A New Modification of the Frost Little Plate for the Detection of Heat Resistant Bacteria in Milk*

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Army contracts and milk ordinances contain an item specifying the maximum allowable bacteria count of milk. The reason for this is obvious, since a healthy cow's milk, that is handled in sanitized utensils and properly cooled and stored, has a low bacteria count. Any deviation from this permits either the entrance of bacteria to milk or the growth of those that are present. In each case a high bacteria count of the milk results.

The majority of the bacteria, in nature, that may find their way into milk are readily destroyed during the pasteurization of milk. On the other hand, milk may become contaminated on the farm or in the pasteurizing plant with bacteria that are heat resistant. The heat resistant bacteria may be thermoduric—be able to survive pasteurization but not able to grow at that temperature, or thermophilic—be able to survive and grow at pasteurization temperature. The presence of excessive numbers of heat resistant bacteria results in a high bacteria count of the finished product and indicates a defect in the system of milk production or processing. In either case corrective efforts should be started. The need for a rapid yet accurate means of detecting the presence of heat resistant bacteria is obvious.

Laboratory pasteurization test for the detection of heat resistant bacteria

The only reliable method for detecting heat resistant bacteria in milk has been the laboratory pasteurization test. Briefly, this test consists in determining the bacteria counts before and after laboratory pasteurization. This technic is being used extensively and is an excellent means of tracing the origin of these organisms. The data of Table 1

TABLE 1

The Effect of Cleaning Farm Dairy Equipment as Measured by the Laboratory Pasteurization Test

	Before Cleaning		
	Bacteria Count of Milk		
Producer	*Raw*	*Lab. Past.*	*Percent Reduction*
1	3,000,000	15,000	99.5
2	1,500,000	500,000	66
3	800,000	500,000	38
4	300,000	8,000	97.5
5	100,000	75,000	25
Composite	850,000	300,000	65
	After Cleaning		
1	70,000	500	99.3
2	10,000	300	97
3	45,000	100	97.8
4	30,000	450	98.2
5	15,000	80	99.5
Composite	50,000	280	99.5

show the poor bacteria count reduction on pasteurization of milk of three producers and demonstrate the detection of the producer's milk containing heat resistant bacteria. The milk of these producers is responsible for a high count of the composite pasteurized milk of one small dairy. This is typical of many data at hand. The beneficial results of a thorough cleaning of the dairy equipment on the farms are shown in the second part of the table.

The pasteurization test is very satisfactory for use in small dairies with few producers but it is not readily ap-

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plicable to dairies with many producers on account of the amount of equipment necessary for conducting the standard plate counts. To overcome this, Meyers and Pence (2) have developed a loop measurement and oval tube technic. The tubes or plates must be incubated for 48 hours before counting. This two-day delay in obtaining the results is an important drawback in correcting the trouble by eliminating the source of the heat resistant bacteria. A need therefore exists for a quick yet efficient cultural method for the detection of heat resistant bacteria in milk.

Frost little plate for determining the numbers of living bacteria in milk

In 1942 Bryan and coworkers (1) reported "The incubation of Frost little plates at 37° C. in a moist chamber for four hours yielded bacteria colony counts of milk and cream comparable to the standard plate count." Thus, a Frost little plate can be prepared from the raw and the laboratory pasteurized milk to locate the milk containing heat resistant bacteria within a period of a few hours. The technic of preparing the Frost little plate has been modified to permit the detection of heat resistant bacteria in milk with a minimum of equipment.

MODIFIED TECHNIC

Instead of discarding the tube containing the milk-agar mixture after making a little plate for the raw count it is placed into a water bath and held at pasteurizing temperature for 30 minutes, returned to the 50° C. water bath (to prevent solidification of the agar by cooling), and a second little plate is immediately prepared for the laboratory pasteurized count. This saves all of the time and equipment of preparing a second tube of milk-agar mixture after pasteurization. The bacteria counts of Table 2 are typical of many obtained upon the laboratory pasteurization of milk; they were obtained from the raw and pasteurized milk by

TABLE 2

A COMPARISON OF BACTERIA COUNTS OBTAINED BY MAKING STANDARD PLATE AND FROST LITTLE PLATE EXAMINATIONS OF MILK BEFORE AND AFTER LABORATORY PASTEURIZATION AND THE BACTERIA COUNT AFTER PASTEURIZATION OF THE MILK-AGAR MIXTURE OF THE MODIFIED FROST PLATE METHOD

Sample		*Bacteria per Ml. of Milk*		*Percent Reduction*
		Raw	*Lab. Past.*	
		Heat Resistant Bacteria Present		
1	Standard plate	10,000,000	900,000	91
	Frost little plate	8,500,000	800,000	90.6
	Modified Frost	8,500,000	850,000	90
2	Standard plate	100,000	40,000	60
	Frost little plate	120,000	45,000	58.8
	Modified Frost	120,000	48,000	60
3	Standard plate	30,000	15,000	50
	Frost little plate	40,000	12,000	70
	Modified Frost	40,000	18,000	55
		Heat Resistant Bacteria Absent		
4	Standard plate	15,000,000	10,000	99.9
	Frost little plate	18,000,000	10,000	99.9
	Modified Frost	18,000,000	15,000	99.9
5	Standard plate	50,000	1,200	97.6
	Frost little plate	65,000	900	98.7
	Modified Frost	65,000	850	98.8
6	Standard plate	25,000	500	98
	Frost Little plate	30,000	450	98.9
	Modified Frost	30,000	600	98

the standard plate and Frost little plate methods, and compared with those obtained in the modified technic. As a result of the agreement of results these cultural methods can be used interchangeably for the detection of heat resistant bacteria in milk.

Further to check the comparison of the laboratory pasteurization test and the modified Frost technic, a series of 1375 milk samples was subjected to both examinations. The results that were obtained are presented in Table 3. The close agreement of the percentage of reduction in bacteria count as obtained by the two methods is an item of real practical value. That is, it allows the substitution of the modified Frost technic for the laboratory pasteurization test, thereby permitting the use of a culture method that requires very little agar and equipment, and yields results in a period of about *five hours* (incubation and time necessary for performing technic) instead of the 48 hours required for the plating and oval tube methods.

TABLE 3

A COMPARISON OF RESULTS OBTAINED UPON EXAMINATION OF 1,375 MILK SAMPLES FOR HEAT RESISTANT BACTERIA BY THE LABORATORY PASTEURIZATION TEST AND THE MODIFIED FROST METHOD

	Percent Reduction of Bacteria Count Upon Heating				
	0–25	*26–50*	*51–75*	*76–95*	*96–99*
Laboratory pasteurization test:					
Number of samples	200	100	125	350	600
Percentage of samples	14.5	7	9.5	25.5	43.5
Modified Frost method:					
Number of samples	188	110	112	360	605
Percentage of samples	13.5	8	8.5	26	44

THE MODIFIED FROST TECHNIC

1. Place a test tube rack, containing enough sterile tubes for the number of samples to be tested, into a water bath at 45 to 50° C.
2. Place 1.0 ml. of milk into a sterile test tube in the water bath. Do not allow more than 15 minutes between step 2 and completing step 4.
3. Add 1.0 ml. of sterile tryptone-glucose-extract agar, cooled to 50° C., into each tube in the water bath, and mix by shaking.
4. Using a sterile pipette place 0.1 ml. of the agar milk mixture onto a sterilized microscope slide (sterilized in the naked flame) and spread evenly over an area of 4 square centimeters with the tip of the pipette. (Two or three such films can be put on the ordinary microscope slide.) (See Fig. 1.)
5. Place the tube containing the milk-agar mixture into a water bath at pasteurizing temperature for ½ hour.
6. Remove to a 50° C. water bath and prepare a second little plate immediately (this will give the living bacteria count after pasteurization).

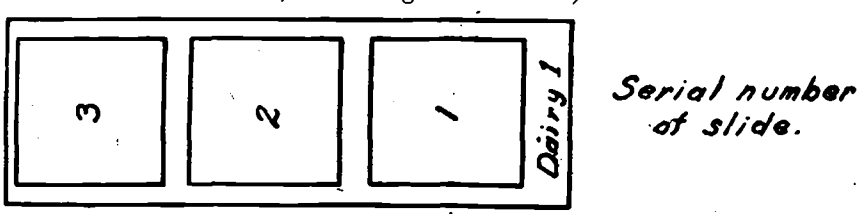

FIG. 1—*Guide for making Frost little plate. Transfer 0.1 ml. of the milk-medium mixture on to the slide and use the tip of the pipette to distribute evenly over the 4 sq. cm. area outlined.*

7. The little plate is allowed to harden and then placed into a moist chamber at 37° C. for a minimum of *four hours of incubation for milk and cream.* The incubation period may be extended to 24 hours without materially affecting the bacteria count. A moist chamber can be made out of any container with a tight-fitting cover by filling the container approximately ¾ full of water. A wire platform must be built above the level of the water to hold the slides during incubation. The proper temperature of bath is maintained by leaving the moist chamber in the 37° C. incubator at all times.)
8. After incubation the plates are dried over a flame, in an incubator, or in a drying oven at a temperature slightly under 100° C. (Agar should not melt; plates should be dried slowly to prevent cracking the medium.)
9. Stain for approximately 1 minute with a methylene blue solution. (To prepare, add 10 ml. of a saturated alcoholic solution to 90 ml. of 30 per cent alcohol.) This should stain the colonies deeply and leave a faint blue background.
10. Wash slides to remove excess stain, being careful not to wash the film off the slides. (Best results are obtained by allowing the water to strike the reverse side of slide—enough water will flow over the preparation to remove the excess stain.) Dry preparations.
11. Examine by using lower power (if few colonies), high dry power or oil immersion (if many colonies) to determine the average number of colonies per field (count *50 fields* when few colonies are present, and *10 fields* when many colonies are present) and note bacteria count by referring to Table 4.

SUMMARY

A rapid and accurate modification of the Frost plate technic is presented for determining the presence of heat resistant bacteria in milk. This is accomplished by pasteurizing the milk-agar mixture after a Frost little plate has been made and then making a second little plate for the pasteurized count.

The results of the modified Frost technic compared favorably with those of the pasteurization test upon examination of a series of 1375 milk samples. In addition, the final results were ready within five hours as compared to 48 hours in the laboratory pasteurization test.

LITERATURE CITED

1. Bryan, C. E., *et al.* A Comparative Study of the Frost Little Plate and Standard Methods for the Bacteriological Examination of Milk, Cream, and Ice Cream. *J. Dairy Sci.*, 25, 827–835 (1942).

2. Meyers, R. P., and Pence, J. A. A Simplified Procedure for the Laboratory Examination of Raw Milk Supplies. *J. Milk Tech.*, 4, 18–25 (1941).

TABLE 4

FROST LITTLE PLATE COUNTS

(This table can be used with all microscopes having a factor of 300,000 in the direct microscopic counting of bacteria in milk)

	Colonies	Fields	Bacteria per cc.		Colonies	Fields	Bacteria per cc.		Colonies	Fields	Bacteria per cc.
Low Power Objective	1	50	50	*High Dry Objective*	1	1.6	30,000	*Oil Immersion Objective*	2	1	480,000
	1	25	100		1	1	42,000		3	1	720,000
	1	12.5	200		2	1	84,000		4	1	960,000
	1	5	500		3	1	126,000		5	1	1,200,000
	1	2.5	1,000		4	1	168,000		6	1	1,440,000
	1	1	2,500		5	1	210,000		7	1	1,680,000
	2	1	5,000		6	1	252,000		8	1	1,920,000
	4	1	10,000		7	1	294,000		9	1	2,160,000
	6	1	15,000		8	1	336,000		10	1	2,400,000
	8	1	20,000		9	1	378,000		15	1	3,600,000
	10	1	25,000		10	1	420,000		20	1	4,800,000