Measurement of Microbial Growth

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Measurement of Cell Numbers

- **Petroff-Hausser counting chambers can be used for counting procaryotes; hemocytometers can be used for both procaryotes and eucaryotes.**
- Procaryotes are more easily counted in these chambers if they are stained, or when a phase-contrast or a fluorescence microscope is employed.
- These specially designed slides have chambers of known depth with an etched grid on the chamber bottom (**figure 6.5**).
- The number of microorganisms in a sample can be calculated by taking into account the chamber's volume and any sample dilutions required.
- There are some disadvantages to the technique.
- The microbial population must be fairly large for accuracy because such a small volume is sampled.
- It is also difficult to distinguish between living and dead cells in counting chambers without special techniques.

Figure 6.5 The Petroff-Hausser Counting Chamber.

(a) Side view of the chamber showing the cover glass and the space beneath it that holds a bacterial suspension.
(b) A top view of the chamber. The grid is located in the center of the slide.
(c) An enlarged view of the grid.

- The bacteria in several of the central squares are counted, usually at 400 to 500 magnification.
- The average number of bacteria in these squares is used to calculate the concentration of cells in the original sample.
- Since there are 25 squares covering an area of 1 mm^2, the total number of bacteria in 1 mm^2 of the chamber is (number/square)(25 squares).
- The chamber is 0.02 mm deep and therefore, bacteria/mm^3 = (bacteria/square)(25 squares)(50).

The number of bacteria per cm^3 is 10^3 times this value. For example, suppose the average count per square is 28 bacteria:

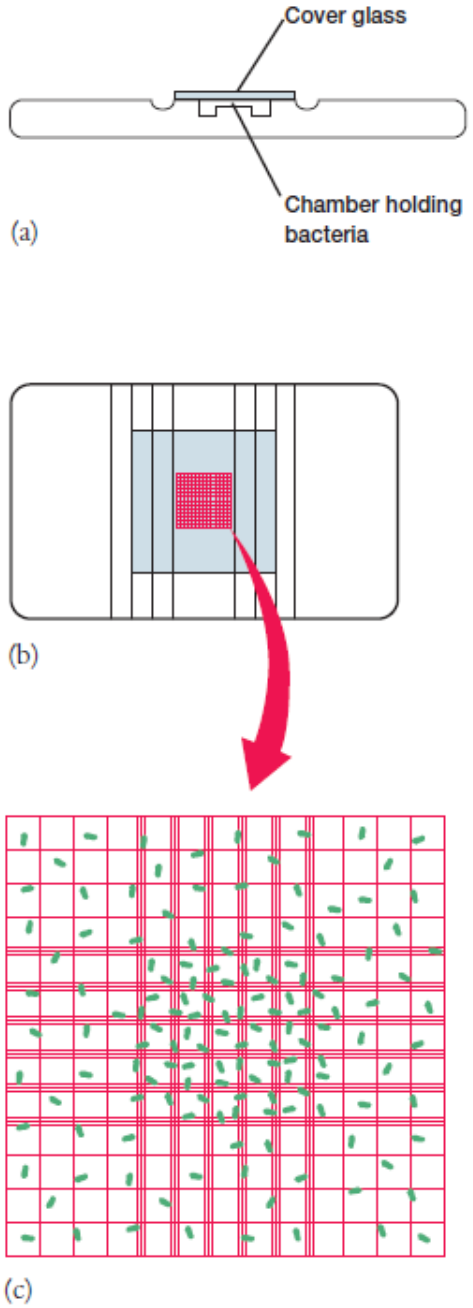
$$\text{bacteria/cm}^3 = (28 \text{ bacteria})\ (25 \text{ squares})(50)(10^3) = 3.5 \times 10^7.$$

Where, 50 is conversion factor; how?
The ruled counting area is:
Length = 1 mm
Width = 1 mm
Therefore, area = 1mm2
Chamber depth = 0.02 mm
So the volume above the entire ruled area is:
1mm×1mm×0.02mm =0.02mm^3
Thus, the entire counting grid contains: 0.02mm^3

We want bacteria per 1mm^3.

Since:

$$\frac{1}{0.02} = 50$$



Coulter Counter

- Larger microorganisms such as protozoa, algae, and nonfilamentous yeasts can be directly counted with electronic counters such as the Coulter Counter.
- The microbial suspension is forced through a small hole or orifice.
- An electrical current flows through the hole, and electrodes placed on both sides of the orifice measure its electrical resistance.
- Every time a microbial cell passes through the orifice, electrical resistance increases (or the conductivity drops) and the cell is counted.
- The Coulter Counter gives accurate results with larger cells and is extensively used in hospital laboratories to count red and white blood cells.
- It is not as useful in counting bacteria because of interference by small debris particles, the formation of filaments, and other problems.

Colony forming units (CFU)

- **CFU (Colony-Forming Unit) method** is another common method for determining the number of **viable microorganisms** in a sample. Unlike the Petroff–Hausser chamber, it measures organisms that are capable of **forming colonies**.
- **Principle**
- A diluted bacterial suspension is spread or poured onto solid agar. After incubation, each viable cell (or sometimes a small cluster of cells) gives rise to a visible colony.
- Therefore:

$$\text{CFU/mL} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated (mL)}}$$

- CFU/mL=Volume plated (mL)Number of colonies×Dilution factor
- Plating techniques are simple, sensitive, and widely used for viable counts of bacteria and other microorganisms in samples of food, water, and soil.
- Several problems, however, can lead to inaccurate counts. Low counts will result if clumps of cells are not broken up and the microorganisms well dispersed.
- Because it is not possible to be absolutely certain that each colony arose from an individual cell, the results are often expressed in terms of **colony forming units (CFU)** rather than the number of microorganisms.
- The samples should yield between 30 and 300 colonies for best results.
- The hot agar used in the pour-plate technique may injure or kill sensitive cells; thus spread plates sometimes give higher counts thanpour plates.

Membrane Filter Technique

- Microbial numbers are frequently determined from counts of colonies growing on special membrane filters having pores small enough to trap bacteria.
- In the membrane filter technique, a sample is drawn through a special **membrane filter**.
- The filter is then placed on an agar medium or on a pad soaked with liquid media and incubated until each cell forms a separate colony.
- A colony count gives the number of microorganisms in the filtered sample, and special media can be used to select for specific microorganisms.
- This technique is especially useful in analyzing aquatic samples.
- Membrane filters also are used to count bacteria directly.
- The sample is first filtered through a black polycarbonate membrane filter to provide a good background for observing fluorescent objects.
- The bacteria then are stained with a fluorescent dye such as acridine orange or DAPI and observed microscopically.
- Acridine orange–stained microorganisms glow orange or green and are easily counted with an epifluorescence microscope.

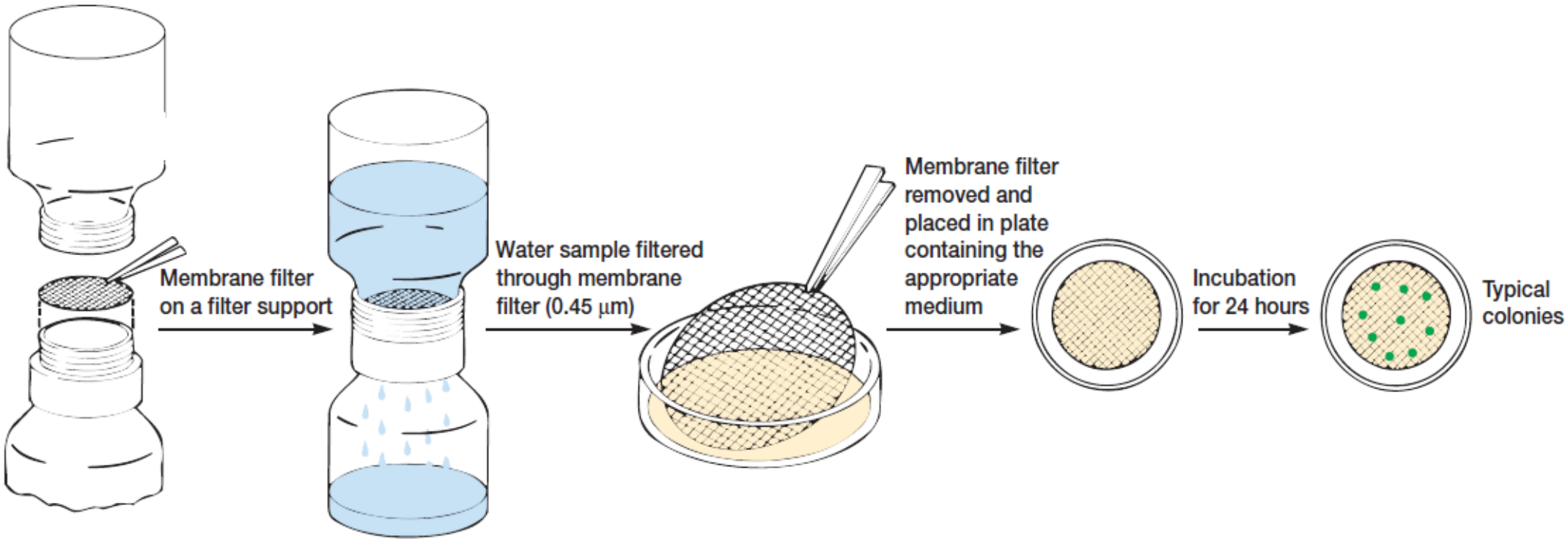


Figure 6.6 The Membrane Filtration Procedure. Membranes with different pore sizes are used to trap different microorganisms. Incubation times for membranes also vary with the medium and microorganism.

Measurement of Cell Mass

- The most direct approach is the determination of microbial dry weight.
- Cells growing in liquid medium are collected by centrifugation, washed, dried in an oven, and weighed. This is an especially useful technique for measuring the growth of fungi.
- More rapid, sensitive techniques depend on the fact that microbial cells scatter light striking them. Because microbial cells in a population are of roughly constant size, the amount of scattering is directly proportional to the biomass of cells present and indirectly related to cell number.
- When the concentration of bacteria reaches about 10 million cells (10^7) per ml, the medium appears slightly cloudy or turbid.
- Further increases in concentration result in greater turbidity and less light is transmitted through the medium.
- The extent of light scattering can be measured by a spectrophotometer and is almost linearly related to bacterial concentration at low absorbance levels.