COMPARISON OF PLATE LOOP AND AGAR PLATE METHODS FOR BACTERIOLOGICAL EXAMINATION OF MANUFACTURING GRADE RAW MILK[1] [2]

SITA RAMAYYA TATINI, ROGER DABBAH[3], AND J. C. OLSON, JR.

Department of Food Science and Industries
University of Minnesota, St. Paul 55101

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SUMMARY

Plate loop counts and standard plate counts on each of several manufacturing grade raw milk samples (handled in cans or in farm bulk tanks) have been compared. On the average, the plate loop count (PLC) was lower than the standard plate count (SPC) regardless of the type of handling of milk on the farm, can or bulk tank. Agreement between the SPC and PLC seemed to depend upon the bacterial-count levels present in milk. Statistical analyses indicated significant differences, at 1% level of probability, between the average bacterial count by SPC and PLC methods regardless of count level ($\leq$100,000/ml or $>$100,000/ml) in case of can milk samples. On the other hand, in case of farm bulk tank milk samples, no significant differences, at the 1% level of probability, between the average bacterial count by SPC and PLC methods were obtained, when the counts were equal to or less than 100,000 per ml; when the counts exceeded 100,000 per ml, significant differences were present. Since the bacterial counts of manufacturing grade raw milk samples are likely to exceed 100,000/ml, the data presented in this investigation indicate that, until the bacteriological quality of manufacturing grade milk supplies undergoes substantial improvement, the PLC method does not appear to be a suitable substitute for the SPC method for routine bacteriological examination of such milk supplies.

Viable bacterial counts by agar plate method (SPC) are commonly used for routine bacteriological examination of raw milk supplies. The plate loop method (PLC), which also gives viable bacterial counts, is more rapid and less expensive than the agar plate method. Thompson et al. *(5)*, the originators of the plate loop method, reported close agreement between the bacterial counts by the PLC and SPC methods on raw milk samples using 35 C for plate incubation. Thus, according to "Standard Methods" *(1)* the PLC may be substituted for SPC for routine bacteriological examination of low count ($\leq$200,000/ml) raw milk. With the exception of the work published by the originators of the PLC method *(5)*, no other studies of this nature were found in the literature. Because of the simplicity of the PLC method, the present investigation was undertaken to compare the counts by these two methods and to evaluate the PLC method for bacteriological examination of manufacturing grade raw milk supplies.

EXPERIMENTAL METHODS

Three hundred and sixty-three can milk and 323 farm bulk tank milk samples (manufacturing grade) from 152 and 124 randomly selected individual producers, respectively, were collected in the Minnesota-Wisconsin-Iowa area during the period from February through October 1963. Can milk samples were taken from the weigh-tanks at the processing plants using a chlorinated dipper. The bulk tank milk samples were taken directly from the bulk tanks at the farms using sterile disposable pipettes after the milk was agitated for at least 3-5 minutes. Samples were taken in sterile screw-cap tubes, placed in ice water, and were transported in an ice chest to the laboratory and were stored overnight at 3.3 C. The next day each was mixed well, the foam was allowed to break up, then each sample was plated by the PLC method *(5)* and the SPC method *(1)*. Rather than being flamed between platings, the loop was rinsed by discharging one ml of sterile phosphate buffered dilution water over the shank and the loop. The loop was tested as described by Thompson et al. *(5)* and was found to be free rinsing. The same individual performed both the SPC and PLC methods using the same lot of plate count agar (Difco). Furthermore, this same individual was given instruction in use of the loop by one of the originators of the PLC method. The plates were incubated at 32 C for 48 $\pm$ 3 hours. At the end of the incubation period the colonies on the plates were counted in accordance with "Standard Methods" *(1)*. Logarithms of bacterial counts by the SPC and PLC methods on each producer's milk were placed on IBM cards to facilitate statistical analyses. Of the 363 can milk and 323 farm bulk tank milk samples examined, 231 and 218 samples, respectively, yielded 300 or fewer colonies ($\leq$300,000/ml) on plates prepared by the PLC method. Only samples which yielded 300 or fewer colonies by the PLC method were included in the analyses of the data. Significance of differences between SPC and PLC was tested (analysis of variance, two-way classification with fixed treatments and random blocks, i.e. counts by SPC and PLC methods as treatments and each milk sample as a block) using log bacterial counts, as described by Steel and Torrie *(4)*. Actual computations were performed by the Control Data Corporation 1604 computer using a "UMSTAT-51" program *(3)*.

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[3]Present address: Market Quality Research Service, Field Crop and Animal Products Branch, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Maryland.

TABLE 1. FREQUENCY DISTRIBUTION OF SAMPLES[a] IN BACTERIAL COUNT RANGES BY THE SPC AND PLC METHODS

Bacterial count range	All samples		Can milk		Farm bulk tank milk	
	SPC (%)	PLC (%)	SPC (%)	PLC (%)	SPC (%)	PLC (%)
<30,000	21.1	24.7	15.6	18.2	28.9	31.7
30,000-100,000	34.5	40.1	34.6	42.0	34.4	38.1
>100,000-200,000	16.7	21.8	18.6	26.8	14.7	16.5
>200,000-300,000	4.0	13.4	4.8	13.0	3.2	13.8
>300,000	22.7	—	26.4	—	18.8	—
Average bacterial count	90 T[b]	63 T	110 T	73 T	72 T	54 T

[a]Based on 231 can milk and 218 farm tank milk samples (231 + 218 = 449 all samples).
[b]T = thousand.

RESULTS AND DISCUSSION

Table 1 shows the average bacterial counts and frequency distribution of samples in count ranges by the SPC and PLC methods. The average counts by the PLC method were lower than the corresponding counts by the SPC method regardless of the type of handling of milk on the farm, can or bulk tank. However, the magnitude of difference between the average count by the PLC and SPC methods was higher for can milk samples. The frequency of occurrence of samples in each bacterial count range, from <30,000 - 300,000, was higher with the PLC method than with the SPC method.

In general, as the bacterial count increased, the difference between the percentage of samples falling in each class by the SPC and PLC methods also increased. For example, 22.1% and 24.7% of all samples were in the range of <30,000 by the SPC and PLC methods, respectively, i.e. a difference of 2.6% between the methods. There was a difference of about 9% between the percentages of samples by the SPC and PLC methods in the range of >200,000-300,000. On the other hand, a substantial percentage of samples (about 23) with PLC's ≤300,000/ml had SPC's of >300,000/ml.

Table 2 shows the frequency distribution of samples in bacterial count ranges by the SPC method within each count range of PLC method. A close agreement between SPC and PLC was obtained for milk samples with counts equal to or under 100,000 per ml, while this was not the case for milk exceeding 100,000 per ml.

For example, about 70% of samples with PLC's under 30,000 per ml also had SPC's under 30,000 per ml. On the other hand, only 31% of samples with PLC's over 100,000 per ml but under 200,000 per ml had SPC's in the same range. A large number of clumps is more likely to be found in milk with a high bacterial count than in milk with a low bacterial

TABLE 2. FREQUENCY DISTRIBUTION OF SAMPLES[a] IN BACTERIAL COUNT RANGES BY THE SPC METHOD WITHIN EACH COUNT RANGE OF PLC METHOD

Bacterial count range by PLC method	No.	Distribution of percent samples by SPC method							
		<30T[b]	30-100T	>100-200T	>200-300T	>300-500T	>500T-1M[c]	>1-3M	>3M
All samples									
<30T	111	70.3	26.1	2.7	—	0.9	—	—	—
30-100T	180	10.6	60.0	21.1	0.6	3.3	3.3	0.6	0.6
>100-200T	98	1.0	14.3	30.6	11.2	16.3	19.4	4.1	3.1
>200-300T	60	1.7	6.7	6.7	10.0	20.0	35.0	13.3	6.7
Can milk samples									
<30T	42	66.7	26.2	4.8	—	2.4	—	—	—
30-100T	97	6.2	61.9	21.7	1.0	4.1	4.1	—	1.0
>100-200T	62	1.6	12.9	32.3	12.9	16.1	21.0	1.6	1.6
>200-300T	30	3.3	3.3	—	6.7	20.0	40.0	20.0	6.7
Farm bulk tank milk samples									
<30T	69	72.5	26.1	1.5	—	—	—	—	—
30-100T	83	15.7	57.8	20.5	—	2.4	2.4	1.2	—
>100-200T	36	—	16.7	27.8	8.3	16.7	16.7	8.3	5.6
>200-300T	30	—	10.0	13.3	13.3	20.0	30.0	6.7	6.7

[a]Based on 231 can milk and 218 farm bulk tank milk samples (231 + 218 = 449 all samples).
[b]T = thousand.
[c]M = million.

TABLE 3. TEST OF SIGNIFICANCE OF DIFFERENCE IN LOG. BACTERIAL COUNTS BETWEEN SPC AND PLC PROCEDURES ACCORDING TO THE TYPE OF HANDLING

Bacterial level/ml	Number of samples		F test	
	Can	Bulk	Can milk samples	Bulk tank milk samples
≤100,000 by PLC	139	152	17.05**	3.5ns
>100,000 by PLC	92	66	43.73**	23.18**
≤100,000 by PLC or SPC	150	161	8.48**	1.32ns

**Significant at the 1% level of probability.

nsNon-significant at the 1 and 5% levels of probability.

TABLE 4. AGREEMENT AND DISAGREEMENT BETWEEN THE SPC AND PLC METHODS IN GRADING MANUFACTURING GRADE RAW MILK SAMPLES[a]

Standard[b]	Unsatisfactory by SPC		Classification by PLC[c]			
			Unsatisfactory		Satisfactory	
	(No.)	(%)	(No.)	(%)	(No.)	(%)
100,000	195	43.4	138	70.8	57	29.2
200,000	120	26.7	51	42.5	69	57.5

	Unsatisfactory by PLC		Classification by SPC[d]			
			Unsatisfactory		Satisfactory	
	(No.)	(%)	(No.)	(%)	(No.)	(%)
100,000	158	35.2	138	87.3	20	12.7
200,000	60	13.4	51	85.0	9	15.0

[a]Based on 449 samples.

[b]Samples which exceeded the standard indicated were considered unsatisfactory.

[c]of samples unsatisfactory by SPC

[d]of samples unsatisfactory by PLC

count. It is likely that the shaking in the preparation of dilutions for SPC procedure tends to break up clumps and to give higher SPC's.

Table 3 shows the results of a test of significance of difference in counts between SPC and PLC procedures for can milk and for farm bulk tank milk. Differences between counts by SPC and PLC for low (under 100,000 per ml) as well as for high (over 100,000 per ml) count milk were statistically significant (1% level) for milk handled in cans. Differences between the counts were statistically significant for high count milk handled in farm bulk tanks, while differences between counts were not statistically significant (5% level) for low count milk handled in farm bulk tanks. For milk samples with a bacterial count equal to and under 100,000 per ml by either SPC or PLC methods, differences were significant between counts by SPC and PLC for can milk samples, while differences between counting methods were not significant (5% level) for farm bulk tank samples.

Assuming that the differences between SPC's and PLC's were due to the breakage of bacterial clumps, then the reason for significant differences in case of can milk samples and not in case of farm bulk tank milk samples, when the counts were ≤100,000/ml, might be explained on the basis of the possible differences in the bacterial flora of these supplies. The bacterial flora of can milk samples might be expected to be predominantly micrococci and streptococci (enterococci as well as lactic streptococci). Psychrophilic bacteria might be expected to predominate in farm bulk tank milk supplies. The micrococci exist largely as irregular masses of bacteria while the streptococci exist as short or long chains. The psychrophilic bacteria tend to be in singles, pairs, and clumps (2). However, the clumps of micrococci or chains of streptococci may contain more individual cells within each clump or chain than would be the case with the psychrophilic bacteria. Therefore, when clumps break, more individual cells may arise from the clumps of micrococci or streptococci than from the clumps of psychrophilic bacteria. However, no evidence supporting this hypothesis is presented in the present study.

Table 4 shows the application of SPC and PLC methods for grading of manufacturing grade raw milk samples. If a standard of 100,000/ml is used for both methods, about 43% of the samples were unsatisfactory by SPC and only about 35% by PLC, i.e. a difference of 8% between the methods. Of the samples unsatisfactory by the SPC, about 71% of these were also unsatisfactory by the PLC. In other words, about 29% of the samples unsatisfactory by the SPC were classified as satisfactory by the PLC. The reverse relationship shows that only about 13% of the samples unsatisfactory by the PLC were classified as satisfactory by the SPC. On the other hand, when a standard of 200,000/ml was used, the disagreement between the two methods in terms of the percentages of samples unsatisfactory by one method that were classified as satisfactory and vice versa was greater. For example, about 58% of samples unsatisfactory by SPC were classified as satisfactory by the PLC, whereas, only about 15% of samples unsatisfactory by PLC were classified as satisfactory by SPC at 200,000/ml standard. Thus, it would appear likely that if the same standard is used for both methods, PLC, would upgrade a considerable percentage of samples that might be unsatisfactory by the SPC.

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FOOD PLANT WASTE DISPOSAL*

Waste disposal is becoming a prime factor in choosing the location for a food processing plant. This has not always been true. Once waste disposal was a simple matter of discharging plant effluent into a stream or narby land area. Older plants, originally located away from populated areas, are now often surrounded by industrial manufacturing of all kinds and, in some instances, densely populated residential districts. Many municipal waste treatment plants are already overloaded by this rapid population and industrial growth and cannot provide adequate protection to downstream water users.

According to the Federal Water Pollution Control Administration, the two principal sources of industrial pollution of rivers in the Pacific Northwest are paper and pulp mills and food processing plants. There are over 900 food plants in this area processing meat, dairy products, fruits, vegetables, potatoes, beet sugar, grain, and marine products. Most of these plants are in the Snake, Yakima, Walla Walla, and Willamette River Basins.

Wastes from food plants are high in dissolved organic materials which combine with and use up the dissolved oxygen of the receiving stream. In addition to organic matter, some plants have relatively large amounts of salt brines and alkalis in their wastes which cannot be returned to a stream or earth strata.

There are other pollution problems closely associated with the growing of food. In areas of high animal concentrations, it is necessary to process or contain in some manner the excreta to prevent contamination of irrigation and underground waters. Feed lots, dairies, and large chicken ranches are now considered a possible source of stream pollution during heavy rains. Recently one state passed a regulation that the location of new feed lots after a certain date must be first approved by the State Water Pollution Board.

In 1966, the U. S. Department of Interior established the Pacific Northwest Water Laboratory on the campus of Oregon State University to continue the fight against water pollution. When fully staffed, it will have a complement of 150 scientific and professional people composed of engineers, chemists, biologists, bacteriologists, hydrologists, soil scientists, oceanographers, mathematicians, and geologists. The program is divided into three broad areas of endeavor: research, technical services, and training. The laboratory will help combat water pollution in the Pacific Northwest and also furnish assistance in solving water quality problems of California, Nevada, and Hawaii.

The solution to waste disposal becomes more complex as the food plant becomes larger and more diversified. Stricter State and Federal regulations are on the way. Several years ago, officers and members of the National Canners Association recognized the need for more knowledge on waste treatment. The NCA Laboratory at Berkeley, California has studied composting techniques as a means of utilizing processing wastes. The possibility of changing processing techniques to modify plant effluent is also under consideration at this laboratory. Other organizations and far sighted individuals have been seeking answers to disposal and water pollution problems for some time. For those processors who have not included waste treatment in their long range planning, it may be too late.

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