Preparation and Use of Somatic Cell Count Samples (SCCS) for Comparison of Milk Somatic Cell Counting Methods

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ABSTRACT

Somatic cell count samples (SCCS) for use in comparison of milk somatic cell counting methods were prepared from the cell sediment deposited in a creamery milk separator. Bovine milk somatic cells were resuspended from the sediment, and serial cell dilutions were prepared in bronopol-preserved milk diluent. Over a 1-year period, sets of SCCS were prepared each month and sent to milk-testing laboratories in the U.S.A., Canada and Europe, and counted by the methods in use at those Laboratories: (a) direct microscopic somatic cell count (DMSCC), (b) Fossomatic counter and (c) Coulter counter. Cell counts were normalized to eliminate the effect of month to month variation in the cell content of the SCCS. Counts obtained by the three methods were similar, although Coulter counter results tended to be lower, and significantly lower (P<0.05) in SCCS with cell counts greater than 700,000 cells/ml than those counts by the other two methods. The effect of shipping on SCCS stability was assessed for SCCS samples sent to and returned from other laboratories, and counted by the Fossomatic method on their return. Counts were similar before and after shipping, except that results for SCCS with cell counts greater than 1,000,000 cells/ml were significantly higher (P<0.05) after their return.

Bovine milk somatic cell counts are indicative of the presence, and to some extent, of the degree of inflammation in the bovine mammary gland (1, 3, 4, 8). Because of the recent automation of cell counting methods, there has been growing interest in the use of bovine milk somatic cell counts as a monitor of general udder health and mastitis control practices.

It is becoming more important to compare somatic cell counts determined by different cell counting methods and by different milk testing laboratories. There is considerable disagreement about the accuracy of methods used for counting somatic cells in milk. Because there is no procedure to give ultimate assurance of cell counting accuracy, one approach is to compare results from common reference samples counted in different laboratories and by accepted somatic cell counting methods.

Heald (6) introduced a bovine milk somatic cell reference sample that could be prepared on a small scale to compare different major somatic cell counting methods. These reference samples were used to compare the cell counts of Coulter counter, direct microscopic somatic cell count (DMSCC), and Fossomatic methods commonly used in milk testing laboratories. Heald (6) also reported that the somatic cell reference samples were stable under usual conditions of shipping and storage, a feature that makes it possible to share somatic cell reference samples with other laboratories.

In order to share somatic cell reference samples with many laboratories, it was necessary to develop a simpler method to prepare reference samples in large quantities and improve the stability of the reference samples without altering the ability of the reference samples to be used in different counting methods. After experimenting with a number of modifications to Heald's procedure, a method was developed using raw bulk milk preserved with potassium dichromate (7). Somatic cells were separated by continuous centrifugation, then appropriate cell dilutions were prepared with the dichromate-preserved skim milk. Recovery of somatic cells from bulk tank milk by continuous centrifugation was low and variable (20 to 40%) (7), making it necessary to handle large volumes of bulk tank milk. Additionally, despite efforts to remove much of the cream before continuous centrifugation, the remaining milk fat tended to clog the centrifuge rotors, seriously decreasing the recovery of somatic cells.

The purpose of this study was to refine the procedure for somatic cell reference sample preparation and to compare cell counts obtained in different laboratories and by different counting methods from twelve different lots of somatic cell reference samples shipped on a regular basis to milk testing laboratories in the U.S.A., Canada and Europe.

MATERIALS AND METHODS

The milk somatic cell reference samples prepared in this laboratory are referred to as somatic cell count samples (SCCS). The preparation of SCCS requires a concentrated suspension of bovine milk somatic cells from which a series of cell dilutions can be prepared using an essentially cell-free diluent. In this study, the source of the bovine milk somatic cells was the sediment of a milk separator at the Pennsylvania State University.
Experiment 1

Over a 12-month period, an SCCS lot was prepared each month. From each lot, randomly selected SCCS of each cell concentration were tested for bacterial contamination and counted (7) in this laboratory. After shipment by commercial carriers to cooperating laboratories in the U.S.A., Canada and Europe, the somatic cell counts of each SCCS lot were determined by the methods in routine use in these laboratories. Because the absolute somatic cell count varied among the twelve different SCCS lots prepared throughout the year, it was necessary to mathematically normalize the somatic cell counts to compare the results among SCCS lots.

The average somatic cell counts for each sample of each lot as counted by each method were calculated. These values were normalized by expressing them as a percentage of the average count by all methods of SCCS sample D for that lot according to the equation:

\[
\text{Normalized value (\%)} = \frac{\text{Cell count, Lot x, Method y}}{\text{Average sample D, Lot x}} \times 100
\]

For each method and lot, the expected value for SCCS sample D was 100%, for SCCS sample C 50%, and for SCCS sample B 25%. The normalized count for the SCCS sample A or diluent with no added somatic cells, was expected to be less than 10%.

Experiment 2

To test the stability of SCCS under most possible conditions of shipping, extra sets from four SCCS lots were sent to 20 randomly selected laboratories, then returned to this laboratory via commercial carriers. After their return, the SCCS were cultured to check for bacterial contamination (7) and cell counts were determined by the Fossomatic cell counter. To compare the somatic cell count results from the four different SCCS lots, the somatic cell counts were normalized as described above, by expressing them as a percentage of the average of all counts of the SCCS sample D for each lot.

The normalized data from both Experiments 1 and 2 were analyzed statistically by least squares analysis of variance (9). The model for Experiment 1 included effects of lot and cell counting methods, whereas the model for Experiment 2 included effects of lot and time (before or after shipping). Lot was considered a random effect in both experiments. Scheffe's multiple-comparison confidence intervals (9) were determined to test pairs of individual means. A P value of 0.05 was set to discern statistically significant differences. Statistical analysis systems (SAS; SAS Institute, Cary, NC) available at the Pennsylvania State University Computation Center was used for analysis of these data. After completing the statistical analysis, the data were converted back to somatic cell concentrations (cells/ml).

RESULTS

Experiment 1

The least squares mean and the standard error of the mean of the cell counts for each of the counting methods within laboratories over the entire 12-month period are summarized in Table 1. These data include cell counts on 561 sets of SCCS; of these 233 were by Coulter counter, 102 by DMSCC and 226 by Fossomatic counter. Results obtained by the different methods were similar, although Coulter counter results for SCCS sample D were significantly (P<0.05), lower than for the other two methods. The somatic cell counts for each of the counting methods are plotted in Figure 1. The consistent tendency of Coulter counter results from SCCS sample D to be lower than those of the other two methods is evident in this figure. The standard error of the mean, a measure of the variability of the sample mean between laboratories, is reported in Table 2. No bacterial contamination was detected on culture of SCCS randomly selected from each of the twelve monthly lots.

Experiment 2

The least squares mean and the standard error of the mean of the SCCS cell counts before and after shipment to and from cooperating laboratories are summarized in Table 3. Counts before shipment were determined by the Fossomatic counter on 80 randomly selected sets of
TABLE 2. The standard error of the mean (cells/ml) among laboratories.

<table>
<thead>
<tr>
<th>SCCS</th>
<th>Standard error of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6752</td>
</tr>
<tr>
<td>B</td>
<td>10520</td>
</tr>
<tr>
<td>C</td>
<td>15803</td>
</tr>
<tr>
<td>D</td>
<td>27154</td>
</tr>
</tbody>
</table>

TABLE 3. Fossomatic cell counts (cells/ml) of SCCS from this laboratory before shipment to and after receipt from cooperating laboratories.

<table>
<thead>
<tr>
<th>SCCS</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>92590 ± 4639b</td>
<td>91871 ± 4385b</td>
</tr>
<tr>
<td>B</td>
<td>321426 ± 4765c</td>
<td>310894 ± 6521c</td>
</tr>
<tr>
<td>C</td>
<td>680133 ± 6245d</td>
<td>699788 ± 7275d</td>
</tr>
<tr>
<td>D</td>
<td>1212200 ± 8029e</td>
<td>1321130 ± 13042e</td>
</tr>
</tbody>
</table>

*Least squares mean plus and minus the standard error of the mean.

b,c,d,e Least squares means with a common superscript are not significantly different, (P < 0.05).

cell counts obtained by three counting methods were similar for A, B and C SCCS samples; however, Coulter counter results from SCCS sample D with cell concentrations above 700,000 cells/ml were lower than those of DMSCC and the Fossomatic counter. Whether this disparity would also be evident in milk prepared by other methods or whether it is peculiar to SCCS prepared as described, is unknown. This question will be studied in more detail in another experiment. In this study, the error variance of cell counts from different cell counting methods between different SCCS dilutions is not constant but varies in a systematic fashion, i.e., as the SCCS cell concentration increases the error variance increases. A logarithmic transformation is one way to deal with heterogeneity of variance and improve the precision of hypothesis tests. However, we believe that the important comparison of cell counts is between cell counting methods within each SCCS dilution. The error variance for the different cell counting methods within each SCCS dilution is constant. Therefore, we chose to do a separate analysis of variance for each SCCS dilution. The model included effects of cell counting method and lot. We have analyzed the data using a logarithmic (log_{10}) transformation, and the analysis yielded the same results. The standard error of the means or the standard deviation of the means was computed by the method described by Snedecor and Cochran (9). The least squares mean of the normalized somatic cell counts was first calculated for each SCCS dilution, cell counting method and lot. The standard error of the mean was then calculated for each SCCS dilution and method.

A limitation of the design of this study was the variation of the number and types of cell counting methods used by the cooperating laboratories to determine the SCCS cell counts from month to month. For example,
one month a laboratory may have reported results of DMSCC done by three technicians and two Fossomatics cell counters, then the next month the laboratory may have reported results from only one technician and one Fossomatic cell counter. Also, the cooperating laboratories did not have the same types of milk somatic cell counting methods. In no case were technicians or machines identified because laboratories experienced a change in personnel and added or removed electronic cell counting machines throughout the study. These circumstances do not compromise the main objective of this study, which was to prepare and use a common reference sample to compare bovine milk somatic cell counts among and within different laboratories. The component of variability of particular interest was the variability of the sample means among laboratories. Because the response we received from laboratories was highly variable from month to month, the standard errors were calculated (9) to estimate the variability among laboratories for each SCCS dilution. First, the least squares mean of the normalized somatic cell counts was calculated for each SCCS dilution and laboratory. Then, the standard error of the mean was calculated for each SCCS dilution. This measure of variation represents differences between laboratories and primarily represents differences in cell counting methods, sample handling and technicians. The standard error of the mean provides a weighted measure of the variance, which we believe provides a more accurate estimate of variance because of the unbalanced nature of the data.

The SCCS appear to be relatively stable under most conditions of shipping. After shipment by commercial carriers to and from cooperating laboratories, Fossomatic somatic cell counts of SCCS samples A, B and C were not significantly different from those of SCCS which had not been shipped. Counts of SCCS sample D (> 1,000,000 cells/ml) were significantly higher after shipment than before. However, this difference was small, ca. 48,000 cells/ml, and is of little practical concern.

SCCS prepared as described can be easily produced in large quantities for sharing with many laboratories. Their stability during shipping and storage, and their suitability for use with the commonly used counting methods, make them useful as reference samples. The use of SCCS can help milk testing laboratories identify and correct problems to improve their accuracy and precision of quantitating somatic cells in bovine milk.

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REFERENCES