

Bacterial count

When one decides to count the number of cells in a sample, the issues of viable numbers, and the total numbers should come to mind. There are many methods used for counting cell numbers, some of which count total cell numbers (live cells + dead cells), and others that count only viable, or live cells. Direct plate counting is a method used to count the number of viable cells in a sample. By the very nature of the procedure, the dead cells are unable to be included in the count.

Once the cells to be counted have been isolated, they are to be diluted due to the fact that too many cells will cause the Petri plate to be so densely populated with colonies, that they would be impossible to count. After the cells have been diluted, they are incubated on an agar medium until colonies form. It is at this time that the cells may be counted.

Estimate the number of bacterial:-

There are several ways by which bacterial numbers can be estimated and the most important of these methods are: -

1 - count by dishes (Plate count) as viable count

There are two main methods of direct plate counting: spread plate method and pour plate method.

2 - count the most likely or (most probable number) MPN

3 - direct microscopic count (DMC)

The following is a summary of the main roads of the three :-

1- Calculate the number of bacterial cells by counting the colonies grown in dishes

a- Pour plate method

Depended on it in the process of casting dishes we can isolate the bacteria as a living cell to grow into colonies visible and depending on that we can count the number of bacterial cells in a particular model, and to set the number of bacteria, the model dillutes using a series of tubes containing a solution of dillution or solution sterile (which may be distilled water or

nutrient broth or normal saline or buffer solution K_2HPO_4) and is in the petridishes containing the nutrient broth or any culture media dissolved after that will notice the declining numbers colonies generated by the process of dillution, which will help to get the colonies are well isolated, as well as he can in this way to obtain information on the quality and quantity of bacteria in the original sample.

Materials needed: -

➤ **In the event that the sample liquid**

- 1 - container, a glass beaker on the sample to be tested
- 2- 6 tubes each containing a dillution solution (distilled water and a nutrient broth or physiological salt or buffer sollution)
- 3 - a glass flask containing the culture media dissolved and sterilized and is present in a water bath temperature of $45-50^{\circ}C$
- 4 - sterile Petri dishes
- 5 - sterile pipettes
- 6 - All you need to complete the experiment in sterile conditions

Way of working: -

- 1 - to transfer 1 ml of original sample to the first tube and the container to 9 ml of reducing the use of sterile absorbent
- 2 - using a new sterile pipette mixes suspense in the first tube well and transferred to 1 ml of the second tube
- 3 - hands-on repeat all the existing pipe and using a new pipette at each transfer
- 4 - to transfer 1 ml of each of the diluted solution to a sterile Petri dishes using sterile pipettes for each dillution
- 5 - Add the culture media melted sterile and cooled to the temperature of $45-50^{\circ}C$
for each of the dishes listed.
- 6 - Move the dishes gently and reverse the direction of clockwise to blend suspense diluted with media
- 7 - to leave the dishes that solidifies the media then placed upside down in the incubator for 24-48 hours and a temperature of $37^{\circ}C$
- 8 - Choose dishes that range where the number of colonies between 30-300 colony
- 9 - Calculate the number of bacterial cells by the following equation :-

number of bacterial cells in the original sample = number of colonies in the petridish X invertedof dillution of the sample

The final number of hits in the size of the original sample to extract the total number of bacteria in it. This method is used routinely where they can learn the preparation of bacteria in the original sample regardless of the few or many.

When using the pour plate method, a diluted sample is pipetted into a sterile Petri plate, then melted agar is poured in and mixed with the sample. Using this method allows for a larger volume of the diluted sample. Usually in the range of 0.1 - 1.0 ml. This method yields colonies that form colonies throughout the agar, not just on the surface. Caution must be taken with this method to ensure that the organism to be counted can withstand the temperatures associated with the melted agar.