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Qualitative and quantitative methods of milk testing

Introduction

Microbiological quality control test of milk can be divided into two groups: for example direct tests (Quantitative) and indirect tests (Qualitative).

A-Quantitative Tests

The direct tests are helpful for assessment of the actual number of bacteria present in milk by microscopic examination, direct microscopic count (DMC) or by enumeration of the colonies formed by viable cells of bacteria, Standard plate count (SPC).

The microscopic examination of raw milk sample provides a rapid indication of the quality of liquid milk. It must, however, be noted that, because of the small sample volume which is being examined, any direct microscopic count is insensitive if the bacterial load is less than 500000 per ml.

By the microscopic method, *clump counts* can be determined. When determining the individual count, all bacterial cells within clumps or in isolation are counted. Dead bacteria can also be stained in this method. However, bacteria killed by heat usually disintegrate soon after, or lose their staining ability. In addition, there are stain solutions that permit the recognition of dead cells, e.g. acridine orange only stains viable cells, and then the microscopic count can be applied to heated milk, or reconstituted samples of powdered milk, to furnish the information concerning the history of product.

The microscopic method can also be used for counting the somatic cells. For milk from individual quarters of cows in normal lactation, a somatic cell count of 125×10^3 250×10^3 per ml, and for bulk milk a somatic cell count of 500×10^3 per ml, has been recommended by IDF**,** although many countries accept counts of 500×10^3 to 100×10^4 per ml.

1- Pour Plate Methods

The plate count or pour plate method is often used for estimating the number of viable number of micro-organisms in liquid, reconstituted or suspended dairy products. Due to a wide range of bacterial counts occurring in dairy products, bacteria can often only be counted after diluting the liquid. A number of 10-fold serial dilutions of the sample are prepared.

One ml of the dilution is mixed with the liquefied sterile agar medium in a sterile Petri dish. After solidification of the agar, the Petri dishes are incubated at a specific temperature and for

a suitable period of time. The bacterial cells grow to recognizable colonies that can be counted. Plates with 30-300 colonies are selected for counting. The loop method (plate loop count) is less time-consuming than the traditional pipetting, is adversely influenced by the following factors:

1- Depth and angle of loop penetration
2- Spread of loop removal
3- Temperature of milk and
4- Wetting ability of the loop and bacterial morphology

The manual plate loop count was suitable for routine lab that analyzes 4000 samples per month. Various automated and semi-automated apparatuses have been developed in an attempt to fully standardize the plate loop method with respect to the angle at which the loop is removed from the sample, the depth of immersion, and the speed of movement of the loop. When these precautions are taken, the very small amount (0.1-1) of the sample may not be representative of the product tested, except perhaps for the 0.1- l**,** hook.

2- Colony Counters

These are designed to count colonies on agar plates. Most of these colony counters consist of a television camera to detect the colonies on the illuminated Petri dish, and a small electronic computer for detection, counting and control. Up to 150 plates per hour may be counted using these counters. The counting of impurities, lumps, un-dissolved parts in the media or air bubbles can be avoided, as these only occur due to the carelessness of the personnel. More serious problem is of counting pinpoint colonies, which are sometimes formed by thermodurics. Colonies smaller than 0□20 mm are usually not counted, while the human eye can see and count colonies exceeding 0□10 mm. Another disadvantage is that, automatic counters cannot distinguish colors or colored colonies. Colonies on media that absorb light, e.g. blood agar, are difficult to count, while diffuse media, e.g. agar containing milk, may cause difficulties because the contrast between colonies and background is too limited. When Petri dishes known to be difficult to count by an automatic colony counter are eliminated, the counts obtained are as accurate as those obtained manually, although the counts are usually 10-15% lower, owing to the exclusion of peripheral colonies.

3- Electronic Colony Counters

Another approach to count colonies is electronic micro-colony counting using particle counters (coulter principle) the micro-colonies grow in a solid nutrient medium containing gelatin that is melted before counting. This method can also be applied to bacteria grown on selective media.

4- Surface Count

Surface colonies grow faster and can be counted after 24 h. Where it is desired to produce surface colonies, the spread method or the drop method can be applied. In the *spread method,* 0.1 ml of the 10-fold dilutions are transferred to, and spread over, the dry surface of a solid agar medium. After incubation, plates on which the overall growth has been retarded due to overcrowding of the colonies must be discarded and the rest are counted.

5- Membrane Filtration

When the bacterial count of a sample is low, and the sample or its dilution can be efficiently filtered, the membrane filtration method is most suitable. After the sample is passed through the membrane, the latter is placed on a solid agar medium, or on a filter pad that has been saturated with liquid medium. After incubation, the bacteria grow into colonies on the membrane. The test is only effective, if the number of colonies per membrane is in the range of 10-200 (optimum: 50). When the medium does not contain an indicator, it is advisable to stain the membrane, e.g. by gently flooding the surface with a 0.1 % aqueous solution of malachite green-oxalate, to confirm the count.

6- Most Probable Number (MPN)

MPN test makes use of a statistical technique to determine low counts of bacteria in dairy products. Three sets of three or five tubes, each containing sterile medium, are prepared and inoculated from each of three consecutive, 10-fold dilutions. Tubes showing bacterial growth after incubation are positive. From the number of positive tubes in each set of three or five tubes, the MPN of bacteria are counted per unit of sample, as per MPN table. When more than three dilutions are made, only the results from three consecutive dilutions are significant. The highest dilution which gives positive results in all of the tubes, and the next two succeeding higher dilutions, should be chosen. When the weight or volume of sample in the first dilution is 10 or 100 times less than the weight or volume listed in MPN tables, then the count tabled will be multiplied by 10 and 100, respectively.

7- Counting Single Bacteria

Apparatuses have been developed that are able to, rapidly, count the number of viable single bacteria in milk. The bacteria, stained with a fluorescent dye (acridine orange), are detected by an image analyzer microscope or alternatively, a small quantity of the diluted 'milk' is applied to a rotating disc that spins in front of a narrow slit in the detector. The fluorescent light emission of the bacteria is transformed into pulses of electricity that in order to avoid interference due to other particles, like fat globules, protein micelles and somatic cells, these materials may be initially separated by centrifugation; the bacteria in the 'milk' are then clearly visible. The separation, dilution, chemical treatment and staining are performed automatically. The same principle is also applied for the counting of somatic cells in milk.

B-Qualitative Methods

Dye reduction tests are indirect methods of assessing the microbiological quality of milk. These are based on the metabolic activity of the microorganisms. A correlation is made between the time required for the reduction of dye and probable number of bacteria in milk. The principle of these tests is to add dyes, like methylene blue, resazurin or trimethyl tetrazolium chloride, to milk or liquid dairy products, and to measure the color change after incubation. The color change is based on the dehydrogenase activity of the bacteria present in sample.