

Direct microscopic count

direct microscopic count or DMC

This method is used for liquid food especially milk, which is different from the previous calculated as all live and dead bacteria, unlike the previous methods as it is the only living cells.

Way of working: -

1 - organizes and defines the field microscopy exactly and then transmits the amount of 0.01 ml of milk or food liquid to a glass slide especially clean and publish an area of cm^2 (100 mm^2) and then let the liquid that dries out and prove the membrane by heat and dying by methylene blue and then counting according to the number of bacterial or Microbial clump per field after counting 10-60 field.

2 - can be counted bacteria by using the microscope face contrast microscope using a slide called petroff-Hausser divided into squares exact area of the square ($1 / 400$) mm^2 , covering the colonies by chip glass rising from the count slide ($1 / 50$) mm and this size is above all square between the slide numbers and the cover = $(1 / 400) \text{ mm}^2 \times (1 / 50) \text{ mm} = 1 / 20000$ mm^3 , size per cubic centimeter

$$= 20000 \times 1000 = 1 / 20000000 \text{ cm}^3$$

And by putting a drop of suitable dilution bacteria and known on a slide number and cover slide cover are counted bacteria per square taking the average number of square at least 20 square, and assume that the number of bacteria per square 5 so the number $5 \times (1 / 20000000) = 2 \times 100000000 \text{ cm}^3 = 2 \times 10^8 \text{ cm}^3$

If either factor dilution = 5 (ie, that the solution of bacterial origin diluted 5 times ($1 / 5$)) Suppose the rate of bacteria per square = 4 then the number of = $5 \times 4 \times 20000000$, calculated area of the field area and is equal to the radius squared x constant percent (PI.), then calculates the microscopy factor (MF) = 100×100 area field by millimeter .

Estimated number of microscopy in each 1 ml by calculating the number of bacteria or bacterial communities in a number of fields microscopy is calculated as the rate of the cells in each field and then hit in the microscopy factor (MF).

Total number = average number of bacterial per square X microscopy factor x inverted dillution .
= (Number of bacteria / number of squares) X (1 / 20000000)

Direct microscopic counts are possible using special slides known as counting chambers, consisting of a ruled slide and a cover slip. It is constructed in such a manner that the cover slip, slide, and ruled lines delimit a known volume. The number of bacteria in a small known volume is directly counted microscopically and the number of bacteria in the larger original sample is determined by extrapolation. Dead cells cannot be distinguished from living ones. Only dense suspensions can be counted.

Bacteria can be counted easily and accurately with the petroff-Hausser counting chamber. This is a special slide accurately ruled into squares that are $1/400 \text{ mm}^2$ in area; a glass cover slip rests $1/50 \text{ mm}$ above the slide, so that the volume over a square is $1/20,000 \text{ mm}^3$ i.e. $1/20,000,000 \text{ cm}^3$. If for example, an average of five bacteria is present in each ruled square, there is $5 \times 20,000,000$ or 10^8 , bacteria per milliliter. A suspension of unstained bacteria can be counted in the chamber, using a phase-contrast microscope.

Advantage of Direct Microscopic count

- 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.

Limitations of Direct Microscopic count

1. Dead cells are not distinguished from living cells.
2. Small cells are difficult to see under the microscope, and some cells are probably missed.
3. Precision is difficult to achieve
4. A phase contrast microscope is required when the sample is not stained.
5. The method is not usually suitable for cell suspensions of low density i.e. $< 10^7$ Cells per ml, but samples can be concentrated by centrifugation or filtration to increase sensitivity.