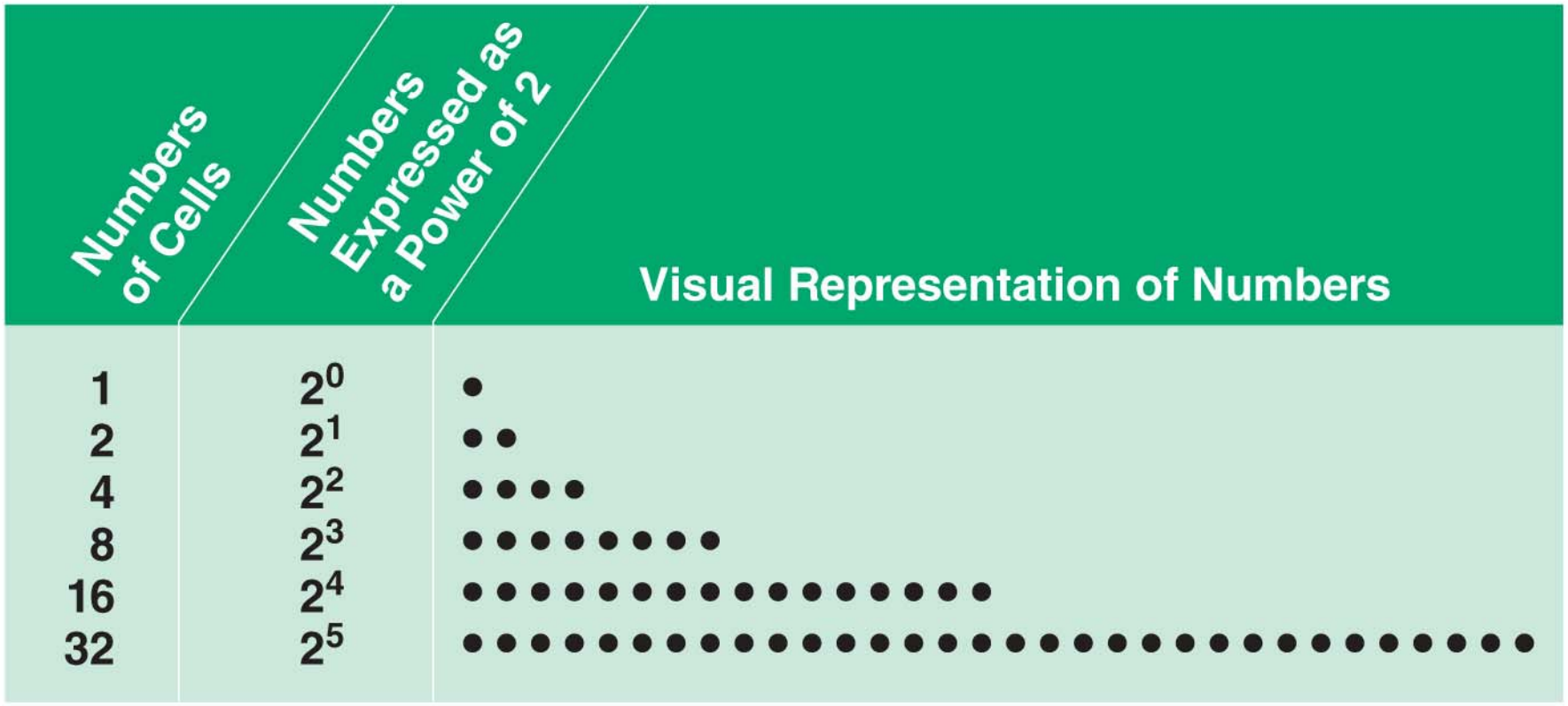
(a) **A diagram of the sequence of cell division**

Binary fission in bacteria.



(b) **A thin section of a cell of *Bacillus licheniformis* starting to divide**

Cell division.



(a)

Cell division

Generation Number	Number of Cells	Log_{10} of Number of Cells
0	$2^0 = 1$	0
5	$2^5 = 32$	1.51
10	$2^{10} = 1{,}024$	3.01
15	$2^{15} = 32{,}768$	4.52
16	$2^{16} = 65{,}536$	4.82
17	$2^{17} = 131{,}072$	5.12
18	$2^{18} = 262{,}144$	5.42
19	$2^{19} = 524{,}288$	5.72
20	$2^{20} = 1{,}048{,}576$	6.02

(b)

Generation Time

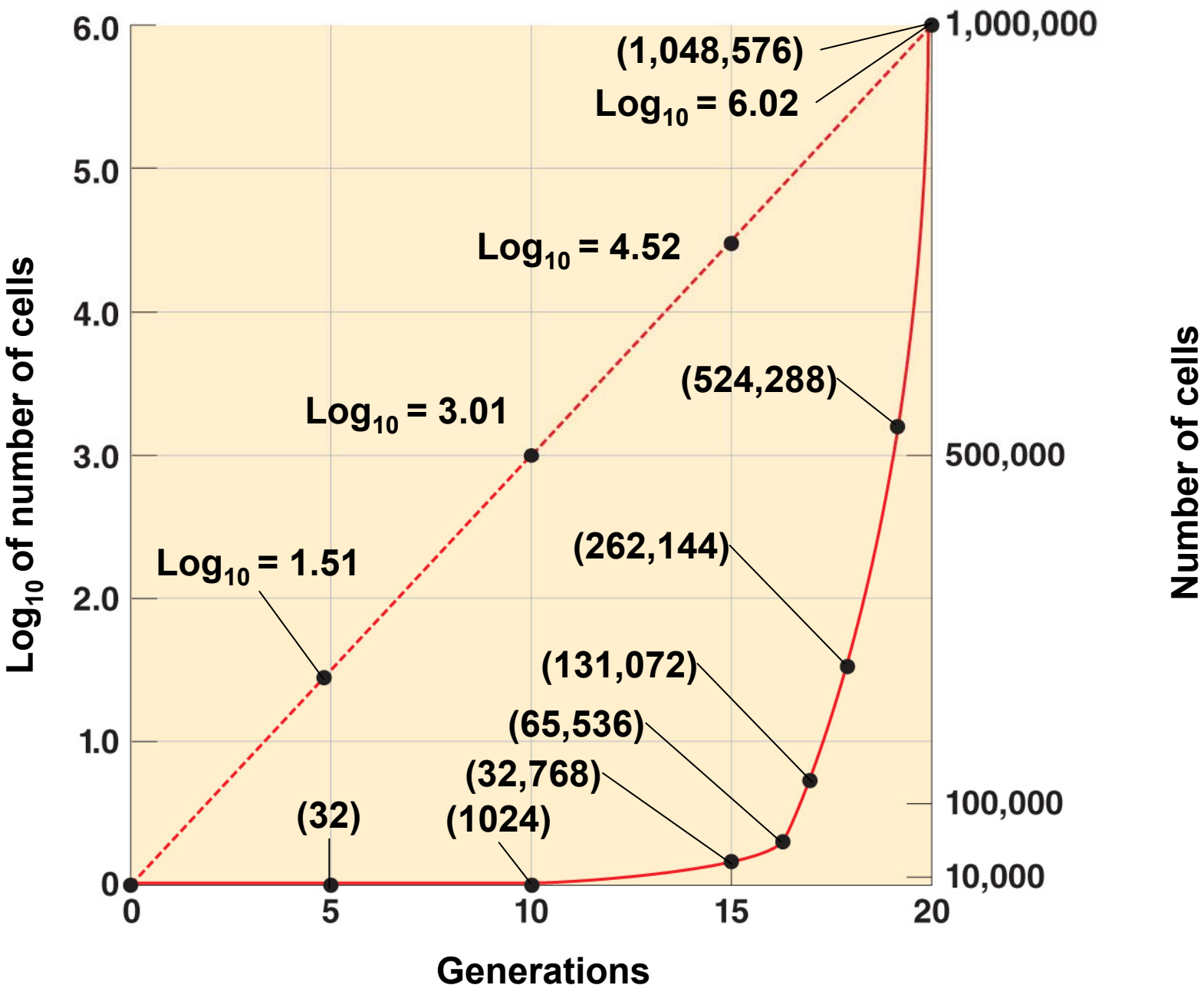
- If 100 cells growing for 5 hours produced 1,720,320 cells:

$$\text{Number of generations} = \frac{\text{Log number of cells (end)} - \text{Log number of cells (beginning)}}{0.301}$$

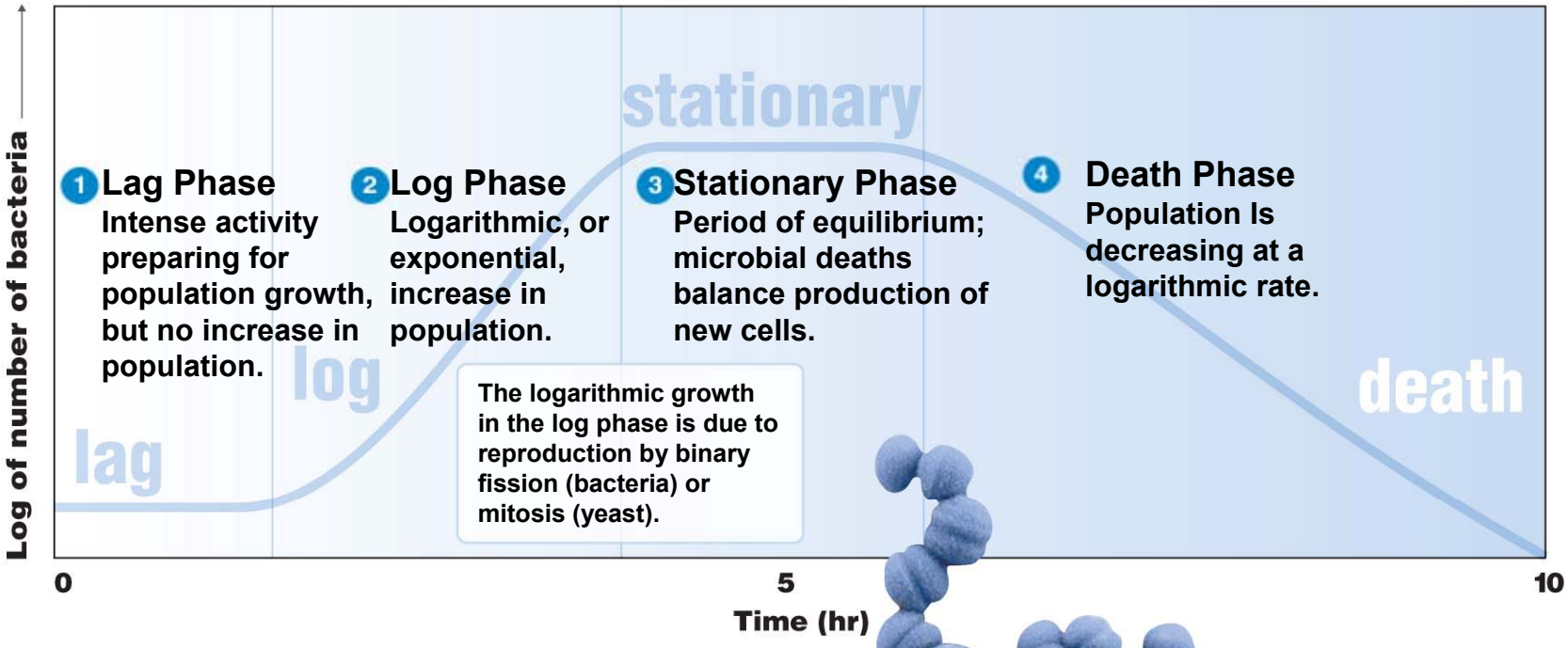
$$\text{Generation time} = \frac{60 \text{ min} \times \text{hours}}{\text{Number of generations}} = \textbf{21 minutes/generation}$$

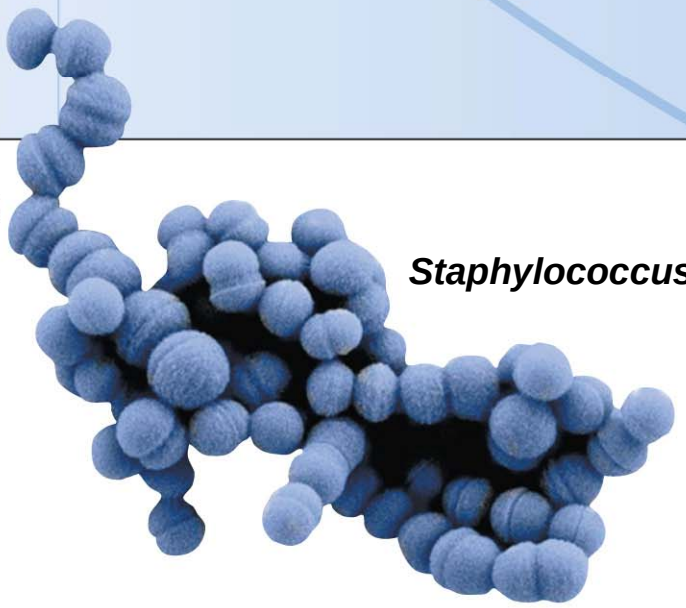
Note: use glucose consumption used to measure metabolic activity.

A growth curve for an exponentially increasing population, plotted logarithmically (dashed line) and arithmetically (solid line).



Understanding the Bacterial Growth Curve.



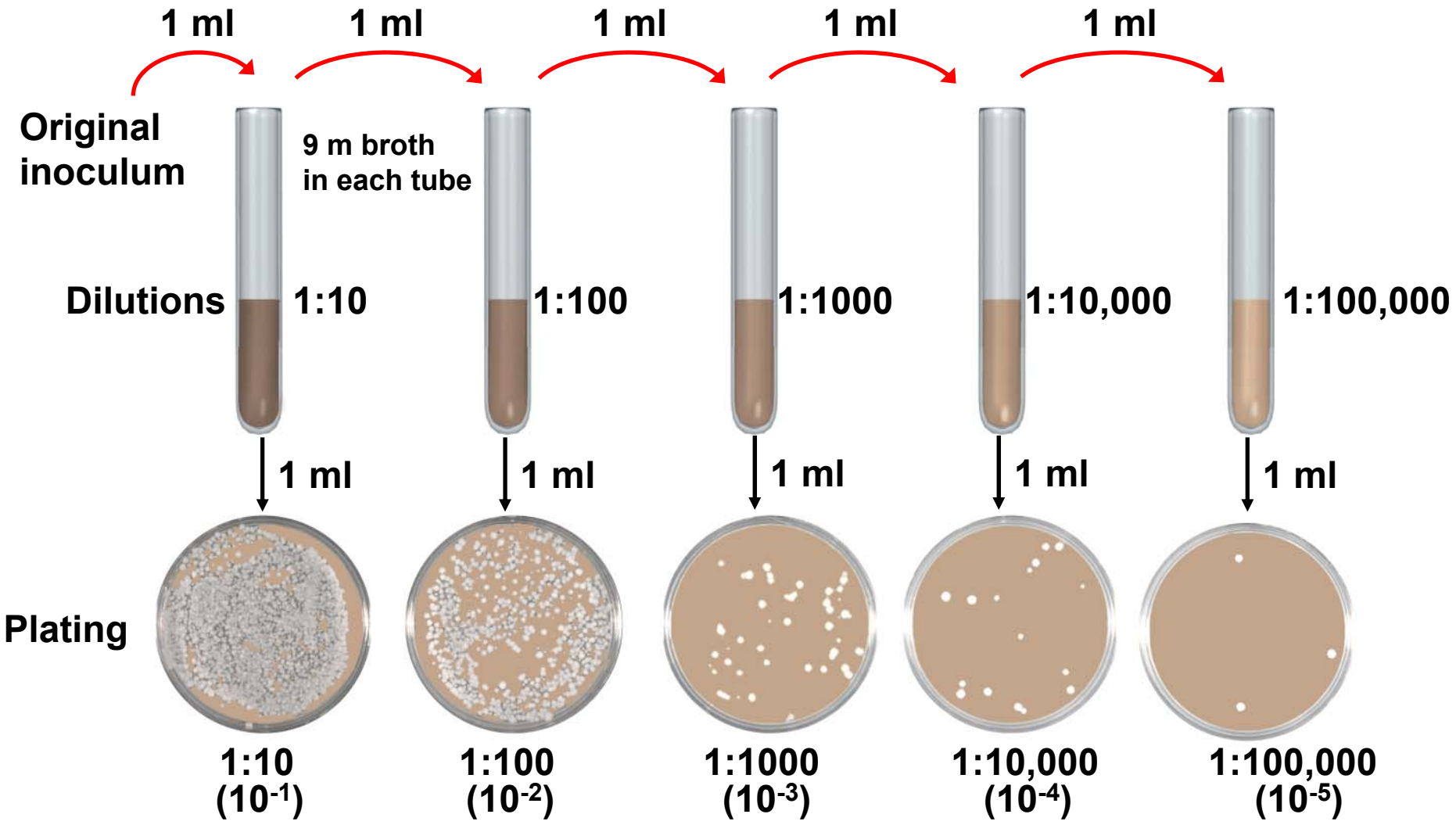


***Staphylococcus* spp.**

KEY CONCEPTS

- Bacterial populations follow a sequential series of growth phases: the lag, log, stationary, and death phases.
- Knowledge of the bacterial growth curve is critical to understanding population dynamics and population control in the course of infectious diseases, in food preservation and spoilage, as well as in industrial microbiology processes, such as ethanol production.

Serial dilutions and plate counts.

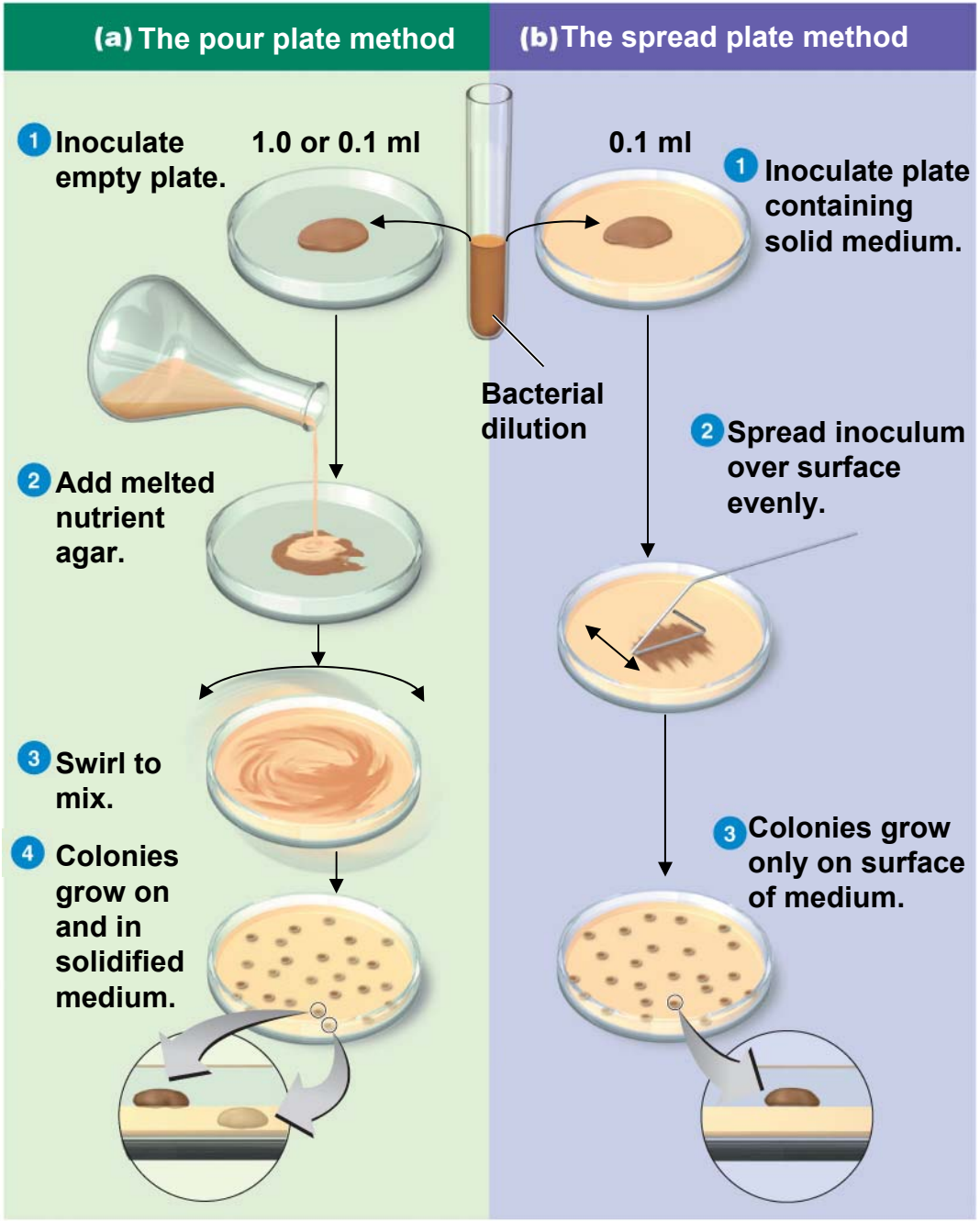


Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 54 colonies are on a plate of 1:1000 dilution, then the count is 54 × 1000 = 54,000 bacteria/ml in sample.)

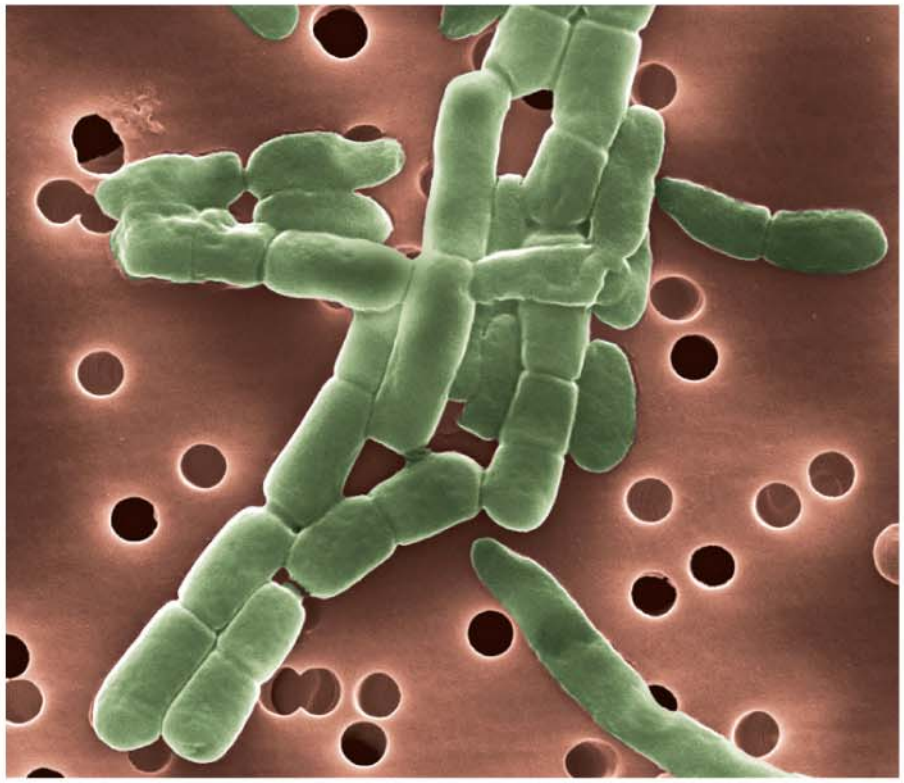
Plate Counts

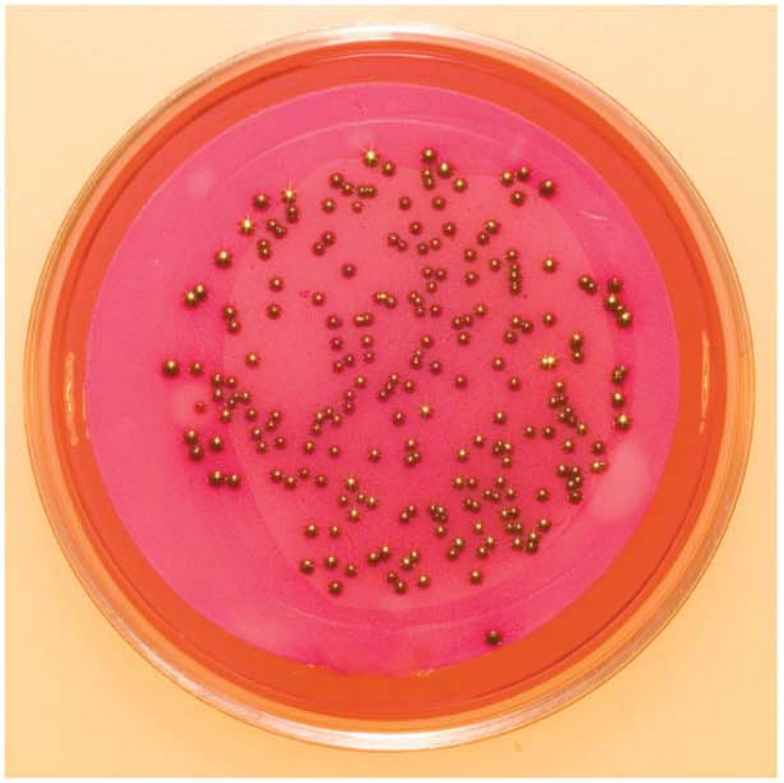
- After incubation, count colonies on plates that have 25–250 colonies (CFUs)

Methods of preparing plates for plate counts.



Counting bacteria by filtration.

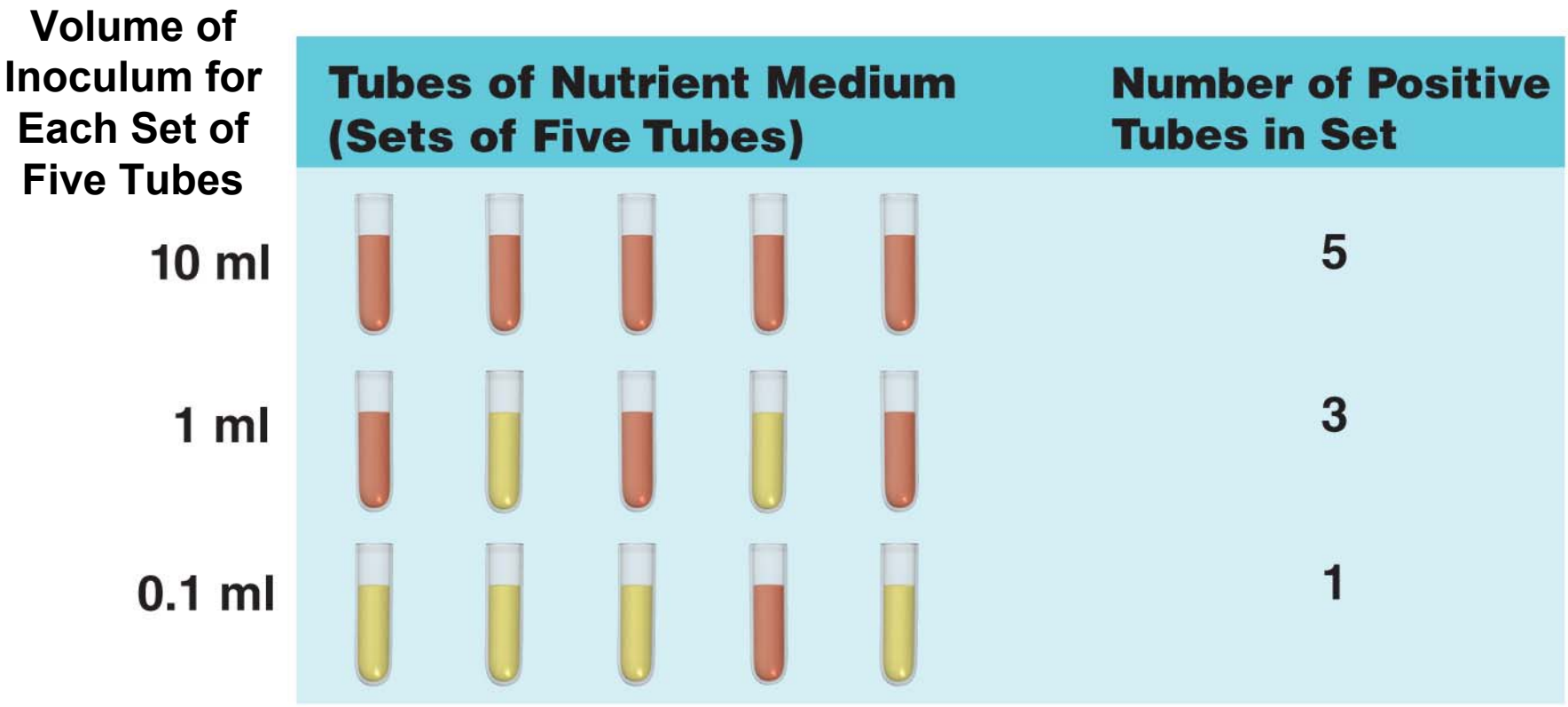




Most Probable Number

- Multiple tube MPN test
- Count positive tubes
- Compare with a statistical table

The most probable number (MPN) method.



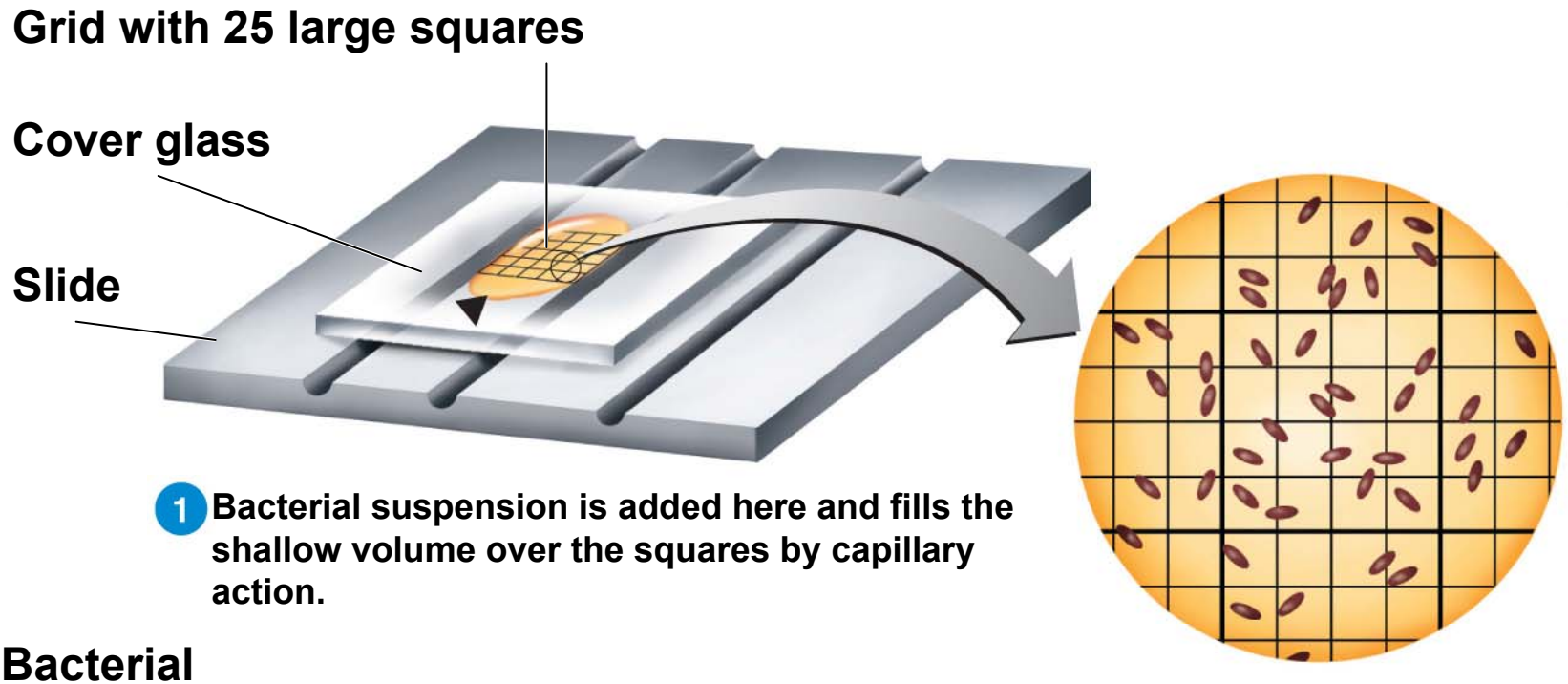
(a) **Most probable number (MPN) dilution series.**

The most probable number (MPN) method.

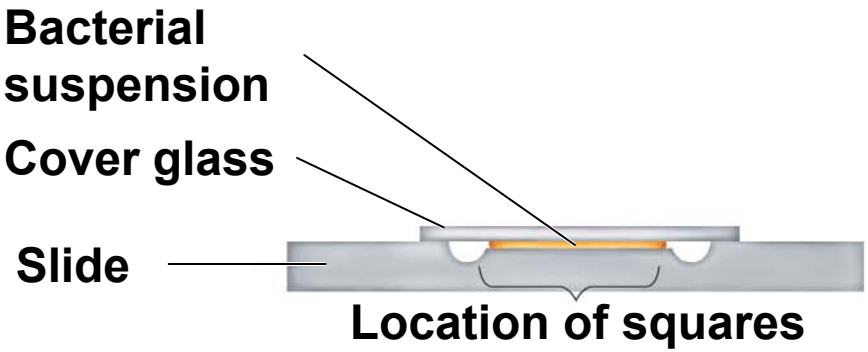
Combination of Positives	MPN Index/ 100 m	95% Confidence Limits	
		Lower	Upper
4-2-0	22	6.8	50
4-2-1	26	9.8	70
4-3-0	27	9.9	70
4-3-1	33	10	70
4-4-0	34	14	100
5-0-0	23	6.8	70
5-0-1	31	10	70
5-0-2	43	14	100
5-1-0	33	10	100
5-1-1	46	14	120
5-1-2	63	22	150
5-2-0	49	15	150
5-2-1	70	22	170
5-2-2	94	34	230
5-3-0	79	22	220
5-3-1	110	34	250
5-3-2	140	52	400

(b) **MPN table.**

Direct microscopic count of bacteria with a Petroff-Hausser cell counter.



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 and the area of the squares are 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.

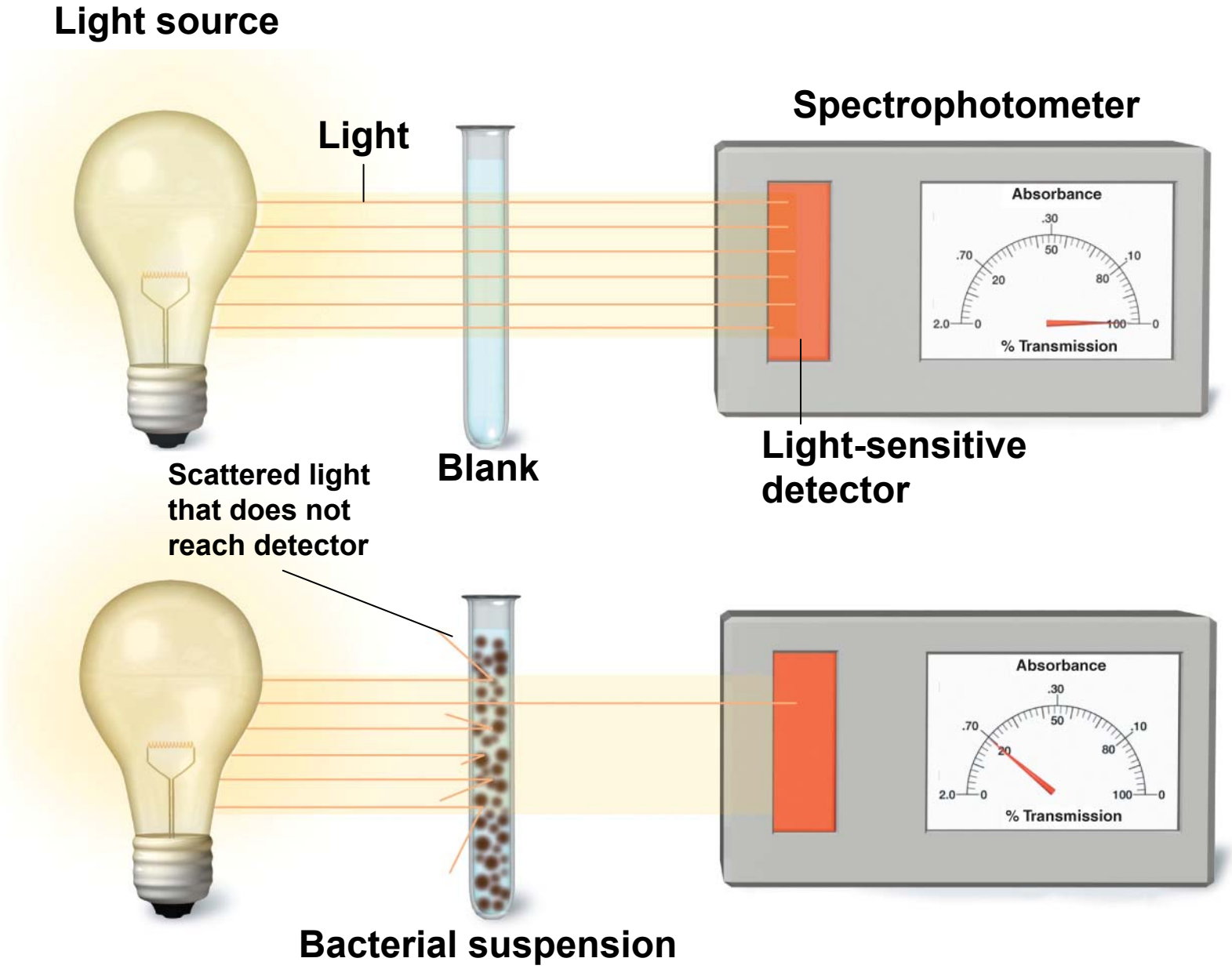
4 The volume of fluid over the large square is 1/1,250,000 of a milliliter. If it contains 14 cells, as shown here, then there are 14 × 1,250,000 = 17,500,000 cells in a milliliter.

Direct Microscopic Count

$$\text{Number of bacteria/ml} = \frac{\text{Number of cells counted}}{\text{Volume of area counted}}$$

$$\frac{14}{8 \times 10^{-7}} = 17{,}500{,}000$$

Turbidity estimation of bacterial numbers.



Measuring Microbial Growth

Direct Methods

- Plate counts
- Filtration
- Most Probable Num.
- Direct microscopic count

Indirect Methods

- Turbidity
- Metabolic activity
- Dry weight

Biosafety Levels

- BSL-1: no special precautions
- BSL-2: lab coat, gloves, eye protection
- BSL-3: biosafety cabinets to prevent airborne transmission
- BSL-4: sealed, negative pressure // Exhaust air is filtered twice

Technicians in a biosafety level 4 (BSL-4) laboratory.