SOME OBSERVATIONS ON THE PLATE-COUNT METHOD OF ENUMERATING BACTERIA IN MILK

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A large number of both scientific and semi-popular papers have been written dealing with the plate-count method of enumerating the bacteria in milk. The greater number of these papers are derogatory to this method and consequently are tending to destroy confidence in it. Extensive personal experience with the plate-count method of enumeration has led the writer to believe that the severity of the criticisms offered is unwarranted.

It is desired in this paper to discuss certain possible sources of the “gross discrepancies” that have been reported in plate counts of milk and to demonstrate that careful technique is important and that a consideration of the principles involved in bacterial growth is essential where agreement in plate counts is desired.

Two types of experiments have commonly been used to demonstrate variations in plate counts on the same sample of milk. In one type the practice has been to pour a large number of plates, usually 100, from the same sample of milk; in the other, portions of the same sample have been plated by workers in separate laboratories. In both types of experiment the counts obtained were used as a basis for judging the accuracy and reliability of the plate-count method. Extremely wide variations have been reported. Wright and Thornton (1927), for example, in reporting upon data obtained on counts of 2,330 plates, found discrepancies as great as 1,500 per cent under conditions of “extreme care.” They state further that “a variation within a series, of less than 100 per cent, can not be depended upon.” Similar discrepancies

269
have been reported by workers employing the two methods above mentioned. It has been stated that these differences have been due to the limitations of the plate-count method.

The efficiency of any determinative method depends upon the skill and care with which it is carried out. It is difficult to understand how such variations in plate counts on the same sample of milk as have been repeatedly reported could occur if the technique employed were consistent with the accuracy required of the method. It has been found, however, that the technique used in many experiments was governed by such factors as the conditions of the experiments, laboratory conditions, laboratory methods, and practicability. The effect upon the plate counts of such factors as accuracy and cleanliness of glassware, care in manipulation, contamination, methods of incubation, and counting, has frequently been discussed. It seems well, however, to point out that when the reliability of a method is to be judged, every factor contributing to its accuracy should be given full consideration.

Where several workers are employed on one experiment, a situation common to both of the test methods under discussion, potential sources of error enter. Personal errors are incalculable. Personal idiosyncrasies, differences in technique, and individual interpretation of values, all contribute to irregular results. In the experiments where a number of workers are engaged independently and in different laboratories in making plate counts on the same sample of milk, there is in addition no coordination of time or exposure or even in the manner of handling the samples. Too little consideration has been given, in both experimental methods, to these sources of error.

Comparisons of plate counts with direct microscopic counts obtained by the Breed method have shown extremely wide variations. This is to be expected because counts obtained by the Breed method include both the living and the dead cells. While this is an elementary criticism, it is nevertheless sound and receives support from the work of Wilson (1922), who demonstrated that even in the logarithmic phase of growth, and under optimum conditions, the number of living cells rarely exceeds 90 per cent
of the total cell population. Other sources of error exist in the Breed method and are generally apparent, yet the Breed counts have frequently been used as a standard upon which to gauge the reliability of the plate-count method.

In making plate counts of milk living organisms are being dealt with. Sherman and Albus (1923) demonstrated that young bacterial cells were more susceptible to certain environmental hazards than were old and mature cells and suggested the possible effect of this susceptibility upon bacterial enumeration. For example, it was shown that exposure to heat or cold caused a marked mortality among growing cells, whereas similar exposures had little or no effect upon old or mature cells. The application of these findings to bacterial enumeration is readily apparent. A sample of milk in which bacteria are actively growing, if packed in ice for a period of time, shows a wide discrepancy in count when both the plate and the Breed methods are used. This discrepancy is due to the fact that many of the more sensitive or young cells are killed by the chilling. The temperature of the water blanks, the time of exposure therein, and the temperature of the agar, likewise have their effect upon young growing cells. A disregard for environmental hazards when making comparative counts is unfair to the plate method, and variable results must be expected.

Bacterial growth may cause wide variations in plate counts. The rapidity of growth of bacteria under favorable conditions has long been known, yet the possible effect of this factor upon plate counts appears generally to be ignored. The importance of a possible increase in the number of bacteria, during the plating process, is given recognition in "Standard Methods of Milk Analysis" (1927) which directs that the plating process is to be completed within fifteen minutes. The time consumed in the plating process is especially important when consistent plate counts are demanded. Considerable time must be consumed in making a large number of plates from the same sample, and a disagreement in count may be expected. The general disregard for such factors as time and growth can readily be appreciated when consideration is given to the manner of making plate counts.
which prevails in public-health and other laboratories where daily counts are made on a number of samples of milk.

**EXPERIMENTAL**

The following experiments are reported to demonstrate the possible effect on plate counts of the factors of time, temperature, and growth. Data representative of the results obtained in many experiments are presented in tables 1 and 2.

The procedure employed in the experiments reported was as follows:

**TABLE 1**

*The effect of time, temperature, and growth on the results obtained by the plate method on milk of low bacterial content*

<table>
<thead>
<tr>
<th>Original count</th>
<th>Plated Immediately</th>
<th>Agar Poured After 15 Minutes</th>
<th>Held in Dilution Bottle 15 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,300</td>
<td>5,900</td>
<td>13,200</td>
<td>16,600</td>
</tr>
<tr>
<td>5,900</td>
<td></td>
<td>13,500</td>
<td>16,000</td>
</tr>
<tr>
<td>Held at room temperature for 2 hours</td>
<td>10,000</td>
<td>9,000</td>
<td>15,400</td>
</tr>
<tr>
<td>Held in ice for 2 hours</td>
<td>17,700</td>
<td>17,800</td>
<td>23,800</td>
</tr>
<tr>
<td>Held at room temperature for 30 minutes</td>
<td></td>
<td></td>
<td>29,000</td>
</tr>
</tbody>
</table>

Platings were made of each sample of milk immediately after it was received. The sample was warmed to room temperature and held for two hours. At the end of this time the bacteria present in the milk had begun active multiplication, and a second plating was made. The sample was then packed in ice for two hours and at the end of this period a third plating was made. Following this the sample was again warmed to room temperature and held for thirty minutes after which a fourth plating was made.

Six plates were poured from the same dilution at each plating with the exception of the first plating shown in table 1. A measured amount was poured, by means of a pipette, into each of
METHOD OF ENUMERATING BACTERIA IN MILK

four plates at the same time. The agar was poured into two of these plates immediately and into the remaining two plates fifteen minutes later. Two plates were poured from the same dilution bottle after it had stood at room temperature for fifteen minutes. Thus there were three duplicate sets of plates made of each milk sample. An inspection of the tables will indicate more clearly the procedure outlined.

The following precautions were taken to obtain uniform results: Previous to sterilizing, all pipettes were washed in a cleansing

| TABLE 2 |
The effect of time, temperature, and growth on the results obtained by the plate method on milk of high bacterial content

<table>
<thead>
<tr>
<th></th>
<th>PLATED IMMEDIATELY</th>
<th>AGAR Poured After 15 Minutes</th>
<th>HELD IN DILUTION BOTTLE 15 MINUTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original count</td>
<td>6,000,000</td>
<td>6,200,000</td>
<td>7,700,000</td>
</tr>
<tr>
<td></td>
<td>5,400,000</td>
<td>7,500,000</td>
<td>6,000,000</td>
</tr>
<tr>
<td>Held at room temperature for 2 hours</td>
<td>28,000,000</td>
<td>35,700,000</td>
<td>35,800,000</td>
</tr>
<tr>
<td></td>
<td>28,100,000</td>
<td>34,000,000</td>
<td>35,600,000</td>
</tr>
<tr>
<td>Held in ice for 2 hours</td>
<td>31,800,000</td>
<td>30,800,000</td>
<td>33,400,000</td>
</tr>
<tr>
<td></td>
<td>30,000,000</td>
<td>29,200,000</td>
<td>33,900,000</td>
</tr>
<tr>
<td>Held at room temperature for 30 minutes</td>
<td>45,700,000</td>
<td>46,800,000</td>
<td>48,000,000</td>
</tr>
<tr>
<td></td>
<td>45,800,000</td>
<td>45,000,000</td>
<td>50,800,000</td>
</tr>
</tbody>
</table>

solution and thoroughly rinsed in water. Only one delivery was made from a single pipette, because it has been observed many times when plating whole milk, especially from the first dilution bottle, that a quantity of material clings to the inside of the pipette when a second delivery is attempted. This is a source of error which has probably been frequently overlooked when checking plate counts. For example, Prescott and Parker (1927), to preserve uniformity and accuracy, employed the same pipette in pouring 10 plates from an identical 1:100 dilution of milk, and in their summary call attention to "the inherent inaccuracy of any plate method of bacterial enumeration."
Dilutions were made in 99 cc. blanks of distilled water which were at room temperature. Standard beef extract agar was used and poured at a temperature of 42°C. After the plates were poured they were placed immediately on a cold plate until the agar had hardened and were subsequently removed before they could become chilled. All manipulations were carried out as quickly as possible and with the greatest care. The plates were incubated for two days at 37°C.

It will be observed from the tables that an appreciable increase in count resulted when the plates were held at room temperature for fifteen minutes before pouring the agar and that a still greater increase resulted from holding the dilution blanks at room temperature for fifteen minutes before completing the plating process. It is recognized that the increases in count may be due to a breaking up of groups or clumps of bacteria because of some unknown action resulting from their being suspended in distilled water—at the same time these increases are observed only when the bacteria are multiplying. In table 2 the first series of counts, which were made before active bacterial growth had begun in the sample, shows practically no increase in number when plates or the dilution blanks were held at room temperature for fifteen minutes. It is believed that lack of appreciation of such changes may be responsible, in a large measure, for the irregular counts often obtained by the plate method.

Table 1 shows clearly what takes place when a sample of milk in which the bacteria are growing is packed in ice. The number of living bacteria in the sample may be reduced materially by this procedure. While a temporary dormancy of the cells may be brought about by the chilling, it is also shown in the tables that when the sample has been held at laboratory temperature for thirty minutes, sufficient growth has taken place to affect the count materially and that the bacteria are again sensitive to the time and temperature hazards of the plating process.

It is to be noted from the data in both tables that the differences in counts due to the various factors employed are not of the same magnitude. This is to be expected when the difference in the bacterial content of the two samples is recognized. When the
bacterial content is low, growth proceeds rapidly and unimpeded, but when it is high, growth is hindered by the accumulated retarding factors that limit cell population. It must be apparent from the data presented in the tables that duplicate plate counts of the same sample of milk can be made that will agree so closely as to satisfy the most exacting bacteriologist, provided all possible sources of error are controlled to the limit permitted by the method.

There is no known method of accurately enumerating bacteria. It appears, however, that the comparability of plate counts and the reliability of the plate-count method of enumeration can be demonstrated, if the counts obtained by successive platings of a sample of milk in which the bacterial population is progressively

![Graph showing bacterial growth in raw milk.](http://jb.asm.org/)
changing give a smooth and characteristic curve when they are plotted.

Figure 1 represents a growth curve obtained by plotting, against time, the logarithms of the number of living bacteria as determined by the counts obtained on a quantity of raw milk held at 37°C. until curdled. The uniform character of the curve is submitted as evidence of the reliability of the plate-count method when it is carefully employed. The severity of the technique used in this experiment to determine the efficiency of the plate-count method can better be appreciated when consideration is given to the fact that the curve was obtained on the mixed bacterial flora of raw milk. A similar curve is easily obtainable when a pure culture is being plated.

As an aid to those who may be unfamiliar with a logarithmic curve, the actual plate counts are shown in the figure.

DISCUSSION

No method of accurately enumerating bacteria has yet been found. Accuracy in bacterial enumeration is highly improbable if not impossible. As far as the writer has been able to find, the claim that bacteriology is an exact science has been set up by those desiring to demolish it. Methods of bacterial enumeration are methods of approximation. The plate-count method when conscientiously employed by trained personnel can, and does, give consistent counts when the sources of all possible error are fully controlled. The looseness of the methods generally practiced in making plate counts would be unthinkable in any chemical analysis, yet analytical results are expected. The assertion that widely discrepant plate counts are due to errors inherent in the plate method is an admission rather than an argument. The methods used in carrying out the experiments on which this assertion is based have been unnecessarily involved. Needful precautions when preparing plates have not been observed, and additional sources of error have been introduced.

The data presented in this paper bear out the contention of the writer, based on extensive experience with the plate-count method,
that when proper care is exercised comparable plate counts can be obtained.

SUMMARY

1. It is pointed out that the experimental methods employed to obtain the data upon which plate counts of milk have been so severely criticized, permit errors that may be responsible for the resulting discrepant counts.

2. It is demonstrated that when a sample of milk in which bacteria are actively growing is packed in ice for a period of time, a number of the organisms are killed.

3. The time consumed in the plating process will markedly affect the accuracy of the plate counts if the bacteria are actively multiplying, especially in milks of relatively low bacterial content.

4. It is demonstrated that with the proper care and a consideration of all the factors involved consistent plate counts of milk can be made.

REFERENCES


