SOMATIC CELL REFERENCE SAMPLES FOR CALIBRATION
OF MILK SOMATIC CELL COUNTING METHODS

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(Received for publication July 12, 1983)

ABSTRACT

Somatic cell count samples (SCCS) for use in calibration of milk somatic cell counting methods were prepared from raw bulk milk preserved with potassium dichromate. Somatic cells were separated by centrifugation, then appropriate cell dilutions were prepared in the dichromate-preserved skim milk. Somatic cell counts from SCCS stored at 4°C were stable over a 23-wk period. No bacterial contamination was detected in these samples. In a collaborative study among eight laboratories, SCCS were not affected by usual conditions by shipping. The SCCS can be used as reference standards for the direct microscopic somatic cell count and the Fossomatic and Coulter Counter somatic cell counting methods.

Recent increased interest in milk somatic cell counts, generated in part by the availability of cell counting as a service of many Dairy Herd Improvement (DHI) programs, has increased the need for accuracy and uniformity of somatic cell counts among laboratories. One means to achieve this is to compare results of common reference samples counted by different methods and in different laboratories.

Several methods for preparation, fixation and storage of somatic cell standards have been proposed (3,5,6). In one of these methods, described originally by Heald (5), somatic cells were separated by centrifugation from dichromate-preserved milk of an individual cow and then diluted in preserved skim milk to appropriate concentrations. After heating to 100°C to reduce bacterial contamination, such samples were suitable for use with either particle counting (Coulter Counter, Coulter Electronics, Hialeah, FL) or fluoro-opto-electronic (Fossomatic, Foss Electric, Hillerod, Denmark) instruments and could be shipped successfully over long distances.

The purpose of the present work was to: (a) modify the original preparation procedure to permit production of larger numbers of samples, (b) characterize the stability of the samples during storage, and (c) compare counts obtained from a set of reference samples after shipment to several laboratories using different counting methods.

MATERIALS AND METHODS

In these studies, the source of milk somatic cells was bulk tank milk rather than milk with a moderately elevated cell count from an individual cow (5). As in the original procedure, potassium dichromate was added to a final concentration of 680 mg/L and mixed thoroughly. The milk was then heated with continuous agitation to 53°C, then stored at 4°C. After overnight refrigeration, the cream layer was removed by skimming. Cells were separated from the skim milk by centrifugation at 5000 x g at 4°C. Some of the supernatant skim milk collected after centrifugation was saved for subsequent use in making cell dilutions. The concentrated cells were diluted in a small volume of skim milk and the cell concentration was estimated with a Fossomatic cell counter. Cells were then diluted with skim milk to make 3 or 4 suspensions of cells at various concentrations. These suspensions were divided into 12-ml volumes in 16 x 100-mm screw-cap polypropylene tubes. Somatic cell counts of randomly selected tubes of each concentration were determined by the direct microscopic somatic cell count (1,2) and Fossomatic (1,2) methods. The remaining samples were then heated to 100°C for 1 min in an autoclave. After cooling, randomly selected samples of each cell concentration were chosen for verification of somatic cell counts by direct microscopic somatic cell count (DMSCC) and Fossomatic methods, and for examination for bacterial contamination as described below. The remaining samples, now designated somatic cell count samples (SCCS), were stored at 4°C until used or shipped.

Somatic cell count methods

DMSCC performed in our laboratory were done by the standard method (1,2). For each sample, two circular 1-cm² films were prepared and stained, and the cells in a horizontal and vertical strip of each film were counted. Fossomatic counts from our laboratory were done using prescribed procedures (1,2) on instruments in the Central Milk Testing Laboratory of the Pennsylvania Dairy Herd Improvement Association, University Park, PA.

Determination of bacterial contamination

At the time of preparation, randomly selected SCCS of each cell concentration were tested for the presence of bacterial contaminants. Also, as stored samples were needed for use, they were similarly tested. This was accomplished by streaking 0.5 ml of each sample on the surface of Trypticase soy agar (Difco) containing 5% washed bovine erythrocytes in a 100 x 15-mm petri plate. Plates were incubated aerobically at 37°C and examined for bacterial colonies after 24 and 48 h of incubation.

SCCS stability at 4°C (Experiment 1)

A batch of SCCS was prepared and stored as described above. At weekly intervals for 23 wk, SCCS of low, medium and high cell concentrations were selected at random, tested for bacterial contamination, and somatic cell counts determined by DMSCC and Fossomatic methods. In this experiment, DMSCC were determined by two techni-
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icians, each counting the same milk films; the count made by each technician was considered an individual determination. Fossomatic counts were done on each of the four instruments at the Central Milk Testing Laboratory. Each sample was counted three times on each instrument; the count from each instrument was the mean of these three counts. The mean count from each instrument was considered a separate determination.

Collaborative study of SCCS (Experiment 2)

A second batch of SCCS, that included standards at four cell concentrations, was prepared. Sets of these SCCS were counted in this laboratory and, after shipment by commercial carriers, in seven cooperating laboratories by the methods in routine use in these laboratories. As some laboratories counted the standards by more than one method, there were available for final analysis four DMSCC, three Coulter Counter and six Fossomatic counts for most SCCS. Least squares means of counts for each SCCS were calculated (7), and differences between means were determined by Student's t test (7). Statistical Analysis Systems (SAS Institute, Cary, NC) GLM procedure (4), available at The Pennsylvania State University Computation Center, was used to do the statistical analysis of these data.

RESULTS

Experiment 1

Randomly selected SCCS of low, medium and high cell concentrations were removed from 4°C storage at weekly intervals for 23 wk. Mean cell counts are shown in Figure 1. Most values plotted are means of two DMSCC counts by two different technicians and four Fossomatic counts, each from a different instrument. However, of the 69 means plotted, 11 include only 1 DMSCC and 13 include only 3 Fossomatic counts. Over the experimental period, mean weekly counts of the low SCCS ranged from 412 to \(487 \times 10^3\) cells/ml; the mean was \(454 \times 10^3\) and the standard deviation was \(51 \times 10^3\). Counts of the medium SCCS ranged from \(550 \text{ to } 797 \times 10^3\) cells/ml, with a mean of \(687 \times 10^3\) and standard deviation of \(78 \times 10^3\). The high reference samples had weekly counts ranging from 1,013 to 1,239 \(\times 10^3\) cells/ml, with a mean of \(1,148 \times 10^3\) and a standard deviation of \(98 \times 10^3\). No upward or downward trend in counts suggestive of deterioration of the SCCS was evident. On culture of SCCS, no bacterial contamination was detected.

Cell counts by the DMSCC and Fossomatic methods are given in Table 1. Mean counts for each of the SCCS samples were similar for the two methods. However, variation in the counts, as indicated by ranges and standard deviations, was greater for the DMSCC than for the Fossomatic.

Experiment 2

Results of the collaborative study of Experiment 2 are given in Table 2. The set of SCCS used in this study included four samples designated as high, medium high, medium low and low. As only one of three laboratories...
with a Coulter Counter provided a count for the high SCCS, this result was not included in the analysis. Mean counts obtained by the different counting methods were not significantly different for any of the SCCS samples. These results indicate that the SCCS may be used in all three counting methods and that they are not affected by conditions of shipping likely to be encountered.

DISCUSSION

SCCS prepared by separation of cells from bulk milk by continuous centrifugation are stable for up to 23 wk when stored at 4°C. They appear to be useful in comparing cell counts determined by both microscopic and commonly used electronic counting methods.

Modifications of the original methods of preparation (3,5,6) included use of bulk tank milk as a source of cells rather than milk with a moderately elevated cell count from an individual cow. Bulk tank milk may have a relatively low cell count and recovery of cells by continuous centrifugation is low and variable (20 to 40%) as compared to the recovery obtained in the original procedure. However, the ready availability of large volumes of bulk tank milk prevents these factors from being serious problems. One difficulty encountered was the tendency of fat from whole milk to clog the rotor head during continuous centrifugation; this was prevented by removing the separated cream after overnight refrigeration before centrifugation.

The experiment was not designed to test the stability of SCCS under all possible conditions of shipping. However, after shipment by air or combined surface-air routings that required up to 15 d for delivery, there was no evidence of deterioration of the SCCS.

ACKNOWLEDGMENTS

We thank R. J. Harmon and Roy Ginn for their technical assistance. We are also grateful for the cooperation of the laboratories that participated in the collaborative study.

Published as paper 6720 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

REFERENCES


