A TRUNCATED SEQUENTIAL PROCEDURE FOR DETERMINING SOMATIC CELL COUNT OF MILK BY THE STRIP METHOD[1]

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ABSTRACT

The Direct Microscopic Somatic Cell Count (DMSCC) "strip" method requires the counting of four strips on two separate milk films. An evaluation of five technicians and 231 milk samples, using a truncated sequential procedure for making cell counts, indicated the potential for significantly reducing the number of strips counted, with little loss in validity of results. For conditions observed in this market, counting of cells could be terminated after the first strip in 85% of the instances with a somatic cell standard set at 1.5 million or higher. Four strips would have to be counted in only 4% of the samples. With the standard set at 1 million or higher, these market conditions would permit one-strip counting 68% of the time, and would necessitate four-strip counting in only 20% of the samples.

The Direct Microscopic Somatic Cell Count (DMSCC) is used as a confirmatory procedure in evaluating milk supplies under the Abnormal Milk Control Program (AMCP) of the U. S. Public Health Service. The method requires that two milk films be prepared and two strips, one horizontal and one vertical, be counted on each film. Because labor costs in the laboratory account for much of the cost of AMCP, procedures for reducing such costs should clearly receive attention.

Since the work of Prescott and Breed *(4)*, considerable research has been done in development and evaluation of screening and confirmatory methods for abnormal milk. The single stain modification of Levowitz and Weber *(2)* was an important step toward reducing the time and complexity of milk film staining. Other innovations, including specially designed glass slides and eyepiece reticles have also been introduced *(3, 5)*. Smith *(8)* found a within-film variance of 130% and a film component variance of 15% of the mean. Schultze et al. *(7)* in a utility-cost study of a number of screening tests assigned the highest rating to the Milk Gel Index, followed by the Wisconsin Mastitis Test when either 1.5 or 1.0 million somatic cell count levels were used as the standard. Also, Schultze et al. *(6)* have developed a statistical procedure, when using the DMSCC as a screening test, for assigning samples to certain categories of test completion depending upon results of a single strip count. Going one step further, and using a slightly different statistical approach, the work reported herein is designed to show the feasibility of count discontinuance after each strip counted when the DMSCC is used as a confirmatory method. As stated previously, current requirements necessitate the counting of four strips, two each on duplicate films.

MATERIALS AND METHODS

During regular testing at the Dairy Quality Control Institute, Inc. laboratories, 10% of those samples showing highest Wisconsin Mastitis Test (WMT) readings (down to but not including a value of 21 mm) are confirmed for somatic cell count by the DMSCC as allowed under the AMCP. For this study, 231 samples were subjected to confirmatory testing. The work was divided among five technicians, each of whom was responsible for preparing and counting her own milk films. No attempt was made to divide the number of samples equally among technicians. Two technicians did 78 tests each, the others, 25, 29, and 19, respectively.

Film preparation and staining were done as described in *Standard Methods for the Examination of Dairy Products (9)*. As recommended by the Subcommittee on Screening Tests, National Mastitis Council *(3)*, glass slides with a clear, circular area of 1 cm^2 permanently outlined on the surface were used. Two films were prepared, and two strips, one horizontal and one vertical, were counted on each film. All technicians used the same microscope and eyepiece. The strip factor was 15,400.

RESULTS AND DISCUSSION

In the discussion that follows, a procedure is presented which provides for discontinuing the cell count, depending upon the results obtained, after the counting of each individual strip. Technically, the procedure can be described as a truncated sequential test, and two assumptions are necessary for its validation: (*a*) that the counts (x_i) per strip within a film follow a common Poisson distribution with mean S,

$$P(x / S) = \frac{e^{-S} S^x}{x!}, \quad x = 0, 1, 2 \ldots \qquad (1)$$

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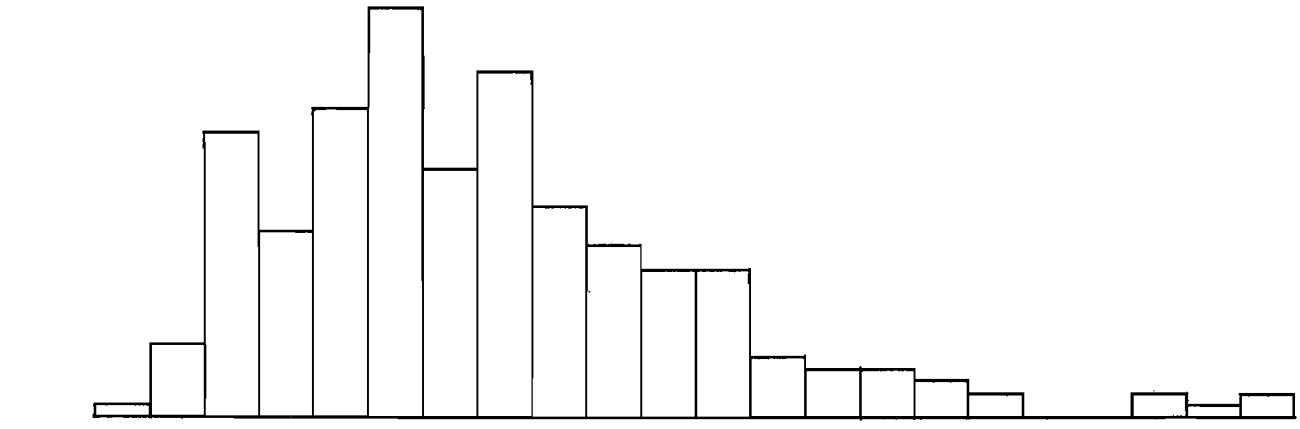

MIDPOINT	21	26	31	36	41	46	51	56	61	66	71	76	81	86	91	96	101	106	111	116	121	126
FREQ.	.004	.026	.10	.065	.109	.144	.078	.12	.074	.061	.05	.052	.022	.017	.017	.01	.009	0	0	.009	.004	.009

Figure 1. Histogram showing midpoint and frequency of single strip counts of 231 different milk samples. (Midpoint figures are based upon a microscope having a strip factor of 15,400.)

and *(b)* that variation between films is small. For a consideration of these assumptions, the reader is referred to the work of Smith *(8)*. In this investigation and for the five technicians who did the tests, the between film variation averaged 3.0% overall, certainly a very reasonable variation.

Once the above two assumptions are satisfied, it may be further assumed that the strip counts over both films follow a common Poisson distribution, and this is the essential requirement of the method that follows.

Proposed procedure

Depending on the width of the strips and size of film, the maximum allowable concentration of somatic cells per milliliter of milk will be equivalent to a maximum allowable concentration (L) per strip. The proposed procedure tests sequentially the hypothesis that H: $S = L$ vs. HA: $S = L - C$ (where $C > 0$ is a constant with a maximum number (4) of strip counts). This choice of constant will be discussed later, but for now it suffices to indicate that the above hypothesis format was selected so that the probability of error could be controlled at $S = L$.

In a fully sequential test (1), that is, one in which maximum sample size is not specified in advance, the decision to stop or continue sampling is usually based on the selection of two numbers (A and B) related to type 1 and type 2 errors. Sampling is continued as long as $B < Z_m < A$; the first time $Z_m \geq A$, accept HA and stop sampling; the first time $Z_m \leq B$, accept H and stop sampling. Here:

$$Z_m = \frac{\prod_{i=1}^{m} P(x_i|\ L\text{-}C)}{\prod_{i=1}^{m} P(x_i|\ L)} \qquad (2)$$

x_i is the ith observation and m is the number of observations taken to that point. For the Poisson distribution and hypothesis in question the criterion $B < Z_m < A$ simplifies to

$$G(m) = \frac{\log \frac{A\text{-}mC}{\left(\frac{L\text{-}C}{L}\right)}}{} < x_1 + x_2 + \ldots + x_m < \frac{\log \frac{B\text{-}mC}{\left(\frac{L\text{-}C}{L}\right)}}{} = F(m) \qquad (3)$$

In the proposed scheme, the above criterion for m=1,2,3 is used, and if sampling has not terminated by the third strip count, then on the fourth count accept H [HA] if $x_1 + x_2 + x_3 + x_4 >$ [$\leq$] $2(2L\text{-}C)$. The constants A and B are chosen to give the desired type 1 and type 2 error values. In general, these constants —and therefore G(m) and F(m)—will be very difficult to determine precisely without the aid of a computer.

In summary, the sampling procedure is implemented as follows: *(a)* Continue counting as long as G(m)

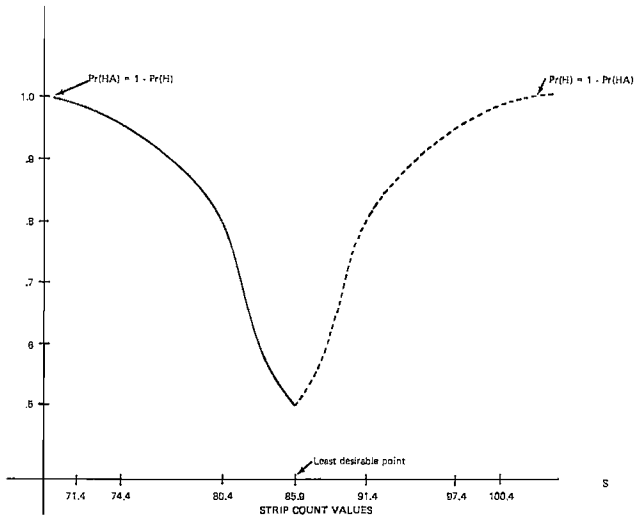

Figure 2. Probability of a correct classification of somatic cell count using a truncated sequential test procedure.

$<x_1+x_2+\ldots+x_m<F(m)$, $m = 1,2,3$. *(b)* The first time $x_1+\ldots+x_m \leq G(m)$, stop counting and accept HA. *(c)* The first time $x_1+\ldots+x_m \geq F(m)$, stop counting and accept H. *(d)* If counting has not terminated by the third strip, then count a fourth strip and accept H [HA] if $x_1+x_2+x_3+x_4 \leq [>]$ 2(2L-C).

Choice of C

The choice of a particular value for C is to some extent arbitary. However, reasonable recommendations can be given.

It is desirable to choose C so that a large percentage, P, (e.g. .70 <P <.90) of all the samples having true S values less than L fall less than L-C. Choosing C in this manner tends to reduce the percentage of samples with true S value between L-C and L. The proposed procedure would be least effective when S is between L-C and L. That is, around the least desirable point, which is S = (2L-C)/2, the probability of accepting either hypothesis is approximately .5. At the same time, it must be pointed out that the value selected for C should not be too small —as C decreases the expected number of strip counts increases. Thus, if C is chosen small, four strip counts will usually have to be taken and the sequential procedure will lose its advantage.

Clearly, an adequate choice for C necessitates knowledge about the distribution of true concentration (S). If this distribution is centered about S = (2L-C)/2 with a moderately small variance, the procedure will have little value. Not only will it be necessary to count four strips much of the time, but the chosen type 1 and type 2 errors will be meaningless; most of the time the true S value will be between L-C and L. The essential first step, then, is to note the kind of distribution that actually exists. From single strip count data acquired during the testing of 231 samples, the histogram in Fig. 1 was developed. This provides a reasonable picture of the distribution of true S values. Based upon this distribution, consider two examples of the use of this procedure, one in which the somatic cell count standard is placed at 1.5 million, the other at 1.0 million.

Example 1

With the maximum allowable concentration of somatic cells established at 1.5 million/ml, using a strip factor of 15,400, the value for L becomes 97.4. The hypothesis in question is H: S = 97.4 vs. HA: S = 97.4 − C.

From the data which served to develop Fig. 1, C was chosen to be 23, which gives P = 0.88. The numbers A and B needed to determine G(m) and F(m) were selected, with the aid of a computer, by adjusting the fully sequential approximations (1) to yield type 1 and type 2 errors of approximately 0.05. This resulted in the following values for G and F:

m	G(m)	F(m)
1	80	91
2	165	176
3	250	261

Terminal point 2(2L-C) = 343

Figure 2 shows how the exact probability of accepting each hypothesis changes as the true concentration, S, changes. Computations for the power curves in Fig. 2 were carried out on the University of Minnesota CDC 6400.

Thus, it would appear that the sequential procedure would be of real value for data of the type illustrated in Fig. 1. In about 85% of the samples, counting would be terminated after the first strip count. Moreover, it could be expected that a small percentage of the samples would have true S values between 74 and 97, such that, in the least desirable of situations, where S = (2L-C)/2 = 85.5, the counting of four strips would be required in only 4% of the samples. This percentage would become smaller as S deviates from (2L-C)/2.

Example 2

Using a standard of 1.0 million somatic cells/ml and the same strip factor (15,400), the hypothesis becomes H: S=65 vs. HA: S=65-C. Now, from the data of Figure 1, C was chosen to be 15, which give P =.65.

For type 1 and type 2 errors of .05, the values for G(m) and F(m) were determined to be:

m	G(m)	F(m)
1	48	66
2	105	123
3	163	180

Terminal point 2(2L-C) = 230

In this situation, counting could be terminated after a single strip in about 68% of the samples. In the least desirable case (S=57.5), four strip counts would be needed for 29% of the samples.

A comparison of this and the previous example indicates that, for the data derived in this laboratory, as expressed in Fig. 1, the procedure becomes less effective as L decreases.

It must be noted that the power of the truncated sequential test can be significantly less than that of the usual technique on which four strips are always counted. For the power levels to be comparable, a maximum sample size greater than four is necessary. However, preliminary investigations indicate that substantial reduction in labor can still be realized with a maximum sample size chosen to yield power levels comparable to the fixed sample size procedure. This point will be considered in greater detail in a future paper which will include a much broader data base.

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