A Reasearch Note

THE DIRECT MICROSCOPIC SOMATIC CELL COUNT AS A SCREENING TEST FOR CONTROL OF ABNORMAL MILK[1]

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ABSTRACT

The Direct Microscopic Somatic Cell Count can be used advantageously in control of abnormal milk to perform simultaneously screening and confirmation of milk samples. Confidence limits computed according to the individual microscope Strip Factor are used to interpret the count made on a single strip of one of the two milk films. The sample is assigned on the basis of the interpreted count to one of three categories: *(a)* less than the legal cell concentration maximum ($P < 0.05$); *(b)* in the region of the cell concentration maximum, and thus subject to confirmation; or *(c)* greater than the legal cell concentration maximum ($P < 0.05$). For samples in categories *(a)* or *(c)* no further counting is required to accept or reject the milk. For samples in category *(b)*, the four-strip confirmatory count may be completed immediately.

The Direct Microscopic Somatic Cell Count in milk (DMSCC) *(2)* was designed to be an accurate confirmatory counting method with good and definable precision. It was to be easily repeatable in exact procedural detail among technicians and laboratories. Specifically, we were acting in response to a need expressed by the National Conference on Interstate Milk Shipments for improved methodology to implement their program for control of abnormal milk. The DMSCC as originally described required the counting of cells in two strips of each of two replicate milk films, and so provided a means of achieving the high precision required of a confirmatory count. This paper reports a way to extend use of the DMSCC. A procedure is described through which a milk sample may be categorized as accepted, rejected, or dubious on the basis of a single strip cell count. Only those samples in the dubious category require additional counting.

According to statistical theory, a single observation (a single strip count, for example) is an estimate of the mean of such observations which may be made on the specified population. The population of concern here consists of stained milk films made from a single sample of milk. The individual observations will vary and provide estimates which are higher or lower than the true mean with equal frequency. If we can obtain a reasonable estimate of the variation among individual observations it is possible to determine the degree of precision achieved by using a single observation or the average of multiple observations as an estimate of the population mean. The precision of an estimate is best described or compared by considering the width of the confidence limits for a selected degree of probability.

In the control of abnormal milk we are interested primarily in determining whether or not the concentration of cells in a milk supply exceeds an established legal limit. The precision of the estimated cell count on milk samples much greater or less than this concentration is of only minor interest. For a given microscope and reticle there is a Strip Factor which can be used to convert the number of cells observed in one strip or the average of several strips to a cell concentration per milliliter. Thus, for any given optical combination the number of cells observed is indicative of the cell concentration in the milk. One such number of cells, the Strip Equivalent (S_E) is indicative of the legal limit established for cell concentration. There is a smaller number of cells which indicates with a high degree of assurance that the cell concentration of the sample is below the legal limit. This number, which we have designated C_L is the lowest single strip count for which confirmation is required. There is also a number of cells larger than S_E which indicates with a high degree of assurance that the cell concentration of the sample is greater than the legal limit. This number, designated C_H, is the highest single strip count for which confirmation is required. The interval C_L to C_H is not great, and it is only within this range that single strip counts must

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TABLE 1. VALUES OF C_L AND C_H APPROPRIATE FOR A RANGE OF STRIP EQUIVALENT (S_E)

C_L	S_E [1]	C_H	C_L	S_E	C_H	S_L	S_E	C_H
44	65	96	64	89	124	84	113	151
45	66	97	65	90	125	85	114	153
46	67	98	66	91	126	86	115	154
47	68	99	66	92	127	87	116	155
47	69	100	67	93	128	88	117	156
48	70	101	68	94	130	88	118	157
49	71	103	69	95	131	89	119	158
50	72	104	70	96	132	90	120	159
51	73	105	71	97	133	91	121	161
51	74	106	71	98	134	92	122	162
52	75	107	72	99	135	93	123	163
53	76	109	73	100	137	94	124	164
54	77	110	74	101	138	94	125	165
55	78	111	75	102	139	95	126	166
56	79	112	76	103	140	96	127	167
56	80	113	77	104	141	97	128	169
57	81	114	77	105	142	98	129	170
58	82	116	78	106	143	99	130	171
59	83	117	79	107	145	100	131	172
60	84	118	80	108	146	101	132	173
61	85	119	81	109	147	102	133	174
61	86	120	82	110	148	102	134	175
62	87	121	82	111	149	103	135	176
63	88	123	83	112	150			

[1] S_E = Legal maximum cell concentration ÷ Strip Factor

be confirmed by counting the additional three strips as originally specified for the DMSCC. The limiting confirmatory numbers C_L and C_H have been so computed that a milk sample for which the single strip count falls just at either number has a probability of less than 5% of being falsely accepted or rejected, respectively. Because the interval for mandatory confirmation (C_L through C_H) is determined by the Strip Factor, it is characteristic of the individual microscope and reticle combination. Table 1 lists the appropriate values of C_L and C_H for a range of Strip Equivalents.

The control values have been developed as follows: We have previously designated the cell count per single strip which is equivalent to the legal limiting cell concentrations as S_E. The lower confirmatory number C_L, for which S_E lies at the upper 95% confidence limit, is expressed through the relationship $S_E = C_L + 1.645 \sqrt{S^2/n}$ *(1)*, where C_L is treated as a mean. But since only one observation has been made, $n = 1$. S^2 is the sample variance appropriate to the value of C_L. Our experience in several large cell counting trials *(3, 4)* indicated that the mean should be multiplied by a factor of 1.45 (reflecting a 12% coefficient of variation). This estimate of variance based on research laboratory experience is apparently too low to forecast performance in the field. A collaborative study performed in routine testing laboratories by the Division of Microbiology, U. S. Food and Drug Administration, produced an average coefficient of variation of 19% (R. B. Read, Jr., *unpublished data*). In conformity with their findings, we have substituted 3.61 C_L for s^2, yielding the equation $S_E = C_L + 1.645 \sqrt{3.61\ C_L}$. This cannot be solved directly for C_L and must be approached by an iterative procedure.

C_H is the upper confirmatory number for which S_E lies at the lower 95% confidence limit. It is computed from the relationship $S_E = C_H - 1.645 \sqrt{3.61\ C_H}$. Again, solution for C_H is by iteration.

In applying this quality control strategy to the control of abnormal milk, samples are prepared for the DMSCC in the standard manner of making, drying, and staining duplicate milk films. But now only the horizontal strip of the first film is counted. The count is immediately compared with the limiting confirmatory numbers C_L and C_H specified in Table 1 for the Strip Equivalent. If the count is less than C_L the milk sample is graded acceptable by the counting procedure. If the count is greater than C_H the sample is graded unacceptable by the counting procedure. Only if the single strip count falls within the range C_L through C_H does the milk sample require the confirmatory count. Since the slide is already in position on the microscope stage, it is a relatively simple matter to count the additional three strips required for the confirmatory count.

When the DMSCC is applied in the manner described to the monitoring of milk samples under the abnormal milk control program, both screening and confirmation of somatic cell concentrations are accomplished simultaneously and rapidly. Very few samples will require the counting of more than one strip across a milk film. All estimations of cell concentration are based upon a theoretical one-tailed 95% confidence limit.

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