count of colony forming unit

Dilution the sample :-

with both the spread plate and pour plate methods, it is important that the number of colonies developing on the plates not be too large because on crowded plates some cells may not form colonies and some colonies may fuse, leading to erroneous measurements. It is also essential that the number of colonies not be too small, or the statistical significance of the calculated count will be low. The usual practice, which is the most valid statistically, is to count colonies only on plates that have between 30 and 300 colonies. The number of bacteria in a given sample may be usually too great to be counted directly. To obtain the appropriate colony number, the sample be counted must almost always diluted in such a manner that single isolated bacteria form visible isolated colonies , the number of colonies can be used as a measure of the number of viable (living) cells in that known dilution. Several 10-fold dilutions of the sample are commonly used. In most cases, serial dilutions are employed to reach the final desired dilution.

However, if the organism normally forms multiple cell arrangements, such as chains, the colony-forming unit may consist of a chain of bacteria rather than a single bacterium. In addition, some of the bacteria may be clumped together. The development of one colony can occur even when the cells are in aggregates. i.e. cocci in clusters (staphylococci), chains (streptococci), or pairs (diplococci), the resulting counts will be lower than the number of individual cells. Each colony that can be counted is called a **colony forming unit** (CFU). By extrapolation, this number can in turn be used to calculate the number of CFUs in the original sample rather than number of bacteria per milliliter. The assumption made in this type of counting procedure is that each viable cell can yield one colony.

There are two ways of performing a plate count: the spread plate method and the pour plate method.

Generally, one wants to determine the number of CFUs per milliliter (ml) of sample. To find this, the number of colonies (on a plate having 30-300 colonies) is multiplied by the number of times the original ml of bacteria was diluted (the dilution factor of the plate counted). For example, if a plate containing a 1/1,000,000 dilution of the original ml of sample shows 150

colonies, then the number of CFUs per ml in the original sample is found by multiplying 150 x 1,000,000 as shown in the formula below:

The number of CFUs per ml of sample = The number of colonies (30-300 plate) X The dilution factor of the plate counted
In the case of the example above 150 x 1,000,000 = 150,000,000 CFUs per ml

This method is used to count only live (viable) cells. A viable cell is defined as one that is able to divide and form offsprings, and the usual way to perform a viable count is to determine the number of cells in the sample capable of forming colonies on a suitable agar medium. For this reason, the viable count is often called the plate count, or colony count. This method is used to enumerate bacteria in milk, water, foods, soils; cultures etc and the number of bacteria are expressed as colony-forming units (CFU) per ml.

PROS AND CONS "

Direct plate counting is a good method of counting cells. A couple of advantages are that it works well for cells that separate in a short amount of time after they divide, and it counts only viable numbers. However, direct plate counting is not without its flaws. It does not work so well for those cells that stick together after cell division. Another problem that may arise is the very specific nutritional needs of some organisms. This is a special consideration when dealing with mixed samples such as those from a soil, because one cannot be sure the media has sufficient nutritional value for all of the cell types present. Sampling error is another possible problem. Sampling error refers to the uneven distribution of the sample on the agar surface. Direct plate counting has been described as the best available method for determining viable numbers, despite the large chance of the occurrence of an error.