COMPARISON OF METHODS FOR GRADING MILK INTENDED FOR MANUFACTURING PURPOSES[1,2]

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SUMMARY

Samples of milk intended for manufacturing purposes were obtained once each season from randomly selected dairy farms in three widely separated geographical locations. Samples (3873) were analyzed by standard plate count (SPC-32 C), direct microscopic clump count (DMCC), methylene blue (MBRT) and resazurin (RRT), (5P 7/4) tests. Correlations for SPC vs. DMCC, MBRT, and RRT were $r = 0.78$, -0.82 and -0.79, respectively; DMCC vs. MBRT, RRT, $r = -0.75$ and -0.68, respectively; MBRT vs. RRT, $r = 0.86$. Prediction of DMCC, MBRT, and RRT on the basis of their regressions with SPC were influenced significantly (99% level of significance) by the geographical source of samples, the type of milk handling on the farm (can or farm bulk tank) and season. On the basis of equivalents (determined by regression) between classifying tests, new standards were developed. The percentage of samples classed the same by the four methods using these new calculated classifications or the classification recommended by the United States Department of Agriculture (USDA) was approximately 50% of all samples. Agreement between the four methods of classifying samples was not close enough to warrant the interchangeable use of the four tests.

Results from calculated classifications varied considerably with geographical location and with the method of handling milk on the farm. Although the use of calculated classifications would give better agreement among the four methods when applied to the supplies from which they were derived, multiplicity of tests methods, their application and interpretation would cause much confusion. Use of the RRT with a 5P 7/4 Munsell color end-point with separate procedures, one for can supplies (RRT: $> 2\ 1/2$, $\leq 2\ 1/2$, and $\leq 1\ 1/2$ hr for Class 1, 2, and 3, respectively) and one for farm bulk tank supplies (RRT: $> 3\ 1/2$, $\leq 3\ 1/2$, and $\leq 2\ 1/2$ for Class 1, 2, and 3, respectively) resulted in grading milk, intended for manufacturing purposes, more uniformly than either use of the four classification methods interchangeably or even use of a single RRT standard based on all samples regardless of type of milk handling on the farm (can or bulk tanks).

Recently a "Minimum Standards for Milk for Manufacturing Purposes and its Production and Processing recommended for adoption by State Regulatory Agencies" was prepared by the United States Department of Agriculture (USDA). These recommendations were published on June 26, 1963 in the *Federal Register (24)*. Included in these recommended standards is a classification of milk supplies based upon bacteriological quality. One of four tests—standard plate count (SPC), direct microscopic clump count (DMCC), methylene blue reduction test (MBRT), and resazurin reduction test (RRT) with a 5P 7/4 Munsell color end-point *(21)* may be selected and used, at the option of the user, as the basis of a quality-maintenance program. It was not recommended that the four methods be used alternately because agreement between the four testing methods has been shown repeatedly to be influenced by the quality of milk supplies under study *(5, 7, 8, 17, 20)*. In general, supplies with a relatively large proportion of individual samples or poor quality show better agreement among the classification methods than those with a relatively large proportion of individual samples of good quality. Furthermore, the type of microflora in milk also has been shown to be a probable factor in the agreement among the four grading methods *(12, 13, 14)*.

In view of the possible extensive application of classifications recommended by the USDA throughout the country, this study was undertaken primarily to determine the extent to which milk produced under different conditions in various parts of the country would be classed uniformly by the four methods of testing. Furthermore, a pertinent statistical basis for the setting of equivalent classes among the grading tests was desired. Other investigators *(1, 2, 3, 4, 9, 10, 11, 15, 16, 22)* have studied the problem of agreement between the test methods, but because of the regional character of the supplies and/or the limited number of samples investigated, extrapolation of their conclusions to more extended regions and supplies did not seem to be warranted.

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TABLE 1 LINEAR CORRELATIONS BETWEEN MILK CLASSIFICATION METHODS ALL SAMPLES – 3,873

	DMCC	MBRT	RRT
SPC	0.78	–0.82	–0.79
DMCC		–0.75	–0.68
MBRT			0.86

TABLE 2. MULTIPLE CORRELATION COEFFICIENTS BETWEEN MILK CLASSIFICATION METHODS AS INFLUENCED BY THE INTRODUCTION OF VARIOUS FACTORS AS INDEPENDENT VARIABLES

Independent variable (s) Introduced	Correlation between the following variables:		
	DMCC[a] vs. SPC	MBRT[a] vs. SPC	RRT[a] vs. SPC
1. None	0.784	0.822	0.790
2. Method of handling	0.784	0.840	0.827
3. Location	0.814	0.835	0.793
4. Season	0.787	0.824	0.792
5. 2, 3 and 4	0.817	0.853	0.831

[a]Dependent variable.

EXPERIMENTAL PROCEDURES

Source of samples.

In an attempt to obtain samples representative of the supplies from several heavy milk producing areas of the United States, samples were obtained as follows: *(a)* three widely separated geographical areas were selected within which a variety of milking practices existed: *(b)* within each area, several plants representative of those engaged in the processing of milk supplies intended for manufacturing purposes were selected; *(c)* producers from each plant within each geographical location were randomly selected from dairy farm load lists of each plant; and *(d)* each selected dairy farm was sampled (with few exceptions) once each season of the year. Sampling was done from winter, 1963 to fall, 1964.

Milk from can shippers was sampled at the weigh-tank in the plant while farm bulk-tank supplies were sampled on the farms. Samples were cooled immediately in ice water, then imbedded in ice in a sample-transport case. On arrival at the laboratory, they were refrigerated below 40 F until analyzed on the following day. The interval between sampling and analysis was not greater than 24 hours.

Statistical procedures for analysis of data.

The corresponding logarithmic values of the bacterial counts were punched on IBM cards. Furthermore, reduction times and other pertinent information relative to each individual sample were punched on the same IBM cards. Linear correlation coefficients and regression equations for the four classification methods were calculated. In order to assess the significance of the influence of factors such as type of milk handling, season, and geographical location of sampling on the statistical relationships between the test methods, multiple regression analysis and equations were determined using these factors as independent variables. Statistical analysis was performed on a Control Data Corporation 1604 computer using an "UMSTAT"-50 program *(18)*.

Methods of analysis.

The SPC[4] (incubation at 32 C), DMCC, MBRT and RRT were performed according to Standard Methods for the Examination of Dairy Products *(23)*; however, some minor modifications were made in the MBRT and RRT as indicated below. All equipment and material necessary for the study were transported from one location to another with the exception of incubators used for plate counts. The Levowitz-Weber modification of the Newman-Lampert stain No. 2 *(23)* was used for the DMCC. In order to minimize variations introduced by different analysts, all SPC's were plated and counted by the same analyst, and all DMCC's were done by another analyst throughout the duration of the investigation. For MBRT, a supplementary reading was made after 1 hour's incubation; the RRT was read every 15 minutes, with inversion every hour after the readings.

RESULTS AND DISCUSSION

A total of 3,873 samples (can, 2,756; farm bulk-tank, 1,117) from approximately 970 producers from 20 processing plants located in 7 states was collected and analyzed by the four test methods. The 7 states were grouped into three widely separated geographical locations.

Correlations.

The linear correlations between the test methods are shown in Table 1. They ranged from –0.68 for DMCC and RRT to 0.86 for MBRT and RRT. Factors such as the type of milk handling (can or farm bulk-tank), geographical location of the supplies, and season of the year modified in various degrees the correlations between the test methods (see Table 2). The method of handling did not modify the correlation between DMCC and SPC, whereas, the season factor generally modified the correlations between the methods to a lesser degree than the other factors.

Comparison of Data and USDA-Recommended Classification.

On the basis of data from all samples and using the appropriate regression equations, a system of evaluating quality levels was established using the SPC, as a reference, at the two levels recommended

[4]Two lots of plate count agar (Difco) were used throughout the study, Control No. 451711 and 463939. The former had a productivity equal to or higher than a lot identified as Control No. 445770 (Difco) which previously had been certified during media certification studies done at the Minneapolis-St. Paul Quality Control Laboratory and sponsored by the Media Certification Commission [see "Standard Methods" *(23)* p. 59.] The second lot, Control No. 463939, was compared in our laboratories with lot 451711 and was found to be equal in productivity.

TABLE 3. USDA[a] AND CALCULATED[b] MILK CLASSIFICATIONS

Class	SPC	DMCC	MBRT	RRT(5P 7/4)
		(A) USDA Classifications — Method		
1	≤ 500,000	≤ 500,000	> 4.5 hr.	> 2.25 hr.
2	≤ 3 millions	≤ 3 millions	≤ 4.5 hr.	≤ 2.25 hr.
3	> 3 millions	> 3 millions	≤ 2.5 hr.	≤ 1.5 hr.
		(B) Calculated Classifications		
1	≤ 500,000	≤ 500,000	> 5.5 hr.	> 2.75 hr.
2	≤ 3 millions	≤ 1.2 millions	≤ 5.5 hr.	≤ 2.75 hr.
3	> 3 millions	> 1.2 millions	≤ 4.5 hr.	≤ 1.75 hr.

[a]Federal Register, June 26, 1963.
[b]Calculated on the basis of regression equations with SPC as an independent variable.

by the USDA: 500,000 and 3,000,000 per ml. All the samples were classified according to the USDA Classification (Table 3, A) and according to the calculated classification based on all samples (Table 3, B). The application of the USDA classifications to all samples (Table 4, column 2) resulted in variations in the percentages of samples placed in Class 1, 2, or 3 depending on the test used. Similar variations were evident when the calculated classifications (based on all samples) were applied to all samples (Table 4, column 3). With USDA classification, percent samples in Class 3 was highest for RRT, followed in decreasing order by SPC, MBRT, and DMCC. With calculated classification, percent samples in Class 3 was highest for MBRT, followed in decreasing order by RRT, SPC, and DMCC. The mean of the differences between the percentage of samples in each class was approximately 7 percent with extremes of 0% for Class 1 milk by DMCC to 19% for Class 3 milk by MBRT.

When the calculated classifications (based on all samples) were applied to all farm bulk-tank supplies, uniformity of classification by the four tests definitely improved over that found when the USDA classifications were applied to the same supplies (Table 4, columns 4, 5). When these same calculated classifications were applied to all can supplies, agreement between the tests did not improve appreciably (Table 4, columns 6, 7).

The percentage of all samples classified the same by all four tests was approximately 50% regardless of the system of classification applied. Some of the disagreement may be the result of the variability of the individual test method. Furthermore, segregation of samples on the basis of their method of handling (can or farm bulk-tank) did not improve appreciably the agreement between the two systems of classification (see Table 5).

Effect of Milk Handling Practices and Geographical Source on Comparisons.

In an attempt to determine the effect of type of milk handling and of geographical source of supplies on the values of DMCC, MBRT and RRT equivalent to SPC (predicted on the basis of regression equations), separate classifications based on data from samples grouped according to method of handling and/or geographical source of supplies were established (see Tables 6, 7, and 8). Differences in reduction times, MBRT and RRT, between can supplies and farm bulk-tank supplies are shown in Tables 6 and 8. For example, the two reduction time standards (MBRT and RRT) consistently differed by 1 hour for the two types of supplies. As shown in Table 7, equivalent values varied for supplies from the three different geographical locations. For example, the Class 1 MBRT standard for locations A, B, and C would be > 6 1/2, >4 1/2, and >7 1/2, respectively.

TABLE 4. PERCENT MILK SAMPLES FALLING IN EACH CLASS WHEN GRADED BY USDA AND CALCULATED CLASSIFICATION (BASED ON ALL SAMPLES)

Class	All samples USDA (%)	All samples Calculated (%)	Farm Bulk-tank supplies USDA (%)	Farm Bulk-tank supplies Calculated (%)	Can samples USDA (%)	Can samples Calculated (%)
			Standard plate count			
1	44.9	same	58.2	same	39.4	same
2	24.3	as	21.2	as	25.4	as
3	30.8	USDA	20.6	USDA	35.2	USDA
			Direct microscopic clump count			
1	56.7	56.7	68.8	68.8	51.5	51.5
2	22.0	16.0	17.0	12.2	25.2	17.6
3	21.3	27.3	14.2	19.0	23.3	30.9
			Methylene blue reduction test			
1	52.2	41.6	77.4	66.3	41.8	31.1
2	20.1	11.7	11.0	10.7	23.6	12.2
3	27.7	46.7	11.6	23.0	34.6	56.7
			Resazurin reduction test			
1	45.2	38.7	70.9	65.5	34.6	27.3
2	17.2	18.0	11.0	14.3	17.2	19.6
3	37.6	43.3	18.1	20.2	48.2	53.1

TABLE 5. RELATIVE AGREEMENT BETWEEN MILK CLASSIFICATION METHODS USING THE USDA LEVELS (SEE TABLE 3, A) AND THE CALCULATED LEVELS (SEE TABLE 3, B).

Category of samples	No. of samples[a]	% of samples in each category classed the same by SPC, DMC, MBRT, and RRT using: USDA class	Calculated class
All samples	3873	48.8	49.7
Class 1	1734	72.6	61.0
Class 2	939	6.3	4.0
Class 3	1200	47.8	69.2
All bulk tank samples	1149	61.6	62.9
Class 1	680	87.6	83.5
Class 2	239	5.4	8.4
Class 3	230	43.0	58.7
All can samples	2724	43.4	44.2
Class 1	1054	62.9	46.5
Class 2	700	6.6	2.6
Class 3	970	49.0	71.6

[a]Number of samples in each class category was established by SPC.

Separate classifications based on data from samples grouped according to geographical source of supplies, season of the year, and method of handling were established. The difference of about 1 hour between MBRT and RRT depending on the type of handling also was observed (data not shown).

The prediction, by regression, of values of DMCC, MBRT, and RRT in function of SPC was shown to be affected significantly (99% level of probability) by geographical source of supplies, type of handling and season of the year. Application of calculated classifications and of the USDA classifications showed that the factors mentioned above should not be ignored, although the season factor does not seem to introduce a difference of practical importance.

The difference in reducing activity of farm bulk-tank supplies as compared to can supplies, especially when the geographical factor and the seasonal factor were removed, strongly suggested the presence of a different type of microflora in each type of supply. Farm bulk-tank supplies were in general picked up every other day (4 milkings). Ninety percent (90%) of the individual suppliers maintained their bulk tanks at 40 F or below, while about 99.8% were maintained below 50 F. The presence of psychrophilic bacteria in raw milk, coupled with storage at low temperatures for rather extended periods, would cause preferential growth of the psychrophilic bacteria, while the growth of other types of bacteria would not be appreciable. Furthermore, due to the temperature (37 C) of incubation used in MBRT and RRT, the reducing activity of such bacteria probably would be much lower than that of a flora consisting of a high proportion of non-psychrophilic type. With can-milk supplies, where every-day pick up was generally practiced, 60% of all samples were, at time of receipt at the plant, above 50 F. Furthermore, several hours often elapsed between milking time and delivery at the plant. This would enable growth of microorganisms of a non-psychrophilic nature. Such a flora likely to be more diversified than that of bulk tank supplies would be more reactive to the reduction tests than the microflora of farm bulk-

TABLE 6. CLASS BASED ON DATA FROM MILK SAMPLES ACCORDING TO METHOD OF HANDLING (CAN OR FARM BULK-TANK)

Class	SPC	DMCC (Can or Bulk)	Calculated Classification: MBRT Can	MBRT Bulk	RRT Can	RRT Bulk
1	≤ 500,000	≤ 500,000	> 5 1/2	> 6 1/2	> 2 1/2	> 3 1/2
2	≤ 3,000,000	≤ 1,200,000	≤ 5 1/2	≤ 6 1/2	≤ 2 1/2	≤ 3 1/2
3	> 3,000,000	> 1,200,000	≤ 3 1/2	≤ 4 1/2	≤ 1 1/2	≤ 2 1/2

TABLE 7. CLASSIFICATION LEVELS BASED ON DATA FROM MILK SAMPLES SEGREGATED ACCORDING TO GEOGRAPHICAL SOURCE OF SAMPLES

Class	SPC	Calculated standards: DMCC A & C	DMCC B	MBRT A	MBRT B	MBRT C	RRT A	RRT B	RRT C
1	≤ 500,000	≤ 100,000	≤ 1,400,000	> 6 1/2	> 4 1/2	> 7 1/2	> 3	> 2 1/4	> 3 1/4
2	≤ 3,000,000	≤ 250,000	≤ 3,500,000	≤ 6 1/2	≤ 4 1/2	≤ 7 1/2	≤ 3	≤ 2 1/4	≤ 3 1/4
2	> 3,000,000	> 250,000	> 3,500,000	≤ 5 1/2	≤ 3 1/2	≤ 5 1/2	≤ 2	≤ 1 1/4	≤ 2 1/4

TABLE 8. CLASSIFICATION LEVELS BASED ON DATA FROM MILK SAMPLES IN LOCATION A SEGREGATED ACCORDING TO METHOD OF HANDLING

	Calculated standards					
		DMCC	MBRT		RRT	
Class	SPC	Can or Bulk	Can	Bulk	Can	Bulk
1	≤ 500,000	≤ 100,000	> 6 1/2	> 7 1/2	> 2 1/2	> 3 1/2
2	≤ 3,000,000	≤ 250,000	≤ 6 1/2	≤ 7 1/2	≤ 2 1/2	≤ 3 1/2
3	> 3,000,000	> 250,000	≤ 4 1/2	≤ 5 1/2	≤ 1 1/2	≤ 2 1/2

TABLE 9. RELATIONSHIP BETWEEN VARIOUS RRT LEVELS AND THE SPC LEVEL

				Calculated RRT based on	
Class	SPC/ml	USDA RRT/hr	Calculated RRT based on all samples	Can	Bulk Tank
1	≤ 500,000	> 2 1/4	> 2 3/4	> 2 1/2	> 3 1/2
2	≤ 3,000,000	≤ 2 1/4	≤ 2 3/4	≤ 2 1/2	≤ 3 1/2
3	> 3,000,000	≤ 1 1/2	≤ 1 3/4	≤ 1 1/2	≤ 2 1/2

tank supplies.

The use of the four tests interchangeably might be applicable if separate standards for each type of milk handling and/or separate standards for each geographical location were established. On the other hand, the use of a single test and exclusion of all other tests in order to achieve a more uniform classification of all milk supplies might be applicable and certainly would be more desirable. A reduction test has a better chance of acceptance than a direct measure of the viable bacterial population in milk (SPC), because few manufacturing milk plants are geared to use the SPC as a routine test. The use of DMCC, at least in its present form, in view of the large differences between analysts and between laboratories would not, in our opinion, be conducive to the uniform grading of milk. The use of other types of stains with a standardization of the counting might improve direct microscopic counting *(6, 19)*. The use of MBRT would, however, neccesitate establishing geographical type of classification as well as separate classification for can and for farm bulk-tank supplies. The RRT was not appreciably affected by the geographical source of supplies, especially when the supplies were segregated on the basis of method of handling. On the basis of these observations, separate RRT classifications, one for can supplies (based on data from all can supplies) and one for farm bulk-tank supplies (based on data from all farm bulk-tank supplies) were determined by the calculation of the values of RRT equivalent to the two SPC levels: 500,000 and 3,000,000 per ml. (see Table 9).

Application of separate classifications, based on respective type of milk handling (see Table 10, last two columns), to each respective type of supply resulted in both cases in a test more severe than SPC. RRT placed 10.5% more farm bulk-tank samples in Class 3 than SPC and placed 13% more can samples in Class 3 than SPC. When a RRT classification, calculated on the basis of all samples was applied to each type of supply (can and farm bulk-tank), RRT placed 18% more can samples in Class 3 than SPC, while it placed approximately the same number of farm bulk-tank samples in Class 3 as SPC; on the other hand, RRT placed 7.3% more farm bulk-tank samples in Class 1 than the SPC.

CONCLUSIONS

The results of four bacterial estimation tests, [SPC, DMCC, MBRT and RRT (5P 7/4)] used for the classification of 3,873 samples of raw milk, intended for manufacturing purposes, from various widely separated geographical locations and from producers handling milk in farm bulk tanks or in cans were compared. The use of four tests caused a number of samples to be upgraded or downgraded, depending on the test used, when either the classification levels proposed by the USDA, or calculated classification levels based on data from all samples analyzed, were applied.

If classification of milk is to be uniform, separate regional standards should be used for can and farm bulk-tank supples; the differences among standards,

TABLE 10. CLASSIFICATION OF FARM BULK-TANK MILK AND CAN-MILK SAMPLES ACCORDING TO LEVELS GIVEN IN TABLE 8

	% distribution of class indicated							
			USDA					
	SPC		RRT		Calculated RRT based on all samples		Calculated RRT based on: Can	Bulk Tank
Class	Can	Bulk	Can	Bulk	Can	Bulk	Can	Bulk
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	39.4	58.2	34.6	70.9	27.3	65.5	31.6	53.1
2	25.4	21.2	17.2	11.0	19.6	14.3	20.2	15.8
3	35.2	20.6	48.2	18.1	53.1	20.2	48.2	31.1

their application, and their interpretation, however, could cause confusion among geographic areas. Possibly use of a single test, such as RRT (5P 7/4), with separate classification levels for can supplies and for farm bulk-tank supplies would be conducive to more uniform classification of milk supplies regardless of their geographical source.

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