Comparing Three Methods for Counting Raw-Milk Pour Plates

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ABSTRACT

Six hundred Grade A raw milk samples were plated on Standard Methods agar and the pour-plate counts compared using three methods. Counts of each sample were determined after incubation at 26, 30 and 32 °C for 72 h with an automatic colony counter (ACC). Quebec colony counter and hand tally (HT) and Quebec colony counter and electronic probe with digital register (DT). No significant differences were found between the counts obtained with the HT and the DT for any of the three incubation temperatures. The ACC counts were lower (P < .01) than the manual counts at each temperature. The ACC counts were .39, .35 and .31 log counts lower than the manual count at 26, 30 and 32 °C, respectively. The ACC counts at 72 h were higher (P < .01) than the ACC counts at 48 h for all temperatures. The difference was about .41 log counts. The two manual counts were totally correlated, while the correlation between the ACC counts and the manual counts was .97. Regression equations were formulated for predicting the 72 h HT count from either the 48 or 72 h ACC count.

Instruments have been developed which automatically count and record the number of colonies on agar plates (2,3,8,9). Automatic colony counters (ACC) were developed to provide an accurate method of counting colonies in agar plates with a significant saving in time and labor. Apparently, most ACC can tolerate variations in agar color, agar depth, optical density, etc. with little detectable effect on counting efficiency (4,9).

A few studies have evaluated the acceptability of using the ACC for counting agar plates prepared from raw milk or food samples, or have compared the ACC results with those obtained using conventional counting methods (2,3,4,8,9,11).

The objective of this study was to compare the results obtained when raw-milk pour plates were counted using three counting methods, the ACC and two manual counting procedures. In addition, the ACC was used to determine differences in counts of agar plates incubated at three temperatures and counted after 48 and 72 h.

MATERIALS AND METHODS

Samples

Six hundred Grade A raw milk samples were analyzed during a 10-month period. Samples were collected by the milk truck driver from individual farm bulk tanks after addition of the fourth milking and just before emptying the tank. Samples were collected in Whirl Pak bags, refrigerated during transportation to the laboratory and held below 4 °C until analyzed.

Plating procedures

Counts were determined on Standard Methods agar (BBL) using the pour plate method (S). Triplicate plates were prepared per dilution plated and one plate of each dilution was randomly selected and incubated at 26, 30 or 32 °C for 72 ± 2 h.

Counting procedures

After incubation, the same person counted each plate using the three counting procedures described below. The automatic colony counter (ACC) used was the Fisher Bacterial Colony counter (Model 480) with a 11.5-cm Hitachi TV monitor. The counter was operated according to instructions furnished by the manufacturer. Based on preliminary studies using Standard Methods agar, the compensation setting was adjusted to give a 37% increase in the scanned plate count. This setting was found to give the maximum counts that were compatible with manual counts. Colonies were counted to a minimum resolution of 0.3 mm and a minimum diameter of 0.2 mm. Before the plates were counted, the bottoms were cleaned with a Kimwipe tissue soaked in 95% alcohol to remove markings, dirt and fingerprints. After each count, plates were rotated approximately 120° to obtain a different counting position, which resulted in three counts per plate. Each plate was counted using the ACC after 48 and 72 h of incubation.

The second counting method (hand tally) used a Quebec Colony Counter and a hand tally to register the count after 72 h of incubation.

The third counting method (digital touch) is based on the principle that when a grounded probe and counting probe both touch a conductive surface, a circuit is completed and a count is registered. The plate was placed on a Quebec Colony Counter, the lid removed and the grounded probe of the AO Automatic Register, Model QR-1 (American Optical Co.) adjusted to permit the arm to make contact with the agar medium. A second probe (counting probe) containing a needle point was moved from colony to colony and automatically registered a count each time the counting probe touched the agar surface. Plates were counted after 72 h of incubation.

Analysis of data

The three colony counts obtained per plate per incubation condition with the ACC were averaged arithmetically and one value used for the analysis. The Statistical Analysis System (SAS) programs were used to analyze the data (1). Duncan's multiple range test was used to determine if the mean counts obtained with the three counting methods differed significantly from each other.

RESULTS AND DISCUSSION

The Standard Plate Counts of the raw milk samples ranged from 20 x 10¹ to 71 x 10² per ml. The mean logarithmic aerobic counts obtained with the three temperatures are given in Table 1. The differences between the counts obtained with the two manual counting methods (Hand Tally and Digital Touch) were not significant for any of the incubation temperatures. The automatic colony counter (ACC) counts were lower.
TABLE 1. Effect of counting method on the mean count of raw milk samples after incubation for 72 h at 26, 30 and 32 C.

<table>
<thead>
<tr>
<th>Counting method</th>
<th>Incubation temperature (C)</th>
<th>Mean count[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td>ACC[a]</td>
<td>5.73[d]</td>
<td>5.62[d]</td>
</tr>
<tr>
<td>Hand tally</td>
<td>6.04[c]</td>
<td>5.97[e]</td>
</tr>
<tr>
<td>Digital touch</td>
<td>6.03[c]</td>
<td>5.97[e]</td>
</tr>
</tbody>
</table>

\[a\]Means of 1792 counts.
\[b\]Each value represents means of 598 counts.
\[c\]ACC = automatic colony counter.
\[d\]Means in columns with different superscripts differ significantly (P < .01).

(P < .01) than the manual counts at each temperature. The differences between the ACC and manual counts decreased with a decrease in incubation temperature. The ACC counts were .39, .35, and .31 log counts lower than the manual counts at 26, 30 and 32 C, respectively.

Several factors seem to be responsible for the differences between ACC and manual counts. Colonies with diameters less than 0.2 mm could not be counted by the ACC used in the study. In addition, colonies not separated by a distance of 0.3 mm were not recognized as individual colonies. The smaller differences in counts at the lower incubation temperatures indicate that a greater percentage of the microflora was able to grow and form colonies countable by the ACC and/or that the colonies formed were larger than at the higher temperature.

When the ACC is used to count pour plates, the portion of the petri dish that extends beyond the inner periphery of the stacking ring located on the bottom of the dish is screened off. The area beyond the stacking ring represents 18 to 20% of the total area of the petri dish. To compensate for the colonies located in this area and not counted directly by the ACC, the counter can be adjusted to automatically compensate for up to 33% of the total area lost because of the stacking ring. In this study, the colonies outside the ACC-viewed area were estimated by using a compensation setting of 33%. This compensation apparently was not sufficient to increase the ACC counts to correspond with the manual counts. Most of the colonies not counted by the ACC would be counted by manual counting methods. Many colonies excluded by the ACC tend to be common in aerobic pour plates prepared from raw milk, especially if incubated at 32 C.

Other investigators have reported differences between ACC and manual counts for raw milk samples to be less than those obtained in this study (2,3,8,11). Plates in other studies were manually screened, before counting, to exclude plates with spreaders and/or with colonies smaller than the resolution of the ACC. Plates were not screened in this study and all values obtained were included in the analysis. While most researchers have reported manual counts to be higher than those of the ACC, some have reported higher counts for ACC (2,11). Differences in results seem to be due to the extent that plates were screened before counting, differences in compensation setting, the resolution capacities of the counters and variations inherent in individual counters.

The mean aerobic count obtained with the Automatic Colony Counter for raw milk samples after 48 and 72 h of incubation at three temperatures are given in Table 2. Manual counts were not made at 48 h because the digital touch method destroyed the plates and prevented them from being incubated an additional 24 h. The mean ACC counts were higher (P < .01) after incubation for 72 h than after 48 h at all temperatures. The differences between the counts obtained at 48 and 72 h were similar for the three temperatures (about .41 log counts).

TABLE 2. Effect of incubation time on mean counts of raw milk samples obtained with automatic colony counter for plates incubated at three temperatures.

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>Incubation temperature (C)</th>
<th>Mean count[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td>48</td>
<td>5.32[c]</td>
<td>5.20[c]</td>
</tr>
<tr>
<td>72</td>
<td>5.73[d]</td>
<td>5.62[d]</td>
</tr>
</tbody>
</table>

\[a\]Means of 1792 counts.
\[b\]Each value represents means of 597 counts.
\[c\]ACC = automatic colony counter.
\[d\]Means in column with different superscripts differ significantly (P < .01).

The higher ACC counts obtained at 72 h were, in part, due to increases in colony size as a result of the longer incubation time. The larger colonies resulted in higher counts because the ACC could detect only those colonies with diameters greater than 0.2 mm as well as the tendency for the ACC to count the larger colonies more than once. The additional incubation time resulted in an increase in the number of colonies with a diameter greater than 0.2 mm. Other studies have shown counts to be higher after 72 h than after 48 h (6,7,12).

Correlation coefficients between the three counting methods combining the results for all incubation temperatures are given in Table 3. The two manual counting methods were totally correlated. The next highest correlation was between the ACC counts and the manual counts after 72 h of incubation (.97). A 0.85 correlation was obtained between the 48 and 72-h ACC counts. The lowest correlation (0.70) was between the ACC count at 48 h and the manual counts at 72 h.

Regression equations which can be used to predict the Hand Tally count at 72 h using either the 48 or 72-h ACC counts are given in Table 4. The regression equation using the 72-h counts is similar to that reported by LaGrange et al. (8). Similar values have been reported by other investigators (2,9), except with a negative intercept. These investigators either manually screened plates or used spread plates of pure cultures.

TABLE 3. Correlation coefficients between three methods used to count raw milk samples.

<table>
<thead>
<tr>
<th>Counting methods</th>
<th>Digital method (72 h)</th>
<th>ACC (48 h)[b]</th>
<th>ACC (72 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand tally (72 h)</td>
<td>1.00 [a]</td>
<td>-.70</td>
<td>.97</td>
</tr>
<tr>
<td>Digital method (72 h)</td>
<td>--</td>
<td>-.70</td>
<td>.97</td>
</tr>
<tr>
<td>ACC (48 h)</td>
<td>.70</td>
<td>--</td>
<td>.85</td>
</tr>
<tr>
<td>ACC (72 h)</td>
<td>.97</td>
<td>.85</td>
<td>--</td>
</tr>
</tbody>
</table>

\[a\]ACC = automatic colony counter.
\[b\]Incubation time.
TABLE 4. Regression equations predicting the 72-hour hand tally count from the 48-hour and 72-hour automatic colony counts for raw milk samples.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log_{10} 72 h hand tally count = 1.94 + 0.77 \times \text{Log}_{10} 48 h ACC^2 count</td>
<td>Predicts 72-hour hand tally count based on 48-hour and 72-hour automatic colony counts.</td>
</tr>
<tr>
<td>Log_{10} 72 h hand tally count = 0.34 + 1.00 \times \text{Log}_{10} 72 h ACC count</td>
<td>Same as above, but with a different intercept.</td>
</tr>
</tbody>
</table>

ACC = automatic colony counter.

The results of this study indicate that Automatic Colony Counters are suitable for counting pour plates from raw milk samples. The ACC seems to be more suitable for use in routine quality control than for use in regulatory work.

REFERENCES


Pennsylvania Association Accepts Abnormal Milk Policy

A “Recommended Abnormal Milk Policy” was prepared by a committee of the Pennsylvania Dairy Sanitarians Association and accepted late last year by the Executive Committee of that association.

Since that time over 450 copies of the policy have been distributed throughout Pennsylvania.

For additional information on the policy, contact: William J. Killough, Secretary-treasurer. Pennsylvania Dairy Sanitarians Association, RD1, Box 393, Conestoga, PA 17516.

Pennsylvania Dairy Sanitarians Association

Recommended Abnormal Milk Policy

The purpose of this policy is to assist dairymen to produce more milk of good quality. It should not be misunderstood as recommending changes in present regulatory standards.

Pennsylvania dairymen shall be notified of all abnormal milk test results. Dairymen shall be informed that a recheck shall be conducted within 14 days of the first abnormal milk count of 750,000 Somatic Cells per ml or greater. The second consecutive count of 750,000 Somatic Cells per ml or greater will be immediately followed by a farm visit by a Pennsylvania Approved Inspector. During this visit the following evaluations shall be made:

1. Herd Survey Sheet shall be completed (NDPC Guideline #18, page 77c).
2. An evaluation of the milking equipment shall be made.
3. An evaluation of the milking procedure shall be made.
4. A cow slide screening test may be conducted.
5. A discussion of the above evaluations shall be held with the dairymen as they relate to abnormal milk and Somatic Cells.
6. Recommendations as to corrective action to be taken shall be made.

If the Pennsylvania Approved Inspector determines that a milking machine dealer’s evaluation is needed for the milking system, forms shall be left with the dairymen for the dealer to complete. The dealer shall be contacted by the dairymen. A copy of the completed form will be returned to the Pennsylvania Approved Inspector.

If a veterinarian is needed to evaluate the herd health, forms shall be left with the dairymen for the veterinarian to complete. The veterinarian shall be contacted by the dairymen. A leucocyte level screening test (CMT or Somatic Cell Count) shall be conducted by the veterinarian. A copy of the completed form will be returned to the Pennsylvania Approved Inspector.

The purpose of this policy is to identify problem dairies before they reach leucocyte levels that require regulatory action or levels that may cause severe hardships to dairymen through loss of production and/or dairy animals. The purpose is not to reduce the regulatory levels of Somatic Cells.

Equivalent levels may be determined by laboratory curve comparing WMT with DMSCC, where applicable.

Osten elected President of Dairy Division of NASA

Orlowe M. Osten, Director, Dairy Industries Division, Minnesota Department of Agriculture, was elected President of the Dairy Division of the National Association of State Departments of Agriculture (NASDA) at the 21st Annual Meeting held in Branson, Missouri, July 23-26.

Other officers elected to lead the dairy organization, which includes membership in 41 states, were: President-Elect - Jess D. Sturm, Wyoming Dept. of Agriculture; Vice President - Jay Boosinger, Florida Dept. of Agriculture; Secy/Treas. - Alfred R. Place, New York Dept. of Agriculture.