THE INFLUENCE OF THE WORKING FACTOR AND CELL CONTENT ON THE PRECISION OF MICROSCOPIC COUNTS OF MILK SOMATIC CELLS[1]

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Summary

Variations in precision of the Breed method for cell counts in milk were investigated by utilizing different working factors (WF) for the same smear and by using the same WF over a major portion of the probable cell count range. A significant inverse relationship was found between precision and the WF. With a constant WF on the other hand, the precision of the count increased very significantly as the actual cell count increased. Formulas showing the relationship between the expected high and low for any given cell count were computed via the least squares method for a WF of 20,000. Evidence was presented that a WF of 5,000 or below would be necessary when a good estimate of cell content is important.

The precision of the Prescott-Breed or direct microscopic cell count of milk *(5)* is dependent upon the working factor (WF). The WF is the product of a formula which utilizes the size of the sample, the smear size, the microscope field diameter and the number of fields counted *(1)*.

Prescott and Breed *(5)*, using a WF of 5,000 found that duplicate somatic cell counts agreed within 15%. Others *(9)* have reported that a ± 50% variation from the mean could be expected while using a WF of 10,000. Recent reports *(3, 4)* have indicated within sample coefficients of variation of 36% and 19%, respectively, when WF's of 15,000 and 7,500 were used. A review of the literature *(6)* disclosed that the WF used by 16 groups of investigators counting somatic cells varied within the range of 10,000 to 50,000.

Since the microscopic cell count is based on probability sampling laws, the WF selected would have a marked effect on the precision of the method. This principle is inherent in the graphic method recently published *(2)* for estimating the precision of all types of direct microscopic counting data based on the Poisson distribution. The precision of the direct microscopic method is also influenced by the number of cells in a sample *(2)*. Within the limits of good counting efficiency, higher cell counts would presumably be less affected by occasional fields which were greatly different from the mean field count.

The variations in precision when different working factors are used and the variations in precision over a major portion of the somatic cell count range using the same working factor, were the subject of this investigation.

Materials and Methods

The procedure as outlined in Standard Methods for the Examination of Dairy Products *(1)* for the direct microscopic method was essentially followed with some modifications *(7)*. Only round smears 1 cm^2 in area made with a 0.01-ml syringe[2] were utilized in these trials.

The effect of different working factors was studied by means of a series of 32 counts made on one smear from a milk sample estimated to contain 8,300,000 cells per ml. A high cell count sample was chosen to minimize the effect of abnormally high or low individual field counts. For each count, 24 fields (WF 20,000) representing a horizontal cross section through the middle of the smear were viewed, and the cells per field recorded in sequence.

Since a count of 6 fields would be required if a WF of 80,000 were used, such counts were attained by utilizing every fourth field from each 24 field count. This provided a total of 128 estimates (24 ÷ 6 x 32) for the WF of 80,000. By adding together every other 80,000 WF estimate, a series of 64 estimates with a 40,000 WF resulted. Since each original count constituted a WF of 20,000, there were 32 such estimates. Adding two 20,000 estimates in sequence produced 16 estimates with a 10,000 WF. The 10,000 WF estimates gave rise to eight 5,000 WF estimates which in turn produced four 2,500 estimates. There were 2 estimates with a WF of 1,250 and one estimate or the group mean based on a WF of 625.

The effect of using the same WF (20,000) over a large portion of the cell count range was also tested. Fifty-six samples of milk were counted. Cell counts ranged from 18,000 to 14,000,000 cells per ml in these samples. On each sample, 8 counts (WF 20,000 each) were made, 2 on each of 4 duplicate smears. The mean ($\bar{X}$), standard deviation(s) and the coefficient of variation ($C = s/\bar{X}$) were computed for each of the 56 samples.

It was found that for a WF of 20,000, 8 separate counts gave a good estimate of the expected extremes of individual counts for any given mean cell count. Hence, using the method of least squares, formulas were computed for the line of "best linear fit," relating the mean to the high, the low and the range of cell count that could be expected for counts when that WF was used. The equation for the line of "best linear fit" as reported here has the formula $Y = bX \pm a$.

[1]This work was part of a thesis submitted by R. Schneider to the Graduate Division, University of California, in partial fulfillment of the requirements for the M.S. degree.

[2]Available from Applied Research Institute, 2 East 23rd Street, New York 10. N.Y.

TABLE 1. THE RELATIONSHIP BETWEEN THE WORKING FACTOR AND THE PROPORTION OF THE SMEAR EXAMINED

Working factor	Per cent of smear examined
1,000	10.00
2,000	5.00
5,000	2.00
10,000	1.00
20,000	.50
50,000	.20
100,000	.10
500,000	.02

RESULTS

The relationship between the WF and the proportion of the smear examined was calculated and is presented in Table 1.

The variation in the range of individual cell counts for different working factors is shown in Table 2. The range of individual counts decreased significantly as the WF grew smaller.

The relationship of cell count, standard deviation and coefficient of variation for a constant working factor of 20,000 is shown over the major part of the cell range in Table 3. It is seen that the amount of variation increases as the cell count decreases. A moderate increase in variability occurred in counts below 1,500,000 cells per ml and a marked increase occurred in counts below 500,000.

For a WF of 20,000, the formulas computed via the least squares method for the "best fit" relating the highest, lowest and range of cell count to the mean were as follows:

1. *In terms of actual cells counted before conversion to cell count*:

High $= 1.103\ \bar{X} + 5.60$
Low $= .902\ \bar{X} - 5.07$
Range $= .201\ \bar{X} + 10.67 - .9k\ (5.63 - \bar{X})$
where $k = 0$ if $\bar{X}$ is over 5.63
$k = 1$ if $\bar{X}$ is under 5.63

2. *In terms of total cell count (cells x WF)*:

High $= 1.103\ \bar{X} + 112{,}000$
Low $= .902\ \bar{X} - 101{,}000$
Range $= .201\ \bar{X} + 213{,}000 - .9k\ (113{,}000 - \bar{X})$
where $k = 0$ if $\bar{X}$ is over 113,000
$k = 1$ if $\bar{X}$ is under 113,000

The formulas for the high and low were symmetrical on either side of the mean. In a random distribution it would be expected that the high and low would be equally distant from the mean. It is noticed that the high was slightly farther away from the mean than the low. However, this variation was not significant since it is an error of only 1 to 2% of the mean.

Tables 4 and 5 indicate the accuracy of the high and low formulas in predicting the expected high and low cell counts for any mean. In table 4, multicounts were made horizontally through the central area of one low cell count smear and one high cell count smear. The cell counts were 440,000 and 8,300,000 cells per ml, respectively. It is seen with both that agreement was close.

In Table 5, a further example of the accuracy of these formulas is shown by a comparison of counts by 4 different counters on smears made by 5 different smear makers of 3 different milk samples. The cell counts of the samples were 130,000, 560,000 and 1,030,000, respectively. Although an additional variable was introduced by using smears by different smear makers, it is seen again that agreement is close.

The formulas for the range were computed from the high and low formulas. The factors .9k (5.63 - $\bar{X}$) and .9k (113,000 - $\bar{X}$) are needed to adjust the cell range because by the low formula, counts below 5.63 and 113,000, respectively, would give negative lows, and this is not possible. All negative lows were considered zero.

The relationship of the WF and cell count to the expected % of error can be shown graphically. Figure

TABLE 2. VARIATION IN RANGE OF CELL COUNTS OBTAINED WITH DIFFERENT WORKING FACTORS ON THE SAME SAMPLE

Working factor	No. of counts	Coefficient of variation[a]	Range of cell counts	Length of range	Length of range as % of group mean
625	1	—	8,300,000[b]	—	—
1,250	2	.0028	8,280,000 - 8,320,000	40,000	.5
2,500	4	.0141	8,150,000 - 8,420,000	270,000	3.3
5,000	8	.0210	8,100,000 - 8,580,000	480,000	5.8
10,000	16	.0377	7,640,000 - 8,740,000	1,100,000	13.3
20,000	32	.0606	7,380,000 - 9,480,000	2,100,000	25.3
40,000	64	.0939	6,840,000 - 10,160,000	3,320,000	40.0
80,000	128	.1340	5,280,000 - 11,200,000	5,920,000	71.3

[a]multiply by 100 for standard deviation as a % of the mean.
[b]group mean.

TABLE 3. THE RELATIONSHIP OF CELL COUNT TO THE STANDARD DEVIATION AND COEFFICIENT OF VARIATION FOR A WORKING FACTOR OF 20,000

Cell count ($\bar{X}$)[a]	Standard deviation (s)[a]	Coefficient of variation ($C = s/\bar{X}$)
250 - 700	25 - 36	.10 - .05
75 - 250	13 - 25	.16 - .10
25 - 75	6 - 16	.23 - .12
Under 25	Under 6	.80[b]- .23

[a]In cells, multiply by 20,000 for the equivalent in cells per ml of milk.
[b]May be exceeded at very low cell counts.

TABLE 4. AGREEMENT OF ACTUAL AND EXPECTED[a] HIGH AND LOW COUNTS (WF 20,000) WHERE MULTIPLE COUNTS WERE MADE

Number of counts	Mean in cells	Low in cells: Actual	Low in cells: Expected[a]	High in cells: Actual	High in cells: Expected[a]
22	22.2	13	15	32	30
32	415.1	369	369	474	463

[a]Expected: Low = .902 X − 5.07; High = 1.103 X + 5.60.

TABLE 5. AGREEMENT OF ACTUAL AND EXPECTED[a] HIGH AND LOW COUNTS (WF 20,000) FOR 4 COUNTERS ON 3 MILK SAMPLES, UTILIZING COUNTS ON SMEARS OF 5 DIFFERENT SMEAR MAKERS

Counter	No. of counts	Mean in cells	Low in cells: Actual	Low in cells: Expected[a]	High in cells: Actual	High in cells: Expected[a]
2	20	6.45	3	1	11	13
3	20	6.50	2	1	14	13
4	20	7.35	1	1.5	14	14
6	20	5.35	2	0	9	12
2	40	27.8	18	20	42	36
3	40	27.3	15	19	35	36
4	40	30.6	19	22	44	39
6	40	26.2	15	18	35	34
2	20	50.5	34	40	68	61
3	20	53.8	42	43	73	65
4	20	54.5	36	44	71	66
6	20	47.4	37	38	67	58

[a]Expected: Low = .902 $\bar{X}$ − 5.07; High = 1.103 $\bar{X}$ + 5.60.

1 denotes the % of errors for computing 95% confidence limits and Figure 2 the % of errors for computing 50% confidence limits (probable error) of 4 selected cell count levels for various working factors.

DISCUSSION

The precision of any Breed cell count on properly prepared smears seems to be the function of 2 factors: *(a)* the number of field counted (WF), and *(b)* the number of cells in a sample (the cell count). This function is in accordance with Poisson distribution principles *(2)*.

The effect of the WF is illustrated in Table 2. The smaller the WF, the better will be the precision. More fields are counted with a smaller WF, hence there is less influence from any individual fields having abnormally high or low counts.

The second factor that affects the precision of the cell count is the number of cells present. The higher the cell count, the more accurate will be the estimate when using a constant WF. This relationship is shown in Table 3. As the count decreases, there is an increase in the variability of individual cell counts as a function of the mean. Thus, the coefficient of variation increases. For the precision to have been the same over the entire cell range, the coefficient of variation would have had to be relatively constant at all levels of cell count.

The actual choice of a WF should be determined by the approximate number of cells in the samples and the reliability required (Figure 1 or Figure 2). A WF within the range of 2,000 to 5,000 appeared

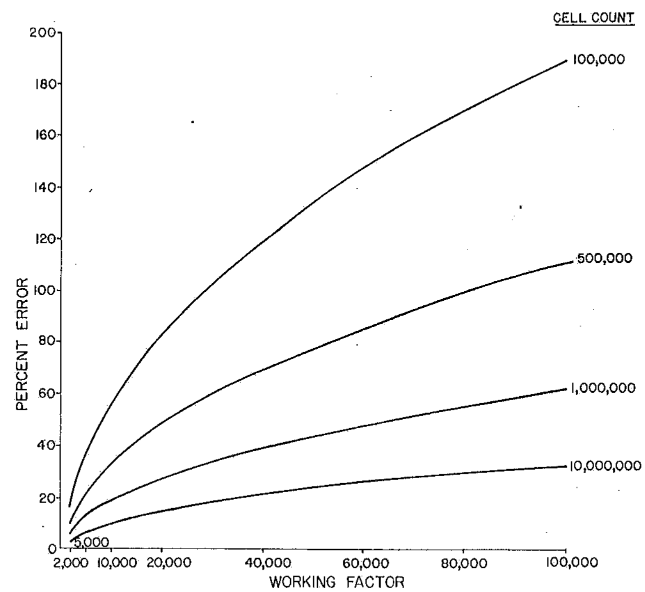

FIG. 1
RELATIONSHIP BETWEEN WORKING FACTOR AND PERCENT ERROR FOR CERTAIN CELL COUNT LEVELS, 95% CONFIDENCE

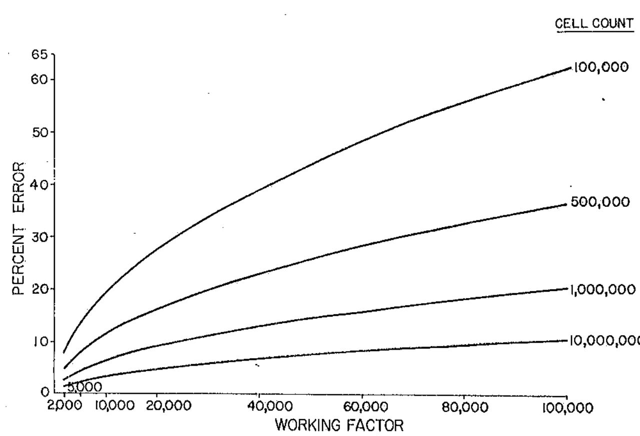

FIG. 2
RELATIONSHIP BETWEEN WORKING FACTOR AND PERCENT ERROR FOR CERTAIN CELL COUNT LEVELS, 50% CONFIDENCE (PROBABLE ERROR)

to give sufficient precision for most purposes, but use of either a higher or a lower WF may be indicated under some circumstances. A method utilizing a WF of 20,000 has been devised *(8)* for application where interest is limited to determining only whether the cell content of a given sample is above or below a certain level.

TABLE 6. THE EFFECT OF THE CONSTANT SECOND FACTOR OF THE RANGE FORMULA[a] ON DIFFERENT LEVELS OF CELL COUNTS (WF 20,000)

Mean cell count	Second factor adjusted	Total range expected	Second factor adjusted as % of mean cell count	Total range expected as % of mean cell count
10,000,000	213,000	2,223,000	2.1	22.2
1,000,000	213,000	414,000	21.3	41.4
100,000	201,000	221,000	201.0	221.0

[a]Range $= .201\ X + 213{,}000 - .9k\ (113{,}000 - \overline{X})$

From the high and low "best fit" formulas, the derived relationship of the range of cell count to the mean illustrates why the Prescott-Breed method does not have the same precision over a large portion of the cell count range (Table 6). Since at all levels the expected cell range is .201 or 20.1% of the mean, the main effect on the range, hence, on the precision of the method at different cell count levels, is caused by the second factor of the formula. For all cell counts, the second factor has the same constant value of 10.67 cells or, when converted, 213,000 cells per ml. The third factor of the formula, the adjustment, has only a minor effect. Thus, at the 10,000,000 cell count level, the constant second factor is merely 2.1% of the mean and accounts for only a small proportion of the expected range. At the 100,000 cell count level, however, the second factor after adjustment is 201.0% of the mean and accounts for practically all of the expected range.

CONCLUSIONS

1. The precision of a given Breed cell count on milk will vary inversely with the WF, and directly with the actual cell count of the milk assuming a constant WF.

2. A definite relationship exists between the expected high and low counts on a given sample and the "true" cell content of that sample.

3. Where a good estimate of cell content is important, a WF of 5,000 or below is necessary, depending upon the number of cells in the sample and the reliability desired.

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