BACTERIAL TEST RESULTS OF GRADE-A RAW MILK SAMPLES AS A MEASURE OF FARM PRODUCTION CONDITIONS[1]

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Abstract

Milk from 30 grade-A farms was subjected to bacteriological tests including the Standard Plate, total, coliform, psychrophilic, thermoduric, and enterococcus count, resazurin reduction time, and leucocyte count to determine the correlation between these tests and farm production conditions. Farm conditions were evaluated at sample collection time by a farm score, which was based mainly on sanitation.

After collection and immediate transportation to the laboratory, half of each sample was stored at 3.3 C for 72 hr, the remainder was preincubated at 12.8 C for 18 hr after storage at 3.3 C for 54 hr; determinations were then performed. The leucocyte count was determined on the fresh sample.

The psychrophilic count was the only bacterial test that showed significant correlation with the farm score. For samples stored at 3.3 C for 72 hr, all comparisons among bacterial counts showed significant correlation except: psychrophilic count vs. resazurin reduction time; coliform count vs. resazurin reduction time; and, coliform count vs. enterococcus count. For preincubated samples, all comparisons among bacterial counts showed significant correlation except: psychrophilic count vs. resazurin reduction time; coliform count vs. resazurin reduction time; and, coliform count vs. thermoduric count. Higher correlations were obtained on the preincubated samples for all bacterial tests except the thermoduric count. Within this experimental design, preliminary incubation did not improve the ability of the bacterial tests to show statistically significant correlation with the farm score. The leucocyte count showed significant correlation with the farm score, but not with the bacterial test results.

Evaluation of data shows that the bacterial test results are not highly correlated with farm production conditions as measured by farm score. Milking-time inspections are necessary to assure that recommended practices are used in grade-A milk production.

In view of the changes in the production of grade-A milk during the last 10 to 15 years, it is necessary to evaluate testing methods and determine how closely they correlate with farm-production conditions. Dilution of microorganisms, resulting from increased milk production per farm, and modern refrigeration systems have limited the reliability of the Standard Plate Count. Present methods of bacterial testing, with lenient maximum limits and frequent lack of milking-time inspections, may permit many undesirable sanitary violations to go undetected.

Emphasis on esthetically acceptable production conditions makes it mandatory to check on production practices. Milking-time inspections provide an ideal method because they can serve an educational as well as a regulatory purpose. Also, they detect undesirable practices that otherwise might go unnoticed. Since only a few milking-time inspections are made, it would be desirable to designate a species or group of microorganisms to serve as a sanitary indicator that could supplement farm inspection, but no such indicator organism has yet been recognized.

Much past work has attempted to correlate results of bacterial tests with each other by using samples of unknown history without regard to age and previous storage temperature. Often, production conditions were not known. Consequently, the significance of a count could not be ascertained. In this study, there was complete control over the sample beginning at the farm. The senior author, a former fieldman, inspected the farms at milking time, collected the samples, and analyzed them personally. During his visits, scores were also assigned for production conditions. After analysis, the results of seven bacterial tests and the leucocyte count were correlated with the farm inspection score to determine the ability of each to evaluate production conditions. The results of the bacterial tests and the leucocyte count were also correlated.

Methods

Milk produced on 30 grade-A dairy farms located within 50 miles of Ames, Iowa, was sampled during a 20-month period. Each farm was visited at least 30 min before milking began on a day when the bulk tank was empty. The individual farms were numerically rated using the score card shown in Fig. 1; the card emphasized cleanliness and sanitization of milk-contact surfaces. The highest possible score was 100 points, and violations were scored according to their seriousness. For example, the penalty was more severe for moisture remaining in equipment if the equipment was not sanitized before it was used. If no violation was observed for an item, the maximum possible score was assigned. The maximum score was six for all items except use of the strip cup and cleanliness of the cows, for which the maximum score was five. If there was a serious violation of an item, the minimum score of 1 was assigned. Each farm received

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DAIRYMAN _____ SAMPLE NUMBER _____

	Very Poor 1	2	3	4	5	Excellent 6
Bulk Tank						
Solids						
Milking Machines						
Solids						
Moisture						
Inflations						
Milk hoses						
Pipeline (tote pails)						
Solids						
Moisture						
Sanitation						
Bulk tank						
Pipeline (tote pails)						
Milking machines						
Udders						
Inflations between cows						
Udders						
Use strip cup						
Individual udder towels						
Clean cows						
Clean udders						
Miscellaneous						

TOTAL POINTS _____

Figure 1. Example of score card used in determining numerical rating of grade-A dairy farm milking facilities and procedures.

a score of six for the miscellaneous item, unless a sanitary violation, not represented elsewhere on the score card, was observed.

Sample collection and dispensing

After milking was completed, a sample was aseptically collected from the bulk tank and placed in a sterile quart fruit jar for prompt refrigerated transportation to the laboratory. At the laboratory, the sample was immediately dispensed by a 10-ml manual continuous syringe into four sterile 15 x 125 mm rubber-stoppered test tubes and seven sterile 20 x 125 mm screw-capped test tubes. After each test tube was filled, it was immediately placed in an ice-water bath. After all test tubes were filled, they were placed in a 3.3 C air incubator, except for one screw-capped tube used for a leucocyte count. The tubes were divided into two equal sets. One set was stored at 3.3 C for 72 hr, and the other set was stored at 3.3 C for 54 hr and then at 12.8 C for 18 hr. The latter treatment constituted preliminary incubation (PI). The storage at 3.3 C simulated the storage treatment that milk would be subjected to in industry before being analyzed.

Analysis of samples

After the 72-hr storage period, the milk samples were removed from the air incubators and analyzed. Procedures given in the 11th edition of *Standard Methods for the Examination of Dairy Products (1)* were followed unless a different procedure is specified. The bacterial tests performed were: thermoduric (TdBC), coliform (CC), enterococcus (EC), psychrophilic (PBC), Standard Plate (SPC), and total (TC) counts. The resazurin reduction time (RRT) also was determined. The bacterial counts were made on duplicate tubes of milk, and the RRT was determined on a single tube of milk. Leucocyte numbers were determined on the fresh milk sample using the Levowitz-Weber method *(1)*.

Thermoduric count. Milk for the TdBC was laboratory pasteurized as recommended by Anderson and Meanwell *(2)*.

Coliform count. Violet Red Bile Agar (VRBA) was used. Plates were incubated at 32 ± 1 C for 18-24 hr.

Enterococcus count. Citrate Azide Agar (CAA) of Saraswat, Clark, and Reinbold *(20)* was used.

Psychrophilic count. The PBC plates were incubated at 7 ± 1 C for 10 days.

Total count. The TC was determined by using Eugonagar (EA) *(6)*. Plates were incubated at 21 ± 1 C for 7 days.

Statistical analysis

For statistical analysis, counts of <1 were recorded as 0. The counts were transformed by taking $\log_{10}$ (count + 1). The PI test was not conducted on the first six samples, and leucocyte counts were not determined on the first five samples because of a change of plans after the experiment was started. This was taken into account in the data comparisons. The RRT was not determined on one sample because of a laboratory accident. Consequently, there is one less degree of freedom for statistical comparisons involving the RRT. Critical values were obtained from *Principles and Procedures of Statistics (23)*.

RESULTS AND DISCUSSION

Correlation coefficients (r) among the seven bacterial tests, the leucocyte count, and the farm score for the two storage treatments are presented in Table 1.

Comparisons with the farm score

The only statistically significant r value between a bacterial test and the farm score was with the PBC. Improperly cleaned and sanitized milk-contact surfaces, especially the bulk tank, are conducive to the development of a large psychrophilic population. Since an unclean surface cannot be effectively sanitized, it may become a reservoir for psychrophiles. The bulk tank has a much larger surface area than other equipment, and the milk is in continual contact with it, whereas the milk is only in transitory contact with much of the other equipment. Since the bulk tank often has milk in it when the inspector or fieldman visits, it may be in an unsanitary state and yet not be detected. For this purpose, the score card was heavily weighted for cleanliness and sanitizing of milk-contact surfaces. Hence, a significant relationship would be expected between the farm score and the PBC. If many of the psychrophiles had not also been enumerated by the SPC and TC, one would expect much closer correlation of the farm score with the PBC than with the SPC or TC.

The agreement between the PBC and the farm conditions was better than with the SPC or the TC.

TABLE 1. CORRELATION COEFFICIENTS OF 30 GRADE-A RAW MILK SAMPLES[a] RECEIVING SPECIFIED STORAGE TREATMENTS

Comparison	Sample storage: 3.3 C for 3 days	Sample storage: PI[b]
Farm score vs.:	r[c]	r[d]
Psychrophilic count	−0.48	−0.43
Total count	−0.35	−0.33
Standard Plate Count	−0.35	−0.34
Coliform count	−0.28	−0.32
Resazurin reduction time	−0.27	−0.29
Enterococcus count	−0.18	−0.19
Thermoduric count	−0.18	−0.18
Leucocyte count	—	−0.64
Standard Plate Count vs.:		
Total count	0.91	0.95
Thermoduric count	0.82	0.57
Psychrophilic count	0.80	0.89
Enterococcus count	0.62	0.70
Coliform count	0.51	0.73
Resazurin reduction time	−0.49	−0.56
Leucocyte count	—	0.13
Total count vs.:		
Psychrophilic count	0.87	0.90
Thermoduric count	0.73	0.57
Coliform count	0.47	0.69
Enterococcus count	0.41	0.56
Resazurin reduction time	−0.42	−0.56
Leucocyte count	—	0.14
Psychrophilic count vs.:		
Thermoduric count	0.57	0.49
Coliform count	0.48	0.69
Enterococcus count	0.38	0.59
Resazurin reduction time	−0.30	−0.39
Leucocyte count	—	0.26
Thermoduric count vs.:		
Enterococcus count	0.52	0.47
Resazurin reduction time	−0.57	−0.51
Coliform count	0.43	0.20
Leucocyte count	—	0.39
Enterococcus count vs.:		
Resazurin reduction time	−0.49	−0.67
Coliform count	0.26	0.45
Leucocyte	—	0.20
Resazurin reduction time vs.:		
Coliform count	−0.05	−0.34
Leucocyte count	—	0.14
Coliform count vs.:		
Leucocyte count	—	0.02

[a]The counts were transformed by taking logarithm$_{10}$ (count + 1) and the resazurin reduction times were transformed by taking logarithm$_{10}$ (reduction time + 1).
[b]Preliminary incubation samples were stored at 3.3 C for 54 hr, and then at 12.8 for 18 hr before being analyzed.
[c]Critical value of r (5%, 28 degrees of freedom) = 0.36.
Critical value of r (5%, 27 degrees of freedom) = 0.37.
[d]Critical value of r (5%, 22 degrees of freedom) = 0.40.
Critical value of r (5%, 21 degrees of freedom) = 0.41.

However, the PBC is relatively expensive and time-consuming. Even though there was better recovery of microorganisms with the TC than with the SPC, neither count showed a significant correlation with the farm score.

The lack of significant correlation of the farm score with the EC and the CC is of interest because both groups of microorganisms have been proposed as indicators of production conditions. Some workers have reported that the CC is not a reliable index of production conditions (*5, 12, 21, 26*), but Sherman and Wing (*21*), Johns (*13*), and Fay (*9*) believed that, with efficient cooling, the coliform results would be suitable for this purpose. However, results of our study show that the CC is not highly correlated with production conditions on the farm. The low correlation between the EC and the farm score obtained in this study agrees with the conclusions of White and Sherman *(25)*. There was no significant relationship between the two indicator groups on the sample stored at 3.3 C for 72 hr. The bacterial flora varies from farm to farm so that no specific group can be expected to be present in relation to the level of farm sanitation. When the milk samples were preincubated, there was significant correlation between the EC and CC. The coliforms grow much more rapidly than the enterococci during PI. The significant correlation indicates that higher CCs were obtained on PI samples that had the higher ECs.

The r value was not statistically significant between the farm score and the TdBC. This was expected since continued neglect in cleaning and sanitizing over an extended period is necessary for a thermoduric population to become established *(8, 17)*. Such continued neglect should not be common on grade-A farms.

The RRT can not be relied upon to evaluate high-quality milk. Only one of the 29 samples stored at 3.3 C for 72 hr had a RRT other than 4 hr. Only two of the 24 preincubated samples had a RRT other than 4 hr. This should be remembered when comparing the RRT with other bacterial test results. Also, the RRT was not significantly correlated with the farm score. The inability of the RRT to measure the quality of adequately cooled milk has been recognized for some time *(3, 16, 19, 22, 24)*.

A significant r value was observed between the leucocyte counts (determined on fresh milk samples) and the farm scores. This indicates that the dairyman who ignores recommended cleaning and sanitizing procedures also has difficulty in maintaining herd health.

The multiple correlation coefficient of the coliform and psychrophilic count with the farm score gave a more comprehensive measure of production conditions. This value was only slightly higher than the

simple correlation of PBC with the farm score for the sample stored at 3.3 C for 72 hr. The correlation was not significant on the PI sample, probably because fewer samples were analyzed for this treatment.

Low correlation between certain bacterial test results and production conditions also has been observed recently by Atherton *(4)*, and Johns et al. *(14)*. The low correlation of the bacterial test results with farm scores could be the result of many factors, the chief being the interaction between different microbial groups in a mixed population, as influenced by physical and chemical factors. Also there are limitations of the tests themselves, and a lack of guidelines for the development of proper weightage in the score card.

Correlation between counts

The r value was significant between the SPC and the other five bacterial counts and the RRT. The high correlation between results of the two tests may be because many of the same microorganisms are enumerated by both methods. Correlation between the SPC and TC was higher than the correlation between the SPC and PBC. These observations were probably related to the temperature of incubation employed for the different tests. In the same light there was a significant correlation between the SPC and CC. The coliforms were enumerated by both counts.

There are reports of correlation *(7, 10)* and lack of correlation *(11, 15, 18, 26)* between coliform and total bacterial counts. Correlation coefficients were higher on the preincubated samples than on the samples stored at 3.3 C for 72 hr for all bacterial tests except TdBC. This was expected because the temperature used during the preincubation (12.8 C) is approaching the optimum growth temperature of most microbial groups found in milk in contrast to 3.3 C storage. The lower correlation coefficient of the SPC with the TdBC on the PI sample indicates variable SPC and TdBC responses to PI. In every comparison with other bacterial tests, the agreement with the TdBC was lower on the PI sample.

Correlation values between the TC and the other six bacterial tests results were statistically significant. As explained earlier, these correlations were probably governed by the temperature of incubation for the various tests. Except for the TdBC, the correlation between the TC and the other bacterial tests was higher for the PI sample than for the sample stored at 3.3 C for 72 hr.

The agreement between the PBC and results of bacterial tests that measure many nonpsychrophilic microorganisms was lower, but still significant. Their association probably results from the common origin of contamination. There was no significant correlation between the PBC and the RRT. Since most psychrophilic microorganisms are poor reducers, good agreement would not be expected.

The correlation coefficient between the EC and the RRT was statistically significant for both sample-storage treatments. Enterococci, with the exception of *Streptococcus durans*, are active reducers so one could expect a relationship between their numbers and the RRT.

The r value was not significant between the RRT and the CC. One would expect a high correlation in high-coliform count milk since the coliform organisms rapidly reduce resazurin dye. Milk used in this experiment had a low coliform count and hence the low correlation. The degree of agreement was much higher, but still not significant, with the preincubated sample. Closer agreement between CC and RRT on the PI sample is probably associated with rapid multiplication of actively reducing coliforms during storage at 12.8 C for 18 hr.

The only significant r value between the leucocyte count of the fresh milk sample and the bacterial test results of the preincubated milk samples was with the TdBC. The reason for this relationship is not clear. High bacterial counts frequently result from contamination, especially from the milk handling equipment, rather than from the udder of the cow.

Effect of preliminary incubation

Within this experimental design, PI did not improve the ability of the bacterial tests to show significant correlation with the farm score. It cannot, therefore, be considered of much practical value for application in grade-A raw milk evaluation. With the PBC (the only bacterial test that showed significant correlation with the farm score), correlation with the farm score on the PI sample was lower than the correlation on the sample stored at 3.3 C for 72 hr. Correlation of the PBC with all bacterial tests except the TdBC was higher on the PI sample. This resulted from the ability of psychrophilic microorganisms to grow rapidly when the incubation temperature was increased. The microorganisms enumerated by tests other than the TdBC multiplied in proportion to those enumerated by the PBC. Some of the organisms enumerated by the other bacterial tests could have been psychrophiles. Although PI may increase the agreement between two bacterial tests, if those results are not an accurate appraisal of production conditions, the higher correlation is of no significance in evaluating production conditions.

Results of several bacterial tests, when evaluated with farm scores, indicate that they would not reflect failure in adhering to recommended procedures for the production of grade-A milk. Farm inspection still is a necessary part of quality control. The relationship of farm inspection to laboratory control

is quite correctly stated in *Standard Methods for the Examination of Dairy Products* (*1*, p. 10-11) which emphasizes the importance of milking-time inspections, and further states that laboratory tests in themselves do not constitute a complete quality control program.

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