

COLLABORATIVE STUDY OF SOME SCREENING TESTS FOR DETECTION OF ABNORMAL MILK¹

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(Received for publication May 22, 1972)

ABSTRACT

The California Mastitis Test, Modified Whiteside Test, Wisconsin Mastitis Test, tube Catalase Test, Milk Gel Index, and Direct Microscopic Somatic Cell Count (NMC Method) were done according to a detailed protocol in five laboratories. Each laboratory tested about 250 bulk tank milk samples in blind duplicate. Screening tests were compared at various critical scores with respect to their identification of milk samples with cell concentration above (positive) and below (negative) 1.0 and 1.5 million/ml. The percent of positive samples correctly identified is the *Utility* of the screening test; the percent of negative samples mis-identified is the *Cost*.

At the 1.5 million cells per milliliter limit, and using U.S.P.H.S. recommended critical scores, the mean Cost/Utility estimates were: CMT 68/98, MWT 64/97, WMT 13/84, and CAT 38/94. Lowering the critical score for WMT to > 20 increased its Utility to an acceptable 89% at 17% Cost. At the 1.0 million/ml cell concentration limit the ranking of tests did not change materially. Laboratories varied widely in Cost of screening with all tests, and, particularly for subjectively-scored tests, in the critical score required for equivalent Utility. In all comparisons, the MGI was the test of choice, with the WMT ranking next.

The Subcommittee on Screening Tests was charged by the National Mastitis Council to investigate currently used indirect tests for screening milk for excessive concentration of somatic cells, and to report its conclusions. The results of our studies of five indirect tests in each of five laboratories are reported here.

¹A contribution from the Subcommittee on Screening Tests, National Mastitis Council, Inc.

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MATERIALS AND METHODS

Testing procedures

Five indirect tests were selected for study. The California Mastitis Test (CMT) was conducted as specified by the originators (8) with the additional precautions that 2.0 ml volumes of milk and reagent were delivered by automatic syringe and cannula, and reactions were graded with reference to color photographic reproductions supplied by Dr. Schalm. The Modified Whiteside Test (MWT) was conducted according to Temple's procedure (13). The Wisconsin Mastitis Test (WMT) was performed according to the brochure written by D. I. Thompson⁸, which does not differ significantly from the procedure recommended by the U.S. Public Health Service (13). Our only procedural variation was that both milk samples and reagent were brought to room temperature before mixing. The Milk Gel Index (MGI) was done according to the method specified for its official use in the Province of Ontario, Canada (7). The method is similar to the WMT with respect to reaction mixture and measurement of height of residual liquid column after timed outflow through a small orifice. It differs in that milk and reagent are mixed in one vessel and then transferred to a viscosity tube, a 5 ml plastic syringe barrel marked at intervals of 0.2 ml. Outflow time is 10 sec. Equipment for the MGI was loaned to each participating laboratory by the Laboratories Division, Ontario Department of Health. The Catalase Test (CAT) procedure was provided by Postle (4) and deviated from the published method in that 2 ml of hydrogen peroxide were used. The reference method for somatic cell concentration was the Direct Microscopic Somatic Cell Count (3) (DMSCC).

The test protocol called for each laboratory to test 250 samples of milk from farm bulk tanks. Fifty samples were to fall within each of five cell concentration ranges, clustering around 0.5, 0.75, 1.0, 1.5, and 2.0 million/ml, respectively. Two to four ounces of well-mixed, unfiltered milk were collected from full tanks (usually the composite of four successive milkings of the herd) after at least 3 min agitation. Samples were immediately subdivided among four screw-capped tubes and refrigerated overnight. Tests were done the next morning after bringing subsamples to room temperature: one milk film for DMSCC, one CMT, and one MWT on each subsample A; then one WMT, CAT, and MGI on each subsample B; the first series of tests repeated on subsamples C; and finally the second series repeated on subsamples D. CMT and MWT reactions were coded and recorded as 1 through 5, CAT scores as percent O₂, MGI scores as (20 × height of residual reaction mixture to nearest 0.1 ml), and WMT to nearest millimeter. The DMSCC was performed using the wide band of the original Subcommittee reticle (10). Since, in a preliminary study, we had experienced difficulty in assuring ourselves of the equivalence of reagents used in the several laboratories, we this time secured a single batch of each reagent and distributed portions of

TABLE 1. SOURCES AND DISTRIBUTIONS OF CELL CONCENTRATION OF SAMPLES USED IN EVALUATING SCREENING TESTS

Lab	Number	Somatic cell concentration		Distribution of cell concentrations (millions/ml)						
		Mean	Median	.25	.25-.49	.50-.74	.75-.99	1.0-1.49	1.5-1.99	2.0-2.49
A	260	.941	.850	2	15	21	23	25	12	3
B ¹	321	1.040	.950	0	3	21	29	31	12	3
B ²	288	1.058	.977	0	3	19	29	34	11	3
C	220	.627	.561	4	36	33	15	11	1	0
D	250	.591	.558	8	33	35	16	8	0	0
E ³	240	1.068	.980	0	12	22	19	26	12	9

¹Not used for WMT (non-standard caps used in tests of early samples)

²Used for WMT only

³Not used for MGI (test not performed)

TABLE 2. COMPILATION OF A COST-UTILITY TABLE FOR WMT SCORES ON BULK TANK MILK SAMPLES¹

WMT score	Cell concentration categories (millions/ml)			Screening at 1.0 million/ml				Screening at 1.5 million/ml			
	<1.0	1.0-1.5	>1.5	True positive	Utility ²	False positive	Cost ³	True positive	Utility ²	False positive	Cost ³
	(No. samples)			(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
< 4	5	0	0	206	100	314	100	78	100	442	100
4	16	1	0	206	100	309	98	78	100	437	99
5	17	0	0	205	100	293	93	78	100	420	95
6	20	1	0	205	100	276	88	78	100	403	91
7	26	0	0	204	99	256	82	78	100	382	86
8	22	0	0	204	99	230	73	78	100	356	81
9	19	0	0	204	99	208	66	78	100	334	76
10	32	0	0	204	99	189	60	78	100	315	71
11	25	0	0	204	99	157	50	78	100	283	64
12	20	0	0	204	99	132	42	78	100	258	58
13	23	1	0	204	99	112	36	78	100	238	54
14	24	7	0	203	99	89	28	78	100	214	48
15	14	5	0	196	95	65	21	78	100	183	41
16	16	4	0	191	93	51	16	78	100	164	37
17	15	6	1	187	91	35	11	78	100	144	33
18	6	12	0	180	87	20	6	77	99	123	28
19	6	27	1	168	82	14	4	77	99	105	24
20	3	22	1	140	68	8	3	76	97	72	16
21	2	8	7	117	57	5	2	75	96	47	11
22	2	14	8	102	50	3	1	68	87	37	8
23	1	11	3	80	39	1	0	60	77	21	5
24	0	7	12	66	32	0	0	57	73	9	2
25	0	1	11	47	23	0	0	45	58	2	0
26	0	1	14	35	17	0	0	34	44	1	0
≤27	0	0	20	20	10	0	0	20	26	0	0
Total	314	128	78								

¹Data from laboratory A

²Numbers and percentages indicate the samples correctly judged as positive if screening were at indicated level.

³Numbers and percentages indicate the samples incorrectly judged as positive if screening were at indicated level.

these to each participating laboratory.

The laboratories found it impossible to adhere to the planned distribution of cell concentrations during sample collection. Table 1 shows the numbers and distributions of cell concentrations of samples from which screening test results were actually submitted. As may be seen from Fig. 1, the sample population available to a given laboratory may be characterized as low-skewed, high-skewed, or symmetrical.

Cost-utility analysis

In a program for control of abnormal milk, screening tests are used to identify positive milk samples, i.e. those with a

content of somatic cells in excess of a specified maximum concentration. Berkson's Cost-Utility analysis (1) fits these circumstances, for it was designed for situations in which "a test result or measure X applying to an individual is used to 'predict' . . . whether the individual belongs to A or B of two mutually exclusive categories." We here define Utility as the effectiveness of a screening test in indicating which milk samples are positive, and we measure it as the percent of true positives which are correctly identified. Two categories of Cost can be described: (a) the cost to the laboratory, and eventually to the producer, in time and ma-

terials of carrying out the confirmatory procedure on milk samples falsely identified as positive by the screening procedure (Type I error); and (b) the cost to equitable and effective functioning of the control program of missing milk samples in violation of the standard (Type II error, consisting of false negatives). We consider the first category of greater importance. The second cost is impossible to quantify, and its urgency is mitigated by the fact that our purpose in abnormal milk control is to monitor sources of milk rather than discrete batches of milk. Cost, then, for this analysis is defined as the extent to which a screening test falsely identifies negative milk samples as positive, and is measured as the percent of true negative samples which are so misidentified.

For a given screening test, Cost and Utility estimates are positively related and are contingent upon the critical score at which they are measured. As the critical score is lowered, both statistics increase in numerical value. Optimum use of this analytical method for choosing among screening tests entails (a) determination for each test of that critical score at which the Utility estimate approximates some predetermined standard, and (b) comparison of Cost estimate for each test at the point of equivalent Utility. We have chosen 90% Utility (i.e. 10% false negatives) as an appropriate criterion for screening test performance. Because critical scores have been established for official use of these tests (14) without regard to this criterion, we have made Cost-Utility comparisons at these specified scores also.

RESULTS AND DISCUSSION

The bulk tank sample data from each laboratory were categorized on the basis of DMSCC results as follows: ≤ 1.0 million cells/ml; > 1.0 million and ≤ 1.5 million cells/ml; and > 1.5 million cells/ml. For each screening test we tabulated the distribution among the three cell concentration categories of samples which yielded each test score. Progressing from the highest score to the lowest, we then listed the cumulative sum of true positive samples and of false positive samples at each test scoring level. These sample sums were converted to percent of total positive or false positive samples and listed as the Utility and Cost estimates, respectively, relevant to the use of that scoring level as the critical score for the test. A sample compilation of Cost-Utility data, for one laboratory's study of the WMT, is shown in Table 2. In the analysis, duplicate screening test scores were treated as independent observations, and both were related to the single DMSCC made on the milk sample. Because of increasing demand that the limiting cell concentration for the national abnormal milk control program be lowered from 1.5 to 1.0 million somatic cells per milliliter, we have applied the analysis of Utility and Cost to simulated screening of milk samples against both concentration limits.

The information in Table 2 is interpreted in the following manner. At the 1.0 million/ml cell concentration limit (shown in the middle columns), there was a total of 206 (128 + 78) positive milk

samples. All of them yielded WMT scores of ≥ 4 . Thus, the choice of $WMT > 3$ as the critical score would result in detection of all the over-limit samples, for a Utility estimate of 100%. Only 140 of the positive samples yielded WMT scores of ≥ 20 , so the choice of $WMT > 19$ as critical score would result in only 68% Utility. Similarly, 309 of the 314 negative samples yielded WMT scores ≥ 4 . Thus, 98% of the total would be considered positive (requiring confirmation) by mistake if $WMT > 3$ were accepted as the critical score. Using a critical score of $WMT > 19$, the Cost would drop to only 3%. These results are unusually good, with fairly sharp separation of WMT scores between the three cell concentration categories. Other participating laboratories experienced less favorable relationships between test scores and cell counts. The Cost-Utility analyses of all five screening tests are shown for each laboratory in Tables 3 through 7.

To compare the various screening tests we averaged the Costs estimated for equivalent Utility in the participating laboratories. The critical test score was not necessarily the same for all laboratories. Selecting the 1.5 million cells/ml concentration limit and demanding 90% Utility, the average Costs of the tests were: CMT = 68%, MWT = 53%, WMT = 17%, CAT = 39%, and MGI = 10%. Because of the limited number of high cell count samples available to laboratories C and D, their results could not be included. Since laboratory E did not perform the MGI, the average given is for laboratories A and B only. Possibly more extensive testing would have caused an increase in our estimate of its Cost. The significance of these large values for Cost is more apparent if one considers the absolute numbers of samples involved. Thus, in identifying 100 of the total 102 positive samples by the WMT, laboratory B also misidentified 464 of the total 548 samples with cell concentration not in excess of 1.5 million cells/ml.

Although the percentage Costs determined in this study are free from bias because of the proportion of positive samples in the population, they do reflect the distribution of cell concentrations within the negative sample group. Our sample populations were accumulated in a conscious attempt to achieve a rather flat distribution across the cell concentration range studied. In a field situation we would expect all percentage Costs to be lower because of a marked skewing of sample distribution toward the lower cell concentrations. The relative values, however, are valid for comparing tests. Excluding the MGI, which is not used in the United States to our knowledge and for which equipment is difficult to obtain, the advantage among the tests studied lies clearly with the WMT.

TABLE 3. UTILITY AND COST ESTIMATES USING THE CMT TO SCREEN BULK TANK MILK SAMPLES

CMT score	a) Screening at 1.0 million somatic cells/ml									
	Lab A		Lab B		Lab C		Lab D		Lab E	
	Util.	Cost	Util.	Cost	Util.	Cost	Util.	Cost	Util.	Cost
	(%)									
3	14	0	71	18	90	43	0	1	3	0
2	92	21	94	59	100	85	33	14	100	90
1	100	93	99	88	100	98	90	56	100	100
tr	100	98	100	99	100	100	95	85	100	100
	b) Screening at 1.5 million somatic cells/ml									
CMT score	Lab A		Lab B		Lab E					
	Util.	Cost	Util.	Cost	Util.	Cost				
	(%)									
3	27	2	79	36	5	0				
2	100	40	93	72	100	93				
1	100	95	96	93	100	100				

TABLE 4. UTILITY AND COST ESTIMATES USING THE MWT TO SCREEN BULK TANK MILK SAMPLES

MWT score	a) Screening at 1.0 million somatic cells/ml									
	Lab A		Lab B		Lab C		Lab D		Lab E	
	Util.	Cost	Util.	Cost	Util.	Cost	Util.	Cost	Util.	Cost
	(%)									
3 +	17	0	82	37	38	12	18	3	2	0
2 +	87	18	98	77	90	46	58	18	88	60
1 +	100	89	100	96	100	75	88	49	99	97
tr	100	99	100	100	100	94	100	78	100	100
	b) Screening at 1.5 million somatic cells/ml									
MWT score	Lab A		Lab B		Lab E					
	Util.	Cost	Util.	Cost	Util.	Cost				
	(%)									
3 +	27	2	98	51	4	0				
2 +	100	40	100	85	92	68				
1 +	100	95	100	97	100	98				
tr	100	99	100	100	100	100				

Under the national abnormal milk control program, the critical score to be used for each screening test is constant, derived from a national survey by the U. S. Public Health Service (13). If each laboratory used these scores rather than its own best determination, the Utility of the tests ranged as follows: CMT (>1) = 93 to 100%; MWT (> + 1) = 92 to 100%; WMT (>21) = 77 to 87%; and CAT (>30% O₂) = 91 to 96%. The lowered Utility of the WMT at that critical score was accompanied by a decrease in its Cost to 13%. To the extent that assignment of uniform critical scores for use in all laboratories is justified at all, our results support those currently specified, with the exception that the score for the WMT should be lowered to >20 to achieve equivalent recovery of positive samples. This conclusion is, of course,

contingent upon exercise of similar care in collection and storage of bulk tank samples and upon their testing within 24 hr. Analysis of milk samples after greater delay or less careful handling may be expected to further reduce the Utility and magnify the Cost estimate for each screening test.

Our data are useful in predicting the efficiency of operation of these screening tests at a somatic cell concentration limit of 1.0 million/ml. Determined as before at that test score which in each laboratory yielded at least 90% Utility, the average Costs of the tests were: CMT = 54%; MWT = 77%; WMT = 45%; CAT = 47%; and MGI = 31%. The increase in proportion of false positive determinations for the WMT and MGI at this lower concentration limit reflects both a real increase in Cost determined for

TABLE 5. UTILITY AND COST ESTIMATES USING THE WMT TO SCREEN BULK TANK MILK SAMPLES

WMT score	Lab A		Lab B		Lab C		Lab D		Lab E	
	Util.	Cost	Util.	Cost	Util.	Cost	Util.	Cost	Util.	Cost
	(%)									
18	87	6	77	34	85	41	83	48	81	20
17	91	11	82	39	92	50	83	51	84	25
16	93	16	87	50	94	57	85	53	87	29
15	95	21	94	63	98	65	90	56	88	36
14	99	28	97	71	100	71	90	60	90	44

score	Lab A		Lab B		Lab E	
	Util.	Cost	Util.	Cost	Util.	Cost
	(%)					
22	87	8	77	16	87	14
21	96	11	83	23	88	17
20	97	16	94	36	93	23
19	99	24	98	41	95	29
18	99	28	98	47	97	41

TABLE 6. UTILITY AND COST ESTIMATES USING THE CAT TO SCREEN BULK TANK MILK SAMPLES

CAT score	Lab A		Lab B		Lab C		Lab D		Lab E		CAT score
	Util.	Cost	Util.	Cost	Util.	Cost	Util.	Cost	Util.	Cost	
	(%)										
37-38	45	3	59	23	58	12	80	23	55	12	50
35-36	56	5	69	31	62	17	90	32	67	20	45
33-34	67	8	72	37	69	19	90	35	77	37	40
31-32	75	16	80	44	85	24	95	41	83	54	35
29-30	84	23	87	55	96	32	98	49	90	75	30
27-28	90	32	90	66	100	37			95	85	25
25-26	92	46	95	77							

CAT score	Lab A		Lab B		Lab E		CAT score
	Util.	Cost	Util.	Cost	Util.	Cost	
	(%)						
39-40	63	7	81	26	73	22	50
37-38	77	10	84	31	83	32	45
35-36	83	15	90	41	90	47	40
33-34	87	21	91	47	93	61	35
31-32	91	30	95	55	96	79	30

the laboratories and also the participation in the averages of two additional laboratories. These two tests would still be the preferred choices for screening at 1.0 million cells/ml.

Although Read et al. (5) approached the analysis of screening test performance in a somewhat similar manner, and apparently balanced their sample population similarly to ours both in range and symmetry, the form of their data presentation precludes direct

comparison of the results. Data presented by Smith and Schultze (10) and Schultze and Smith (8) in earlier studies of the CMT match closely the results of their laboratory in the present study. In the three separate studies, using CMT at >1 to screen for milks in excess of 1.0 million cells/ml, they achieved between 90 and 95% Utility at a Cost of 28, 21, and 21%, respectively. Results of Kowalczyk (1) can be expressed in this form, assuming a cell concentration

limit of 1.0 million/ml. Excluding samples with concentrations greater than 5 million/ml, he found CMT scores of ≥ 1 to detect 94% of positive samples at a Cost of 57% false positives, and MWT scores of $\geq 1+$ to detect 88% of positives at a Cost of 52% false positives. His sample distribution was also unlike that to be expected in the field, with 0.5 to 1.0 million cells/ml the most frequent subclass. Data of Postle (4) can be recalculated to provide information on screening bulk tank samples at 0.5 million cells/ml. The Utility and Cost, respectively, were 83% and 37% for the CAT at $\geq 30\% O_2$, and 91% and 22% for the WMT at ≥ 15 . These results, although fragmentary, support our conclusion as to the relative superiority of the objectively-scored tests based on the gel reaction. The fact that the scoring level of $30\% O_2$ in the CAT has been treated as equivalent to either 0.5 or 1.5 million cells/ml by different experimenters without much difference in Cost or Utility speaks clearly of the extreme imprecision of this test for estimating cell concentration.

The general superiority of results from the MGI, coupled with the fact that it really differs only in hardware from the runner-up WMT, tempts us to call for development of a new screening test, based on the same principle as these but with mechanical improvements to make the advantages of the MGI more readily accessible. If an indirect screening test is to be employed for routine milk monitoring, the

potential saving in operating cost offered by the WMT and MGI should be maximized.

Differences in the performance and calibration of the various screening tests are apparent in our results. Laboratory A, for example, used the WMT (Table 5) to achieve greater than 90% Utility at either cell concentration level at a Cost of only 11%. The Cost among the other laboratories ranged from 23 to 63%, despite the more favorable sample distributions for laboratories C and D. Laboratory A performed each of the screening tests at the lowest Cost, and in most instances laboratory E at the highest Cost. Since the relative ranking of Cost for the tests was similar among the laboratories, it is tempting to conclude that a major cause of the disparity was differences in magnitude of random error in DMSCC performance. We computed for each laboratory's DMSCC data the mean value for relative variance of sample means according to the method of Smith (11). For laboratories A, B, C, D, and E, respectively, the computed values for $s^2\bar{y}$ were .436 \bar{y} , .552 \bar{y} , .445 \bar{y} , .225 \bar{y} , and .919 \bar{y} . The high variance found for laboratory E is consonant with its relatively high Cost estimates for the screening tests. The variance estimated for laboratory D is sufficiently smaller than the expected .4 \bar{y} to alert one to the possibility that the technician performing the DMSCC tended to smooth the differences among individual strip counts.

TABLE 7. UTILITY AND COST ESTIMATES USING THE MGI TO SCREEN BULK TANK MILK SAMPLES

<i>a) Screening at 1.0 million somatic cells/ml</i>									
MGI score	Lab A		Lab B		Lab C		Lab D		
	Util.	Cost	Util.	Cost	Util.	Cost	Util.	Cost	
	(%)								
32-33	89	10	62	8	42	19	88	26	
30-31	92	13	65	9	42	20	88	26	
28-29	93	16	71	14	46	23	95	31	
26-27	95	20	73	18	56	24	95	32	
24-25	98	24	81	26	65	28	95	40	
22-23			86	30	73	28			
20-21			90	38	77	34			
18-19			93	45	87	36			
16-17			96	57	90	42			

<i>b) Screening at 1.5 million somatic cells/ml</i>					
MGI score	Lab A		Lab B		
	Util.	Cost	Util.	Cost	
	(%)				
48-49	92	4	42	2	
46-47	94	7	47	2	
44-45	95	9	59	3	
42-43	96	11	62	4	
40-41	100	16	72	7	
38-39			75	9	
36-37			87	14	
34-35			90	16	

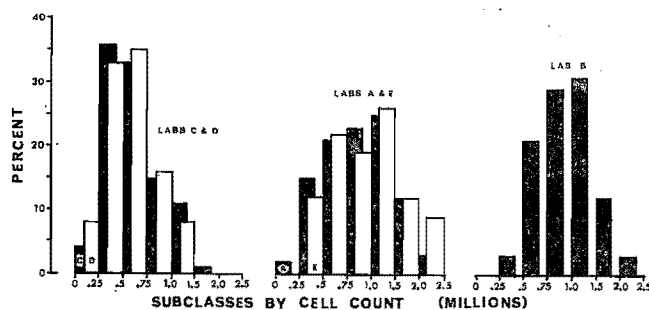


Figure 1. Distributions by cell concentration of milk samples used to evaluate screening tests.

Another aspect of the problem of interlaboratory differences is illustrated by the variation in scoring level required to achieve equivalent Utility. This variation is apparently systematic, and could reflect bias in performance of either the screening test or the DMSCC, or both. An example can be seen in the MGI results, for which critical scores (90% Utility) against 1.0 million cells/ml were, respectively, 30, 20, 16, and 28. The two subjectively read screening tests yielded extreme illustrations of this problem. When the CMT was used to screen against the 1.0 million cells/ml level, a score of 3 as assigned in the respective laboratories resulted in Utility estimates of 14, 71, 90, 0, and 2%. The MWT was not much better, achieving 17, 82, 38, 18, and 2% Utility, respectively. Clearly, scoring judgements were not equivalent, for we cannot conceive of such an extreme bias in cell counting. Distributions of high cell count samples among laboratories would lead us to expect a different ranking of percentages, namely mid, high, low, low, and high, for the respective laboratories, if distribution were the controlling factor.

In this collaborative study we exercised extreme care to achieve uniformity both in collection and storage of relatively fresh samples and in the details of testing procedure. Despite our efforts, large differences appeared in the relation between screening test scores and cell counts for the several laboratories. Since even greater discrepancies are likely to obtain among the many dairy laboratories engaged in abnormal milk control testing, we strongly believe that each laboratory should develop control data required to establish and justify a working critical score based on its own circumstances of milk supply and laboratory performance.

On the basis of these studies, the Subcommittee on Screening Tests has come to the following conclusions:

(a) That all the indirect screening tests included in this investigation may be expected to identify an inconveniently large proportion of false positives when used to screen milk samples for cell concentration above or below a limiting somatic cell concen-

tration.

(b) That among the tests investigated, the WMT and MGI can be so used with the least Cost.

(c) That these two tests would still be the methods of choice if the limiting cell concentration for the national abnormal milk control program were lowered to 1.0 million/ml.

(d) That individual laboratories may be expected to vary widely in the efficiency with which they operate any screening test, and to a lesser degree in the test score required to detect a similar proportion of over-limit samples.

(e) That the currently-accepted test scores for screening milks against 1.5 million somatic cells/ml are valid generalizations, except that the WMT score should be lowered to > 20 to make its Utility equivalent to that achieved with the other indirect tests.

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