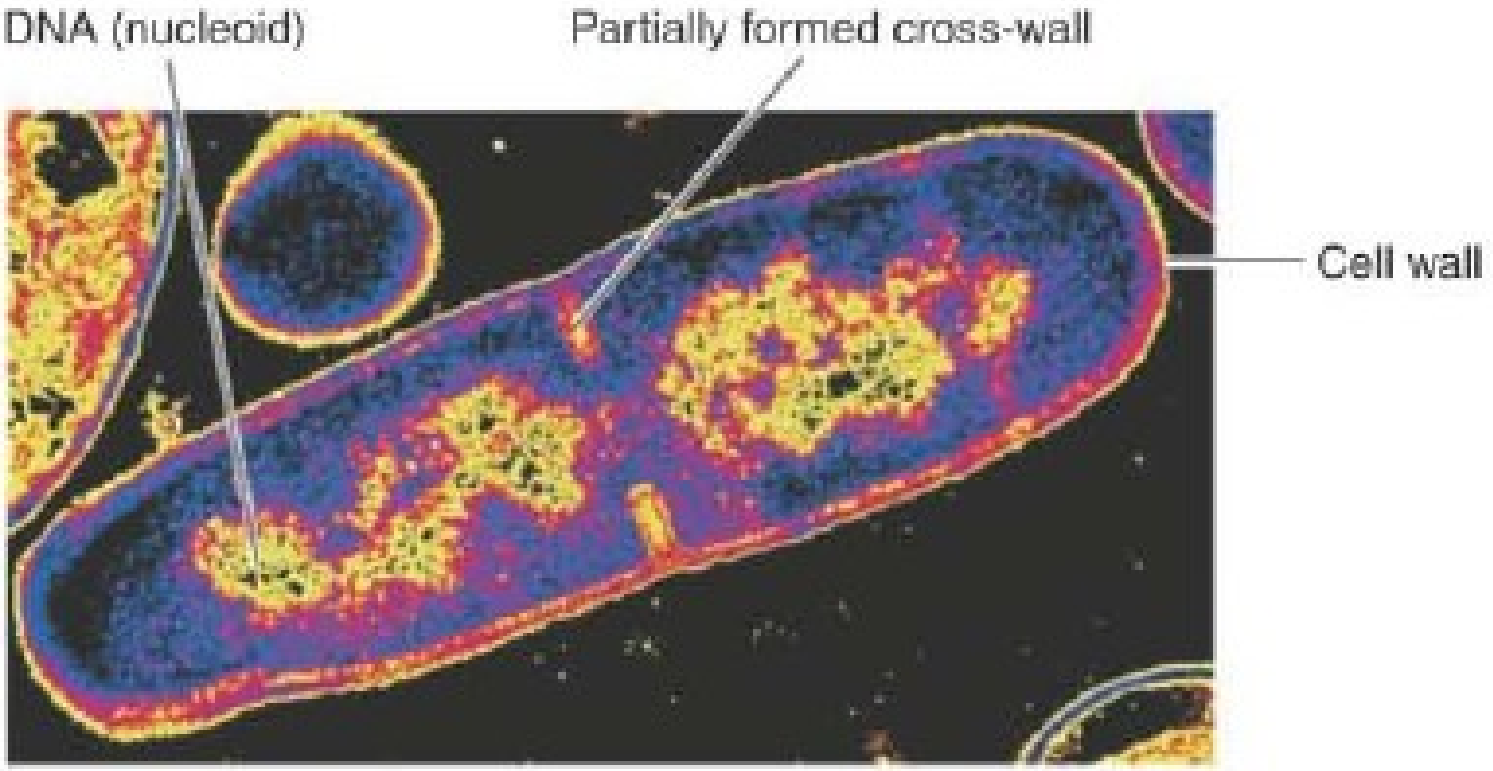
The Growth of Bacterial Cultures

Learning outcomes

1. Bacterial division
2. Generation time
3. Growth curve
4. Direct Measurement of Microbial Growth
5. Estimating Bacterial Numbers by Indirect Methods
6. Construct a growth curve for a batch culture

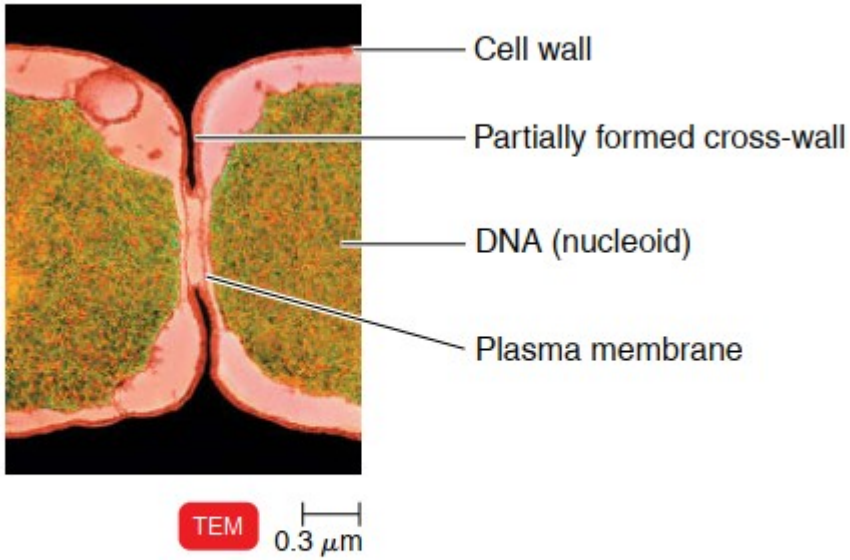
Bacterial division



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

Bacterial division

(a) A diagram of the sequence of cell division

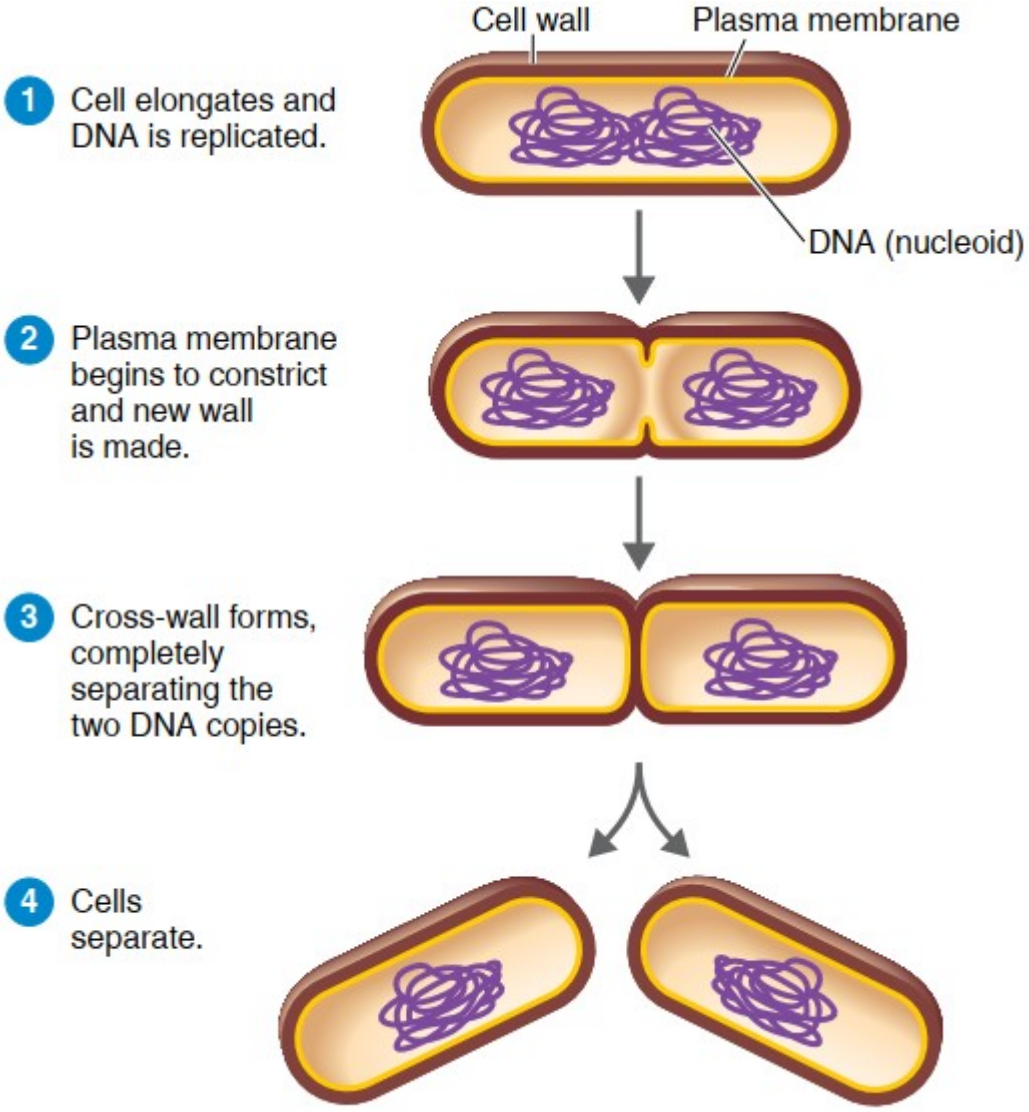


(b) A thin section of an *E.coli* cell starting to divide

Figure 6.12 Binary fission in bacteria.

Q In what way is budding different from binary fission?

Bacterial division



(a) A diagram of the sequence of cell division

Generation time

- Generation time is the time required for a cell to divide (and its population to double).

- The generation time varies considerably among organisms and with environmental conditions, such as temperature and/or culture conditions.

- Most bacteria have a generation time of 1 to 3 hours; others require more than 24 hours per generation.

Generation time

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	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

Generation time

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

Calculation of generation time

t_o: time at which the number of cells is N_o

t: time at which the number of cells is N_t

n: number of cell generations in the $(t-t_o)$ time interval

$$N_t = N_o . 2^n$$

$$\log N_t = \log N_o + n.\log 2$$

$$n = (\log N_t - \log N_o)/\log 2$$

$$g = (t-t_o)/n$$

$$g = [\log 2 * (t - t_o)]/(\log N_t - \log N_o)$$

Calculation of generation time

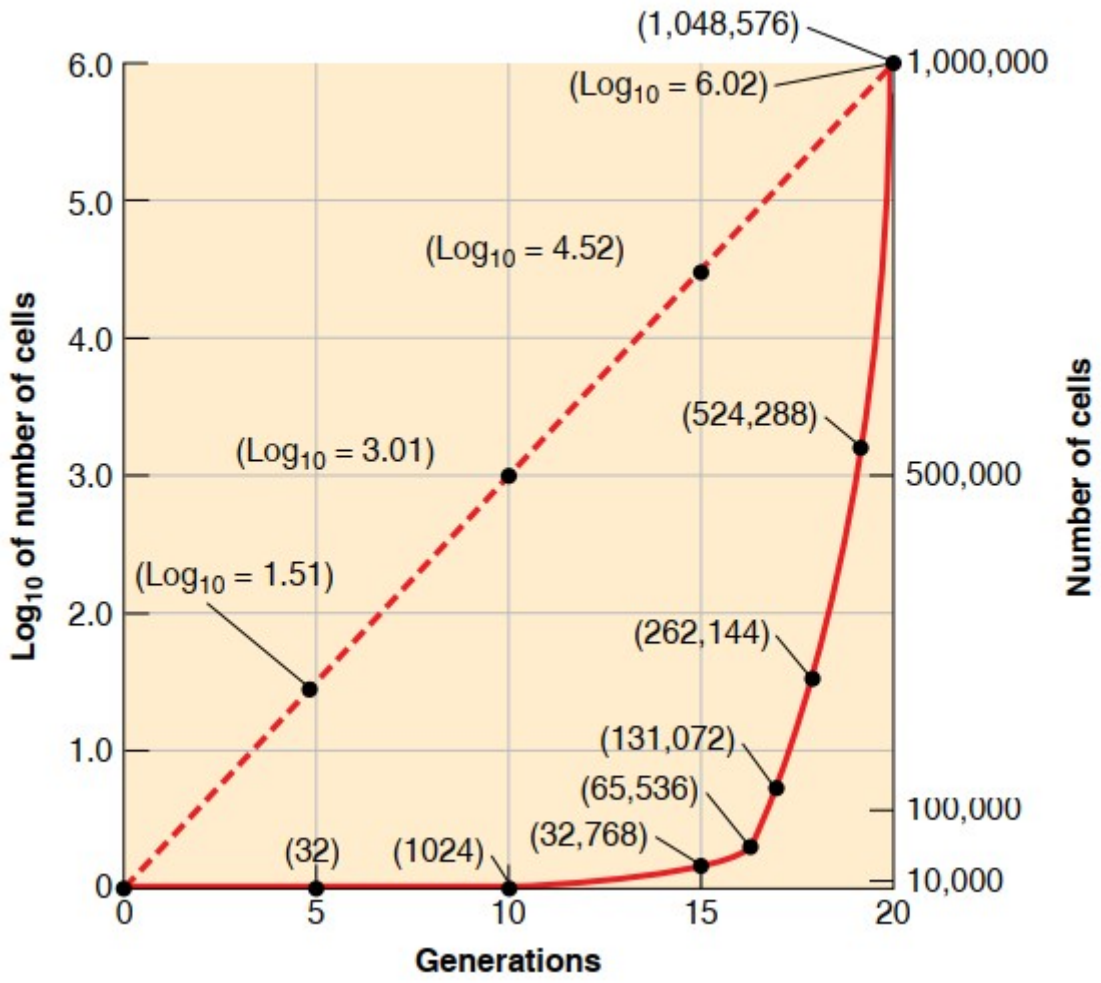
Example: A fermenter with bacteria in the growth phase contains 10^4 cells/ml at 6h and 10^8 cells/ml at 10h. Calculate the generation time.

$$g = [\log 2 * (t - t_o)]/(\log N_t - \log N_o)$$

$$g = 0{,}301 * (10-6)/(8-4)$$

$$g = 0{,}301 \text{ (h)}$$

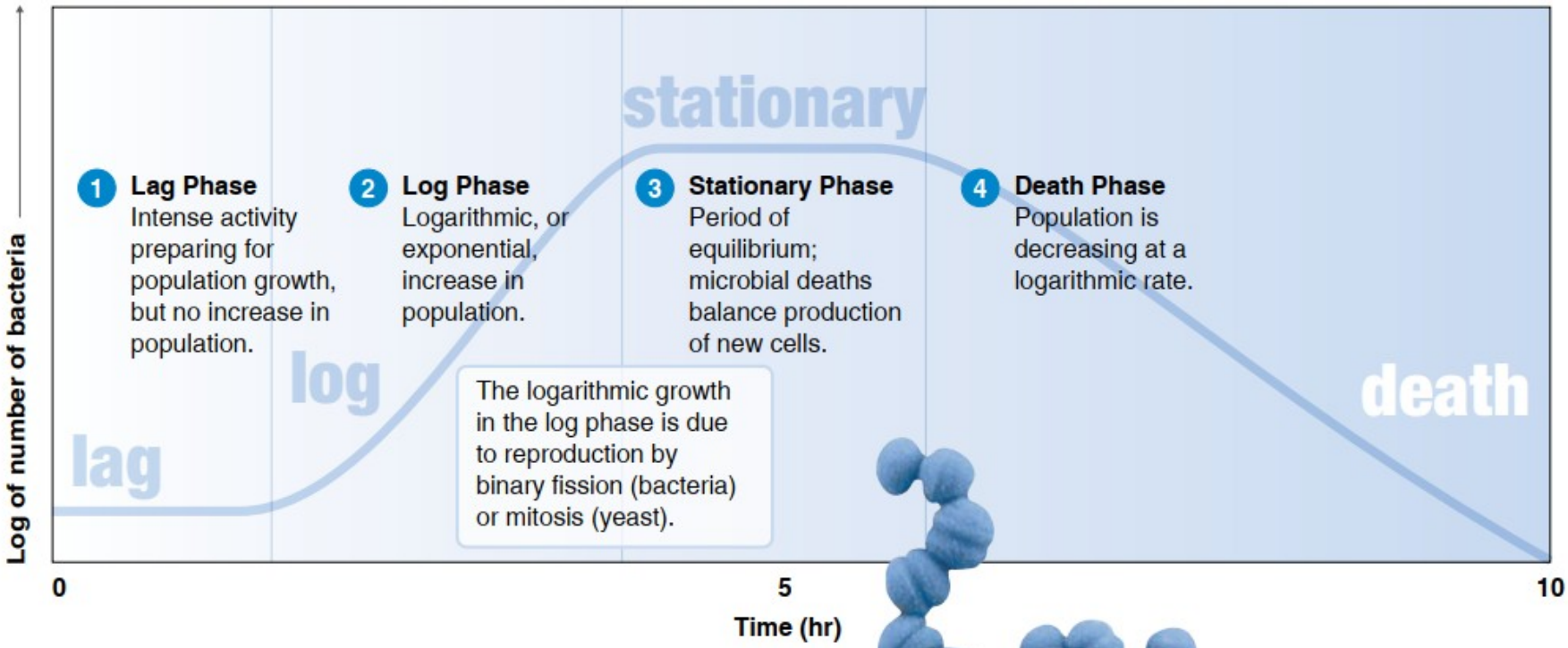
Growth curve

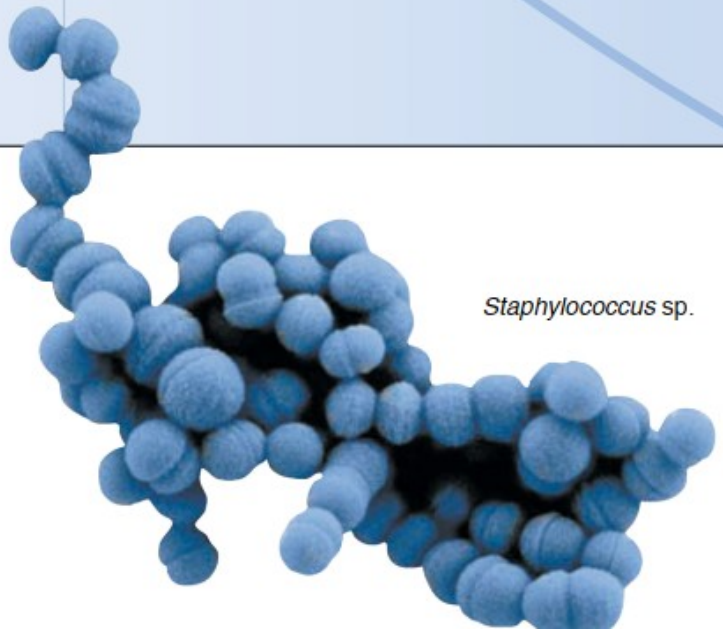


Growth curve

(The math required to calculate generation times is presented in Appendix B.) If binary fission continues unchecked, an enormous number of cells will be produced. If a doubling occurred every 20 minutes—which is the case for *E. coli* under favorable conditions—after 20 generations a single initial cell would increase to over 1 million cells. This would require a little less than 7 hours. In 30 generations, or 10 hours, the population would be 1 billion, and in 24 hours it would be a number trailed by 21 zeros. It is difficult to graph population changes of such enormous magnitude by using arithmetic numbers. This is why logarithmic scales are generally used to graph bacterial growth. Understanding logarithmic representations of bacterial populations requires some use of mathematics and is necessary for anyone studying microbiology. (See Appendix B.)

Growth curve & phases of growth





Staphylococcus sp.

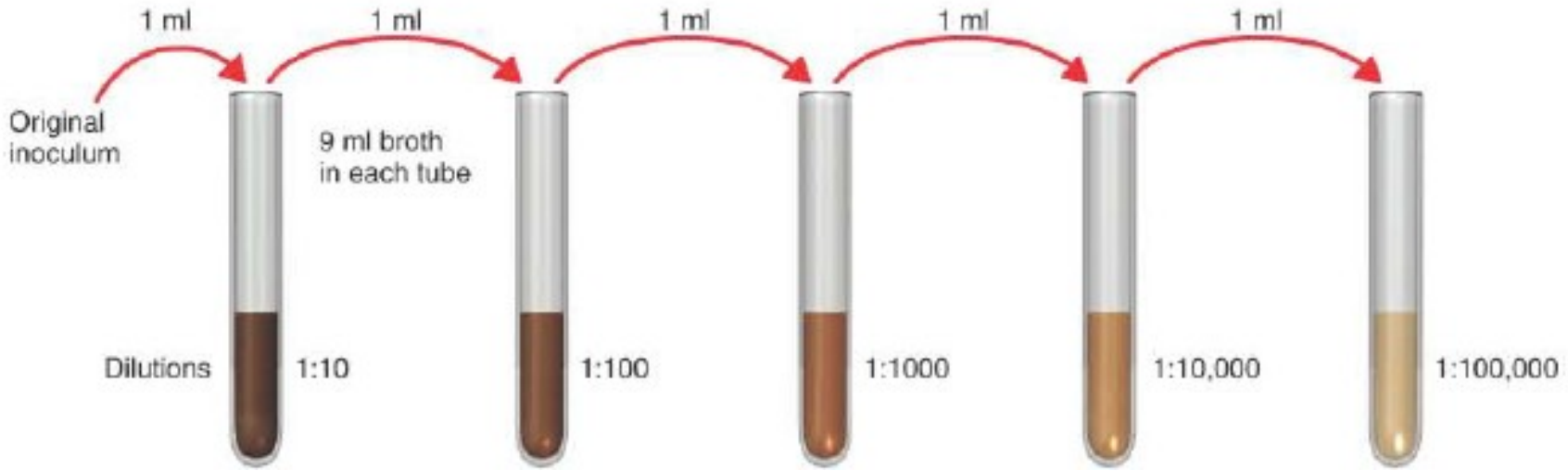
KEY CONCEPTS

- 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, and as well as in industrial microbiology processes, such as ethanol production.

Direct Measurement of Microbial Growth

Direct Microscopic Count

- Calculated on the basis of the number of cells counted in a (defined) volume of cell-containing fluid.
- The cell fluid must be diluted to an appropriate concentration to be counted.

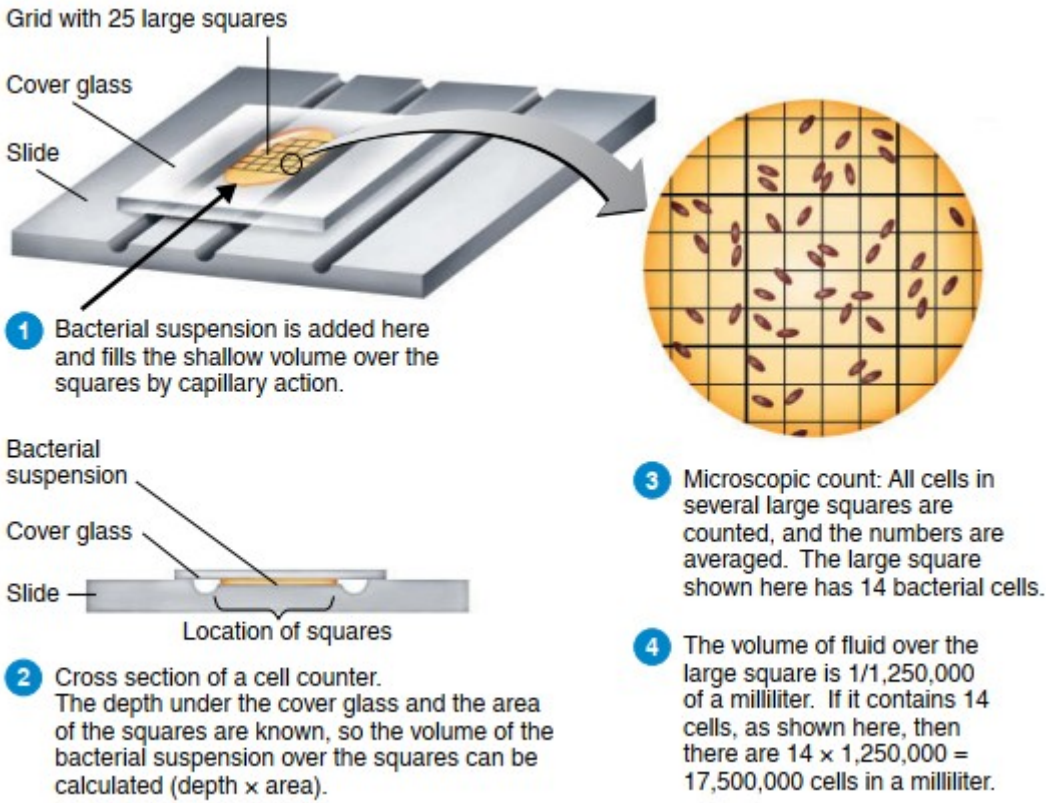


Direct Measurement of Microbial Growth

Direct Microscopic Count

Petroff-Hausser counting chamber

There are 25 large cells x 16 small cells = 400 small cells; Total area = 1 mm2; height = 0.02 mm.

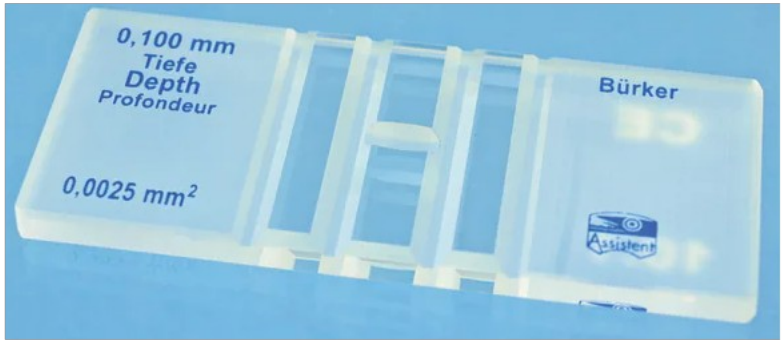


Direct Measurement of Microbial Growth

Direct Microscopic Count

Counting chambers

Hemocytometer



h_c = 0.1 mm

$V_c = 10^{-4}$ mL

Petroff hausser chamber

h_c = 0.02 mm

$V_c = 2 \times 10^{-5}$ mL

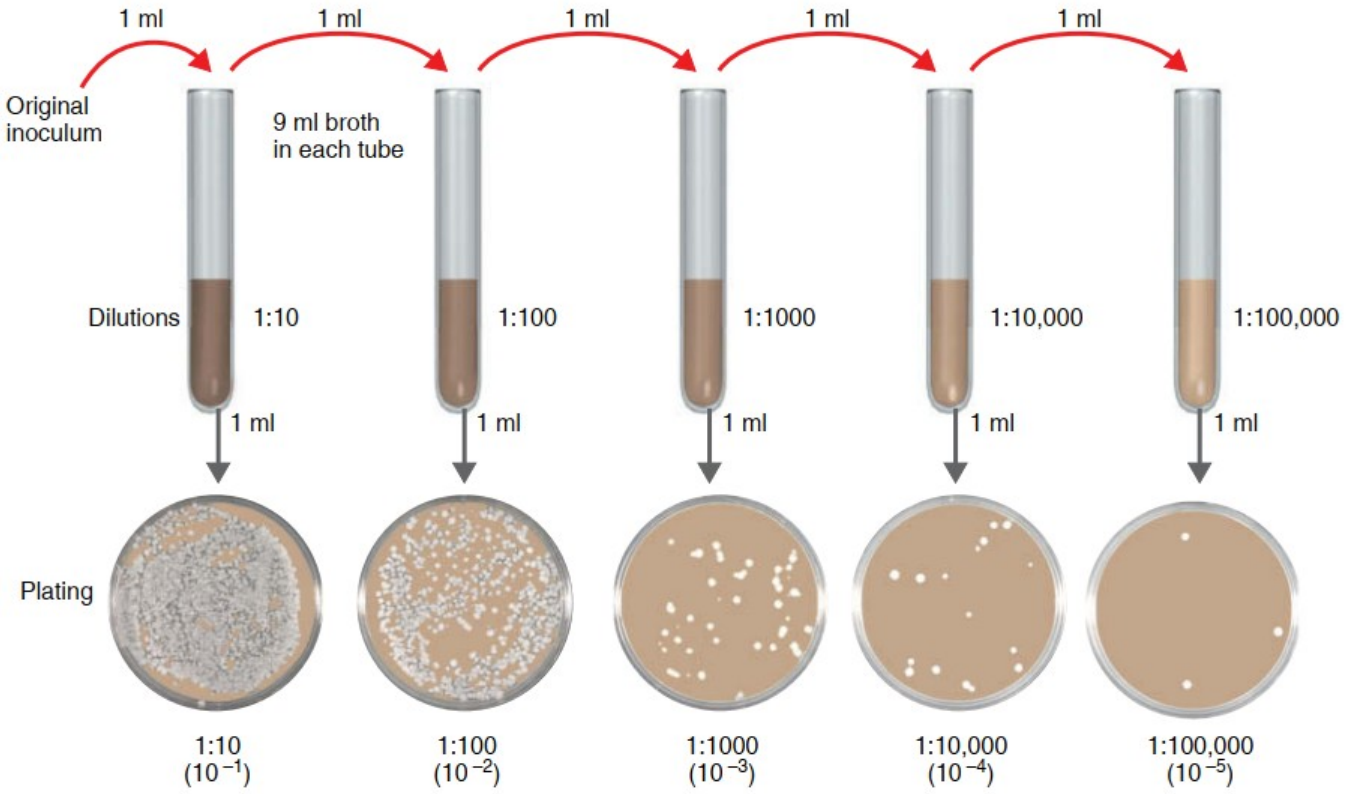
Direct Measurement of Microbial Growth

Plate count (this method can be used for both bacteria and yeast)

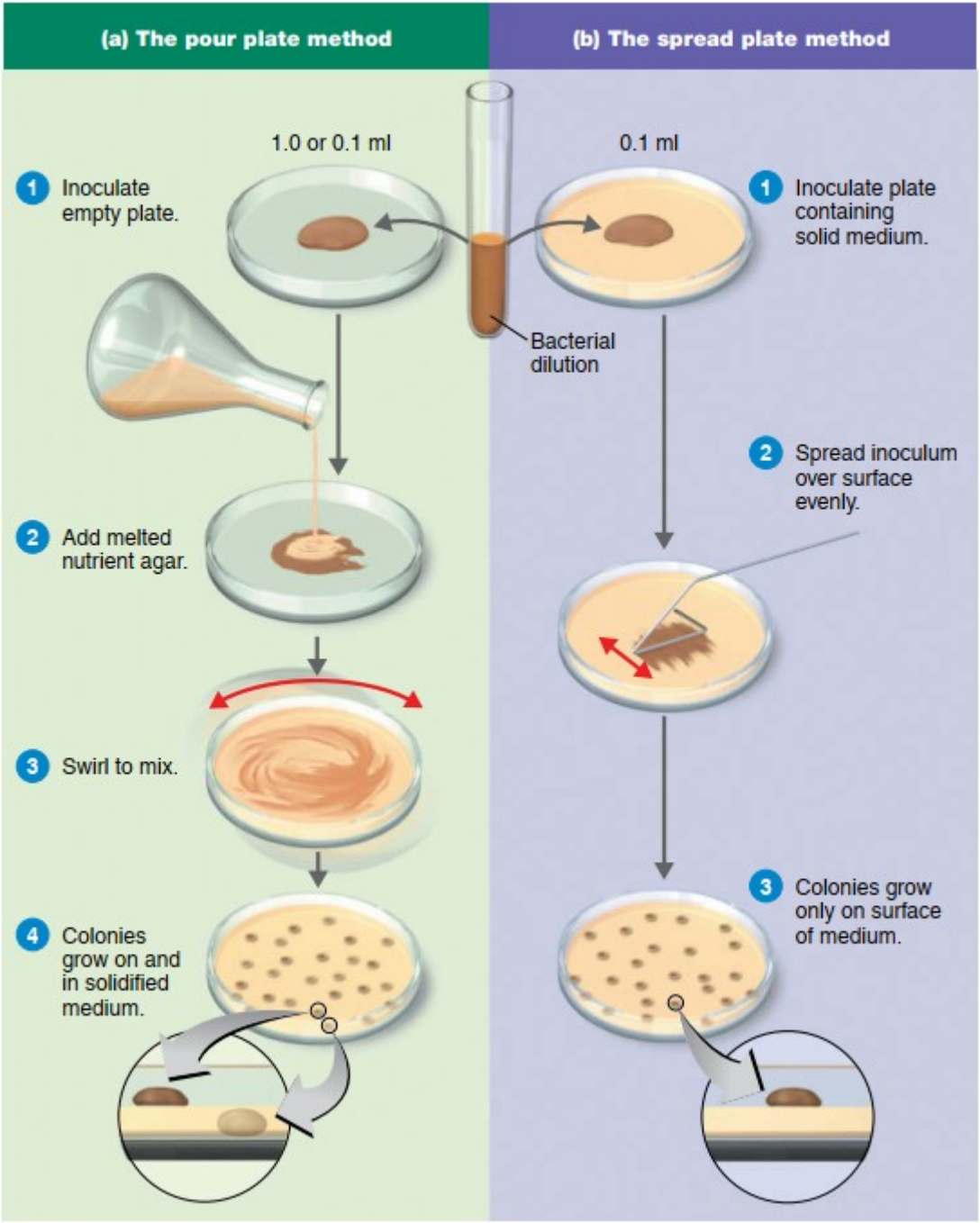
- ✓ **Method principle:** a living cell will grow and divide through many generations to form a colony (CFU - Colony Forming Unit).
- ✓ Count the number of colonies on the agar plate -> the number of living cells in a volume of cell-containing liquid.
- ✓ It is necessary to dilute the cell solution to an appropriate concentration to be able to count.

Direct Measurement of Microbial Growth

Serial dilutions and plate counts

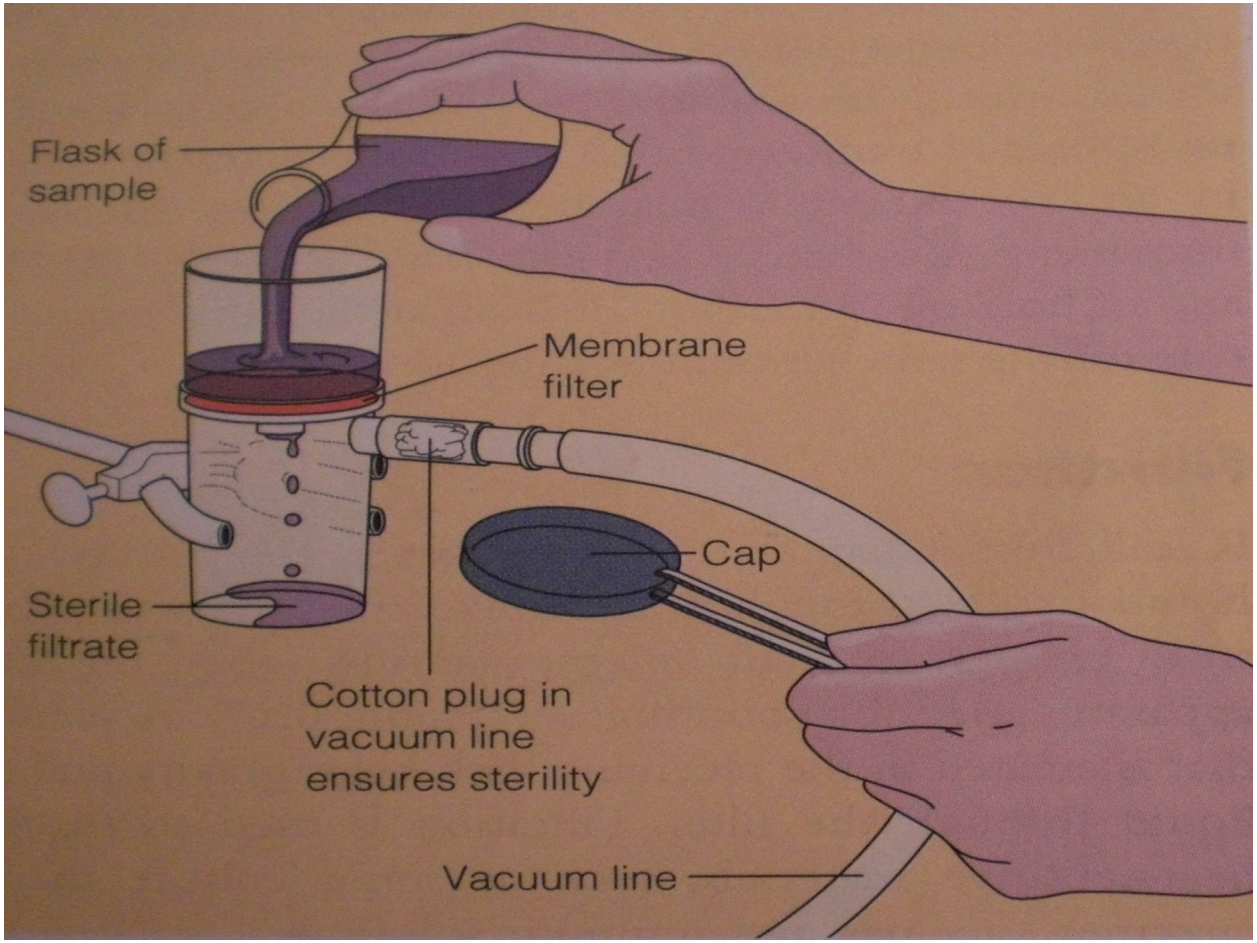


Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 54 colonies are on a plate of 1:1000 dilution, then the count is 54 × 1000 = 54,000 bacteria/ml in sample.)


(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate.
1 Inoculate plate containing solid medium.
Bacterial dilution
2 Add melted nutrient agar.
2 Spread inoculum over surface evenly.
3 Swirl to mix.
3 Colonies grow only on surface of medium.
4 Colonies grow on and in solidified medium.

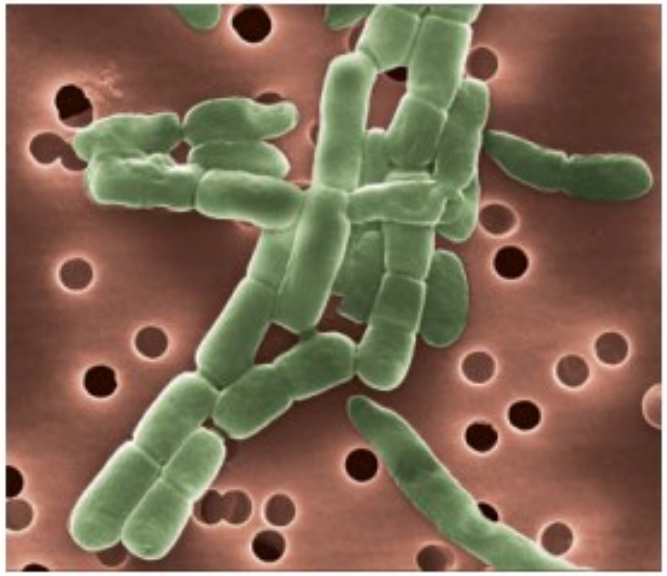
Direct Measurement of Microbial Growth

Filtration

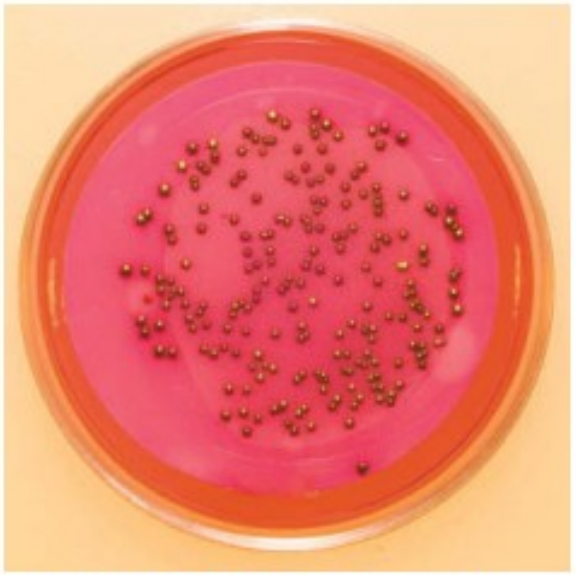


Direct Measurement of Microbial Growth

Filtration



(a) The bacterial populations in bodies of water can be determined by passing a sample through a membrane filter. Here, the bacteria in a 100-ml water sample have been sieved out onto the surface of a membrane filter. These bacteria form visible colonies when placed on the surface of a suitable medium.



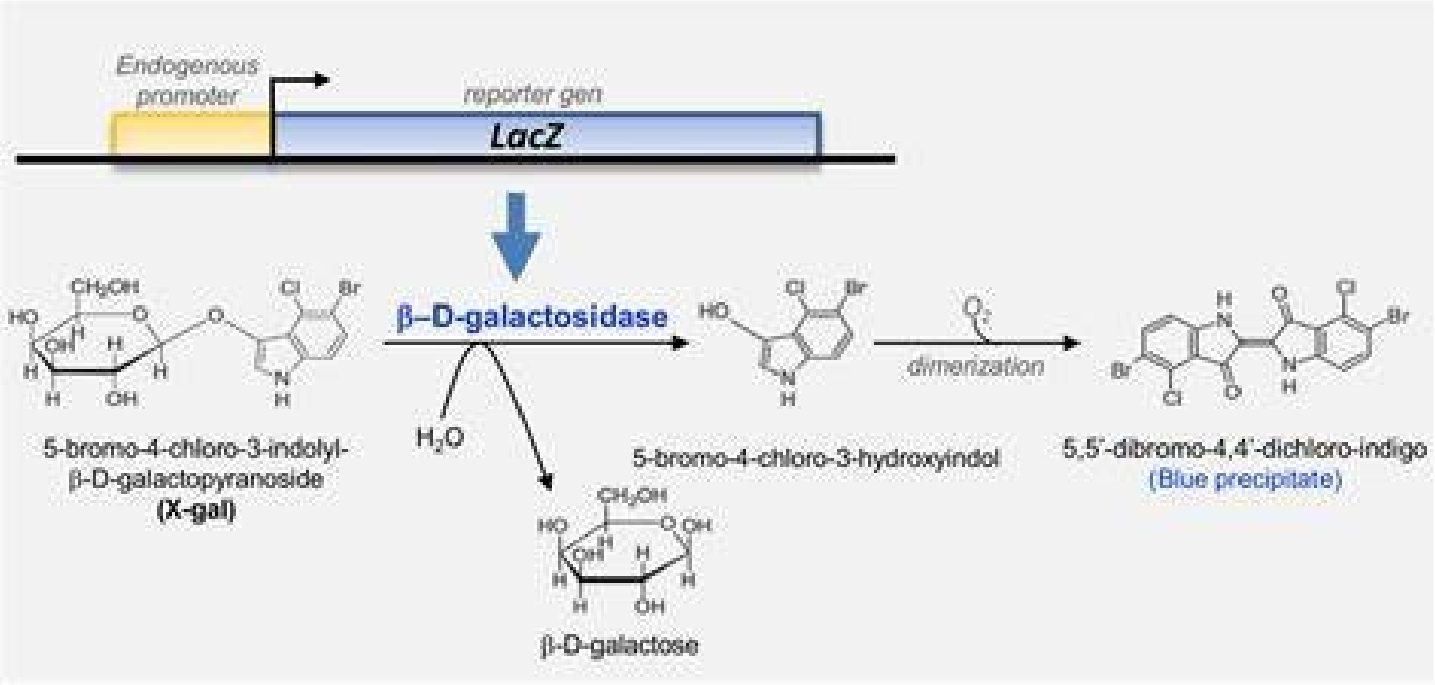
(b) A membrane filter with bacteria on its surface, as described in (a), has been placed on Endo agar. This medium is selective for gram-negative bacteria; lactose fermenters, such as the coliforms, form distinctive colonies. There are 214 colonies visible, so we would record 214 bacteria per 100 ml in the water sample.

Direct Measurement of Microbial Growth

The Most Probable Number (MPN) Method

Method principle: the greater the number of bacteria in a sample, the more dilution is needed to reduce the density to the point at which no bacteria are left to grow in the tubes in a dilution series.

Signs of growth?



Direct Measurement of Microbial Growth

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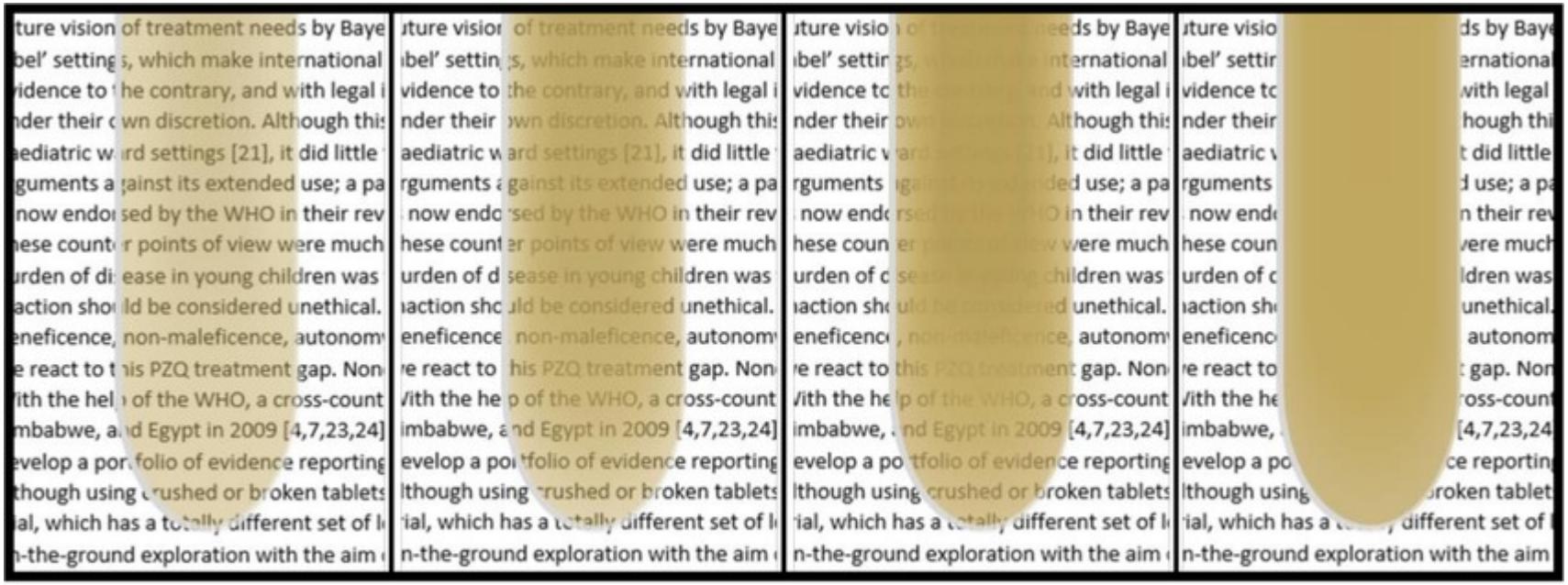

Clear	Slightly Cloudy	Cloudy	Turbid
-	+	++	+++

Direct Measurement of Microbial Growth

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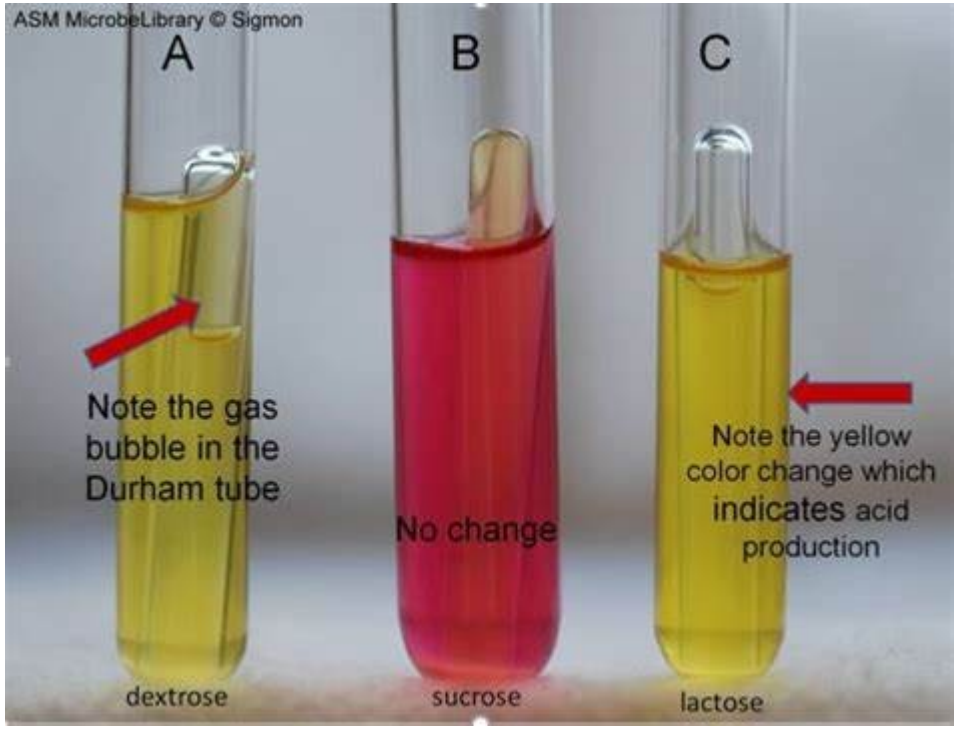
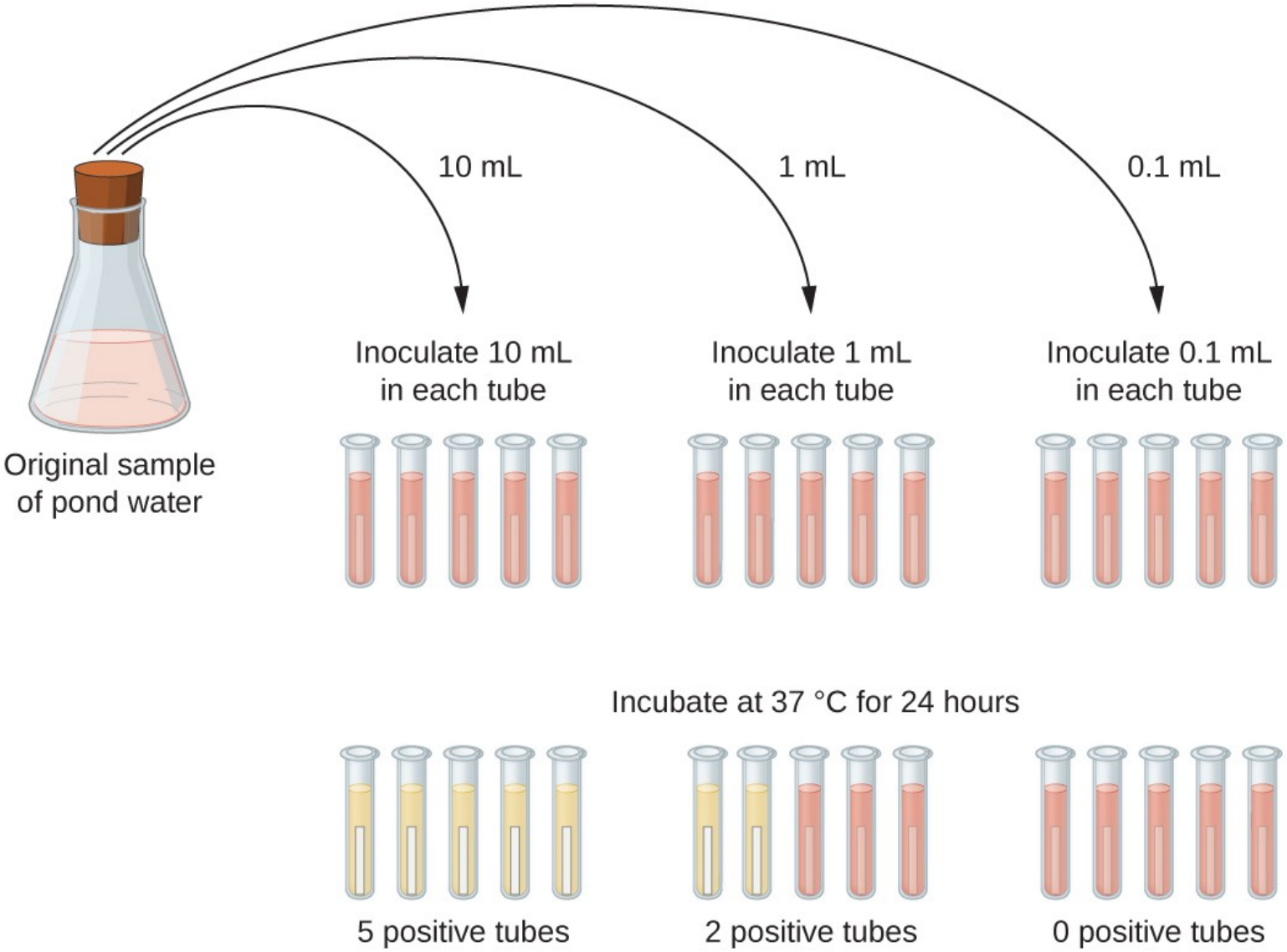


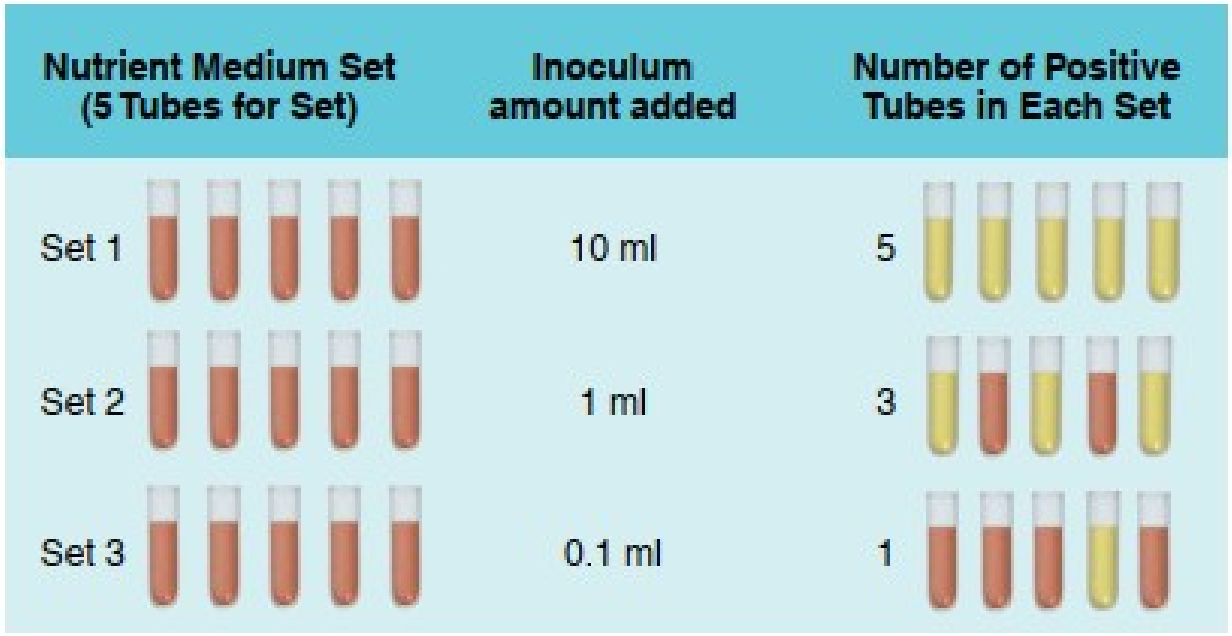
Direct Measurement of Microbial Growth

The Most Probable Number (MPN) Method



Direct Measurement of Microbial Growth

The Most Probable Number (MPN) Method



(a) Most probable number (MPN) dilution series

Direct Measurement of Microbial Growth

The Most Probable Number (MPN) Method

Combination of Positives	MPN Index/ 100 ml	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		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. MPN tables enable us to calculate for a sample the microbial numbers that are statistically likely to lead to such a result. The number of positive (yellow) tubes is recorded for each set: in the shaded example, 5, 3, and 1. If we look up this combination in an MPN table, we find that the MPN index per 100 ml is 110. Statistically, this means that 95% of the water samples that give this result contain 34–250 bacteria, with 110 being the most probable number.

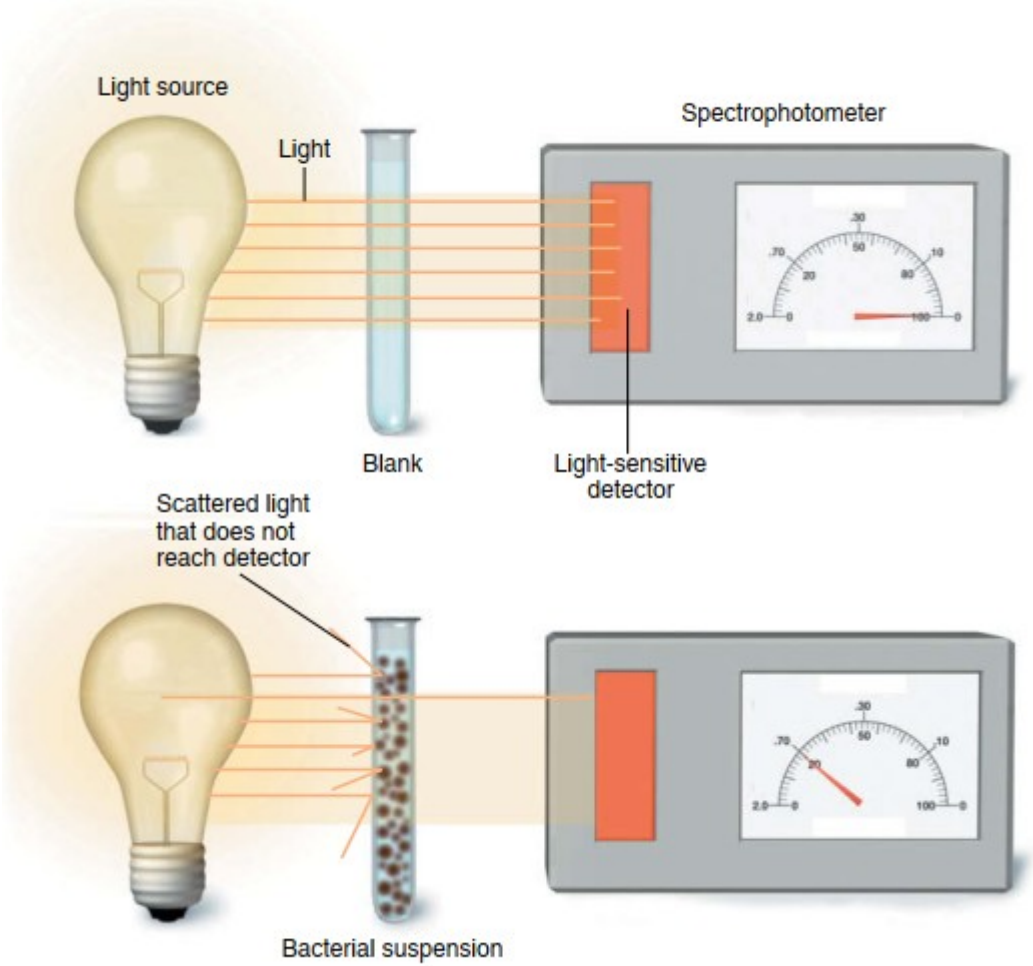
Estimating Bacterial Numbers by Indirect Methods

Turbidity

A spectrophotometer

Estimating Bacterial Numbers by Indirect Methods

Turbidity



Estimating Bacterial Numbers by Indirect Methods

Turbidity

- ✓ The light beam is passed through the cell-containing solution to the transilluminator: the more cells there are, the less light reaches the transilluminator. The change in light is converted to a percentage of transmittance (% transmittance).

- ✓ Absorbance (also known as obtical density - OD) is calculated as % transmittance: OD = 2 – log (% transmittance)

- ✓ Dilution is required, usually when $OD_{600\ nm} > 0.7$.

- ✓ $OD_{600\ nm} = 1$ if the *E. coli* cell suspension has a concentration of about 8×10^8 cells/ml (*S. cerevisiae*: about 3×10^7 cells/ml).

Estimating Bacterial Numbers by Indirect Methods

Dry Weight

- ✓ Centrifuge the cell-containing solution -> separate & collect the cell biomass.
- ✓ Wash the cell biomass.
- ✓ Dry the biomass at a suitable temperature so that the cells only lose water to a constant mass.

Estimating Bacterial Numbers by Indirect Methods

Methods based on metabolic activities

$$\text{ATP} + \text{D-luciferin} + O_2 \xrightarrow[Mg^{2+}]{\text{luciferase}} \text{Oxyluciferin} + \text{AMP} + PP_i + CO_2 + \text{light}$$

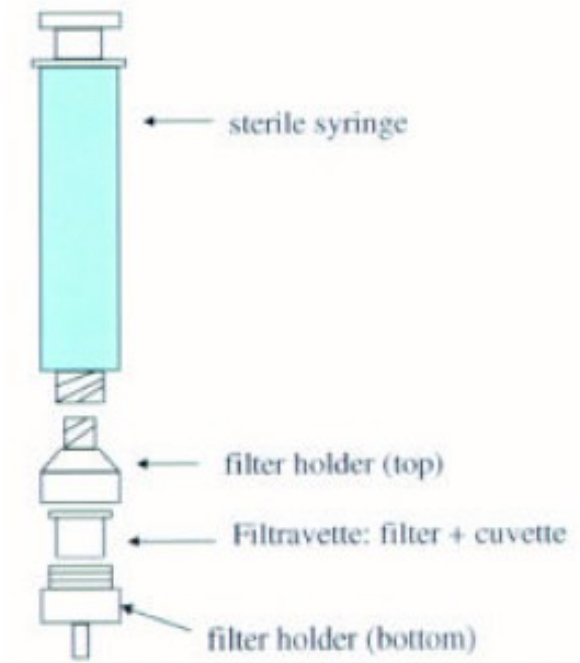


Figure 1 *Filtration of a water sample. The water sample is pressed through a Filtravette, which is inserted into a filter holder.*

Construct a growth curve for a batch culture

1) Prepare activated bacterial cells for inoculation;

2) Prepare liquid medium in a suitable vessel for culture growth;

3) Inoculate the culture into the medium;

4) Cultivate the culture under suitable conditions;

5) Take samples periodically and determine the density of cell biomass produced in the sample by a suitable method until the amount of biomass decreases with the culture time.

6) Construct a curve showing the dependence of the amount of biomass produced on the culture time.