Measurement of Bacterial growth (direct microscopic count).

Different methods are employed for measuring the cell growth of microorganisms. Cell growth is indicated by increase in the number of cells or increase in weight of cell mass. There are direct and indirect methods of measuring microbial growth.

Direct Measurements

Total count and viable count are the two methods widely employed to count cell numbers.

Total count:

The total number of cells in a population can be measured by counting a sample under the microscope. This is called direct microscopic count. This is done by using a specialized counting chamber called Petroff Hausser chamber which is a specially designed slide with a grid. The liquid sample is placed on the grid which has a total area of $1mm^2$ and divided into 25 large squares. The number of cells in large square is counted and the total number of cells is

calculated by multiplying it with a conversion factor based on the volume of the chamber

Petroff-Hausser counting chamber

Petroff-Hausser cell counter/ counting chamber/ cytometer/ hemacytometer (because it was devised originally for counting blood cells) calibrated to accept a tiny sample that is spread over a premeasured grid.

In such a counting chamber, a grid with squares of known area is marked on the surface of a glass slide. There are 25 large squares and each large squares have 16 smaller squares in them. The area of each small square is 1/400 mm^2 (total area 1.0 mm^2) and a glass cover slip tests 0.02 mm above the slide. So, total volume of the area is 0.02 mm^3 or 0.00002 cm^3 (ml). Once the number of cells has been counted in several different fields, the average number of cells per unit area of grid can be calculated under the phase-contrast microscope without staining, giving a measure of the number of cells per 0.00002 cm^3. Microscopic counts can be done on either sample dried in slides or on samples in liquid. Dried samples can be stained to increase contrast between cells and their background.

The total number of cells in solution is

= (N_c× 10^3/ (0.05 × 0.05 × 0.02 × 1/D))

Here,

N_c= Counted bacterial cell

D= Dilution factor

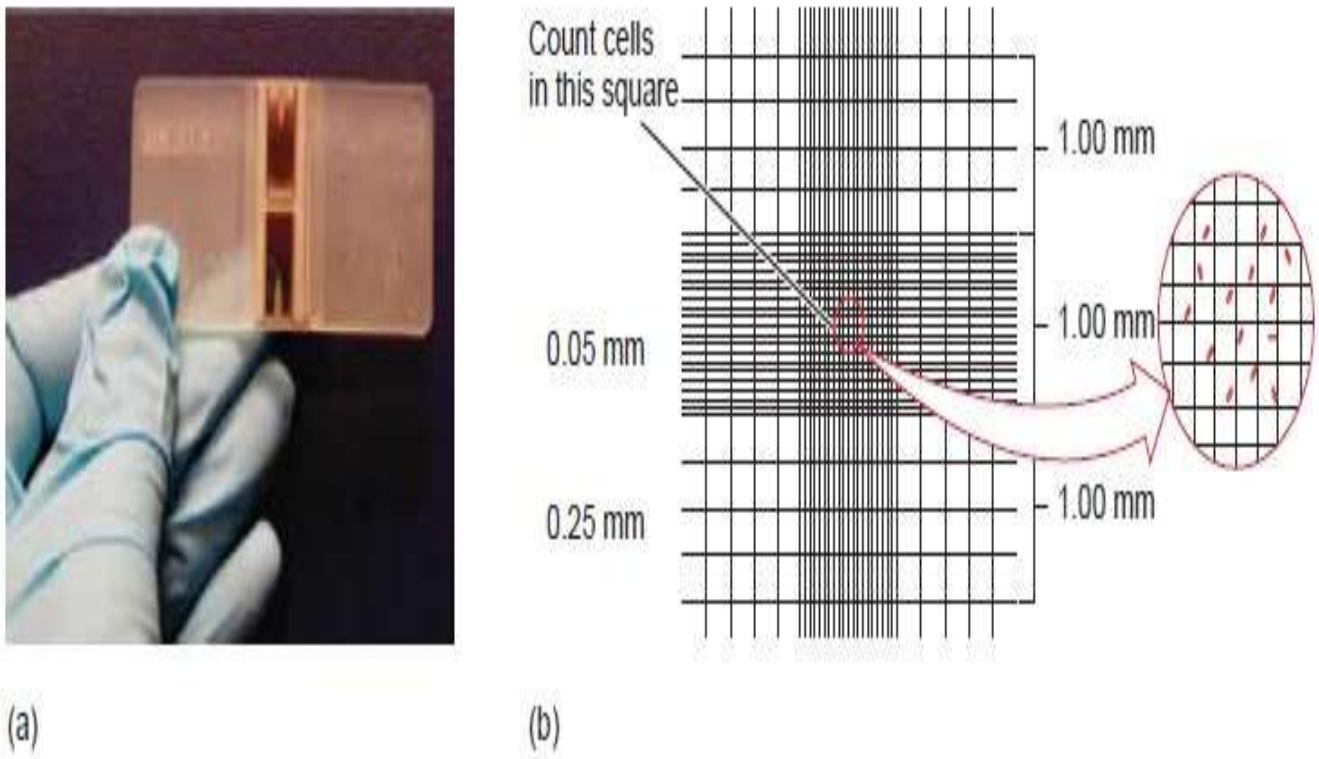
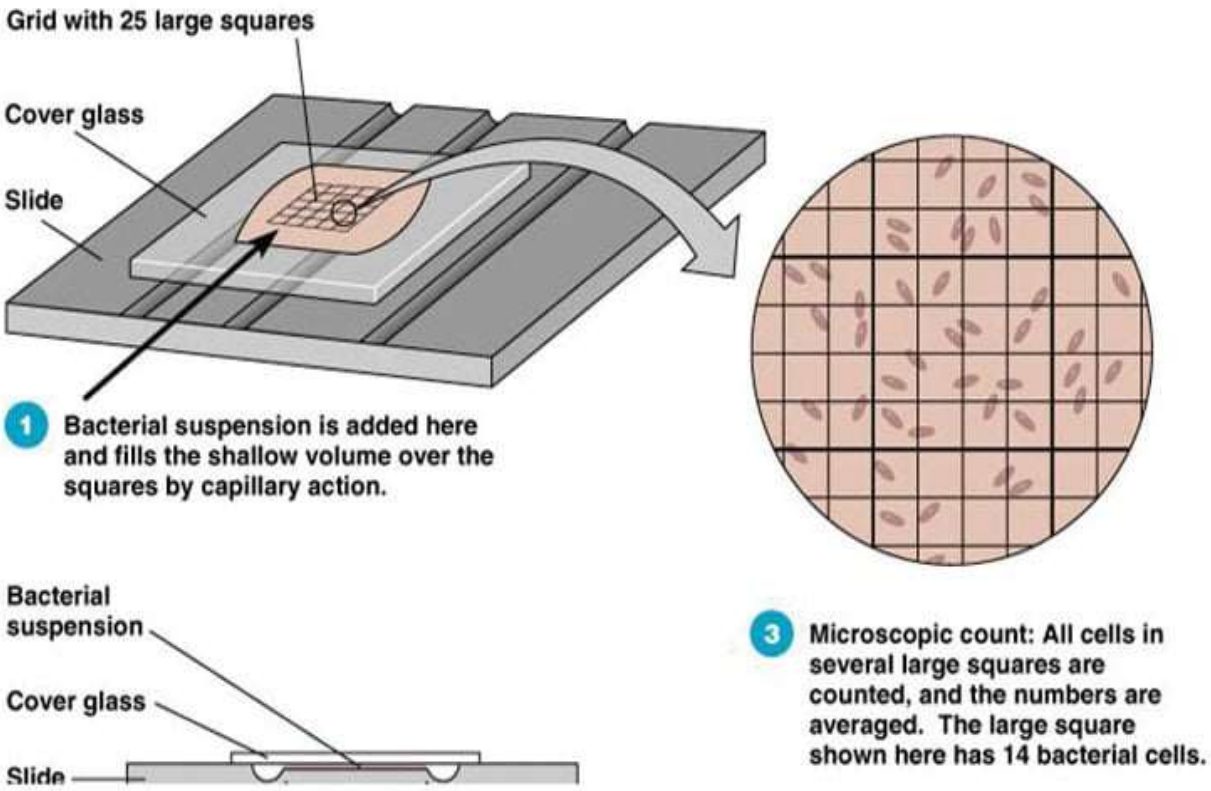


Figure 6.8: (a) Petroff-Hausser counting chamber
(b) Microscopic observation of bacterial cells



Advantages

- **Rapid, Simple and easy method requiring minimum equipment.**
- **Morphology of the bacteria can be observed as they counted.**
- **Very dense suspensions can be counted if they are diluted appropriately.**

Disadvantages

1. **Without special staining technique dead cells can not be distinguished from live cells.**
2. **Small cells are difficult to see under microscope and some cells are inevitably missed.**
3. **Precision is difficult to achieve.**
4. **A phase-contrast is required if the sample is not stained.**
5. **Cells suspension of low density (less than about 106 cells/ milliliter) have few if any bacteria in the microscope field unless a sample is first concentrated and re-suspended in a small volume.**
6. **Motile cells must be immobilized before counting.**
7. **Debris in the sample may be mistaken for microbial cells.**

The procedure:

1. Sample Preparation:

- Dilute the bacterial suspension to a countable concentration.
- If necessary, use a staining solution (like methylene blue) that allows for distinguishing between live and dead cells.

2. Loading the Chamber:

- Place a coverslip over the counting chamber.
- Carefully introduce the diluted sample under the coverslip, ensuring it fills the etched grid area.

3. Microscopic Examination:

- Place the loaded chamber on the microscope stage.
- Focus on the grid using a suitable objective (e.g., 40x).
- Count the cells within specified squares of the grid.
- In some labs, the four corner squares and the center square are counted.

- **For cells touching the boundary lines, some labs count those touching the top and left boundaries.**

4. Calculations:

- **Determine the average number of cells per square.**
- **Calculate the total number of cells per milliliter using the known volume of the counting chamber and the dilution factor.**

Formula Example:

Bacteria/ml = (total bacteria counted x dilution factor x volume factor) / number of squares counted

Example:

If you count an average of 50 cells in the five counted squares (four corners and center), and the dilution factor is 1:10, and the volume of each small square is 1/1,250,000 cc, then:

Bacteria/ml = (50 cells * 10 * 1,250,000) = 625,000,000 cells/ml