

Enumeration of bacteria in a population



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Scientists use a number of different methods to determine the number of micro-organisms that are present in a given population. This can be accomplished by using the spectrophotometer to measure the optical density of the population, by directly counting the microorganisms using a haemocytometer, or by serial diluting the bacteria and plating the diluted bacteria on media that supports the growth of the micro-organisms. The latter method is somewhat more time consuming, but provides statistically accurate and repeatable results. This method is also the ideal method for enumerating microorganisms in a given population because it only identifies the living organisms in that population.

Microbial counting is useful in the basic sciences and is used to determine the number of bacteria present for physiological or biochemical studies. Food or water microbiologists test food, milk or water for the numbers of microbial pathogens to determine if these products are safe for human consumption.

A serial dilution is used for plating and counting of live bacteria to determine the number of bacteria in a given population. Serial dilution of a solution containing an unknown number of bacteria, plate these bacteria and determine the total number of bacteria in the original solution by counting the number of colony forming units and comparing them to the dilution factor. Each colony forming unit represents a bacterium that was present in the diluted sample. The numbers of colony forming units (CFU's) are divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria per mL that were present in the original solution.

2. 1. Standard Plate Count Method: A Viable and Direct Count

A standard plate count method is used to determine the number of viable bacterial cells per unit volume of a sample using agar plate media. For example, to determine the number of viable bacterial cells per millilitre of a liquid sample, a fixed volume of sample would be transferred to a plate, the solution would be spread across the plate and the colonies would be counted after incubation. The colonies are referred to as colony forming units (CFU). Once the number of CFUs on the plate is determined it can be divided by the volume plated to determine the concentration of cells in the sample. If a sample contains over one thousand cells per unit volume then it will produce too many CFUs to count accurately on the plate. These samples should first be diluted in sterile media before transferring to plate media so that a countable number of colonies appear. Since the actual concentration of the sample is unknown it is common practice to dilute the sample serially (for example 1/10, 1/100, 1/1000, etc.) then spread-plate the multiple serial dilutions. The highest dilutions will produce the lowest number of CFUs and the lowest dilutions will produce the highest number of CFUs. The plate with the countable number of colonies should be selected to count. When using standard size Petri dishes, a countable plate would be one with between 30 and 300 CFUs. Dilutions with fewer than 30 colonies are easy to count, but often produce inaccurate results since one or two contaminating colonies can cause a significant overestimate of the cell count. After the colonies are counted the concentration of cells in the plated dilution can be determined by dividing by the amount plated. Once the concentration of cells at the

specific dilution is determined, the concentration in the original sample can be calculated after dividing by the total dilution.

1. Transfer one millilitre from the sample to 9 ml of sterile media in tube A and mix. This will be a 1/10 serial dilution.
2. Transfer one millilitre from tube A to 9 ml of sterile media in tube B and mix. This will be another 1/10 serial dilution. The total dilution up to this point is 1/100.
3. Then transfer 1 ml from tube B to 9 ml of sterile media in tube C and mix. This is a 1/10 serial dilution. The total dilution up to this point is 1/1000.
4. 0.1 ml from each tube should be plated. Plates A and B contained too many colonies to count (Figure. 1). Plate C is countable. If plate C contained 43 colonies we can determine the concentration in the sample. a. 43 CFU/0.1 ml = 430 CFU/ml, b. 430 CFU/ml / 1/1000 or 430 CFU/ml / 1×10^{-3} = 4.3 $\times 10^5$ CFU/ml

A

B

C

Figure: 1. Number of colonies forming units in different plates

2. 1. 1. Calculating the number of bacteria per mL of serially diluted bacteria:

To calculate the number of bacteria per ml of diluted sample the following equation should be used:

For example, if for the 1×10^{-8} dilution plate 0.1 ml of the diluted cell suspension is plated and counted 200 cfu, then the calculation would be:

$200/0.1 \text{ mL} \times 10^{-8}$ or $200/10^{-9}$ or 2.0×10^{11} bacteria per mL.

2. 2. Spectroscopy Enumeration Method: Optical Density

In indirect method of enumerating the cell concentration in a bacterial culture involves using a spectrophotometer. Bacterial cells absorb light well at the wavelength of 686 nm when grown in standard media. A spectrophotometer can be used to measure the amount of light at a wavelength of 686 nm that is transmitted through a bacterial culture. The more bacteria in the culture, the more light will be absorbed by the culture and the less light transmitted through the culture. The spectrophotometer will measure the percentage of light transmitted through the culture and this number can be converted to optical density (OD). OD is a quantitative way of describing the turbidity of a culture. OD is inversely proportional to the percent transmittance ($OD = 2 - \log\%T$). As the turbidity of a culture increases the cell concentration increases and the OD increases (the percent transmittance decreases). In order to relate OD to an actual cell concentration a standard curve has to be set up. A culture is serially diluted and the OD is calculated for each dilution using a spectrophotometer. Then a viable count is made for the original culture that was diluted. Calculations are made to determine the viable count of cells for each serial dilution. A graph is used to plot the OD of each dilution on the X axis and the log viable cell concentration on the Y axis.