Comparison of the Automated with the Semi-Automatic Coulter Counter Method and the Direct Microscopic Somatic Cell Count (DMSCC) on Raw Milk Samples

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(Received for publication November 9, 1978)

ABSTRACT

The automatic Milk Cell Counter (MCC) and semi-automatic electronic cell counter (ESCC) of Coulter Electronics were compared with each other and with the direct microscopic cell count (DMSCC) on raw milk samples with various cell counts. The average DMSCC count on 241 samples of milk with Wisconsin Mastitis Test (WMT) results of 22 mm and higher was 55,000 cells/ml above the average MCC count when calibrated to a 4.4-μm minimum particle diameter. This difference is statistically significant at the 1% level. On 24 different raw milk samples of widely varying somatic cell count analyzed in replicate six times per sample, the standard deviations for replicate samples were 34,300, 34,900, and 136,000 for the MCC, ESCC and DMSCC, respectively. For these tests, the MCC had been calibrated to a 4.3-μm minimum particle diameter. The average difference between counts by the MCC and ESCC methods was only 6080/ml, but this was statistically significant at the 5% level. The average MCC count with the equipment set at 4.3-μm minimum particle diameter was 58,000 above the average DMSCC count.

Coulter Electronics Limited has developed a fully automated device capable of analysing milk samples for somatic cell count at a rate of about 210 samples per hour. Previous work (2-6, 8, 9) has shown a similar though semi-automatic method to yield acceptable results for this kind of analysis. While other automated procedures have been developed for this purpose, the Coulter Counter offers the advantage of a calibration technique based upon particle size. It can also be calibrated against a reference test such as the Direct Microscopic Somatic Cell Count (DMSCC). As a particle counter, the automated Milk Cell Counter (MCC) offers some advantage in speed over the semi-automatic counting device (ESCC). The procedures for making the two analyses are identical except for one specific step. The MCC would thus be expected to yield essentially the same results as the ESCC. This work was done to establish the validity of this concept.

MATERIALS AND METHODS

Standardization of Coulter Counters

A semi-automatic Model ZP Coulter Counter (Coulter Electronics, Hialeah, Florida) has been in use in the Dairy Quality Control Institute, Inc. Laboratory for some time. This device was standardized using a Size Distribution Analyzer and latex particles of 3.40 μm diameter. This technique is explained in a previous paper (4).

The fully automatic Coulter Electronics Milk Cell Counter (MCC) was standardized on organic chicken blood cells (Coulter Electronics, Hialeah, Florida 33010) using the half-count procedure suggested by the manufacturer. Average size of these cells was 4.71 μm in diameter. In preliminary work the lower threshold setting, below which particles will not be counted, was fixed at 4.4 μm in diameter. Results of 241 determinations checked against the Direct Microscopic Somatic Cell Count suggested that the equipment was biased on the low side. To bring results into better agreement with the DMSCC, the equipment was re-calibrated to count particles larger than 4.3 μm in diameter.

The MCC method can be calibrated either on standard particles of matter or against counts of a reference standard such as the DMSCC. Use of particulate matter provides a way of avoiding errors inherent in the reference standard procedure. This is important when the reference standard is less precise than the test method, which appears to be the case with the DMSCC.

The ESCC procedure used in this study was that given in reference (5). The MCC method is capable of making 210 determinations per hour. Up to 50 samples can be accepted at one time on a rotating sample rack. Though done automatically, the basic steps in the MCC analysis are almost the same as in the ESCC procedure. While on the rotating rack, milk samples are stirred, an aliquot is automatically removed, then diluted 1:100 with Somaton fixative (Coulter Electronics, Hialeah, Florida), and dispensed into a reaction tube. Reaction tubes also progress through the counting process in a rotating handling rack. During this time samples are heated to 80 °C in a polyethylene glycol bath. Duration of heating is 10 min. At this point the MCC and ESCC methods vary. The ESCC samples are cooled to room temperature before counting to avoid physical changes in the cells. The MCC counting takes place immediately following heat treatment; therefore cooling is not necessary.

The MCC is programmed to count a diluted sample every 17 sec. The machine takes 0.3 ml of diluted sample, divides the results by three, corrects for coincidence, subtracts the background count, and prints results on a teleprinter.

The method of making DMSCC determinations was the single strip technique as described in Standard Methods for the Examination of Dairy Products (1). Preliminary test

In a preliminary trial extending over a 7-week period, 3,362 raw milk samples were analyzed with the MCC. Samples were taken from the ongoing operation of Dairy Quality Control Institute, Inc. In addition, all samples having a Wisconsin Mastitis Test value of 22 or higher were analyzed by DMSCC, and results compared with MCC analyses. A total of 241 samples were thus compared. Based upon results of this study, both the MCC and ESCC were re-standardized to count particles (somatic cells) of 4.3 μm and larger in diameter.
RESULTS AND DISCUSSION

In the preliminary trial in which 241 samples of raw milk were analyzed by both the MCC and DMSCC methods, the average difference between the counts by the two methods was 55,000 cells/ml. A t-test showed that difference to be significant at the 1% level. For this reason the electronic devices were re-standardized to a 4.3-μm minimum diameter.

Data in Table 1 show mean and grand mean results of somatic cell counts made on 24 samples analyzed in replicate six times by MCC and ESCC methods, and four times by DMSCC (once each by each four technicians). These data show good agreement between the three methods over a wide range of somatic cell counts. Grand means were 554,000, 560,000 and 496,000 for the MCC, ESCC and DMSCC methods, respectively. Standard deviations were 34,300, 34,900 and 136,000, respectively.

The average difference between the MCC and ESCC methods was 6080. An analysis of variance indicated that this difference was significant at the 5% level, but only because the variance within replicates was so small; i.e., 1,200. When DMSCC, MCC and ESCC results were compared, the method, sample and interactions between them were all found to be highly significant at the 0.1% level. The difference between the electronic count means and the DMSCC mean is of the same magnitude, but of opposite sign from the previous test. Thus 4.4- and 4.3-μm particle sizes bracket the optimum value for standardization of electronic devices.

 Except for automation and a single step in the analysis, the MCC and ESCC methods are identical. Results by the two methods were expected to agree rather well, and this work validates that hypothesis. The observed differences are of small magnitude. The data also seemed to suggest that better agreement between MCC, ESCC and DMSCC will be achieved when the electronic devices are standardized at a 4.35-μm lower threshold value.

A collaborative study of the ESCC method has been previously reported (4). The procedure used in the study was recommended for approval at the International Dairy Federation meeting in June of 1978.

Because of the similarity in the ESCC and MCC methods, the good agreement between the two methods observed in this investigation, and the recommended approval of the ESCC method, the authors wish to urge official adoption of the MCC procedure.

REFERENCES


