A TECHNIQUE FOR LABORATORY PASTEURIZATION USING DISPOSABLE CONTAINERS

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The laboratory pasteurized test as described in Standard Methods *(1)* is only used to a limited extent because it is too time-consuming and requires too much additional equipment. Attempts to reduce the time required in setting up the test and to eliminate the need for additional glassware by using the glass collection vial as the heating container have not proven satisfactory. One of the major difficulties arises from the fact that droplets of milk remain on the under surface of the closure after shaking and proper thermal processing is impossible since the container cannot be immersed because of leakage. Flaming the upper region of the tube to "burn off" droplets of raw milk increases the likelihood of leakage as the teflon or rubber seal in the closure becomes misshapen upon contact with the hot glass.

The advent of a sterile plastic tube[1] with a leakproof closure has made it possible to utilize a single tube to collect the raw milk sample on the farm and to carry out the laboratory pasteurization test; the need for additional sterile glassware is eliminated and the time required for the test is considerably reduced. The certainty of the seal makes it possible to completely submerge the tube during laboratory pasteurization and complete heating of every particle of milk is assured.

This work was undertaken to study the utilization of a plastic tube for the collection of raw milk for a standard plate count and to determine the feasibility of using this same container and sample for the laboratory pasteurization test. In the first phase of this study the come-up times of milk in glass and plastic tubes were compared. In the second phase pure cultures of heat-resistant and heat-sensitive bacteria were subjected to pasteurization in glass and plastic tubes. In the third phase producer samples were collected and the results obtained following laboratory pasteurization were analyzed statistically for comparative purposes.

[1]Manufactured by Falcon Plastics Division of B-D Laboratories.

MATERIALS AND METHODS

Come-Up Studies

Since the volume of milk in the tube as well as the number of tubes under test at one time markedly influences the come-up time, control over these variables is mandatory. To control the quantity of raw milk in each tube a 10 $\pm$1-ml volume was selected since this represents a practical volume if the hauler or sanitarian uses a standard dipper. To control the number of samples under pasteurization at one time, a group of 40 samples was selected as a working unit. The results reported in the come-up trials and in the comparative laboratory pasteurization tests are based on a working unit of 40 tubes each containing 10 $\pm$1-ml of milk. In all cases, the plastic tubes (17 x 100 mm) were completely submerged in a covered wire basket weighted to prevent floating. The screw cap glass tubes (20 x 150 mm) containing a similar volume of milk were immersed so that the water level was approximately 7.5 cm above the level of the milk.

Come-up time studies were undertaken using a 5.2-gal Unitherm water bath containing 4.7 gal of water. The temperature was adjusted to 143°F using a 300-watt heater. In the come-up trials the temperature of the milk in the tube in the center of the cluster was taken at minute intervals using a thermocouple. The results are shown in Figure 1.

Pure Culture Studies

Corynebacterium filamentous, Micrococcus sp. and *Micrococcus* strain MS102 were used in this study. One ml of a 24-hr nutrient broth culture of each organism was added to 9 ml of sterile milk in plastic and glass tubes. The initial levels of inoculum in the milk with *C. filamentous* and *Micrococcus* strain MS102 were 100,000 and 990,000 per ml, respectively. With *Micrococcus* sp. studies were made using average initial inoculations of 52,000, 164,000, and 299,000 per ml. These levels were selected as they are representative of the range which might be encountered

Figure 2.—Apparatus used in making laboratory pasteurization tests showing raw milk samples in plastic tubes and weighted covered wire basket.

TABLE 1—COMPARISON OF LABORATORY PASTEURIZATION IN PLASTIC AND GLASS TUBES USING *Micrococcus* SP.

Sample		Initial count	Lab. past count Plastic	Lab. past count Glass	% Destruction Plastic	% Destruction Glass
		(No/ml)	(No/ml)	(No/ml)		
1	Repl. A	160,000	250	190	99.84	99.88
	Repl. B	170,000	400	370	99.75	99.86
2	Repl. A	63,000	110	140	99.83	99.78
	Repl. B	41,000	120	100	99.71	99.76
3	Repl. A	320,000	430	380	99.87	99.88
	Repl. B	280,000	470	410	99.83	99.85

in actual practice. Laboratory pasteurization was carried out as described above using a 40-tube working unit. Heating during come-up and exposure for the 30-min holding period was undertaken in a water bath at 143°F. On the basis of the findings in the come-up trials, the samples in plastic tubes were held in the bath for 40 min; samples in glass tubes were held for 36 min; timing commenced at the moment of immersion. After laboratory pasteurization the samples were cooled rapidly by placing them in a water bath to cover the milk level and plated in duplicate. Counts were made after incubation at 35°C for 48 hours. The results obtained with *Micrococcus* sp. are given in Table 1.

Comparative Studies on Producer Samples

Twenty duplicate producer samples were tested to ascertain whether there was a significant difference in bacterial count following laboratory pasteurization in plastic and in glass tubes. Blank tubes containing 10 ±1 ml of water were used to adjust the unit to 40 tubes. Laboratory pasteurization was carried out as described previously. Standard plate counts were made in duplicate and the results compared statistically. The apparatus used in this study is shown in Fig. 2.

RESULTS AND DISCUSSION

The data presented in Figure 1 show the come-up rate when 40 glass tubes each containing 10 ±1 ml of milk at 45°F were placed in a water bath at 143°F. The time required to reach 143°F was 6 min with glass tubes. Under similar conditions using plastic tubes and complete immersion, the come-up time was 10 min. Although the come-up times in plastic in both instances the samples were held at temperatures between 139-143°F for approximately four min. Because of the similarity of the curves throughout this temperature range, the bacterial kill in this phase of heating is quite similar in plastic and glass. Exposure periods below 105°F (0.5 min in glass and 1.5 min in plastic tubes) would not contribute materially to the lethal process. The exposure period required to raise the temperature from 105°F to 139°F (4 min and 1.5 min in plastic and glass, respectively) represents the area of greatest diversity between these two come-up curves. Because of differences in the thermal destruction rates of various

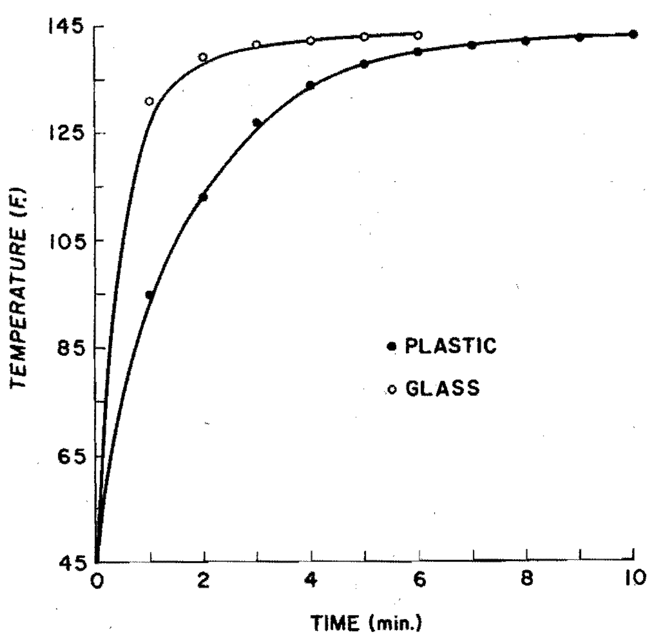

Figure 1.—Come-Up Curves For Milk In Glass and Plastic Tubes In A Water Bath at 143°F.

and glass differ somewhat it is interesting to note that organisms an interpolation as to the significance of this come-up difference is impossible.

In an attempt to obtain some information relative to the importance of this difference in come-up time with respect to the laboratory pasteurization test, *C. filamentous*, *Micrococcus strain* MS 102 and *Micrococcus* sp. were carefully laboratory pasteurized in milk using plastic and glass containers. In the absence of definite endpoints with *C. filamentous* it was impossible to determine the effect of the different come-up curves on bacterial destruction. The bacterial destruction in either instance appeared to be similar to the extent that neither glass nor plastic showed any survivors after heating when samples were plated at the one ml level. With the extremely heat resistant *Micrococcus* strain MS102, at an initial average concentration of 990,000 cells per ml, average survivor levels of 930,000 and 855,000 per ml. were obtained in glass and plastic containers, respectively. The close agreement between these values indicate that the come-up rate did not appreciably influence the total destructive effect of the complete heat treatment. The results obtained with *Micrococcus* sp. are given in Table 1. After heating at 143°F for 36 min in glass and 40 min in plastic tubes, the per cent destruction, based on the grand average, was 99.84% and 99.81%, respectively. The range of the actual count observed between replicates within a single sample indicated that the difference in per cent reduction based on the grand average value is insignificant.

To study a more heterogenous bacterial spectrum and to gain some information as to the practical application of this technique under field conditions, twenty producer samples were collected by a commercial hauler and tested in plastic and glass containers. An analysis of variance of the data presented in Table 2 indicated no significant difference at the 99% level between the counts obtained on samples in glass and plastic tubes. In comparing the data, the correlation coefficient was 0.9936. It is apparent from this that the laboratory pasteurization test as carried out in plastic tubes as described gives results comparable to that obtained with glass tubes when the milk samples are exposed at 143°F for 40 and 36 min respectively.

The use of a plastic tube as a container for collecting raw milk as well as a vial for carrying out the laboratory pasteurization test saves considerable time as no transfer of milk to a clean sterile tube is necessary. This also eliminates the need for additional screw-caps tubes and transfer pipettes, both of which require cleaning and sterilizing. Plastic tubes have been used as sampling tubes in our laboratory for approximately one year. The haulers using these tubes have registered no complaints. Haulers are instructed to replace the plastic cap so as to close the tube but not to seat the cap completely; removal of the cap for making a raw milk count is thus facilitated. For laboratory pasteurization the cap is seated completely to prevent leakage during immersion.

TABLE 2—COMPARISON OF COUNTS ON MILK SAMPLES PASTEURIZED IN PLASTIC AND GLASS TUBES

Producer Sample	Raw milk SPC at 35°C	Lab. past. count	
		Plastic	Glass
	(No/ml x 1000)	(No/ml)	(No/ml)
1	43	620	790
2	100	120	130
3	280	50	30
4	66	230	160
5	2,700	900	880
6	87	240	130
7	50	30	90
8	250	40	20
9	380	2,200	2,300
10	80	40	60
11	41	5,500	5,200
12	16	1,600	1,100
13	9	560	380
14	25	110	120
15	8	220	190
16	4	40	25
17	17	960	690
18	5	150	110
19	4	180	140
20	23	6,000	6,500

CONCLUSIONS

A plastic container has been found to be satisfactory for the collection of raw milk samples for bacterial counts and for the laboratory pasteurization test.

A procedure is described for making a laboratory pasteurized test using the same tube in which the milk sample was collected; the method saves time and requires less equipment.

ACKNOWLEDGMENT

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REFERENCES

1. American Public Health Association. Standard methods for the examination of dairy products. 10th ed. Am. Public Health Assoc., Inc., New York. 1953.