Welcome Students,

This module is from Unit 2 Methods in Microbiology, from the Paper Microbiology and Plant Pathology.
The name of the module is Enumeration of Microorganisms. Module number 9.
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In this module, we will discuss the methods used to determine viable and total cell counts in microbial population. The two methods which we will discuss are direct methods and indirect methods.

Learning outcomes
At the end of this module, the students will be able to explain several laboratory methods which are used to determine viable and total cell counts in microbial population.

Now let us begin with the topic Enumeration of microorganisms.
The term enumerate means to count.
Now, the number of microbial cells present in a sample is called a cell count. The number of microbes which are present in a clinical sample indicates the extent of infection in the sample. The quality control of drinking water, food, medication and even cosmetics depends on the estimation of microbial count to detect the contamination and to prevent the spread of disease. There are several laboratory methods which can be used to detect cell count. The choice of the method depends on factors such as nature of the microorganism, whether it is a bacteria, virus, etc., characteristics of the microorganism and the purpose of study.

Microorganisms multiply at a very rapid rate. Therefore, their numbers increase exponentially. Therefore, there is a need to dilute the sample before estimating the number of individuals in the population. In the serial dilution method 1 mL of sample is suspended in 9 mL of sterile broth to obtain a microbial suspension of 1:10 dilution. This sample is further serially diluted in multiples of 10 by transferring 1 mL of the previous dilution to 9 mL of sterile broth to obtain the next dilution. These dilutions are then used for counting. As the dilution increases, the cell count decreases.

Now microbial population can be determined in two ways, either by using the direct methods or the indirect methods. Under the direct methods we have total count and viable count. Total count can be carried out using either microscopic method or electronic method. Under indirect methods we have determination of cell mass and the second one is spectrophotometric method. The determination of cell mass can be carried out either by measuring the dry weight or measurement of cell nitrogen.

Now let us study about each one in detail.
First are the direct methods.
This method involves the counting of cells in a liquid culture or colonies on a plate. This is a direct way to estimate the number of organisms in a sample. There are two methods which can be used to determine cell numbers. These are total count, which is also called as the non viable count. In this method, the total number of cells i.e., both living as well as dead cells are counted. Therefore, it is also known as the

non-viable count method. The total count can be determined using two methods, either the microscopic method or electronic method.

So first let us study about the microscopic method. This is the simplest way to count microorganisms. In this a special type of slide called the Haemocytometer is used to count the number of cells. Now here we can see a picture of the haemocytometer. So on this a grid is present which facilitates precision in counting of the microorganisms. The culture is first transferred to the haemocytometer and then the cells in several small squares are counted. Then an average is taken to obtain a reliable measurement. The advantages of the microscopic method are that it is easy to use, relatively fast, inexpensive and the number of cells in each dilution can be viewed and counted. And the disadvantages are it does not yield an accurate count of the number of live cells. Since, under the microscope it is difficult to distinguish between living, i.e., live cells, dead cells as well as debris of the same size. This method does not work well with dilute cultures, since a sufficient amount of cells need to be present on the slide to be counted.

The next one is electronic method. This is a method in which electronic cell counting device is used to detect and count the cells. The instrument which is used is called a Coulter Counter, which is used for counting the cells in the suspension. Now, this is a more efficient method, since, in a few seconds thousands of cells can be counted. The disadvantage of this method is that it cannot distinguish between living and dead cells.

These two methods, they provide an estimate of the total number of cells. But in many situations it is important to know the number of live or viable cells. Therefore, we use other methods i.e., the viable methods. So, counts of live cells are needed when assessing the extent of an infection, the effectiveness of antimicrobial compounds and medication and contamination of food and water.

So, the next one is viable count. In this method, counting only living cells in a microbial culture is done. So in this method, plating a known volume of the diluted sample is done on an appropriate medium and then counting the number of colonies is done after incubation. The principle of this method is that all viable cells multiply and form a colony under suitable conditions. The number of colonies is equal to the number of viable cells in the sample.

Now coming to the indirect methods. In these methods,the measurement of cell presence or activity is done without actually counting the individual cells.
The first one is determination of cell mass. The increase in the cell weight or cell mass is an indicator of an increase in cell number. So in this method, the cell mass is actually weighed. This can be done either by measuring the dry weight or measuring the cell nitrogen. Now for measuring the dry weight, the cell culture suspension has to be concentrated by centrifuging or by filtering it. And then it is dried. The dried pellet, which is consisting of the cells, is then weighed. This method is most useful for filamentous microorganisms which are difficult to count under the microscope.

Next is measurement of cell nitrogen. In this method, the microbial growth is measured in terms of the quantity of nitrogen which is present in the cells. Now as we know, protein is a major component of cells

and it is made up of nitrogen. Now, this method is useful with dense microbial suspensions, where the amount of growth is large.

The next one is spectrophotometric method. Now this is the most popular indirect method to measure the bacterial and yeast cell count. As the growth takes place the turbidity of the cell suspension increases in the culture medium. Now, this turbidity can be determined at regular intervals. The increase in turbidity is directly proportional to the increasing number of cells in the culture.

Now let us summarize what we have studied in this module.
In this module we have discussed the enumeration of microorganisms. There are two methods, direct methods and indirect methods. In direct we saw how we count the total number of cells. And also the second one was viable count where only living cells were counted. Under indirect, we had determination of cell mass, i.e., measurement of dry weight or the cell nitrogen and the second one was spectrophotometric method.

Now these are the references which I have used for this module.

This is an additional reference to a website which you can browse for more information on this module.

Thank you.