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Methods of Counting Bacteria in Milk.

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Inasmuch as dairy literature is full of references to the number of bacteria in milk and also because many standards are being adopted which make use of the number of bacteria as a means of classifying milk, dairy farmers should understand something of the methods used to determine the number of bacteria present in milk.

Difficulty of making count.

Because of the fact that bacteria are exceedingly small objects which are visible only under the highest powers of the microscope and also because they may occur in enormous numbers in milk, it is physically impossible to count them with absolute accuracy. Only **two methods of counting bacteria** in milk have ever been suggested and until recently only one of these has ever been generally used.

The method in general use is an indirect one which depends upon the ability of bacteria to grow on nutrient jellies composed largely of gelatin or agar, the latter being a sea-weed jelly from Japan. The growth of individual bacteria or groups of bacteria on these semi-transparent jellies produces spots which consist of masses of bacteria easily visible to the naked eye. The number of these masses of bacteria is assumed to be the same as the number of bacteria.

Attempts have been made for many years to count bacteria as seen under a microscope, but it is only within the past few years that a practical method for counting the bacteria in milk by

this means has been devised. Usually, only a small portion of a cubic centimeter of milk is examined by either method, the number of bacteria, or of spots on the gelatin or agar actually counted being rarely more than 500. The number so obtained is multiplied by the numerical factor necessary to transform the results to a cubic centimeter basis. A cubic centimeter contains about 15 drops of milk.

Inaccuracies of count. Anyone who has attempted to secure an accurate count of a large number of objects, small or large, will realize at once that it is unreasonable to expect methods of counting such tiny objects as bacteria to give absolutely accurate results. Such being the case, we must be prepared to find large discrepancies between bacterial counts made from duplicate samples of milk and such expectations are found to hold true in practice. The difficulties involved are different according to the method of counting used and will be briefly explained.

Plate count. Where the **plate method** of counting bacteria is used, certain things prevent an accurate count. They may be outlined as follows:

1. If large numbers of bacteria are present, it is necessary to measure out very small quantities of milk. The amount actually used varies between one-half of a cubic centimeter and one

ten millionth of a cubic centimeter. The method of measurement used for the smaller quantities is the dilution method whereby a definite quantity of milk is added to a measured quantity of sterile water. This is the most accurate method known of measuring small quantities of liquids, but it is evident that relatively large errors must occur where it is necessary to measure very small quantities of milk.

2. Not all bacteria present will grow on the nutrient jellies used. Those which do not grow are not counted in the final result. The size of the error due to this cause is unknown as no method has yet been devised by which it can be measured. Long experience has, however, made it probable that it is not ordinarily a large one where milk is the substance examined.

3. Some bacteria grow so fast on the nutrient jellies used that they obscure or prevent the growth of other bacteria. The error so caused may be a serious one in individual cases but is usually evident to the trained man and so is frequently seen and discounted.

4. A more constantly occuring and variable error is one which has always been recognized but has never been even approximately measured until recently. It is an error due to the fact that individual bacteria tend to cling together in masses. This tendency is more marked in some species than in others, some invariably growing in chains or clusters. Such

groups cannot be separated by shaking or by diluting with sterile water. Thus the colonies or masses of bacteria which originate on the nutrient jellies do not always arise from single individual bacteria but frequently from clusters of two to hundreds of individuals. Inasmuch as there is no way of distinguishing the colonies which arise from clusters of individuals, the count obtained from these plates does not represent the total number of bacteria present as is usually stated but really represents the number of individual groups of bacteria present, a group consisting of from one to hundreds of bacteria. Results secured at the Station by comparison with the microscopic method of counting bacteria show this error to be frequently much larger than 100 per ct. Yet because of the fact that the error seems to be a fairly constant one, plate counts can be compared among themselves.

5. Other less important errors creep in because of necessary manipulations.

Microscopic count.

When the bacteria are **counted by means of a microscope,** the difficulties of getting an accurate count are likewise great, whether greater than those involved in the plate count or not, is not yet known. Some of these difficulties are as follows:

1. It is never possible to examine a large amount of milk with a microscope so that the same difficuly arises here that arises in connec-

tion with the plate method; namely that of measuring small quantities of milk accurately. The method of measurement used is necessarily somewhat less accurate than the one used in the plate method so that a larger error is introduced into the count obtained by this method than is introduced in the similar way into the count obtained by the plate method. Duplicate counts on the same sample of milk show, however, that this error is not so large as to interfere seriously with the results. The amount of milk actually examined varies between one three thousandth and one five hundred thousandth of a cubic centimeter.

2. It is usually impossible to tell the difference between living and dead bacteria under a microscope. Where it is desired to make a count of living bacteria only the presence of dead bacteria causes an error of unknown size. Fortunately comparative counts with the plate method, which shows up living bacteria only, do not indicate that this is a large error where fresh milk is examined. In the case of pasteurized milk, this error is large and apparently uncontrollable. In those cases where the object of the count is to determine the total number of bacteria present whether living or dead, this difficulty disappears.

3. Where very few bacteria are present in milk, it is impossible to examine a sufficiently large quantity of milk to secure an accurate count.

4. Other less important errors creep in because of necessary manipulations.

Comparison between counts.

In spite of these difficulties, experience has shown that the plate method is sufficiently accurate to give results which may be compared with each other and which may serve as a satisfactory basis for interpreting the condition of a given sample of milk. **But it should be understood that the number given does not represent the total number of individual bacteria.** The microscopic count has not been sufficiently used to justify the same confidence in results, but the tests made at the Station and elsewhere, give reason to believe that it gives results which are at least relatively as accurate as those secured by the plate method and possibly more so. **In this case the final count gives the total number of individual bacteria,** and so is usually much higher than the plate count. The two counts cannot be directly compared.

Where use each method of counting.

In general it may be said that either count may be used on fresh milks. Where large numbers of samples are to be examined and the matter of expense is a consideration or where the information must be secured quickly to be of any service, the microscopical method of counting is the one to use. This method is especially applicable for the ex-

amination of samples of milk at a milk station where the bacterial count is made a basis for the payment of premiums to farmers. By its use milk can be readily and quickly classified into as many as three grades. With the plate method, the procedure is more elaborate and requires more time and equipment. Results are not available for 2 or 5 days, according to the time used in growing the bacteria on the nutrient jellies. Yet because of the fact that this is the more generally applicable method and because of the fact that it is the standard method of procedure, it must be used in the greater number of cases.