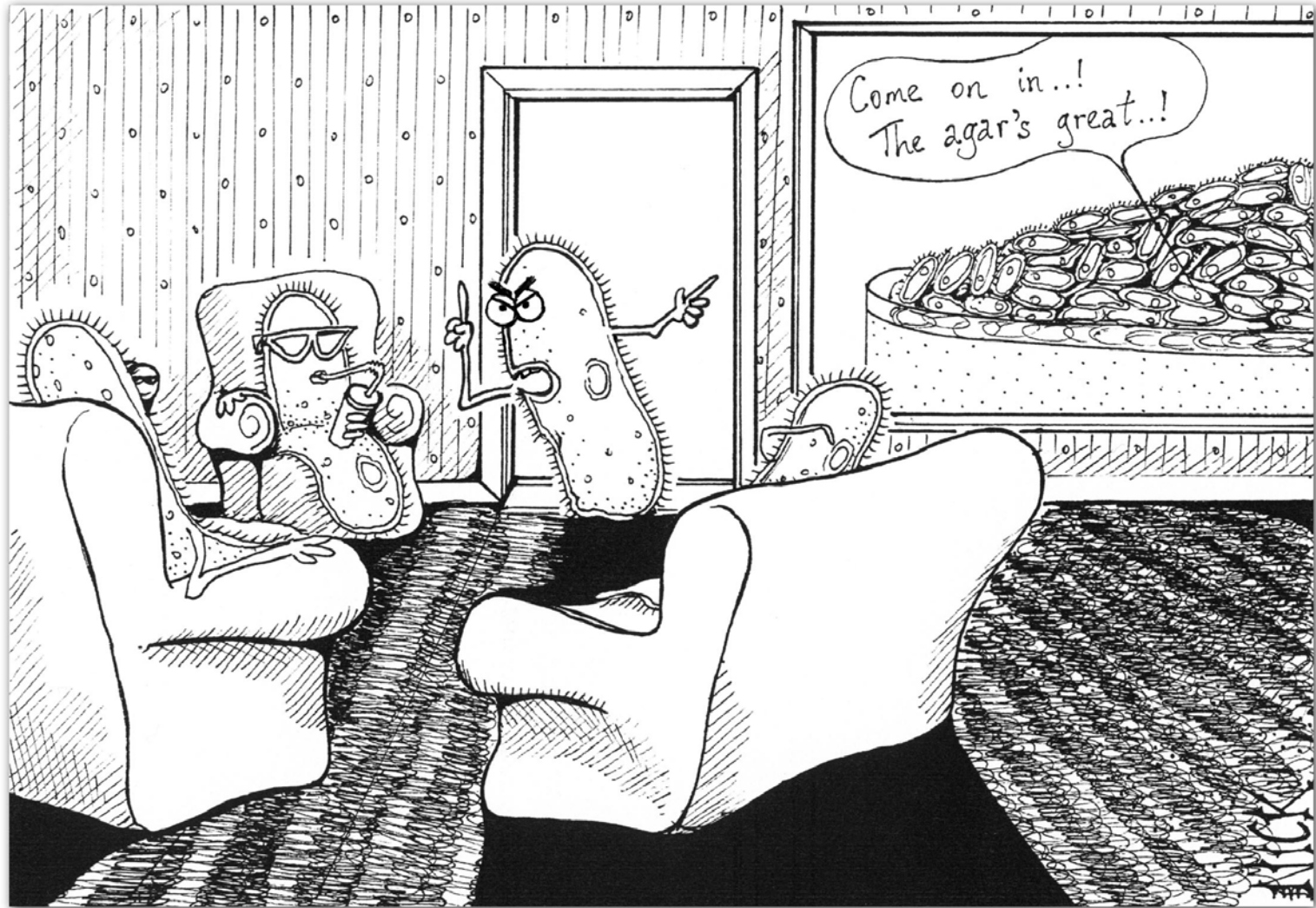
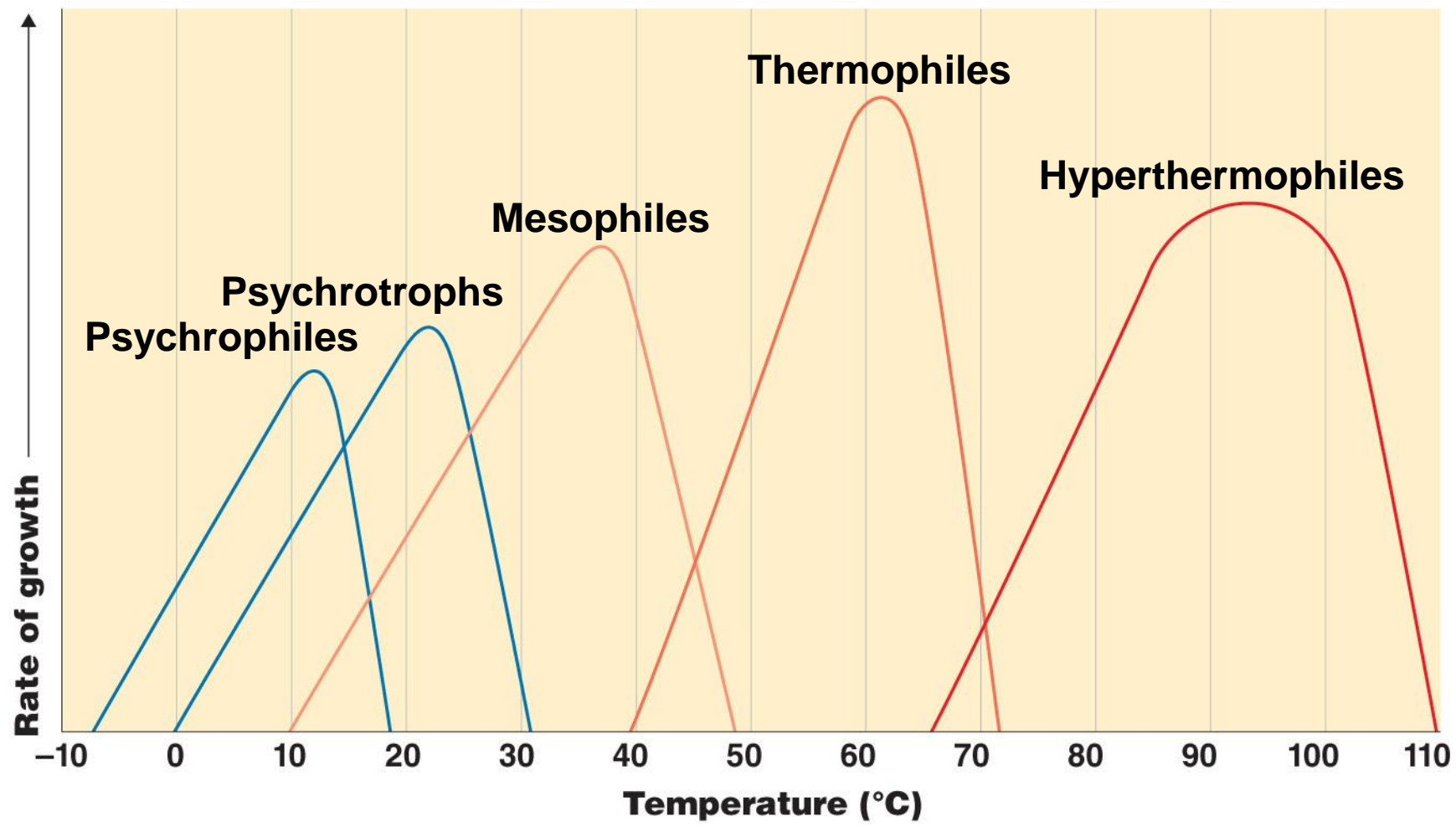


# Microbial Growth (Ch 6)



*"I wish you'd learn to put the lid on your Petri Dish, Harry! We came here today with just four kids but now it looks like we've got several million..!!"*

Figure 6.1 Typical growth rates of different types of microorganisms in response to temperature.



**Applications of Microbiology 6.1 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.**



1 m

**Figure 6.2 Food preservation temperatures.**

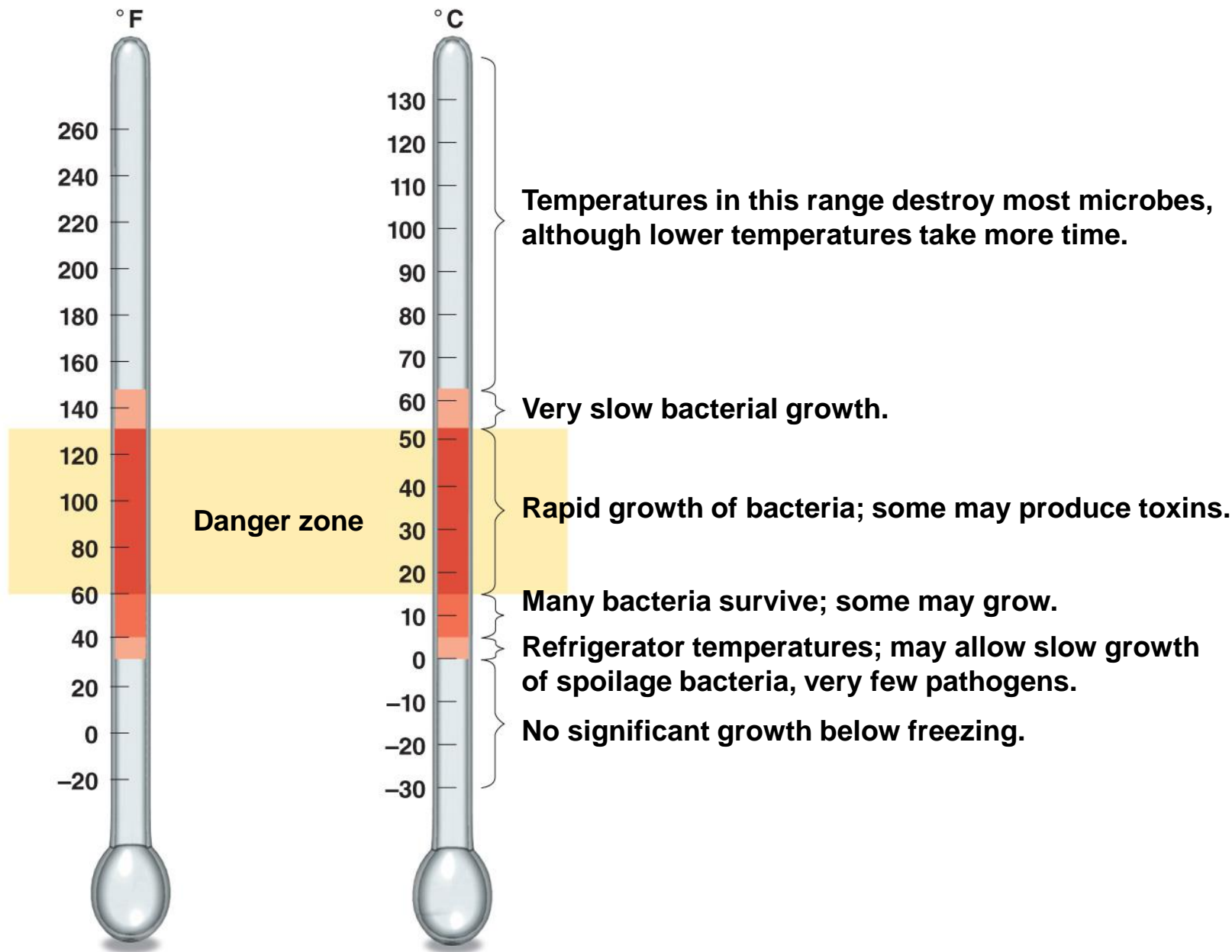


Figure 6.3 The effect of the amount of food on its cooling rate in a refrigerator and its chance of spoilage.

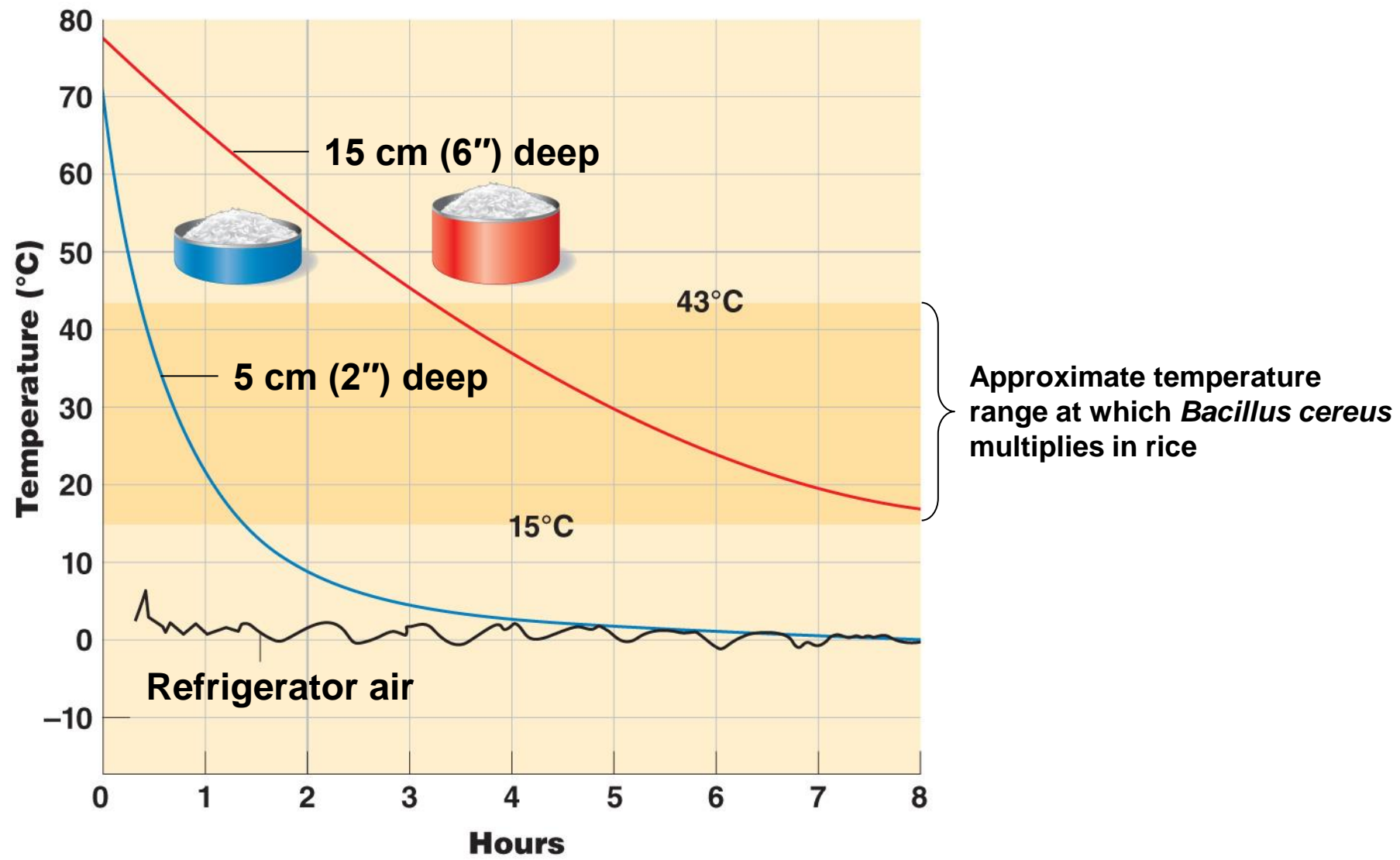
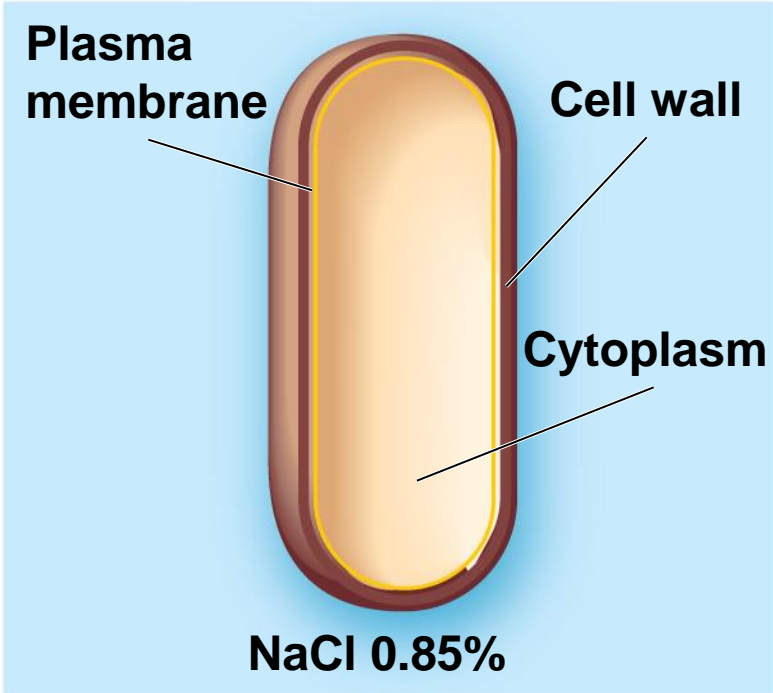
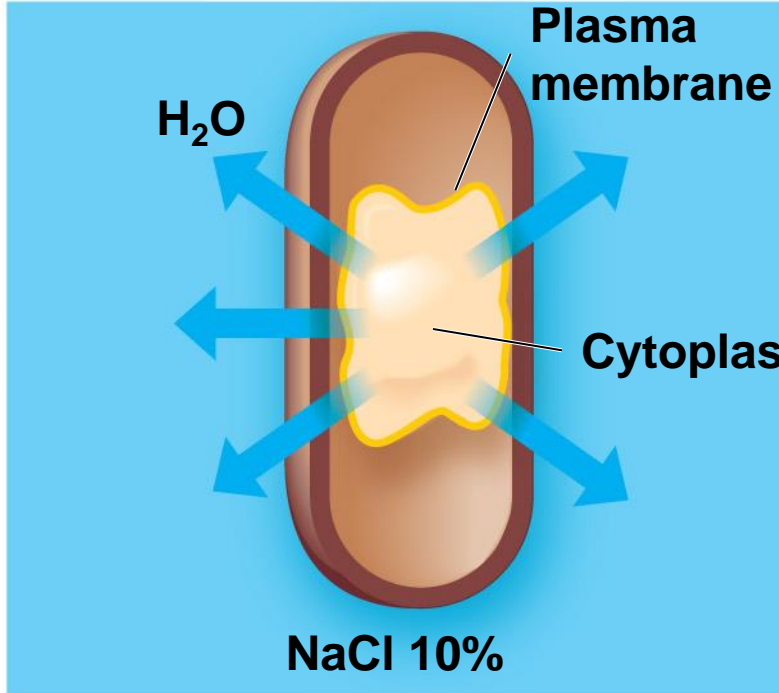


Figure 6.4 Plasmolysis.








(a) Cell in isotonic solution.



(b) Plasmolyzed cell in hypertonic solution.

**Table 6.1 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**

	<b>a. Obligate Aerobes</b>	<b>b. Facultative Anaerobes</b>	<b>c. Obligate Anaerobes</b>	<b>d. Aerotolerant Anaerobes</b>	<b>e. Microaerophiles</b>
<b>Effect of Oxygen on Growth</b>	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.
<b>Bacterial Growth in Tube of Solid Growth Medium</b>					
<b>Explanation of Growth Patterns</b>	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.
<b>Explanation of Oxygen's Effects</b>	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.

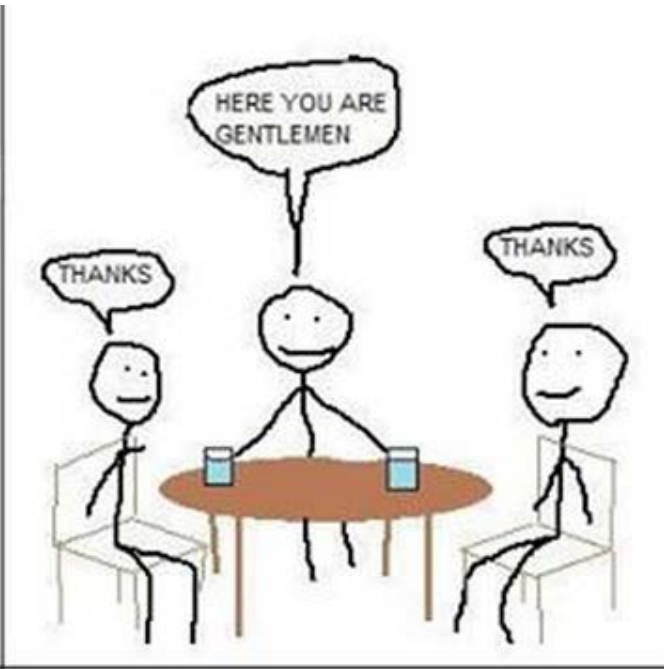




Table 6.2 A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

**TABLE 6.2** A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ( $\text{NH}_4\text{H}_2\text{PO}_4$ )	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ )	0.2 g
Potassium phosphate, dibasic ( $\text{K}_2\text{HPO}_4$ )	1.0 g
Water	1 liter

**Table 6.3 Defined Culture Medium for *Leuconostoc mesenteroides***

**TABLE 6.3** Defined Culture Medium for  
*Leuconostoc mesenteroides*

<b>Carbon and Energy</b>
Glucose, 25 g
<b>Salts</b>
NH <sub>4</sub> Cl, 3.0 g
K <sub>2</sub> HPO <sub>4</sub> *, 0.6 g
KH <sub>2</sub> PO <sub>4</sub> *, 0.6 g
MgSO <sub>4</sub> , 0.1 g
<b>Amino Acids, 100–200 µg each</b>
Alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine
<b>Purines and Pyrimidines, 10 mg of each</b>
Adenine, guanine, uracil, xanthine
<b>Vitamins, 0.01–1 mg each</b>
Biotin, folate, nicotinic acid, pyridoxal, pyridoxamine, pyridoxine, riboflavin, thiamine, pantothenate, <i>p</i> -aminobenzoic acid
<b>Trace Elements, 2–10 µg each</b>
Fe, Co, Mn, Zn, Cu, Ni, Mo
<b>Buffer, pH 7</b>
Sodium acetate, 25 g
<b>Distilled Water, 1,000 ml</b>
*Also serves as buffer.

**Table 6.4 Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria**

**TABLE 6.4** **Composition of Nutrient Agar,  
a Complex Medium for the Growth  
of Heterotrophic Bacteria**

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

# Simmons' Citrate Agar

## Formula / Liter

Ammonium Dihydrogen Phosphate.....	1 g
Dipotassium Phosphate .....	1 g
Sodium Chloride .....	5 g
Sodium Citrate.....	2 g
Magnesium Sulfate.....	0.2 g
Bromthymol Blue .....	0.08 g
Agar .....	15 g

Final pH:  $6.9 \pm 0.2$  at  $25^{\circ}\text{C}$

Formula may be adjusted and/or supplemented as required to meet performance specifications.

# Tryptic Soy Broth

## Formula / Liter

Enzymatic Digest of Casein .....	17.0 g
Enzymatic Digest of Soybean Meal .....	3.0 g
Sodium Chloride .....	5.0 g
Dipotassium Phosphate.....	2.5 g
Dextrose.....	2.5 g

Final pH:  $7.3 \pm 0.2$  at  $25^{\circ}\text{C}$

Formula may be adjusted and/or supplemented as required to meet performance specifications.

# Brain heart infusion broth

From Wikipedia, the free encyclopedia

**Brain-heart infusion broth** (**BHI broth** or **BHIB**) is a highly nutritious general-purpose growth medium for culturing fastidious and nonfastidious microorganisms, such as streptococci, pneumococci and meningococci.<sup>[1]</sup> It is made by boiling cow<sup>[2]</sup> or porcine<sup>[3]</sup> hearts and brains. Boiling releases soluble factors into the broth. The broth can then be turned into powder for easy distribution. BHI broth contains sodium chloride which is used to differentiate enterococci from nonenterococcal group D streptococci.<sup>[4]</sup> BHI broth is often used in food safety, water safety, and antibiotic sensitivity tests.<sup>[3]</sup>

## See also [edit]

- Microbiological culture
- Tryptic Soy Broth
- Lysogeny broth
- SOC medium

## References [edit]

- ↑ US Biological. Brain Heart Infusion Broth (Powder)
- ↑ Bacterial nutrition . *Virtual Microbiology Textbook for Microbiology 102*.
- ↑ <sup>a</sup> <sup>b</sup> Acumedia Manufacturers. Brain-Heart Infusion Broth (7116) . Neogen.
- ↑ BD. BHI (Brain Heart Infusion) Broth, 5 mL

Categories: Microbiological media



# Chocolate agar

From Wikipedia, the free encyclopedia

**Chocolate agar** (CHOC) or **chocolate blood agar** (CBA) - is a non-selective, enriched growth medium. <sup>[1]</sup> <sup>[2]</sup> It is a variant of the blood agar plate, containing red blood cells that have been lysed by slowly heating to 80 °C. Chocolate agar is used for growing fastidious respiratory bacteria, such as *Haemophilus influenzae* and *Neisseria meningitidis*.<sup>[3]</sup> These bacteria need growth factors, like NAD (factor V) and hemin (factor X), which are inside red blood cells; thus, a prerequisite to growth is lysis of the red blood cells. The heat also inactivates enzymes which could otherwise degrade NAD. The agar is named for the color and contains no actual chocolate.

## Variants [edit]

Chocolate agar with the addition of bacitracin becomes selective, most critically, for the genus *Haemophilus*. Another variant of chocolate agar called Thayer-Martin agar contains an assortment of antibiotics which select for *Neisseria species*.

## References [edit]

- ↑ Segen. "Chocolate agar: Definition" ↗. The Free Dictionary. Retrieved 28 September 2012.
- ↑ "Chocolate Agar (CHOC)" ↗. Anaerobe free systems. Retrieved 28 September 2012.
- ↑ Gunn, B.A. "Chocolate agar: A differential medium for gram positive cocci" ↗. PubMed. Retrieved 28 September 2012.

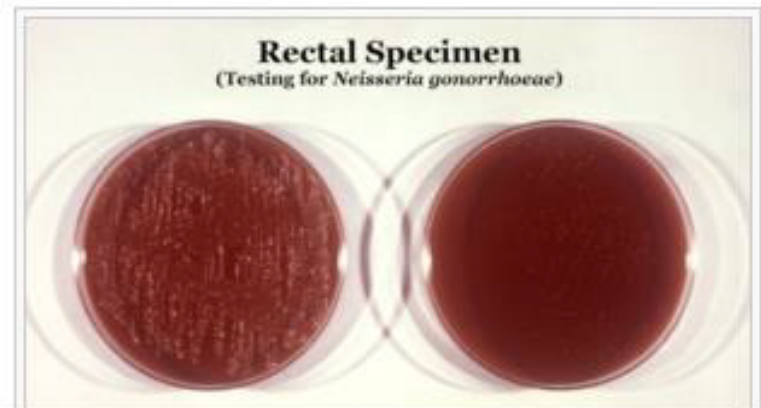
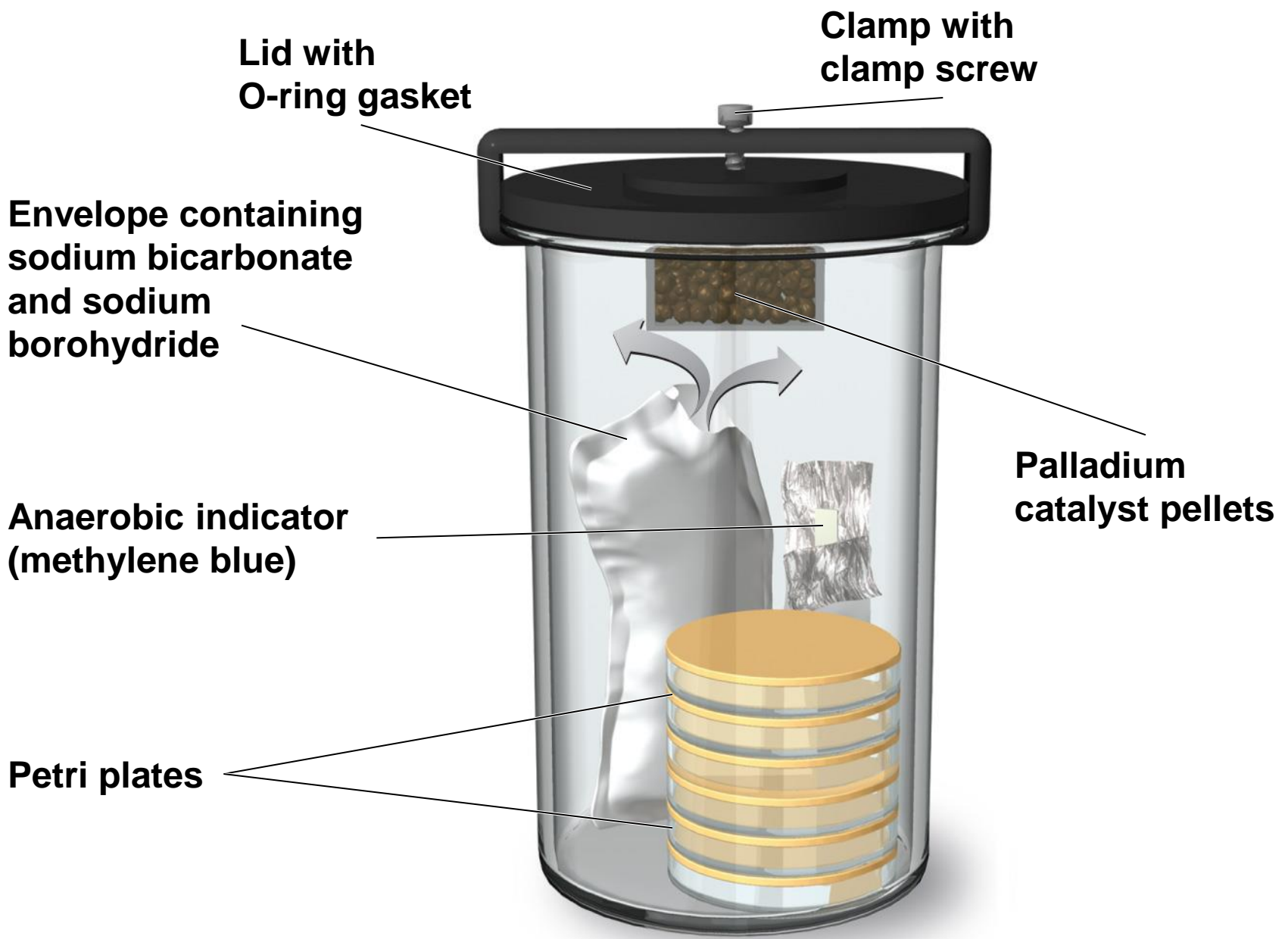


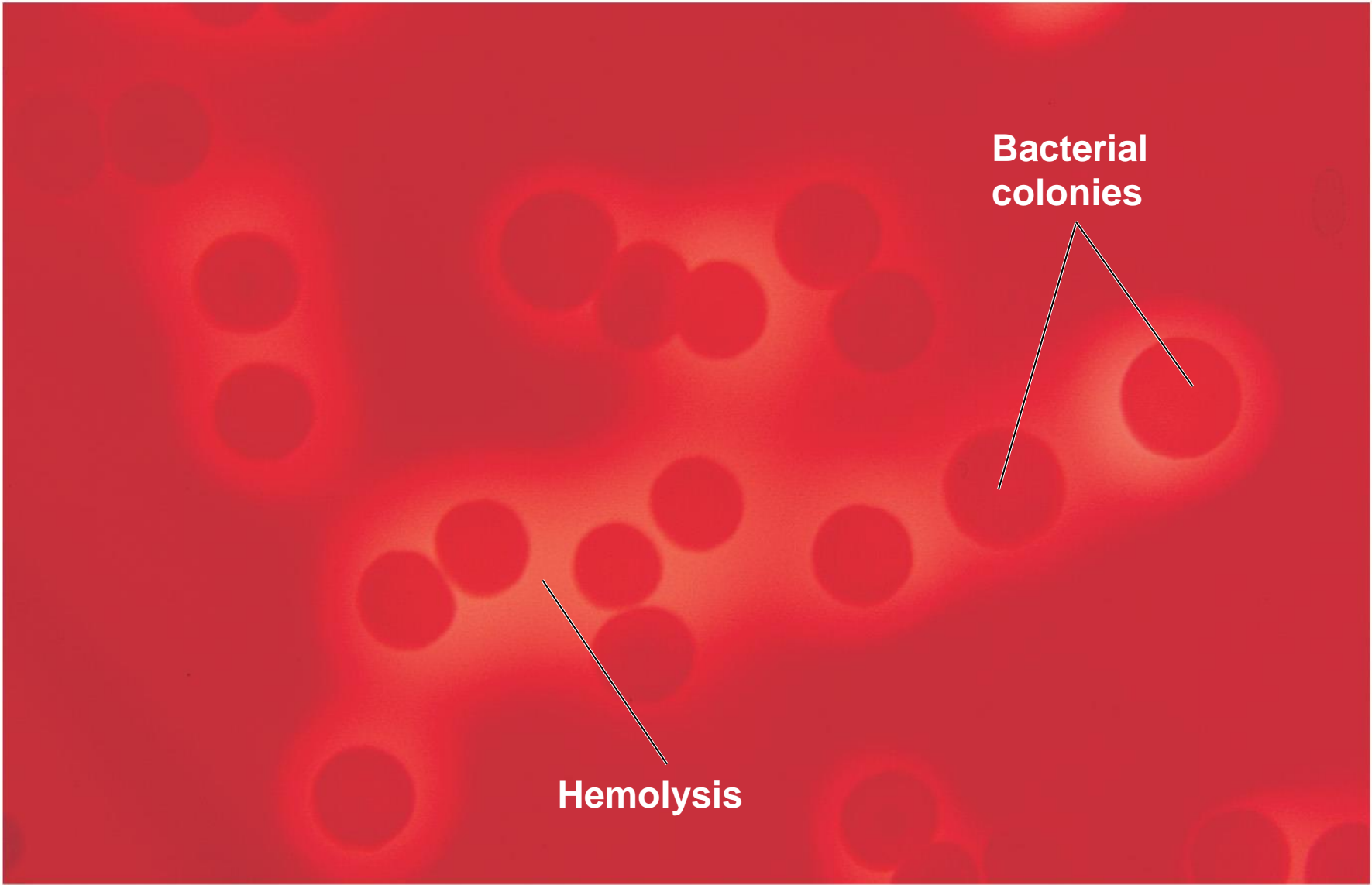
Figure 6.6 A jar for cultivating anaerobic bacteria on Petri plates.





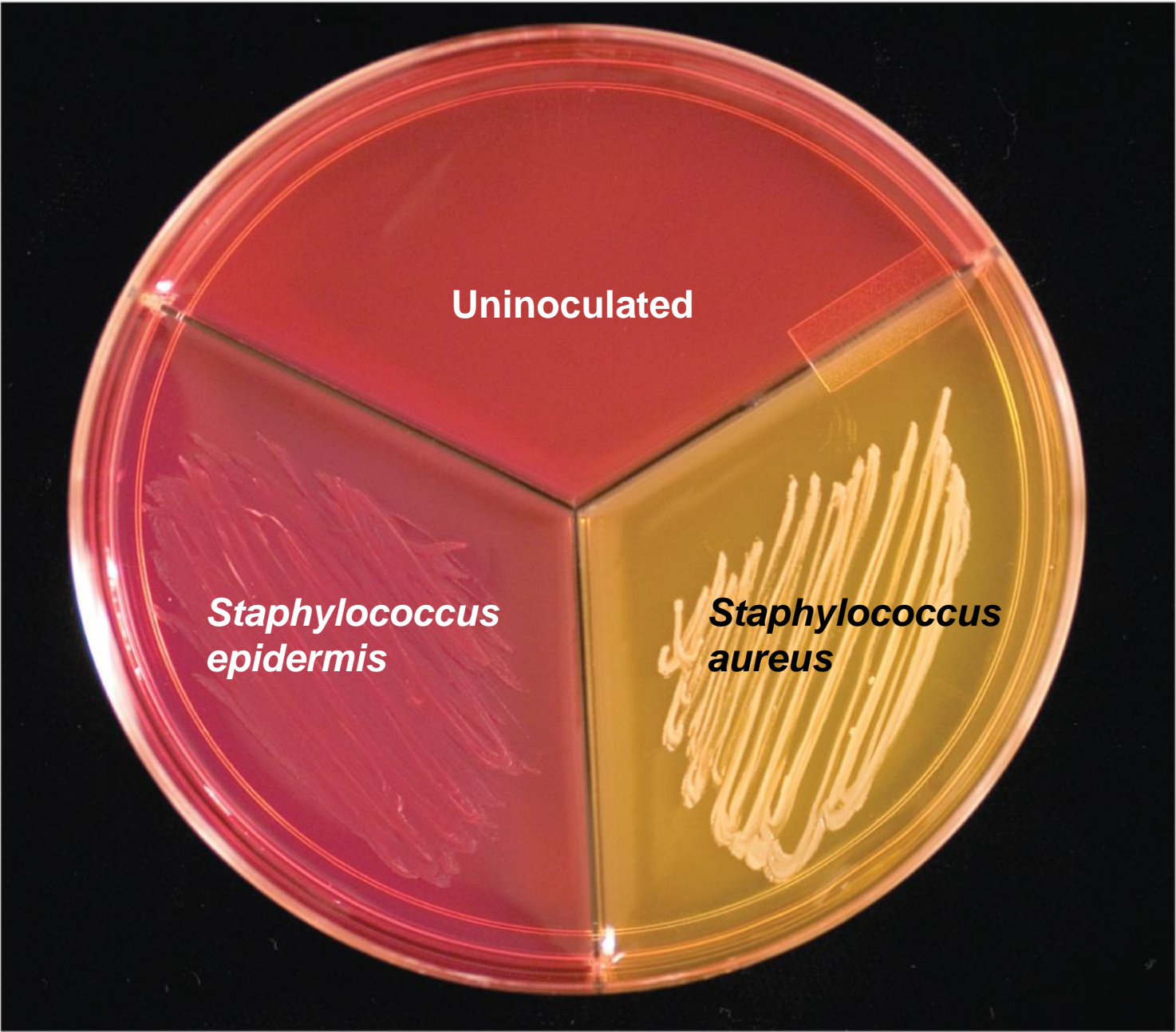


**Figure 6.9 Blood agar, a differential medium containing red blood cells.**



2 mm

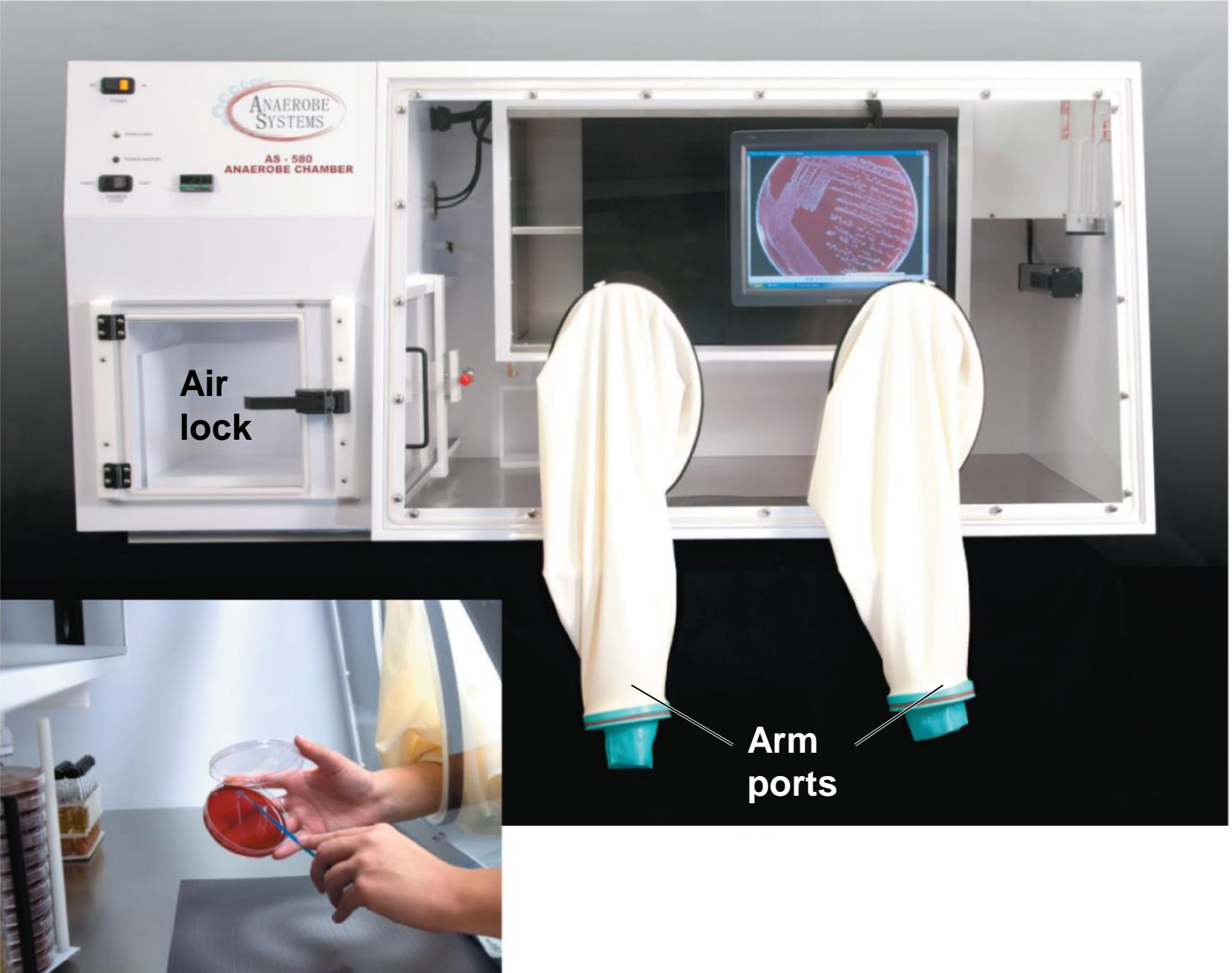
Figure 6.10 Differential medium.



## Biosafety levels

<http://www.cdc.gov/training/quicklearns/biosafety/>

Figure 6.7 An anaerobic chamber.



# Biological Safety Cabinet.



**Biological Safety Cabinet.**



Biological Safety Cabinet.



**Biosafety level 3 (BSL-3)**

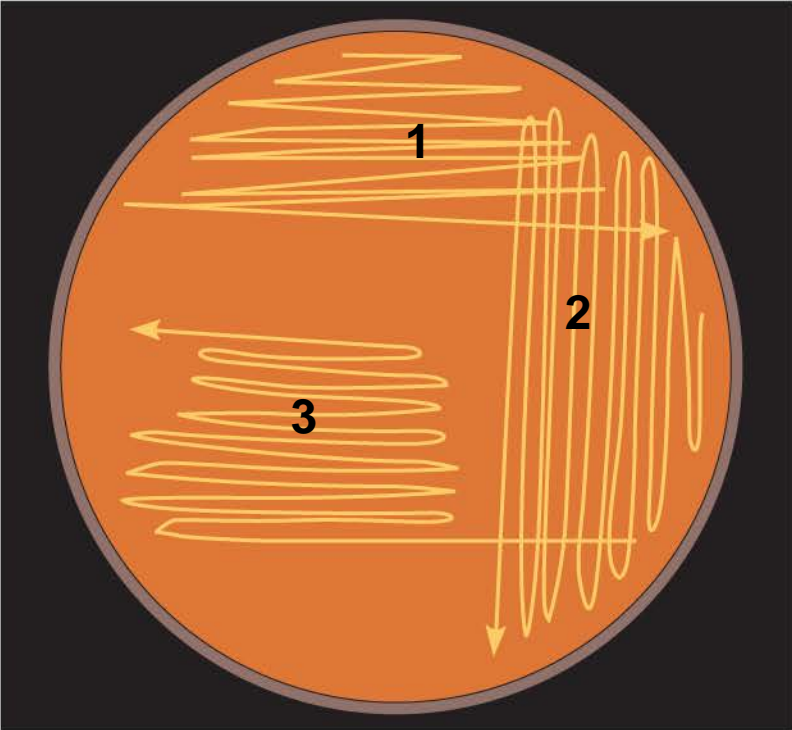




Figure 6.8 Technicians in a biosafety level 4 (BSL-4) laboratory.



Figure 6.11 The streak plate method for isolating pure bacterial cultures.



(a)



(b)

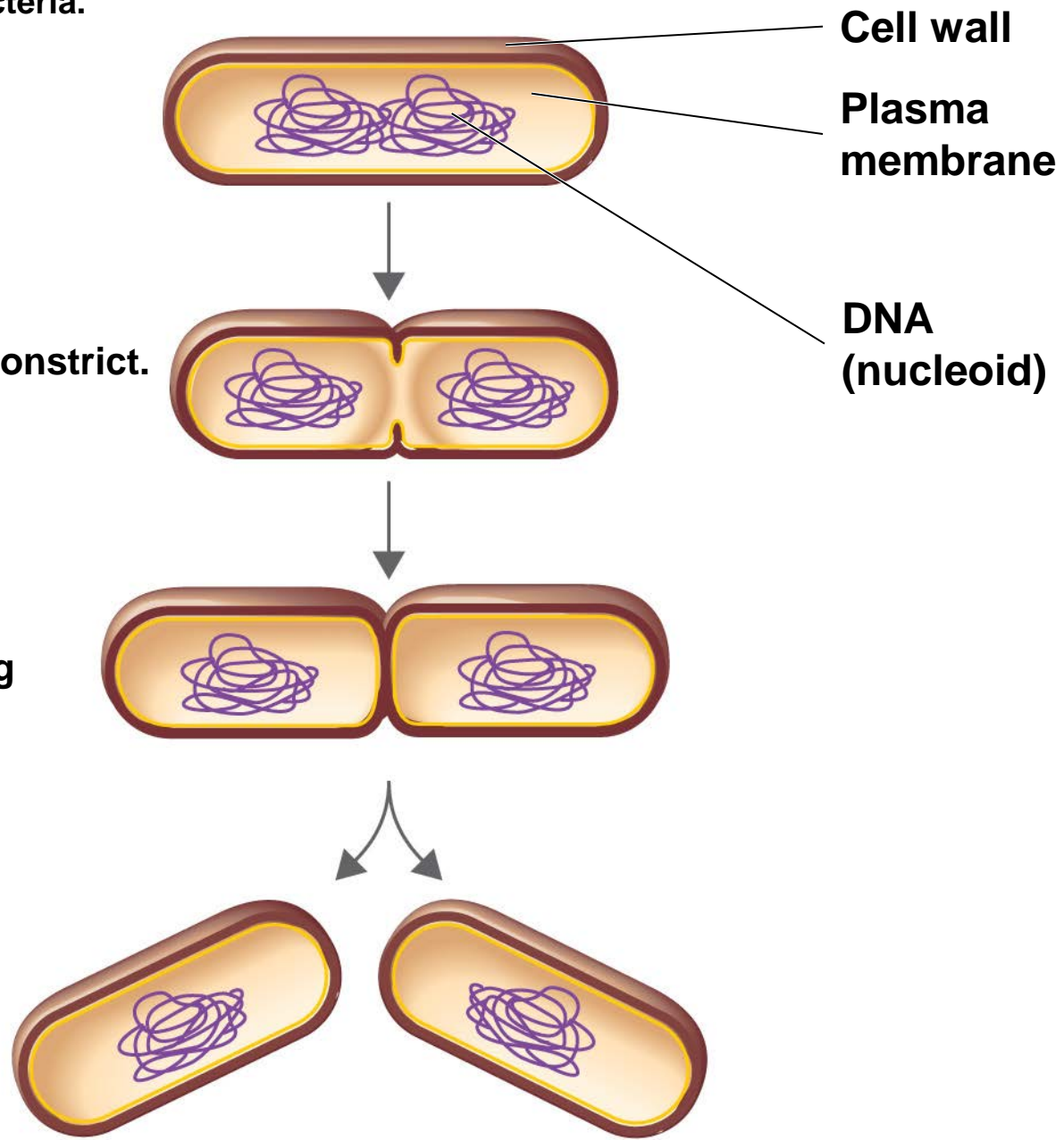
Figure 6.12a Binary fission in bacteria.

1 Cell elongates and DNA is replicated.

2 Cell wall and plasma membrane begin to constrict.

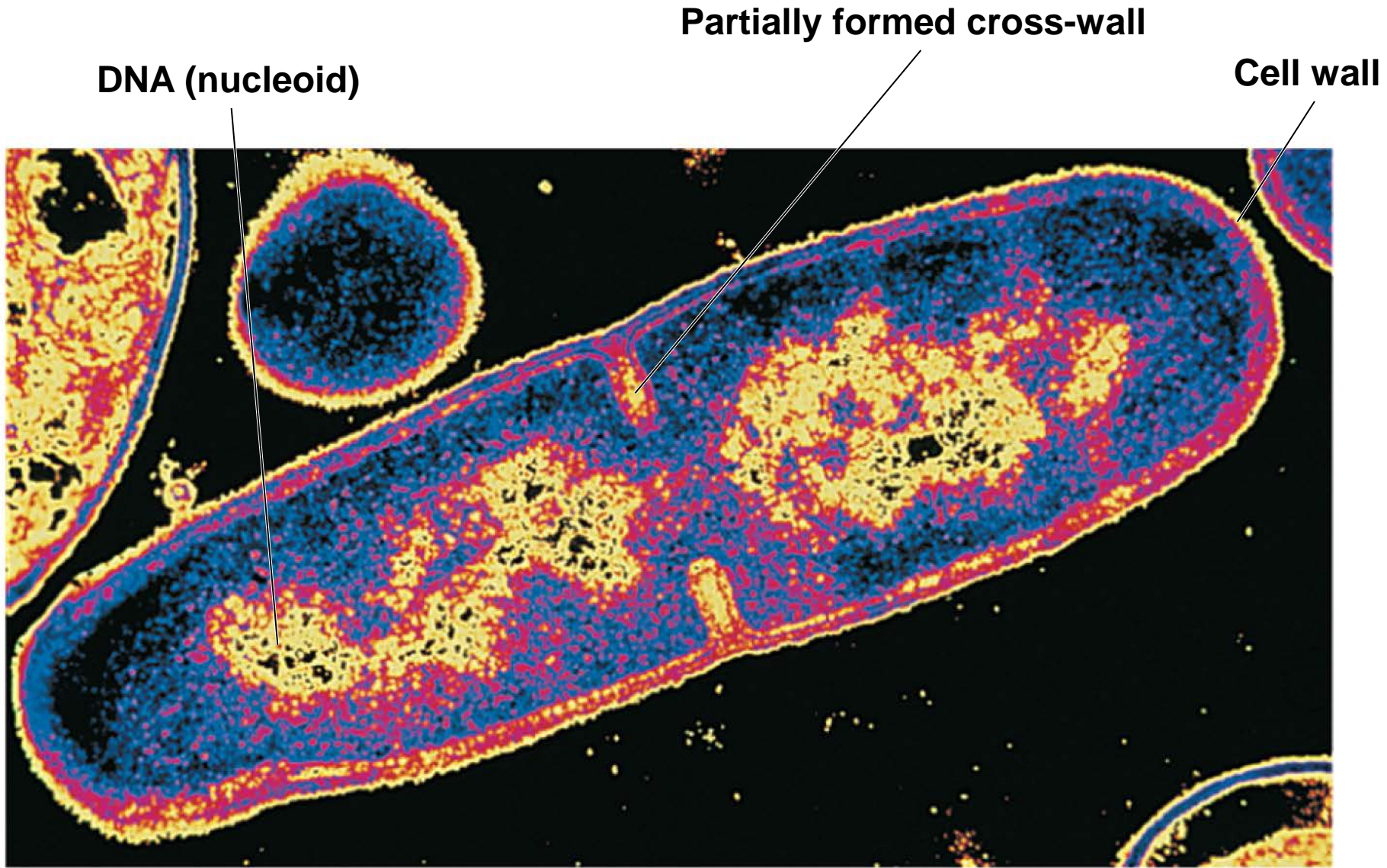
3 Cross-wall forms, completely separating the two DNA copies.

4 Cells separate.



(a) A diagram of the sequence of cell division

Figure 6.12b Binary fission in bacteria.

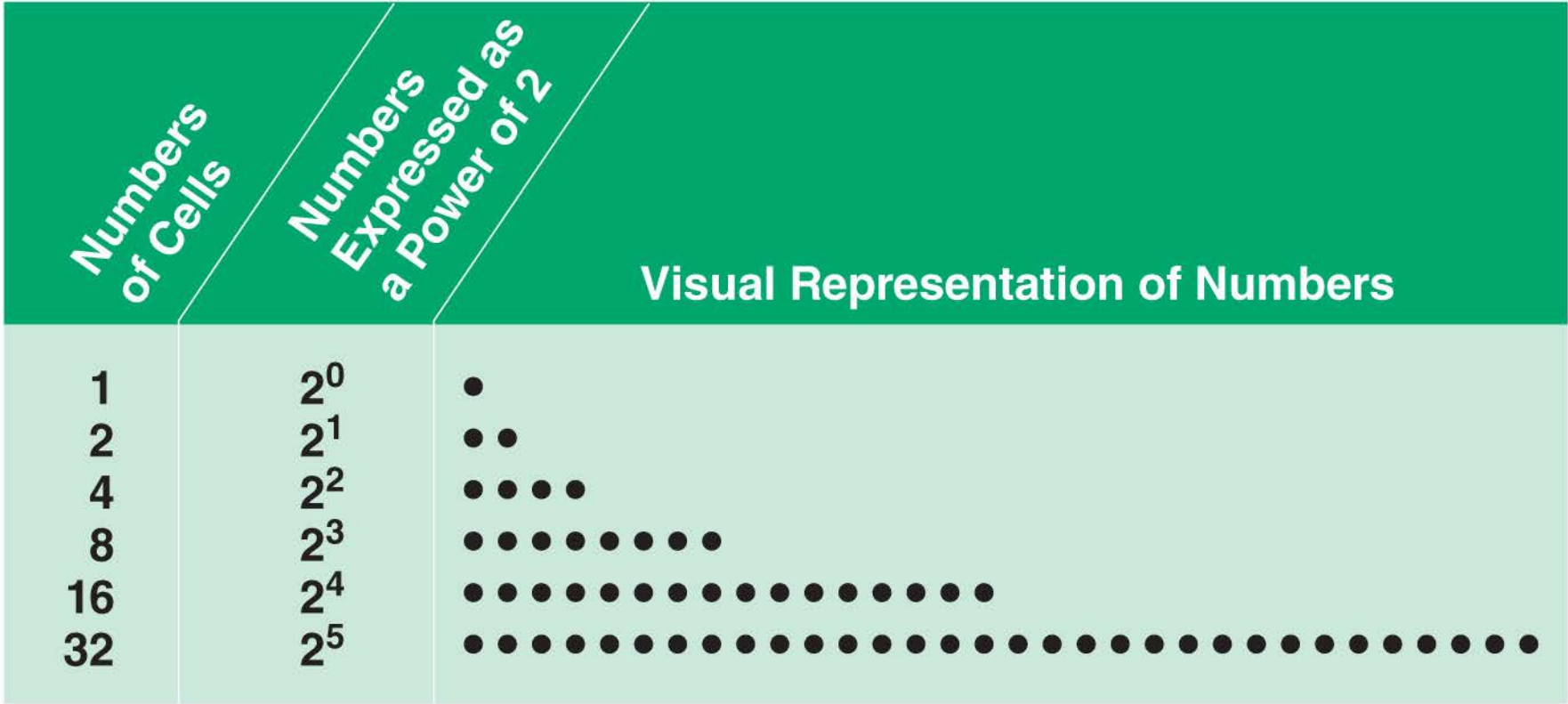


(b) A thin section of a cell of *Bacillus licheniformis* starting to divide

TEM

1.0  $\mu\text{m}$

Figure 6.13a Cell division.



(a)

Figure 6.13b Cell division.

Generation Number	Number of Cells	Log <sub>10</sub> of Number of Cells
0	2 <sup>0</sup> = 1	0
5	2 <sup>5</sup> = 32	1.51
10	2 <sup>10</sup> = 1,024	3.01
15	2 <sup>15</sup> = 32,768	4.52
16	2 <sup>16</sup> = 65,536	4.82
17	2 <sup>17</sup> = 131,072	5.12
18	2 <sup>18</sup> = 262,144	5.42
19	2 <sup>19</sup> = 524,288	5.72
20	2 <sup>20</sup> = 1,048,576	6.02

**(b)**

Figure 6.14 A growth curve for an exponentially increasing population, plotted logarithmically (dashed line) and arithmetically (solid line).

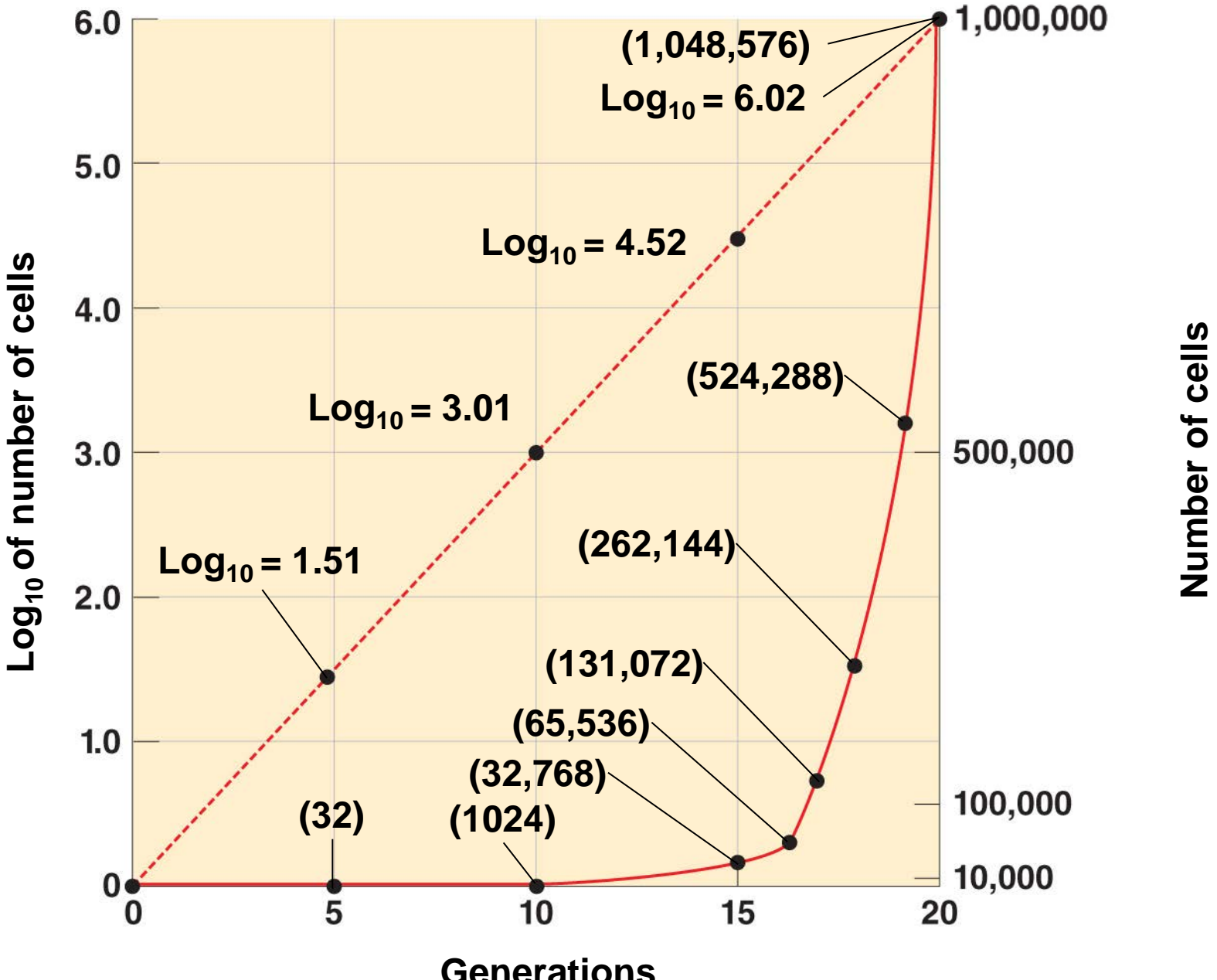
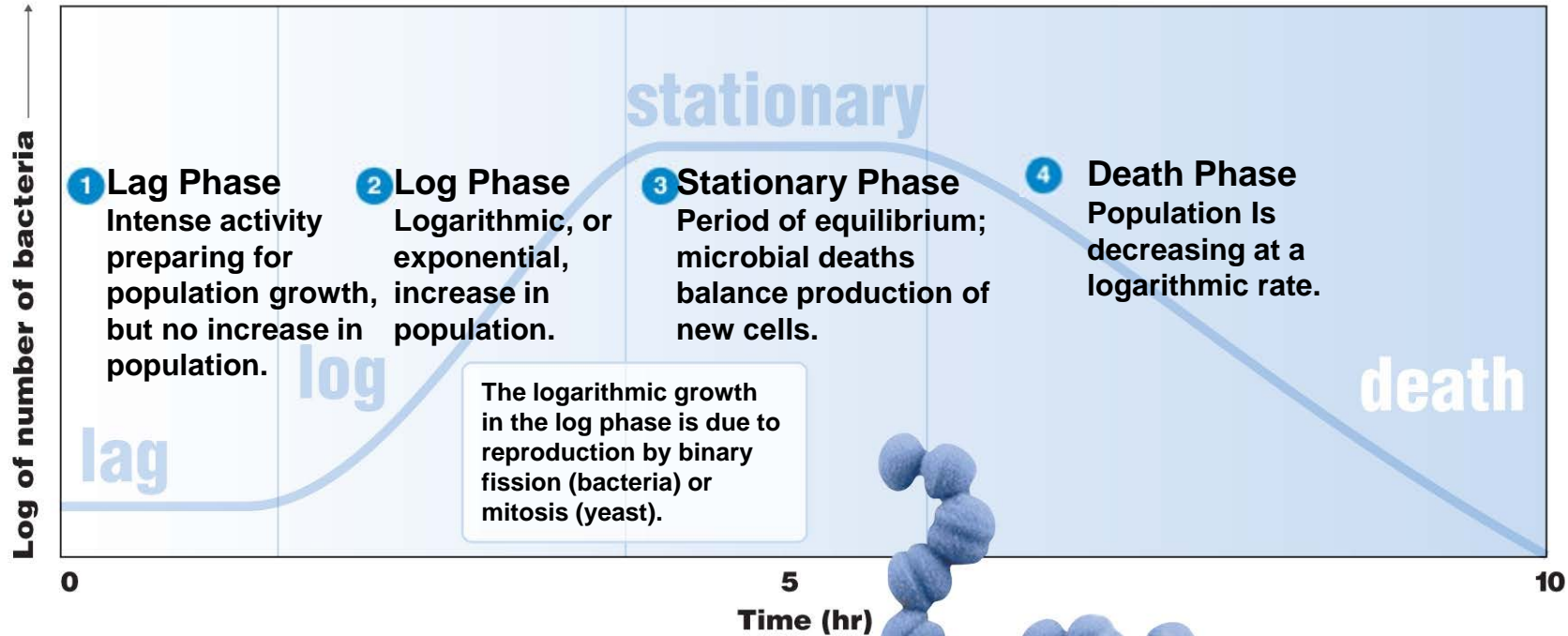


Figure 6.15 Understanding the Bacterial Growth Curve.



**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.

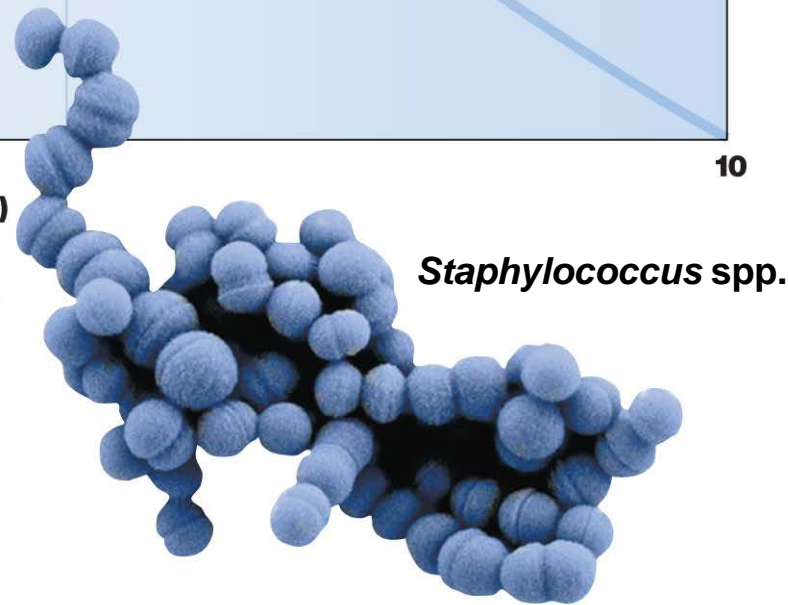
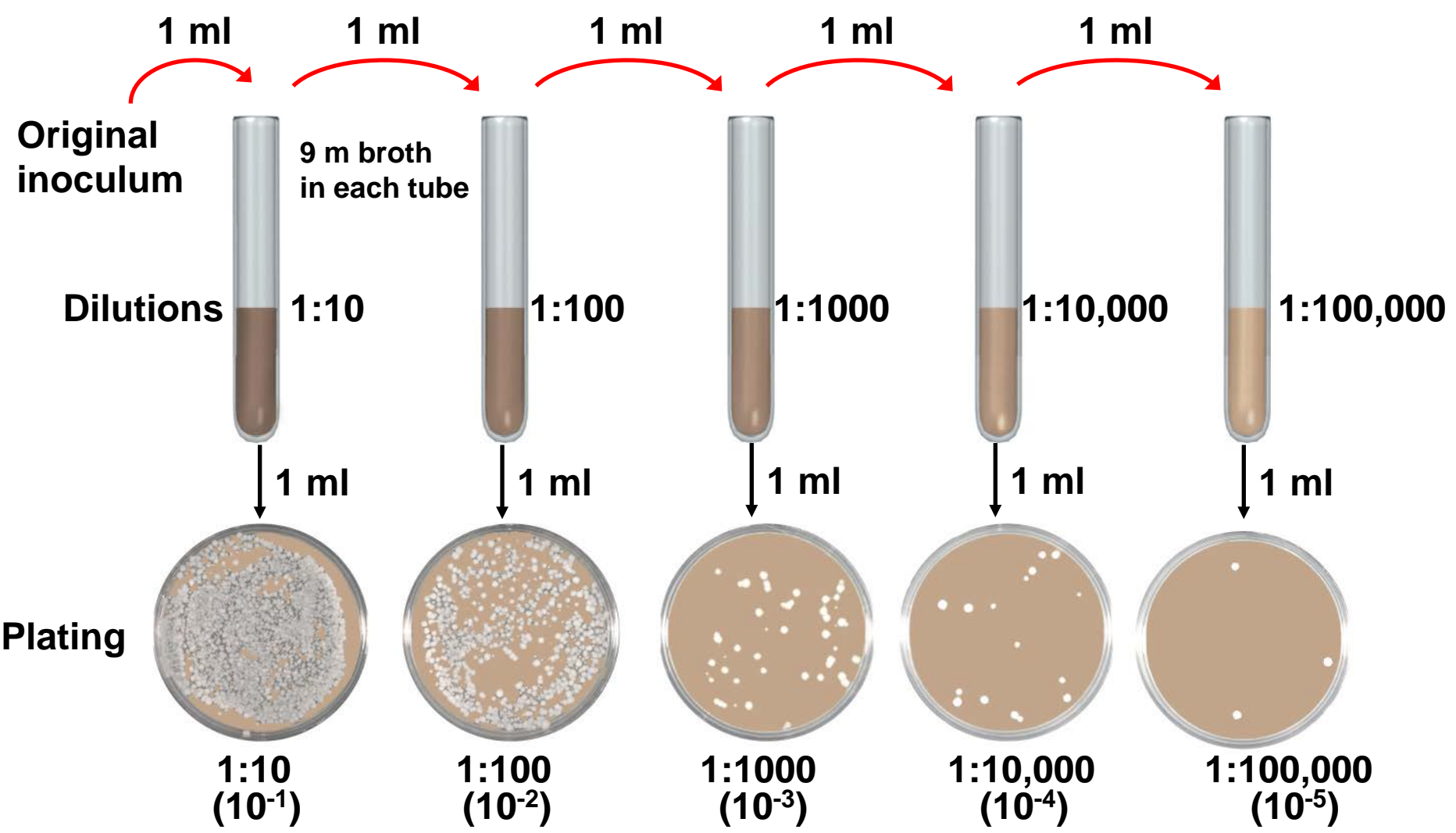




Figure 6.16 Serial dilutions and plate counts.



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.)

Figure 6.17 Methods of preparing plates for plate counts.

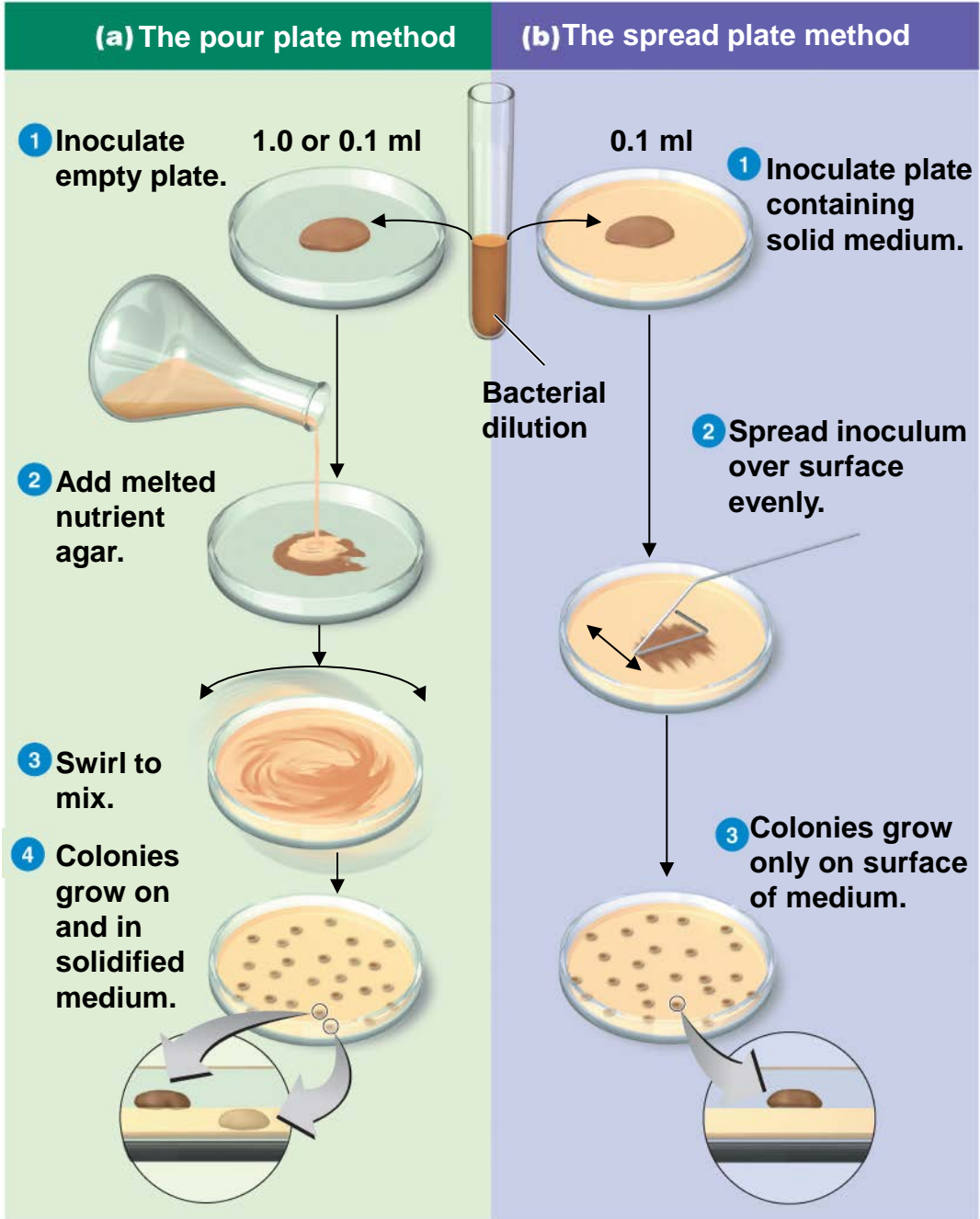
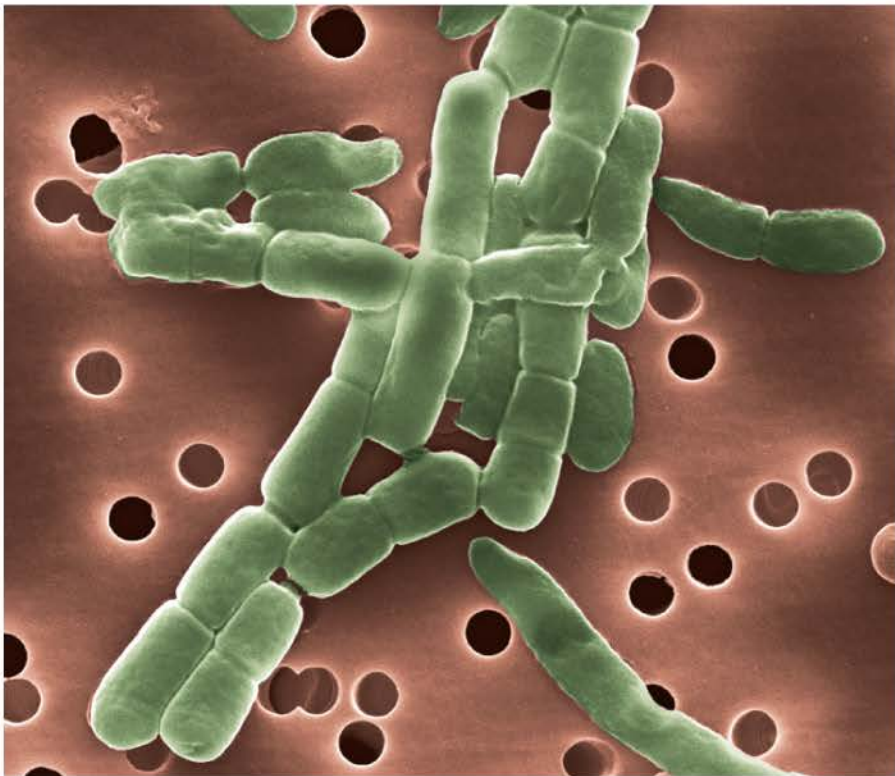
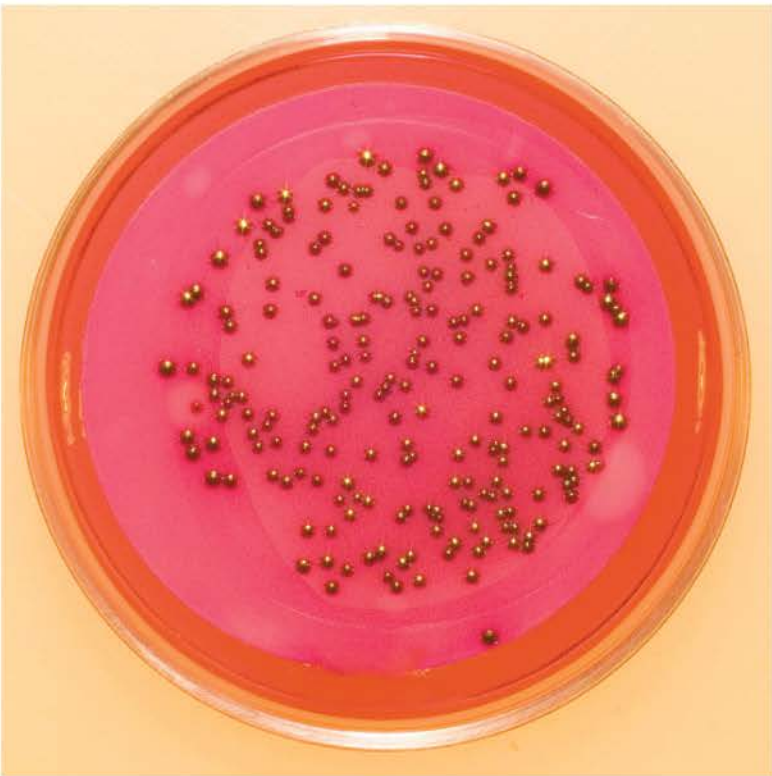


Figure 6.18 Counting bacteria by filtration.



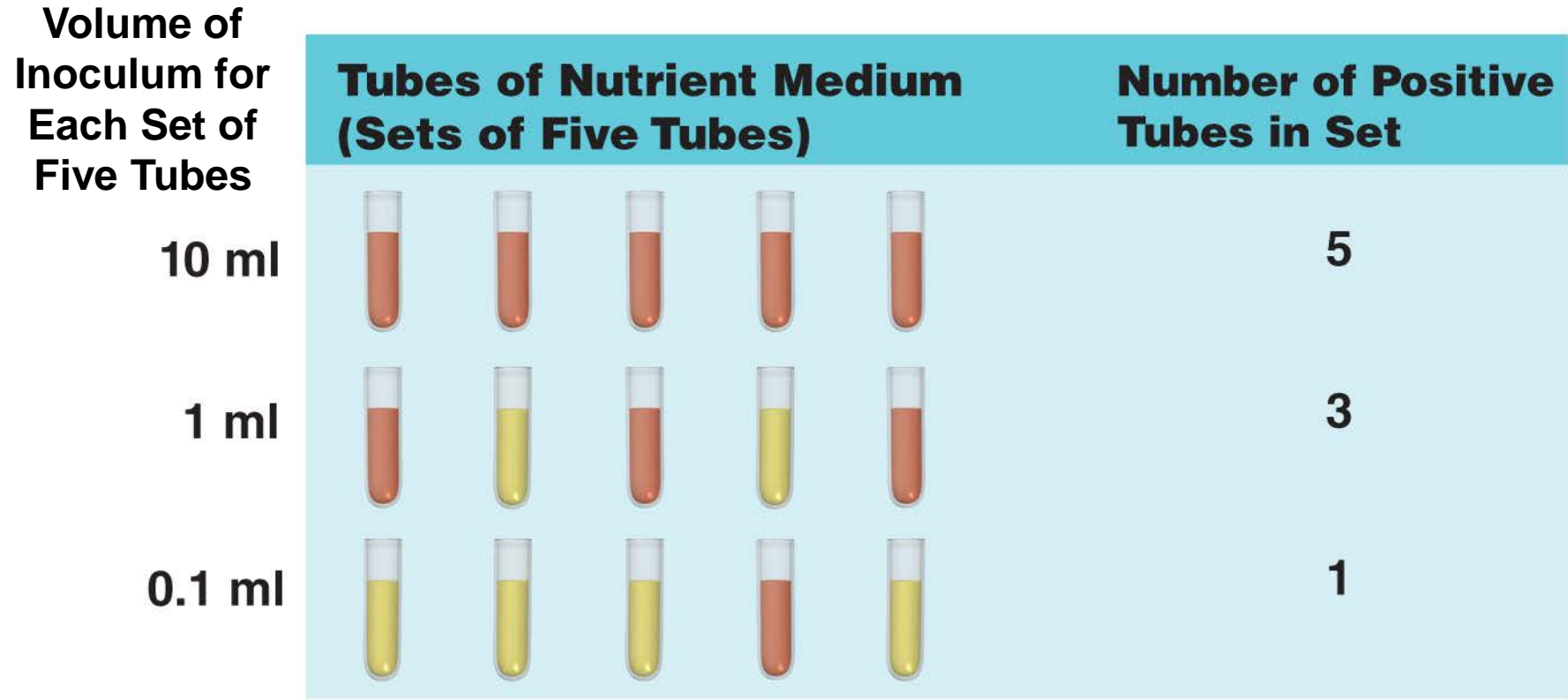
(a)

SEM 1.5  $\mu\text{m}$



(b)

Figure 6.19a The most probable number (MPN) method.



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

Figure 6.19b 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

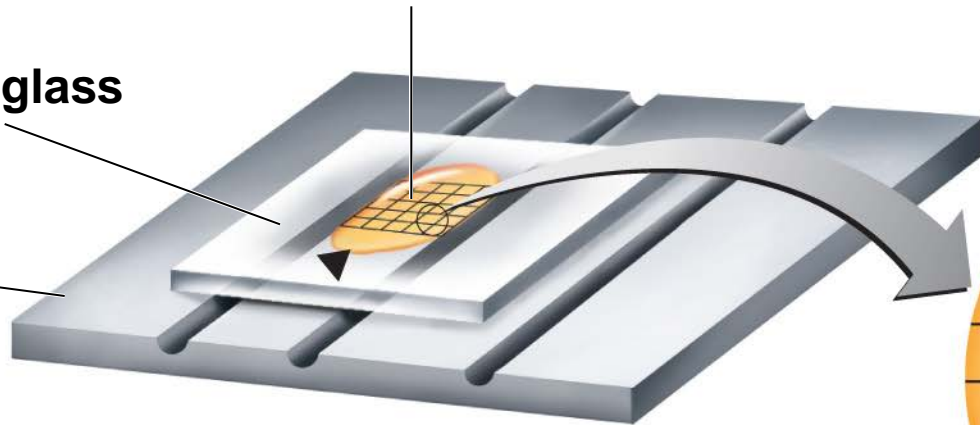
(b) MPN table.

Figure 6.20 Direct microscopic count of bacteria with a Petroff-Hausser cell counter.

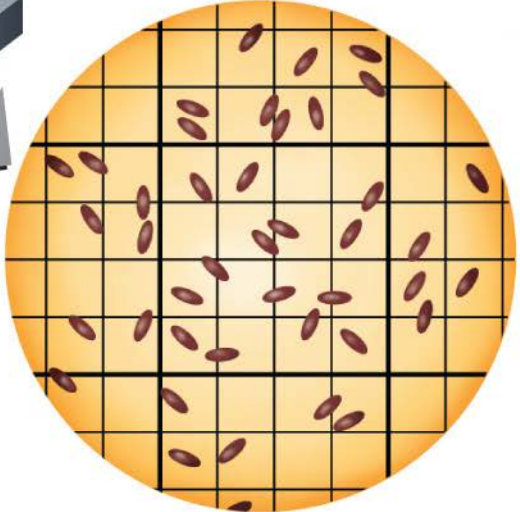
**Grid with 25 large squares**

**Cover glass**

**Slide**



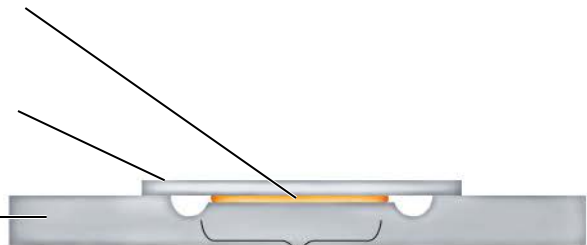
**1** Bacterial suspension is added here and fills the shallow volume over the squares by capillary action.



**Bacterial suspension**

**Cover glass**

**Slide**



**Location of squares**

**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  $\times$  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.

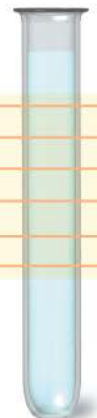
**4** The volume of fluid over the large square is  $1/1,250,000$  of a milliliter. If it contains 14 cells, as shown here, then there are  $14 \times 1,250,000 = 17,500,000$  cells in a milliliter.

Figure 6.21 Turbidity estimation of bacterial numbers.

**Light source**

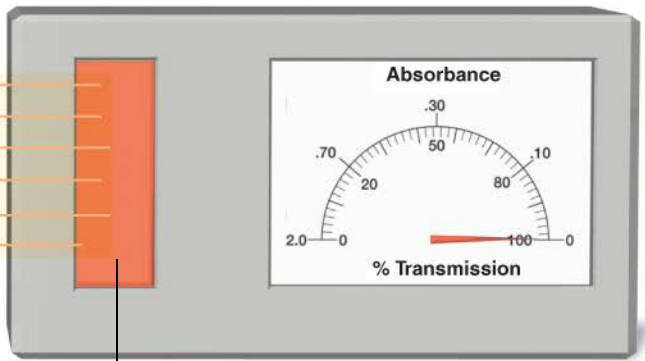


**Light**



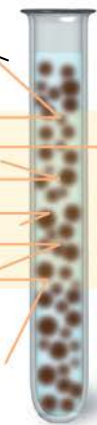
**Blank**

**Spectrophotometer**



**Light-sensitive detector**

**Scattered light that does not reach detector**



**Bacterial suspension**

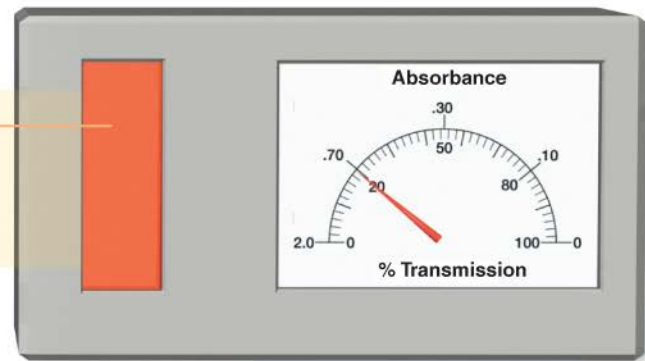
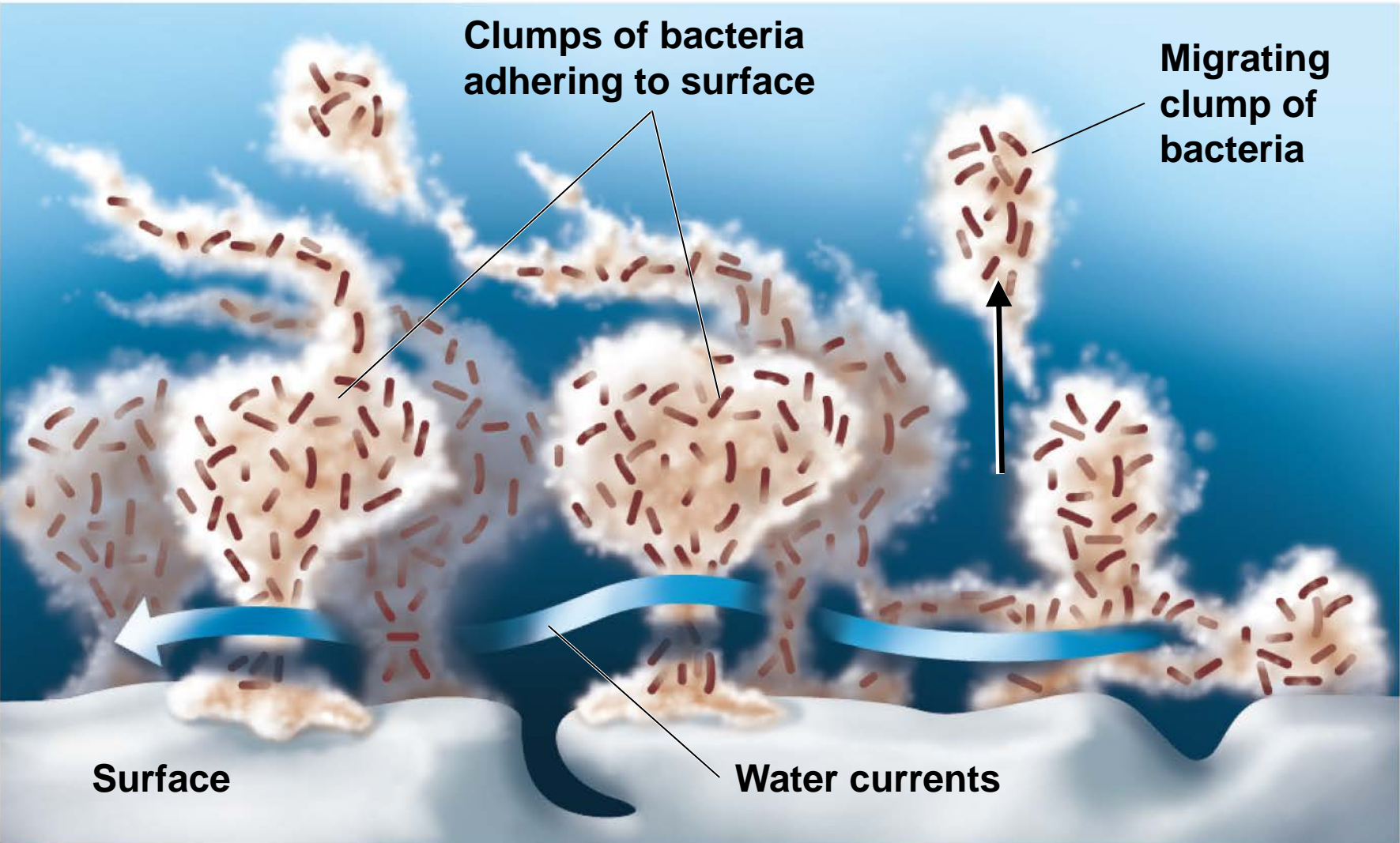


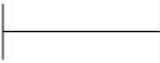
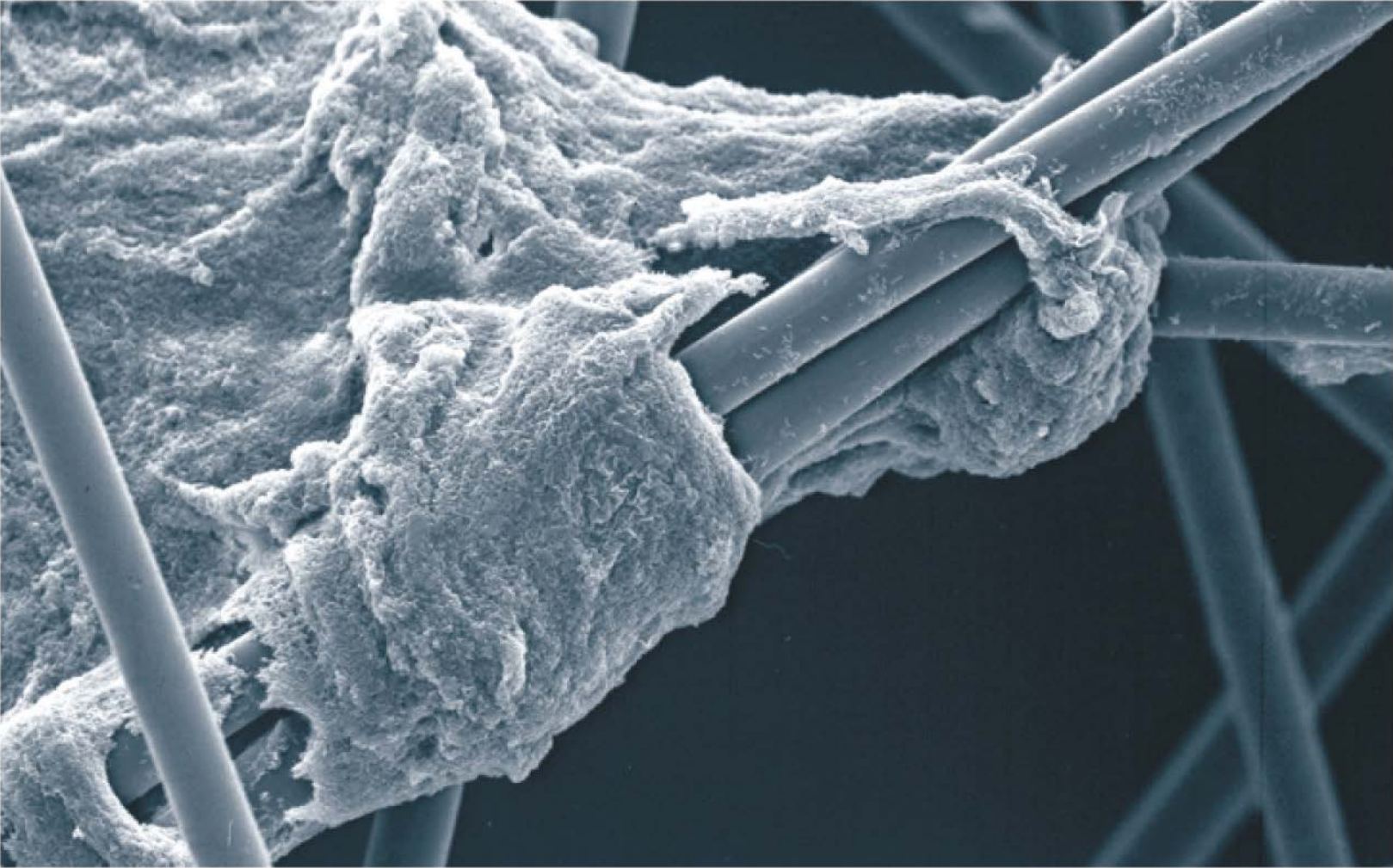
Figure 6.5 Biofilms.



10  $\mu\text{m}$



Applications of Microbiology 3.2 *Pseudomonas aeruginosa* biofilm.



5  $\mu\text{m}$

**SEM**