COMPARISON OF METHODS FOR ESTIMATING SOMATIC CELL LEVELS IN BULK MILK[1]

GILBERT E. WARD AND DAVID T. BERMAN

Department of Veterinary Science
University of Wisconsin
Madison, Wisconsin 53706

(Received for publication April 14, 1971)

ABSTRACT

Various methods of estimating somatic cell levels in milk were compared with the direct microscopic somatic cell count (DMSCC). The electronic cell count (ECC) using centrifugation to separate cells from milk yielded correlation coefficients of 0.870 to 0.907 when compared to DMSCC. The Wisconsin mastitis test (WMT) yielded correlation coefficients of 0.714 to 0.753 when compared to the DMSCC. The diphenylamine filter-DNA method yielded correlation coefficients of 0.988 to 0.991 when compared to the DMSCC. The filter-DNA method was modified by using indole instead of diphenylamine to obtain color. This method reduced the time for color development from 16 hr to 10 min. The correlation coefficients were 0.987 to 0.990 using this method when compared to the DMSCC.

The purpose of this study was a comparison of various methods of estimating somatic cell levels in milk and to compare them with the direct microscopic somatic cell count (DMSCC). When the work was initiated, three methods seemed promising: Wisconsin mastitis test (WMT), electronic cell count (ECC), and DNA-filter method.

Development of the DMSCC *(1)* has provided an accurate method to estimate somatic cells in milk. In our experience it is laborious, requires careful laboratory technic to obtain repeatable results, and does not lend itself to inter-laboratory comparisons.

Reports on the ECC indicate that high correlations with the DMSCC can be obtained *(7, 8, 9, 10)*. A conventional centrifugation method was used *(3)*.

The WMT is a rapid test and has yielded highly significant correlations with the DMSCC *(10)*. It was included in the trial without modification.

The DNA-filter method has yielded highly significant correlations with the DMSCC *(4)*. However, it requires 16 hr for color development. By use of indole this time was reduced to 10 min. Two trials with each of two methods (indole and diphenylamine) were compared in this study.

[1]Research supported by the College of Agricultural and Life Sciences and by PHS Research Grant UI00124 from the National Center for Urban and Industrial Health. Paper No. NS 715.

MATERIALS AND METHODS[2]

DMSCC was performed according to the method of the sub-committee on screening tests of the National Mastitis Council *(1)*.

ECC. The instrument used in these determinations was a Celloscope. Paper mulberry pollen was used to establish the volume of particles counted at various threshold settings. The diameter of the pollen was determined using a carbon arc projector. Pollen particles (1000) were measured on a projection screen (1 inch on the screen was equal to 0.01 mm diameter). The median value was 13 μ. The machine was calibrated each day using the half count method.

Milk was prepared for use by centrifugation for 1 hr at 280 *g* in 0.85% NaCl which had been filtered using a 0.65μ membrane filter. Cells were suspended using a vortex mixer. The final dilution was 1:25. This yielded a working factor of 10,000 (25 × 64/0.164). All counts were made at 6.0 μ, 6.6 μ and 7.1 μ diameter settings. All milk samples were prepared and counted in duplicate. Corrections were not made for coincidence.

DNA-filter method. The diphenylamine filter method is described elsewhere *(4)*. This method was performed in duplicate on all samples.

In addition, indole was used to develop color as a rapid method. Milk (5 ml) was filtered as in the diphenylamine procedure *(4)*. The filter was placed in a capped tube of 5 ml of a solution of 1 part 5N HCl, 1 part 0.06% indole, 2 parts 1% saline *(2)*. This tube was placed in boiling water bath for 10 min then cooled in tap water. Absorbency was determined at 490 nm using a Spectronic 20 spectrophotometer.

WMT. The Wisconsin mastitis test was performed as described previously *(10)*.

Size of trials. The number of samples tested was limited to 5 to 10 per day, with two such trials per week. Batches of ingredients were made in rather small amounts. Therefore, in a random fashion, reagents were aged or made-up as needed. A precaution observed in this regard was that the indole solution and 1% saline solution was stored in the refrigerator (4 C).

RESULTS

Each method except DMSCC was performed in duplicate (trials 1 and 2). All possible correlation coefficients for each method and each trial are presented in Table 1.

Regression equations with standard errors for in-

[2]Equipment: Celloscope, Particle Data, Inc., Elmhurst, Illinois. Spectronic 20, Bausch and Lomb, 820 Linden Avenue, Rochester, New York 14625.

TABLE 1. SIMPLE CORRELATION COEFFICIENTS.

		Filter-DNA				WMT		Electronic count					
		Indole		Diphenyl-amine				Centrifugal method					
	Trial number:	1	2	1	2	1	2	1	1	1	2	2	2
	Diameter:							6.0 μ	6.6 μ	7.1 μ	6.0 μ	6.6 μ	7.1 μ
DMSCC		.987	.990	.991	.983	.714	.753	.904	.905	.907	.870	.873	.871
Filter DNA	Indole		.993	.989	.989	.668	.716	.884	.882	.884	.853	.853	.852
				.992	.990	.674	.717	.886	.885	.885	.856	.859	.856
	Diphenylamine				.994	.677	.722	.905	.906	.908	.875	.876	.875
						.687	.728	.904	.902	.904	.863	.864	.862
WMT							.965	.686	.692	.692	.690	.697	.701
								.717	.721	.721	.739	.743	.745
	Diameter												
Electronic count (Centrifugal)	6.0 μ								.998	.997	.929	.933	.936
	6.6 μ									.999	.931	.935	.938
	7.1 μ										.933	.937	.940
	6.0 μ											.998	.997
	6.6 μ												.999

TABLE 2. REGRESSIONS

Indole filter - DNA

$\overline{\text{DMSCC} \times 10^{-6}} = 4.34 \times \text{O.D.} + 0.042$

O. D. at 490 nm in 1.25 cm cuvette Std. error B = 0.074

Diphenylamine filter - DNA

$\overline{\text{DMSCC} \times 10^{-6}} = 5.49 \times \text{O.D.} - 0.060$

O. D. at 600 nm in 1.9 cm cuvette Std. error B = 0.089

Electronic cell count

$\text{DMSCC} \times 10^{-4} = 2.72 \times \text{ECC} - 54.2$ (diameter = 6.0 μ) Std. error B = .156

$\text{DMSCC} \times 10^{-4} = 2.82 \times \text{ECC} - 48.1$ (diameter = 6.6 μ) Std. error B = .161

$\text{DMSCC} \times 10^{-4} = 2.87 \times \text{ECC} - 41.4$ (diameter = 7.1 μ) Std. error B = .167

dole and diphenylamine filter-DNA, and ECC centrifugal methods are presented in Table 2.

DISCUSSION

The filter-DNA method yielded the highest correlation coefficients with the DMSCC (0.987 - 0.991) of any of the methods. The correlation coefficient between filter-DNA trials was 0.989 to 0.994. These trials were separate from initial measurement of sample to reading on the spectrophotometer. Correlation coefficients of filter-DNA methods with WMT and ECC were nearly as high as the DMSCC with these methods. Thus, one of the filter DNA methods could probably serve as a confirmatory or a single test for somatic cell estimation without the need to conduct other tests. The method with 2 deoxy-D-ribose as a standard has the added advantage of permitting comparison between trials and among laboratories. Preliminary data from work in progress indicate that storage of milk at 4 C is adequate for preservation of samples for 4 days.

The centrifugal ECC yielded correlation coefficients of 0.870 to 0.907 with the DMSCC. Preparations of samples counted at different diameter

settings yielded correlation coefficients of 0.999 to 0.997 (within trials). However, samples prepared in duplicate (from dilution thru centrifugation yielded correlation coefficients of 0.929 to 0.938 (between trials).

These results indicate that sample preparation is important in determining correlation coefficients. Regression equations were different (Table 2) with different diameter settings. The slope of the regression line indicates that particles other than somatic cells are counted at the lower range and that cells are lost in the upper range of counts. The importance of determining the proper regression equation for source of milk samples has been reported (5). These results indicate the need for some method to be developed to compare counts from different laboratories. The effect of length of time of centrifugation and the amount of centrifugal force used has not been reported. Higher correlation coefficients than those reported here have been achieved by others (6).

Highly significant correlation coefficients (0.71 - 0.75) between DMSCC and WMT were obtained. This method is the most economical and rapid of all used in this trial. Results yielded more scatter in relation to DMSCC in milk with 1,000,000 or more cells per milliliter. This test properly identifies abnormal milk, and this is achieved rapidly and economically. Precautions should be observed to use only fresh milk (<24 hr after farm pick-up) which has been properly refrigerated (4 C).

REFERENCES

1. Brazis, A. R. 1968. National Mastitis Council, Sub-Committee on Screening Tests. Direct microscopic somatic cell count in milk. J. Milk Food Technol. 31:350-354.

2. Ceriotti, G. 1952. A microchemical determination of desoxyribonucleic acid. J. Biol. Chem. 198:297-303.

3. Cullen, G. A. 1967. A method of counting cells in milk using an electronic cell counter. Veterinary Record 80:188-195.

4. Hutjens, M. F., L. H. Schultz, G. E. Ward, and S. Yamdagni. 1970. Estimation of somatic cells in milk using membrane filter separation and DNA determination with diphenylamine. J. Milk Food Technol. 33:227-231.

5. Mitchell, W. R., F. H. S. Newbould, and I. Platonow. 1967. Electronic and microscopic counts on bulk milk samples. Veterinary Record 81:298-299.

6. Pearson, J. K. L., C. L. Wright, and D. O. Green. 1970. A study of methods for estimating the cell content of bulk milk. J. Dairy Res. 37:467-480.

7. Phipps, L. W., and F. H. S. Newbould. 1966. Determination of leukocyte concentrations in cow's milk with a Coulter counter. J. Dairy Res. 33:51-64.

8. Phipps, L. W. 1968. Electronic counting of cells in milk: Examination of a chemical treatment for dispersal of milk fat. J. Dairy Res. 35:295-302.

9. Read, R. B. Jr., A. L. Reyes, J. G. Bradshaw, and J. T. Peeler. 1967. Electronic counting of somatic cells in milk. J. Dairy Sci. 50:669-674.

10. Thompson, D. I., and D. S. Postle. 1964. The Wisconsin mastitis test-an indirect estimation of leucocytes in milk. J. Milk Food Technol. 27:271-275.

ECONOMICS LABORATORY ENTERS PROCESSING SYSTEMS MARKET

Economics Laboratory, Inc. is entering more strongly into the marketing of processing systems, according to an announcement from E. B. Osborn, president of the company.

The company has formed an Equipment-Engineering Division—Automation Systems, to serve companies that handle fluid or semi-fluid products—primarily those in the beverage and food processing fields — dairies, breweries, bakeries and processors of various types of souces and dressings.

The division, which will headquarter in Beloit, Wisconsin, will provide engineering design, installation service and equipment for processing systems that feature centralized control and automated CIP (Clean-In-Place) operation. National and international markets will be served.

Tying in closely with the formation of the new division is the company's previously announced agreement-in-principle to acquire Electrol Specialties Co., South Beloit, Illinois, manufacturer of custom-built electrical controls used in automated systems marketed by Econlab's Klenzade Automation Department, and a new marketing agreement with the Computer Concepts Corporation of Knoxville, Tennessee, suppliers of solid-state electronic equipment. The new division will apply computer technology to production problems in processing industries.

Dale A. Seiberling will have overall responsibility for the new division as Assistant Vice President. He has been Manager of the company's Klenzade Automation Department since 1960. Ronald B. Douglas will serve as Manager, and Frank J. Bazo as Western Regional Manager. Ahmad A. Jannoun will coordinate activities of the new division within International Operations.

The new division was formed to capitalize on the company's know-how in equipment engineering for the beverage and food processing industries and the increasing trend toward automation in these fields.