# Bacteriological Control of Milk Quality*

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DURING the past few years, particularly since the adoption of tryptone glucose extract agar as the standard methods medium for making plate counts on milk, there has been increasing research and interest relative to the bacteria count of pasteurized milk. This has resulted in a more intense interest in the raw milk supplies than has previously been shown, with the introduction of laboratory pasteurization as a means of weeding out raw milks which contain large numbers of thermoduric and thermophilic bacteria.

It has been well established by numerous workers that the thermoduric bacteria found in pasteurized milk originate in dirty utensils, milking machines, and the like on the farm. Hence, a high bacteria count in pasteurized milk resulting from thermoduric bacteria is not a reflection on the pasteurizing plant, as has been commonly supposed in the past, but a direct reflection on the supply of raw milk from which the pasteurized milk was obtained.

Macy (1) found thermoduric bacteria to be the chief source of high counts in pasteurized milk; therefore in order to secure uniformly low counts in pasteurized milk, the raw milk containing excessive numbers of thermoduric bacteria must be detected and eliminated from the supply.

Laboratory pasteurization, or some modification of it has been the only method developed to date for the detection of thermoduric bacteria. While there can be little objection to the laboratory pasteurization test itself, the original technique of making standard methods plates on the laboratory-pasteurized samples is so cumbersome that it is not a practical test for the industry. However, several modifications have been proposed which simplify the procedure considerably.

Maack (2) suggests using a standard loop to transfer 0.001 cc. of the laboratory pasteurized sample to previously poured agar plates and streaking several samples on one plate. Hileman and Leiber (3) suggest incubating the laboratory pasteurized samples over night and examining them under the microscope for the presence of micrococci, which indicate thermoduric bacteria.

Mallmann, Bryan, and Fox (4) developed a modified laboratory pasteurization procedure and microscopic examination to detect thermoduric bacteria. In this procedure, only the living cells are stained and counted under the microscope, and a microscopic count over 40,000 bacteria per ml. is considered excessive.

Myers and Pence (5) developed a method involving an oval tube and standard loop, which embodies all the advantages of the standard plate count, and in addition, may be performed as rapidly as any of the accepted tests for grading raw milk supplies. We obtained very close agreement with their technique and the standard plate count; and were able to pasteurize the samples in the laboratory, make the inoculation on the oval tubes, and count the colonies on the tubes in approximately the same time per test as is required for making methylene blue tests.

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In making this study, we were fortunate in having two supplies of raw milk. The graded milk supply is the regulation raw milk, which upon pasteurization, will meet grade A specifications of the Standard Ordinance proposed by the United States Public Health Service. We also had access to an ungraded milk supply which is used for manufacturing purposes and is produced under no inspection at all, the only requirement being that it be delivered to the plant sweet and free from objectionable flavors and odors.

Weekly samples were taken from our graded and ungraded supply of milk for a period of 14 consecutive months. Three 10 ml. portions were taken from each sample, one each for a methylene blue and resazurin test, and the third was used for laboratory pasteurization. The samples were pasteurized at 143° F. for 30 minutes, then immersed in ice water, and held for 3 hours before plating on oval tubes.

The resazurin test was made by reading the samples at 30 minute intervals for 3 hours, a distinct purple pink end point being considered the end point of reduction. At the end of 3 hours, a smear was made and examined under the microscope to determine the type of bacteria predominating. The methylene blue samples were read at hourly intervals for 6 hours. Both resazurin and methylene blue samples were incubated overnight and examined the following morning to determine the type of curd formation.

After plating the laboratory-pasteurized samples on oval tubes, the samples were incubated overnight, then examined under the miscroscope to determine the type of flora prevailing.

In this study, 1,200 samples were collected and laboratory pasteurized. Of this number, 957 were samples of graded milk, and the remaining 243 were from the ungraded supply. Table 1 summarizes the results by months. In this table comparing the laboratory-pasteurization results of graded and ungraded milk, the two grades gave approximately the same percentage of tests between 5,000 and 20,000 bacteria per ml.; the exact percentage being 13.9 percent for the graded samples and 16.5 percent for the ungraded samples. The percentage of samples with oval tube counts over 20,000 per ml. however, revealed an unbelievable difference between the two grades of milk. The total for the graded samples being 8.1 percent, while 63.5 percent of the ungraded samples had an oval tube count over 20,000. From the standpoint of laboratory pasteurization, the chief difference in the two grades of milk is the

TABLE 1

MONTHLY SUMMARY OF 1,200 SAMPLES LABORATORY PASTEURIZED

(957 Samples of Graded Milk, and 243 Samples of Ungraded Milk)

| | Month— | Jan. | Feb. | Mar. | Apr. | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Graded | Samples tested | 196 | 52 | 65 | 119 | 53 | 90 | 62 | 54 | 26 | 52 | 52 | 136 | 957 |
| | % 5,000 to 20,000 | 15.3 | 23.0 | 34.0 | 15.0 | 5.7 | 6.7 | 6.5 | 7.4 | 3.9 | 13.5 | 17.3 | 12.5 | 13.9 |
| | % over 20,000 | 12.2 | 17.3 | 7.7 | 6.7 | 1.9 | 1.1 | 1.6 | 1.9 | 3.9 | 0.0 | 11.5 | 14.7 | 8.1 |
| Ungraded | Samples tested | 20 | 20 | 25 | 20 | 54 | ... | 11 | 18 | 10 | 20 | 20 | 25 | 243 |
| | % 5,000 to 20,000 | 10.0 | 20.0 | 4.0 | 10.0 | 18.5 | ... | 0.0 | 22.0 | 20.0 | 10.0 | 20.0 | 28.0 | 16.5 |
| | % over 20,000 | 65.0 | 70.0 | 64.0 | 75.0 | 65.0 | ... | 64.0 | 61.0 | 30.0 | 90.0 | 35.0 | 60.0 | 63.5 |

number of samples with oval tube counts over 20,000. Accordingly, the following data will deal with the samples which gave oval tube counts in excess of 20,000 bacteria per ml.

The months of November, December, January, February, March, and April gave relatively high percentages of samples that did not pasteurize to a count below 20,000; while the months of May, June, July, August, September, and October gave an almost insignificant percentage of counts over 20,000. This is the exact reverse of what one would anticipate, since all our quality tests have indicated that our supply of milk is of much higher quality during the winter months.

There is no such trend in the ungraded milk samples, but the entire monthly summary shows that an ungraded supply of milk such as ours would never supply milk which could be pasteurized to a low count at any month of the year. This contradicts the early criticism of pasteurization, that it merely covered up faulty practices on the farm, and did nothing to improve the sanitary quality. Pasteurization may cover up certain faulty practices on the farm, if the plate count is taken as the criterion of quality of pasteurized milk, but the careless handling of utensils and milking machines on the farm shows up in the form of high bacteria counts in the pasteurized product.

In Table 2, we have the microscopic classification of both graded and ungraded milk supplies for the samples which gave oval tube counts over 20,000 per ml. The table for the graded milk supply is based on 47 tests, or 6.3 percent of the total of 742 samples examined. The microscopic examinations were made after 3 hours incubation, and classified according to the method advanced by Lazarus (6). Unclean utensils, external contamination, and poor production accounts for 72.3 percent of the samples containing over 20,000 bacteria per ml. The direct microscopic examination of graded milk samples would have been effective in weeding out samples containing large numbers of thermoduric bacteria were it not for the fact that in 19.2 percent of the samples with oval tube counts in excess of 20,000, no bacteria were found in the ten fields examined. Thus, in many cases, raw milk with a low bacterial content is responsible for high counts after the milk is pasteurized.

TABLE 2

MICROSCOPIC CLASSIFICATION OF SAMPLES CONTAINING OVER 20,000 THERMODURIC BACTERIA PER ML.

| Graded Milk Samples | | | | | | Ungraded Milk Samples | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No Bacteria | Lack of Cooling | Dirty Utensils | External Contamination | Poor Production | Mastitis | No Bacteria | Lack of Cooling | Dirty Utensils | External Contamination | Poor Production | Mastitis |
| 19.2 | 4.3 | 17.1 | 23.4 | 31.8 | 4.2 | 7.5 | 42.4 | 4.2 | 6.7 | 39.2 | 0.0 |

The data for the ungraded milk is based on 189 tests, of which 62.5 percent or 118 samples gave oval tube counts over 20,000 per ml. For this type of milk, the samples giving high oval tube counts which showed no bacteria in the microscopic examination dropped to 7.5 percent. However, in 42.4 per cent of the high counts, lactic acid types of bacteria predominated, according to the microscopic examination. Thus, the microscopic examination did not reveal any unsanitary condition on the farm in approximately 50 percent of the ungraded milk samples which contained excessive numbers of thermoduric bacteria. We noticed that many of the thermoduric micrococci encountered are very similar in appearance to the common types of lactic acid diplococci. Undoubtedly a number of the samples heavily contaminated with thermoduric bacteria were considered to be lactic acid diplococci when viewed under the microscope. There is also the possibility of error due to the large number and variety of bacteria present, since it is more difficult to find the troublesome organisms when the bacterial content is extremely high than when it is lower.

on the fermentation test. For each of the grades of milk, more than 50 percent of the samples with oval tube counts over 20,000 gave a lactic type curd on the fermentation test. We have been able to develop considerable interest among our producers with the fermentation test, and it might be of value in a sanitary program for its visual effects, but it was of no value in detecting milk containing excessive numbers of thermoduric bacteria.

Table 4 shows the methylene blue classification of samples with oval tube counts over 20,000 bacteria per ml. There were 49.0 percent of the graded samples over 20,000 that did not re-

TABLE 3

FERMENTATION TEST CLASSIFICATION OF SAMPLES CONTAINING OVER 20,000 THERMODURIC BACTERIA PER ML.

| Graded Milk Samples | | | | Ungraded Milk Samples | | | |
|---|---|---|---|---|---|---|---|
| Not Coagulated | Lactic Type Curd | Gassy Curd | Peptonized Curd | Not Coagulated | Lactic Type Curd | Gassy Curd | Peptonized Curd |
| 8.5 | 55.4 | 12.8 | 23.3 | 4.2 | 53.4 | 19.5 | 22.9 |

TABLE 4

RESULTS OF METHYLENE BLUE TEST ON SAMPLES CONTAINING OVER 20,000 THERMODURIC BACTERIA PER ML.

| Hours on Methylene Blue — | 1 | 2 | 3 | 4 | 5 | 6 | Over 6 |
|---|---|---|---|---|---|---|---|
| Graded samples (percent)...... | 6.4 | 4.2 | 6.4 | 8.6 | 16.9 | 8.5 | 49.0 |
| Ungraded samples (percent)... | 46.7 | 12.8 | 10.9 | 9.4 | 6.0 | 3.5 | 10.7 |

Table 3 shows the results of the fermentation test on 47 graded milk samples which gave oval tube counts over 20,000. In using this test, we have presupposed that a digested or peptonized curd indicated the presence of bacteria originating in improperly cleaned and sterilized utensils. Since thermoduric and thermophilic bacteria originate in unclean utensils, we thought this test might be of value in detecing thermoduric bacteria. However, only 23.3 percent of the graded samples with oval tube counts over 20,000 showed peptonization, and only 22.9 per cent of the ungraded samples with oval tube counts over 20,000 bacteria per ml. gave a peptonized curd

duce methylene blue in 6 hours, while only 10.7 percent of the ungraded samples with oval tube counts over 20,000 failed to reduce in 6 hours. Although there was more widespread contamination of the ungraded samples with thermoduric bacteria than was present in the graded samples, there was also a much more prevalent contamination of other types of bacteria that reduced methylene blue, whereby most of the samples with high oval tube counts also reduced methylene blue in 6 hours. This would indicate that methylene blue should be a very effective test in starting a quality improvement program, but after definite progress has been made, it is necessary to supplement it with some other test, such as laboratory pasteurization or some of its modifications.

Table 5 shows the resazurin classification of both graded and ungraded samples over 20,000. There were 38.3 per cent of the graded samples over 20,000 which did not reduce in 3 hours, while 16.9 percent of the ungraded samples failed to reduce. This gave comparable results to those obtained with methylene blue; however resazurin was considerably more effective than methylene blue on the graded samples of milk, and slightly less effective on the ungraded samples. There is not sufficient difference, however, to give it consideration over methylene blue on this type of work, since both methylene blue and resazurin are extremely ineffective in weeding out milk containing thermoduric bacteria.

In our reference to the bacteria surviving pasteurization, we have grouped them all together and classed them as thermoduric. We recognize of course, that this also includes the thermophilic bacteria, if any are present; and since the term thermoduric means any bacteria which is heat resisting but will not grow at pasteurizing temperatures or above, we were interested in obtaining some information on the temperature necessary to reduce the high thermoduric counts to an acceptable number. Accordingly, 54 samples of our ungraded milk supply were prepared in duplicate and one sample pasteurized a 143° for 30 minutes, the other heated to 190° and immersed in ice water immediately. Of the samples pasteurized at 143° for 30 minutes, 16.7 percent had oval tube counts between 5,000 and 20,000; while 50 percent of the samples had oval tube counts over 20,000. Of the samples pasteurized at 190°, however, 14.8 percent had counts between 5,000 and 20,000, and none of the samples were over 20,000. From the commercial viewpoint then, it may be said that the bacteria resisting pasteurization encountered in this study resisted 143° for 30 minutes, yet were killed by 190° momentarily.

TABLE 5

RESULTS OF RESAZURIN TEST ON SAMPLES CONTAINING OVER 20,000 THERMODURIC BACTERIA PER ML.

| Hours on Rasazurin — | ½ | 1 | 1½ | 2 | 2½ | 3 | Over 3 |
|---|---|---|---|---|---|---|---|
| Graded samples (percent) | 6.4 | 6.4 | 8.5 | 8.5 | 12.8 | 19.0 | 38.3 |
| Ungraded samples (percent) | 61.8 | 5.1 | 5.1 | 5.9 | 4.3 | 1.8 | 16.0 |

## SUMMARY

Thermoduric bacteria are prevalent in both graded and ungraded milk supplies. They are of little consequence in graded milk during the summer months, but become of sufficient magnitude during the winter months to make it extremely difficult consistently to obtain low counts on the pasteurized products.

Uninspected and ungraded milk supplies are so thoroughly contaminated with thermoduric bacteria throughout the year that it would be impossible to pasteurize milk of this description so that it would meet the requirements of the standard ordinance from the standpoint of the bacteria count of the finished product.

The microscopic examination of the incubated samples of raw milk failed to detect samples with large numbers of thermoduric bacteria. Even in samples where large numbers of thermoduric bacteria are present in the raw milk, they are so similar to some of the types of lactic acid bacteria that their identification is difficult, if not impossible.

Although the fermentation test is of value in building interest among the producers, it is in no way able to detect samples that are heavily contaminated with thermoduric bacteria.

The methylene blue and resazurin tests are ineffective in weeding out milk with large numbers of thermoduric bacteria. Heat-resisting bacteria as a group are very poorly reducing bacteria, and even when present in extremely large numbers have little effect on either the methylene blue or resazurin test. These tests are successful in grading out milk containing large numbers of thermoduric bacteria only when there is also a high contamination of other bacteria which are capable of reducing the dye. In this connection, they are very effective for the first quality improvement work when the producers are lax from most every standpoint of quality, but as the sanitary practices are improved and the source of large numbers of highly reducing bacteria eliminated, the thermoduric bacterial content is not reflected in the results of the tests.

Laboratory pasteurization alone is not a satisfactory test on which to base the sanitary control of a milk supply, but on the other hand, none of the other present tests have proven satisfactory to be used without some supplementation from other tests. However, the laboratory pasteurization test definitely detects a type of unsanitary condition on the farm that is not detected by any of our present tests. Furthermore, this unsanitary condition perhaps has more direct influence on the plate count of the pasteurized product than all the other sanitary qualities obtained with older tests. Laboratory pasteurization is the one test that provides a carry through in the inspection of both raw and pasteurized milk. With our present method of inspecting and grading raw milk supplies, there is no assurance whatsoever that a graded raw milk supply will give a sufficiently low count after pasteurization to meet the requirements for pasteurized milk. In a well balanced sanitary control of raw milk supplies, it should be assured that raw milk meeting the requirements for its grade would likewise meet all the requirements of its grade after being pasteurized.

The oval tube technique developed by Myers and Pence, which we used in this study, has proven its worth both in connection with the laboratory pasteurization test, and also in the sanitary control work in the plant. We have found it capable of replacing the standard plate count altogether for routine control work with equally satisfactory results, and requiring only a small fraction of the labor, equipment, and space required for the standard plate count.

REFERENCES

1. Macy, H. High Bacterial Counts in Pasteurized Milk—Some Factors Involved. Intern. Association Milk Dealers. Bull. No. 8, 179 (1939).

2. Maack, A. C. Control of Heat Resisting Bacteria. *The Milk Dealer,* **30**, No. 4, 84 (1941).

3. Hileman, J. L., and Leber, Henry. High Temperature Short Time Pasteurization and Its Practical Application to the Dairy Industry. *J. Milk Technol.,* **4**, 128 (1941).

4. Mallmann, W. L., Bryan, C. S., and Fox, William K. A New Microscopic Procedure for the Detecting and Locating of the Source of Thermoduric Organisms in Milk. *Ibid.,* 4, 195 (1941).

5. Myers, R. P., and Pence, J. A. A Simplified Procedure for the Laboratory Examination of Raw Milk Supplies. *Ibid.,* **4**, 18 (1941).

6. Lazarus, N. E. Quality Control of Market Milk. Olsen Publishing Co. (1935).