COMPARISON OF MODIFIED ASTELL ROLL-TUBE COUNT WITH STANDARD PLATE COUNT OF THERMODURIC BACTERIA IN BULK RAW MILK

(i) Effects of Pasteurization Methods on Counts,

(ii) Relative Cost of Roll-tube Count in Respect to S.P.C.

by

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A Major Thesis submitted to the
Faculty of Graduate Studies and Research
The University of Manitoba
in candidacy for the degree of
Master of Science
1961

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One hundred and fifty-three samples of bulk raw milk were laboratory pasteurized in lots of 10 samples by the H.T.S.T. and holder methods. The thermoduric counts were determined by a modified Astell Roll-tube method and the standard plate count method. Four comparisons were made and while variations in the counts were apparent, statistical analysis using the "t" test indicated that, on the basis of the limited data, the difference between any of the four comparisons was not significant at the 1% level.

The H.T.S.T. laboratory method gave the same result as the "holder" laboratory method, and was performed more economically.

The modified Astell Roll-tube method was as accurate as the S.P.C. method, in addition, it was simpler, less time consuming and less expensive than the S.P.C. method. Consequently, on these basis, the modified Astell Roll-tube method could be used to determine thermoduric counts in raw milk.

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INTRODUCTION

In recent years there has been a general reapprasial of methods used to determine the quality of raw milk. As a result, the thermoduric count has gained favourable attention, among sanitarians, as one of the tests used to assess milk for quality.

Thermoduric organisms survive pasteurizing temperatures and consequently contribute to counts in excess of the legal limit for pasteurized products. These organisms enter milk chiefly from inadequately cleaned milking machines and other equipment; thus their presence in pasteurized products indicates unsanitary conditions on the farm. However, most of these organisms are not pathogenic and have little bearing on the keeping quality of the pasteurized product (Corash, 1948; Sawartling, 1953).

The official method for determining the thermoduric count consists of laboratory pasteurization of samples by the holder method and plating on standard plate count medium. However, this method is complex, time consuming and expensive; and as a consequence, because of these difficulties various simplified procedures have been reported.

The purpose of this investigation was two-fold:

(a) To compare the efficiency of the holder and H.T.S.T. method of laboratory pasteurization and

(b) To determine the value of a simplified roll-tube method on milk pasteurized by these methods in respect to counts and cost.

REVIEW OF LITERATURE

The official method for determining thermoduric counts is given in Standard Methods for the Examination of Dairy Products (1953). In this method the milk is pasteurized by the holder method. However, some investigators have pasteurized milk by the H.T.S.T. method, and obtained, in general, higher counts.

A comprehensive review of thermoduric bacteriae in pasteurized milk has been presented by Hileman (1940).

Hileman and Leber (1941 a), in a study involving 125 samples, found that low count milk pasteurized by the H.T.S.T. method, gave counts of the same level as similar milk pasteurized by the holder method; and high count milk pasteurized by the H.T.S.T. method gave higher counts.

Hileman et al (1941 b) reported that thermoduric organisms were the primary cause of the high counts in milk pasteurized by the H.T.S.T. method. In a study on 484 samples, involving 48 producers, laboratory pasteurized at 161° F for 16 seconds, 79.3% of the surviving bacteria were micrococci, 7.4% streptococci, 8.1% sarcinae and 5.2% bacilli. Certain species of micrococci survived the H.T.S.T. method in greater numbers than was the case with the holder method. The most common of these were _M. candidus_, _M._

epidermicus, and M. varians. They considered these to be the predominant micrococci in the thermoduric flora of improperly cleaned milking utensils.

Hiscox (1943), in routine weekly bacteriae tests made over a period of two years on commercial samples from a single plant, showed that H.T.S.T. laboratory pasteurization greatly reduced the number of thermophilic bacteria. In the same tests, thermoduric organisms were destroyed to a degree equal to that of the holder process. This had been demonstrated previously by Yale and Kelly (1933) and by Workman (1941).

In a study of H.T.S.T. laboratory pasteurization Galesloot (1951), found that high thermoduric counts were caused chiefly by certain species of Streptococcus: St. thermophilus, St. bovis and St. durans.

Both Macy (1949) and Doetsch (1939) found excessive numbers of thermoduric bacteria in milk to be significant because they indicated unsanitary conditions of production.

Mallmann et al (1940) collected milk samples from milking machines which received little or no inspection. A large percentage of the bacteria were resistant to pasteurization. Further, milk produced under strict sanitary inspection showed only few heat resistant organisms.

Anderson et al (1933) found that excessive numbers of thermoduric organisms correlated well with unsanitary production practices. Laboratory pasteurization proved a most useful test to detect these organisms.

Levowitz (1958) demonstrated that high total counts associated with high thermoduric counts, indicated inefficient washing or mere rinsing; and that high total counts associated with low thermoduric counts indicated that equipment had not been washed.

Little (1942) demonstrated that laboratory pasteurization detected unsanitary conditions on the farm that were not detected by any of the methods in use. He found that thermoduric flora generally consisted of poor reducers, and consequently they were not easily detected by dye reduction methods.

Claydon (1955) reported that milk from teat-cup liners, in reasonably good physical condition but having microscopic breakdown of inside surfaces had higher thermoduric counts than milk from new liner units. Consequently he concluded that thermoduric counts could be used to detect defective inflations on milking machines.

Fabian (1946) reported that neither the resazurin nor the methylene blue test was an accurate index of thermoduric bacteria.

Johns (1954) postulated that the resazurin reduction test did not detect thermoduric organisms because these organisms were weak reducers. Hence, he proposed the adoption of the thermoduric count as an index of faulty farm procedures.

Sommer, as reported by Johns (1959), immersed paper strips saturated with plate count agar in laboratory pasteurized milk. These strips absorbed a definite volume of milk. These were incubated and the thermoduric bacteria counted.

Mallmann et al (1941) suggested incubating samples at 58 - 60° C for two hours before direct microscopic examination. Samples with more than 40,000 per ml indicated thermoduric organisms were present and were classified as unsatisfactory.

Hileman and Lieber (1941) suggested incubating laboratory pasteurized samples overnight and examining microscopically for micrococci, which would represent an index of thermoduric organisms.

Maak (1941) suggested using a standard calibrated 0.001 cc loop to streak several laboratory pasteurized samples on a previously poured agar plate as a screening test for thermoduric bacteria.

Myer and Pence (1941) employed an oval tube technique to determine the thermoduric count on a large number of samples. Five ml milk aliquots in vials were pasteurized by the holder method. By means of a welded, calibrated loop (0.01 ml or 0.001 ml) a loopful of laboratory pasteurized milk was transferred to an oval tube containing 5 ml of sterile and tempered medium. After inoculation the tube was swung in a small arc for a minimum of 5 seconds and then allowed to solidify on the flat side in an inclined position. Incubation was at 32° C for 48 hours. Counts by this method agreed closely with counts made by the standard plate method.

Johns et al (1942) made a comprehensive comparison of simplified methods used to determine the number of thermoduric organisms in laboratory pasteurized milk and concluded that results with the oval tube method of Myer and Pence showed close agreement with those of the standard plate method. Other simplified methods did not compare as favorably.

Thompson (1960) employed a plate-loop count method to determine counts on raw milk. He used a 0.001 ml calibrated loop attached to a continuous volume syringe for rinsing the sample into a standard petri dish prior to pouring the agar. Results of 51 samples with this method compared closely with standard plate counts.

A modification of the tube technique appeared in 1947 in Britain. This was the Astell Roll-tube method. It was designed to replace the petri dish method. The apparatus consists of Roll-tube bottles, bacteriological seals, a spinner, water bath, pipettes, wire racks and a colony counter. A description of the apparatus and also a comparison of results obtained by it compared with the standard plating method were presented by an anonymous author (1957). In this investigation three separate trials involving 90 samples of milk were used. The results of the Roll-tube method agreed closely with those of the plating method. Earlier workers using similar tubes had reported essentially the same results (Prouty et al, 1944; Howard and Fischer, 1950; and Clegg et al, 1951).

PROCEDURE

The whole project involved 153 samples of raw milk obtained from 32 bulk milk shippers in the Winnipeg milk-shed. The milk samples were taken by qualified bulk-tank truck operators. Before a milk sample was taken from a bulk tank, the agitator of the tank was set in motion for a minimum of five minutes to ensure thorough mixing. A dipper sterilized in hypochlorite (200 ppm) was used to dispense not less than 50 ml of milk to a sterile glass jar. The jar was sealed with a plastic cap lined with a non-toxic disposable liner. These samples were placed in the refrigerated compartment of the truck. Upon arrival at the dairy, usually at noon and again late in the afternoon, the samples were refrigerated and delivered to the laboratory the following morning, when they were tested in duplicate by the Standard Plate Count method, and also by the Astell Roll-tube method, modified as follows.

Four-ml aliquots of Difco Standard Plate Count medium in Astell Roll-tubes were sterilized at 250° F for 15 minutes, sealed and stored at room temperature. For each experiment the appropriate number of tubes was selected and the agar melted and tempered to 45° C in a thermostically controlled water bath.

Instead of using the 1:10 and 1:100 dilutions prescribed in the Astell method, 0.1 ml and 0.01 ml milk were delivered directly to the medium. A one-ml pipette graduated in 0.1 ml was used for the 1:10 dilution and a certified 0.01 ml loop for the 1:100 dilution. When inoculating the roll-tube, care was taken to deliver the sample directly into the medium. The loop was shaken back and forth several times to ensure complete detachment of milk from the loop. After inoculation, each tube was shaken once to ensure mixing of the milk and agar, and rotated in the spinning apparatus until the medium solidified. Tubes were incubated in an upright position, at 32° C for 48 hours. A Quebec colony counter was used and proved to be satisfactory.

<u>Pasteurization and Platings of Milk Samples</u>

Milk samples were pasteurized according to Standard Methods (1953). Ten samples were tested at a time. Pasteurized samples were plated in duplicate in two dilutions (1:10 and 1:100). Incubation was at 32° C for 48 hours. A Quebec colony counter was used.

RESULTS

Count Studies.

Counts by the two methods of pasteurization are presented in table I. Counts on 53 of the samples were either lower or higher than accepted permissable counts and accordingly were not considered further.

Four comparisons were made:

(a) Counts by the roll-tube and plate methods on H.T.S.T. pasteurized samples.

(b) Counts by the roll-tube and plate methods on holder pasteurized samples.

(c) Counts by the roll-tube method on the two methods of pasteurization and

(d) Counts by the plate method on the two methods of pasteurization.

While evidence of considerable variation in the counts is apparent, statistical study using the "t"-test indicated that on the basis of these limited data the difference between any of the four comparisons was not significant at the 1% level. The mean counts and the "t" values for the four comparisons are shown schematically as follows:

H.T.S.T.		Holder	"t" value	Critical value
S.P.C.	vs	S.P.C.	0.55	2.326
Roll-tube	vs	Roll-tube	0.88	2.326

H.T.S.T.			"t" value	Critical value
S.P.C.	vs	Roll-tube	0.53	2.326

Holder			"t" value	Critical value
S.P.C.	vs	Roll-tube	0.83	2.326

Mean count on 100 samples.

H.T.S.T.		Holder	
S.P.C.	Roll-tube	S.P.C.	Roll-tube
6600	6000	6000	5100

Cost Studies.

The Roll-tube method involved fewer hours of labour when the H.T.S.T. laboratory pasteurization method was used, and also when the accepted holder method was used. The summarized results follow.

Method	Holder	H.T.S.T.
S.P.C.	65 hours	56 hours
Roll-tube	42 hours	37 hours

It may be observed that the time involved in these tests appears high. This can be accounted for on the basis of the small number of samples tested at one time. The comparison, however, is valid because this factor affected the two methods in the same way.

Likewise, the Roll-tube method involves less medium, less incubation space, and less labour for preparing supplies and cleaning equipment. This is shown in summary below.

	Roll-tube	S.P.C.
Medium	4 ml/tube	10 ml/plate
Incubation space for 10 samples, 2 dilutions	5600 c.c.	8000 c.c.
Time required for preparing supplies and cleaning equipment (ratio)	2	3

Finally, the total cost is less. This involves labour for preparation of media and cleaning equipment, cost of supplies, and time for making the tests. Again, this is summarized below.

Method	Holder	H.T.S.T.
S.P.C.	$130	$112
Roll-tube	$ 84	$ 74

THERMODURIC COUNTS DETERMINED BY S.P.C. AND MODIFIED ASTELL ROLL-TUBE METHODS ON MILK PASTEURIZED BY THE H.T.S.T. AND HOLDER METHODS

TABLE I

H.T.S.T.		HOLDER	
S.P.C.	Roll-tube	S.P.C.	Roll-tube
2920	2100	1100	1050
330	370	400	300
2770	2130	1760	1380
1370	1150	1330	840
470	360	320	300
1220	530	1010	300
900	940	1280	1270
1790	1550	1280	900
27900	23600	12600	9800
1330	1180	300	300
21800	19500	12000	8400
770	600	300	300
510	530	370	510
1860	1680	1510	1300

TABLE I cont'd

H.T.S.T.		HOLDER	
S.P.C.	Roll-tube	S.P.C.	Roll-tube
1510	1150	1170	940
1320	1100	1360	980
1590	1580	1680	1620
7200	6800	6800	5000
1130	1290	930	1140
1230	1180	1040	1000
910	900	870	460
1200	1000	1070	850
1000	1030	1150	1110
9300	1030	1000	990
1140	1230	1110	1130
410	360	340	400
4300	3700	3500	4500
1700	1050	1550	1210
9300	7800	8700	5100
5400	5500	4500	4100
920	1040	920	720
380	360	300	300
1070	980	1200	1010

TABLE I cont'd

H.T.S.T.		HOLDER	
S.P.C.	Roll-tube	S.P.C.	Roll-tube
550	690	750	810
27500	28000	27100	26200
28400	27800	28600	25900
16000	18200	1900	1700
26000	20600	21200	16000
3700	300	3000	2800
21500	14700	15900	14800
1140	910	1170	770
9100	7700	7400	4000
2760	2660	2580	1900
21300	19100	18500	11300
1080	940	940	690
11500	10100	10100	11000
390	510	410	390
4200	3400	3500	3000
20900	19600	22200	20300
22100	19900	16300	15500
480	440	410	380
5900	5200	4100	4900

TABLE I cont'd

H.T.S.T.		HOLDER	
S.P.C.	Roll-tube	S.P.C.	Roll-tube
1170	1210	1290	1310
4300	4100	4100	3900
12300	11000	13000	9500
1350	1220	820	780
12200	11700	10700	9400
610	580	450	360
4900	5300	4600	3800
630	700	510	530
460	430	380	300
690	600	580	500
1530	1020	1160	970
1600	1670	1650	1720
14000	11900	13000	11000
740	700	500	490
1000	1410	720	860
11200	9700	7500	5500
14100	14100	15800	13600
330	300	320	300
29000	25900	28100	22700

TABLE I cont'd

H.T.S.T.		HOLDER	
S.P.C.	Roll-tube	S.P.C.	Roll-tube
880	940	600	670
630	610	620	650
1090	1160	1040	830
750	840	610	870
760	810	700	760
680	730	700	740
18500	13900	18700	18500
4800	4300	4600	3500
2760	26700	23500	22300
2840	21800	25400	20300
1130	950	700	530
530	380	450	360
2300	1430	1900	1300
340	300	510	300
2000	1620	2180	1750
28800	24500	24300	22100
510	430	360	320
15400	12300	14400	11800
1260	1030	910	920

TABLE I cont'd

H.T.S.T.		HOLDER	
S.P.C.	Roll-tube	S.P.C.	Roll-tube
14300	9800	12200	12100
7600	5100	5000	4200
7200	4700	7900	5500
12500	1900	4800	6700
11200	9000	13200	8100
1000	910	920	860
11000	9700	7500	5500
11000	14100	12000	13600
330	300	320	300
29000	25900	28100	22700

DISCUSSION

Statistically there was no difference between counts determined by either the modified Astell Roll-tube method or the Standard Plate Count method on milk pasteurized by the H.T.S.T. and holder method. This is probably due to the fact that the bacterial flora from milk samples used in this investigation were such that they were destroyed equally by the two methods of pasteurization. This view is in agreement with results from earlier investigations.

The counts by the modified Roll-tube method were in general lower than the plate method, although statistically there was no difference. The variation can be attributed to the fact that the Roll-tube has only two-thirds the surface area of a standard petri dish, consequently, the colonies were crowded closer together.

A greater number of spreaders developed in the roll-tube method than the plate method. This is probably because moisture is not trapped as efficiently in the roll-tube as in the petri dish.

It would appear, on the basis of the limited data, that the modified Astell Roll-tube method could be adopted to determine thermoduric counts in laboratory pasteurized milk. It is as accurate as the Standard Plate Count method and it has the advantages of being simple, less time consuming and expensive.

SUMMARY AND CONCLUSION

One hundred and fifty-three samples of bulk raw milk were laboratory pasteurized in lots of 10 samples by the H.T.S.T. and holder methods. The thermoduric counts were determined by a modified Astell Roll-tube method and the Standard Plate Count Method. Four comparisons were made and while variation in the counts were apparent, statistical analysis using the "t"-test indicated that, on the basis of these limited data, the differences between any comparison was not significant at the 1% level.

This investigation indicated that the modified Astell Roll-tube method was simpler, less time consuming and less expensive and was as accurate as the plate method. Consequently, on the basis of these results the modified Astell Roll-tube method could be used to determine the thermoduric counts in laboratory pasteurized milk.

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