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Spiral Plate Count Method for the Examination of Raw and Pasteurized Milk

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The spiral plate count method (SPLPC) was compared with the standard plate count (SPC) method by examining 201 samples of raw and pasteurized milk. Although the means of the two methods differed significantly at $\alpha = 0.01$, the difference was <10% and was not considered to be of any practical importance. The pooled replicate variances of both methods were <0.003, indicating good agreement between duplicate plates, with the variance of the SPLPC slightly less than that of the SPC. We believe this study indicates that the SPLPC could be substituted for the SPC in the bacteriological examination of milk.

The standard plate count (SPC) is the method of choice for determining the viable bacterial count of milk, although several simplified methods for estimating the count have been developed and described. The oval tube count method (9), when used with the 0.001-ml loop, was reported by Donnelly et al. (5) to give counts of raw milk that were essentially equivalent to the SPC method. Thompson et al. (12) reported that the plate loop count method, when used with the 0.001-ml loop, gave counts that compared favorably with the SPC. Berridge (2) found that a cylinder (3 mm in length by 2 mm in diameter) delivered 0.01 ml of 5 N hydrochloric acid. Donnelly et al. (6) developed a similar cylinder for use in the cylinder plate count method for examining pasteurized milk products. They found that their cylinder delivered 10.5 mg of milk and gave a geometric mean count of pasteurized milk products that was within ±5% of the geometric mean count of the SPC. Bradshaw et al. (3), using the 0.001-ml loop and the 0.01-ml cylinder in an automatic agar-plate-making machine for examining milk, found that the average difference between the geometric mean counts by the machine and by SPC was <9%.

Gilchrist et al. (7) and Campbell and Gilchrist (4) developed a spiral plating system that uses only one plate per sample. In this system, a machine deposits a small volume of sample (about 35 μl) on the surface of a rotating agar plate. The sample is deposited in ever decreasing amounts from the center to the edge of the plate in the form of an Archimedes spiral. During incubation, colonies develop along the lines where the sample was deposited, in proportion to the number of bacteria the sample contained. The spiral plate is counted with a colony counter similar to a standard model but fitted with a special grid or on a laser electronic counter.

In the study reported here, the spiral plate count (SPLPC) method was compared with the SPC in enumerating bacteria in raw and pasteurized milk. The results of a collaborative study comparing the SPC and the SPLPC for the examination of cream will be analyzed and published at a later date.

MATERIALS AND METHODS

Milk. Raw milk samples were from farm bulk tanks and were picked up and supplied to us by a local dairy in the Cincinnati area. The raw milk samples for the field study were routine samples being examined in a dairy laboratory in St. Paul, Minn. All raw milk samples were in plastic Whirl-Pak bags, and portions for examination were taken directly from the bags. Pasteurized milk, packaged in paper cartons or in plastic bottles, was purchased from local supermarkets and delicatessens in quantities that varied from a half pint (0.237 liter) to one gallon (3.79 liters). The milk in the cartons or bottles was well mixed, and approximately 100 ml of each sample was poured into sterile milk dilution bottles before they were examined.

SPLPC method. The spiral plating machine, its operation, and quantitation in determining bacterial counts have been described (4, 7). A modified machine, similar to the one described, was used in the comparative examination of milk (4).

Standard plate count method. The SPC is described in *Standard Methods for the Examination of Dairy Products* (1).

Agar. Standard methods agar (BBL) was used in both methods in Cincinnati; plate count agar (Difco Laboratories) was used in St. Paul. The spiral plates (150 by 15 mm) were prepoured, and the agar was

allowed to harden and dry before they were inoculated. The pour plates (100 by 15 mm) were inoculated, and the agar was poured in the usual manner.

Examination of milk. In plating by the SPLPC, approximately 3 to 4 ml of the undiluted milk samples was transferred to 5-ml plastic diSPo beakers, and a portion of the sample was drawn up through the Teflon tubing into the syringe by vacuum. The vacuum was closed, and the 35 μl of the sample was spiraled over the agar surface of the prepoured agar plates. For the SPC, appropriate dilutions of the milk samples were made and transferred to the pour plates, which were poured and mixed with agar in the usual manner. The pour plates were allowed to solidify, and both the pour and spiral plates were inverted and incubated at 32 ± 1 C for 48 ± 3 h. All plates were prepared in duplicate.

The pour plates were counted on a standard colony counter (a Hellige dark-field model), and the spiral plates were counted on a similar counter that accommodates petri dishes (150 by 15 mm) and is fitted with a special grid. The grid (Fig. 1) quantitatively relates the colonies on a spiral plate to the volume in which they were contained. It is divided into areas by four concentric circles and octants, with each octant divided into sectors. The grid, therefore, represents the areas of a spiral plate on which a known, constant volume of sample is deposited. In counting the spiral plate, only areas with well-separated colonies are counted. Colonies are counted in one octant from the outer edge of the plate towards the center until at least 25 colonies are observed; then the remainder of that sector where the 25th count occurred is counted. A similar area of the opposite octant is counted, and the counts of the two octants are added together. (For example, Fig. 1 shows that if "3" was recorded, the area counted was made up of the three shaded sectors from each of the octants numbered 3.) The colonies are counted in a known area (thus a known volume), and the count per milliliter is calculated by dividing the colonies counted by the volume in which they were contained (7). As an illustration (4), let us state that the area counted yielded 100 colonies, and the volume deposited in the area was 10 μl; thus, 100/10 = 10 colonies/μl or 10,000 colonies/ml. If an octant contains less than 25 colonies, the whole plate is counted, and the count is divided by 35 μl (the volume of sample that was deposited over the entire surface of the plate) to give the count per milliliter.

The laser counter. In some experiments, the spiral plates were also counted electronically by a laser counter (Fig. 2), which passes a narrow laser beam through an agar plate in a spiral pattern from the outer circumference towards the center. Any particle that interrupts the beam as it spirals towards the center is registered as a count.

The laser counter is designed to count a preset number of colonies, i.e., 200. If the plate contains less than 200 colonies, the laser counts all the colonies, and the plate count is given in a digital readout. This count is divided by the volume − 35 μl (as in the manual counting) − to give the count per milliliter. If the plate contains more than 200 colonies, the counter stops and displays the area in which 200

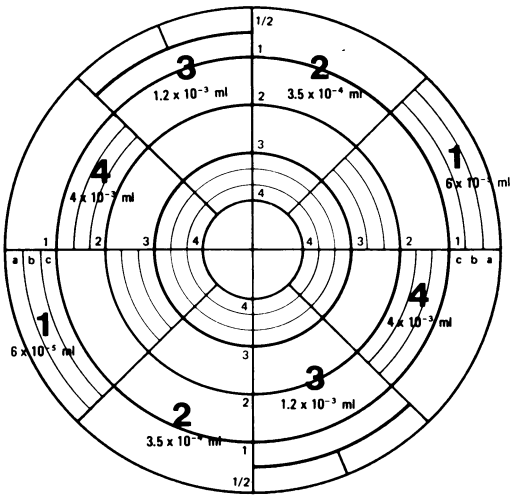

FIG. 1. *Grid for counting spiral plates.*

colonies were counted. The count per milliliter is determined by reading this area from a calibration curve, similar to the one shown in Fig. 3. This curve was generated by plotting the areas (which represent certain volumes of bacterial suspensions) in which 200 colonies were counted against average duplicate pour plate values of the same suspensions.

Statistical methods. The counts by SPC and by SPLPC (using only samples that gave pour plate counts that fell within the 30- to 300-count range or had a count of at least 20 colonies by spiral plate) were converted to $\log_{10}$ counts and compared. The $\log_{10}$ counts were assumed to be normally distributed with a homogenous variance over a range of counts from approximately 500 to 300,000/ml of sample.

The design of the experiments was based on a test to detect at least a 10% difference between the two methods 9 times out of 10. The probability of rejecting the hypothesis that the two methods yield equal results when true was set at $\alpha = 0.01$ or 1 in 100. Using a previous estimate of variance between duplicate counts of 0.005 (6), the number of samples required was computed to be at least 25 for each study, with duplicate counts made on each sample by both methods. An analysis of variance was run on each series of samples, and the geometric means and replicate variances of the $\log_{10}$ counts of the SPC and SPLPC were compared. The interaction of methods with samples was also investigated. The SPLPC manual counts were compared with the laser counts of the same plates by a paired t test. Calculations were made as shown by Ostle (10).

RESULTS

Raw and pasteurized milk (Cincinnati). Table 1 shows the analysis of variance of 97 raw milk samples, and Table 2 shows the comparison of the geometric mean counts per milliliter and replicate variance of $\log_{10}$ counts of the SPC

FIG. 2. *Model 500, Exotech laser beam electronic colony counter.*

and SPLPC. Tables 3 and 4 show a similar analysis of 61 homogenized milk samples, and Tables 5 and 6 show the analysis of 28 chocolate and skim milk samples. As expected, the mean recoveries of bacteria per milliliter differed significantly from sample to sample, and there was a significant difference in the geometric means of the two methods (Tables 1, 3, and 5). However, the difference was small; the geometric mean count of the SPLPC of raw milk samples was only 3% greater than that of the SPC (Table 2), 3% lower for homogenized milk

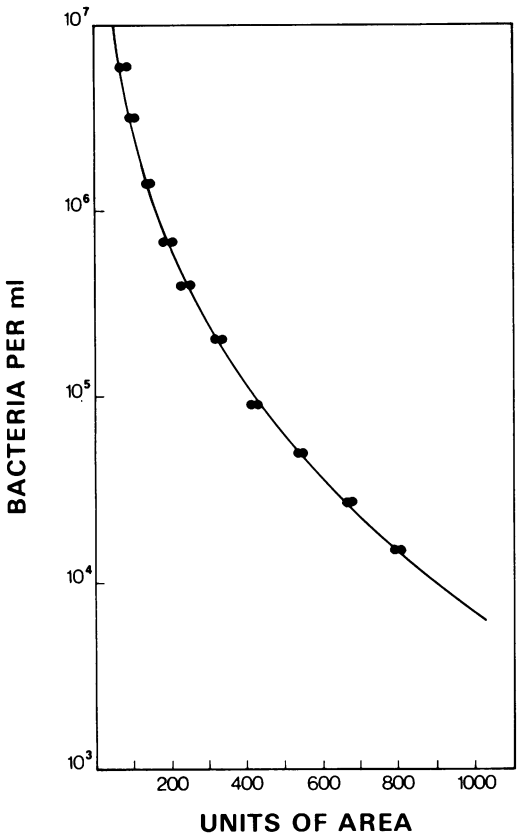

FIG. 3. *Calibration curve for determining the count per milliliter of a sample from the laser spiral plate count.*

TABLE 1. *Analysis of variance of 97 Cincinnati raw milk samples*

Source	Sum of squares	Degrees of freedom	Mean square	*F* ratio
Methods (A)	0.03516	1	0.03516	13.17[a]
Samples (B)	87.68750	96	0.91341	342.10[a]
AB interaction	1.47656	96	0.01538	5.76[a]
Error	0.51852	194	0.00267	
Total	89.71774	387		

[a] Significant at $\alpha = 0.01$.

(Table 4), and 6.7% lower for chocolate and skim milk (Table 6). The replicate variance for the SPLPC was slightly less for both raw and homogenized milk, but it was greater than that of the SPC for chocolate and skim milk.

Raw milk (St. Paul). To evaluate the spiral plating machine in a dairy laboratory examining routine milk samples, a field study was carried out at the Dairy Quality Control Institute in St. Paul, Minn. Fifteen samples of raw milk were compared by SPC (two analysts making individual examinations) and by SPLPC. Table 7 shows the SPC per milliliter of the samples and the SPLPC per milliliter, the latter being determined by manual and laser counting of the spiral plates. The statistical

TABLE 2. *Comparison of geometric means and replicate variances for 97 Cincinnati raw milk samples*

Method	Geometric mean	Difference (%)[a]	Replicate variance
SPC	33,000[b]		0.00289[c]
SPLPC	34,000	+3	0.00246

[a] Percentage of difference of the geometric means = [SPLPC − (SPC/SPC)]100.
[b] Observations per mean = 194.
[c] Degrees of freedom per variance = 97.

TABLE 3. *Analysis of variance of 61 Cincinnati homogenized milk samples*

Source	Sum of squares	Degrees of freedom	Mean square	*F* ratio
Methods (A)	0.03296	1	0.03296	11.56[a]
Samples (B)	146.99487	60	2.44991	859.62[a]
AB interaction	0.61279	60	0.01021	3.58[a]
Error	0.34729	122	0.00285	
Total	147.98790	243		

[a] Significant at $\alpha = 0.01$.

TABLE 4. *Comparison of geometric means and replicate variances for 61 Cincinnati homogenized milk samples*

Method	Geometric mean	Difference (%)[a]	Replicate variance
SPC	3300[b]		0.00305[c]
SPLPC	3200	−3	0.00267

[a] Percentage of difference of the geometric means = [SPLPC − (SPC/SPC)]100.
[b] Observations per mean = 122.
[c] Degrees of freedom per variance = 61.

TABLE 5. *Analysis of variance of 28 Cincinnati chocolate and skim milk samples*

Source	Sum of squares	Degrees of freedom	Mean square	*F* ratio
Methods (A)	0.05371	1	0.05371	15.66[a]
Samples (B)	57.26953	28	2.04534	596.31[a]
AB interaction	0.29102	28	0.01039	3.03[a]
Error	0.19922	58	0.00343	
Total	57.81348	115		

[a] Significant at $\alpha = 0.01$.

TABLE 6. *Comparison of geometric means and replicate variances for 28 Cincinnati chocolate and skim milk samples*

Method	Geometric mean	Difference (%)[a]	Replicate variance
SPC	1,500[b]		0.00238[c]
SPLPC	1,400	−6.7	0.00449

[a] Percentage of difference of the geometric means = [SPLPC − (SPC/SPC)]100.
[b] Observations per mean = 56.
[c] Degrees of freedom per variance = 28.

TABLE 7. *Comparison of the SPC and the SPLPC in determining the bacterial count of 15 St. Paul raw milk samples*

Sample	Count/ml			
	SPC		SPLPC	
	Analyst 1	Analyst 2	Manual count	Laser count
1	73,000[a]	84,000	91,000	65,000
2	14,000	13,000	15,000	24,000
3	7,200	6,900	7,300	6,000
4	19,000	21,000	21,000	25,000
5	72,000	99,000	73,000	65,000
6	19,000	17,000	18,000	12,000
7	35,000	35,000	37,000	39,000
8	9,600	9,200	10,000	8,100
9	19,000	20,000	20,000	23,000
10	47,000	31,000	31,000	27,000
11	6,500	4,800	6,200	7,100
12	26,000	22,000	17,000	10,000
13	15,000	12,000	8,200	13,000
14	57,000	45,000	40,000	46,000
15	15,000	14,000	13,000	14,000

[a] The count per milliliter is equal to the average of duplicate counts rounded to two significant figures.

analysis of the SPC and the SPLPC (which does not include the laser counts) is shown in Tables 8 and 9. There was no difference between the geometric means of the SPC of analyst 2 and that of the SPLPC (Table 9). There was a significant difference between the geometric mean of analyst 1 and the other two means. The latter were 9.1% lower than that of analyst 1. This difference was not considered to be of any practical importance because it was less than 10%.

Sample method interaction. The sample method interaction was significant at $\alpha = 0.01$ for each series of samples examined. The interaction reflects the inconsistent differences between the means of the SPC and SPLPC for each sample. As an example of this, these differences were computed for the Cincinnati raw and homogenized samples and are summarized in Tables 10 and 11. The differences ranged from −44 to 143% of the SPC geometric means for raw milk and −45 to 148% for homogenized milk. Eighty-seven percent of the SPLPC re-

TABLE 8. *Analysis of variance of 15 St. Paul raw milk samples*

Source	Sum of squares	Degrees of freedom	Mean square	*F* ratio
Methods (A)[a]	0.04346	2	0.02173	9.97[b]
Samples (B)	10.20898	14	0.72921	334.50[b]
AB interaction	0.21400	28	0.00766	3.51[b]
Error	0.09828	45	0.00218	
Total	10.56532	89		

[a] Two SPC analyses and one SPLPC analysis.
[b] Significant at $\alpha = 0.01$.

TABLE 9. *Comparison of geometric means and replicate variances for 15 St. Paul raw milk samples*

Methods	Geometric mean	Difference (%)[a]	Replicate variance
SPC (analyst 1)	22,000[b]		0.00277[c]
SPC (analyst 2)	20,000	−9.1	0.00097
SPLPC	20,000	−9.1	0.00281

[a] Percentage of difference of the geometric means = [SPC_2 − (SPC_1/SPC_1)]100; [SPLPC − (SPC_1/SPC_1)]100.
[b] Observations per mean = 30. This mean differed significantly from other two at $\alpha = 0.01$.
[c] Degrees of freedom per variance = 15.

TABLE 10. *Frequency of percent difference of sample SPLPC geometric means compared to SPC geometric means*

Difference (%)[a]	Raw milk	Homogenized milk
>30	16	6
21 to 30	5	4
11 to 20	11	1
1 to 10	10	14
0 to −10	15[b]	16
−11 to −20	21	11
−21 to −30	10	6
< −30	9	3
Total number of samples	97	61

[a] Range = −44 to 148%. Percent difference of the geometric mean = [SPLPC − (SPC/SPC)]100.
[b] Three 0 values.

TABLE 11. *Percent difference of SPLPC geometric means within −40 to 40% of SPC geometric means within four boundaries*

Boundaries (%)	Raw milk	Homogenized milk
−10 to 10	25.8	49.2
−20 to 20	58.8	68.9
−30 to 30	74.2	85.2
−40 to 40	86.6	95.1

TABLE 12. *Comparison of geometric means and replicate variances of SPC and SPLPC of raw and homogenized milk samples over the major areas of the spiral plate*

Method	Area of spiral plate	Geometric mean/ml[a]	Replicate variance of $\log_{10}$ counts[b]
SPC		1,400 (102)	0.00380 (51)
SPLPC	Total	1,400	0.00281
SPC		9,200 (56)	0.00192 (28)
SPLPC	4	8,600	0.00126
SPC		33,000 (122)	0.00231 (61)
SPLPC	3	33,000	0.00232
SPC		120,000 (72)	0.00360 (36)
SPLPC	2	120,000	0.00329

[a] Figures in parentheses are observations per mean.

[b] Figures in parentheses are degrees of freedom per variance.

sults for raw milk and 95% of the results for homogenized milk were between the boundaries of −40 to 40% of the SPC (Table 11).

Deviation of the SPLPC results from the SPC results did not seem to be related to concentration. The linear correlation coefficients between means of the $\log_{10}$ counts for the two methods were $r = +0.964$ for raw milk and $r = +0.992$ for homogenized milk.

Distribution of bacteria over the spiral plate. In using the SPLPC, only certain areas, depending on the density of the colonies, are counted. To determine how proportional the distribution of bacteria over the surface of the spiral plate was, the data from 176 raw and homogenized milk samples, arranged according to the four areas of the spiral plates that were counted, are shown in Table 12. It can be seen that, with one exception, there was no difference between the geometric means of the SPLPC and that of the SPC. The replicate variances from the areas were analyzed by Bartlett's test (10), resulting in a χ^2 of 14.78 for seven degrees of freedom. This was not significant at $\alpha = 0.01$.

Comparison of laser and manual counts of SPLPC plates. The SPLPC plates of 173 raw and homogenized milk samples, which ranged in count from 5×10^2 to 1×10^7, were counted manually and by the laser counter, and the methods were compared by a paired t test. Using the null hypothesis that the average difference equaled zero and setting α at 0.01, a difference was computed between the means of each sample. For the 173 samples, $t_{172} = 1.67$ and was not significant. A linear regression was also run on the data. The $\log_{10}$ laser counts $= y$ and $\log_{10}$ manual counts $= x$ was used to obtain the estimates. A value of the correlation coefficient $r = 0.982$ was obtained, and this was considered satisfactory.

DISCUSSION

The comparison of the SPLPC with the SPC in examining 201 raw and pasteurized milk samples (Tables 1, 3, 5, and 8) showed that the means differed significantly at $\alpha = 0.01$. These differences were <10% (Tables 2, 4, 6, and 9) and were not considered to be of any practical importance in deciding to use the SPLPC rather than the SPC. The interaction term was also significant. The frequency distribution (Table 10) is given as a percentage of the SPC geometric means: [SPLPC − (SPC/SPC)]100. A percentage of difference of 100 in Table 10 means that the geometric mean for a sample examined by SPLPC was twice the SPC value; a −50% difference means that the SPLPC mean was one-half of the SPC value. At least 87% of the SPLPC geometric means were between −40 and 40% of the SPC geometric means.

On a spiral plate, the bacteria are fixed along the spiral track made by the deposited sample, which decreases proportionally from the center to the edge of the plate. The question was thus posed as to whether the accuracy or the variability of the SPLPC depends upon the volume of the deposited sample. A comparison of the results (Table 12) of raw and homogenized milk samples in the four areas counted indicates that this is not the case. The geometric mean counts by the spiral plate, regardless of the area counted, agree with those of the SPC, and the replicate variance of the SPLPC was not significantly different from that of the SPC. Thus, from a practical point of view, it usually takes less time to count a spiral plate (as only the colonies in certain areas have to be counted) than it does to count a pour plate on which all the colonies have to be counted.

Although the laser counter, which is one of several automatic colony counters that are now available (8, 11), was not used in the statistical comparison of the SPLPC with the SPC, it was used on a trial basis in this study. The laser counts of 173 raw and homogenized milk SPLPC plates were compared with the manual counts of the same plates. Statistical analysis of the data by a paired t test and by a linear regression showed that agreement between the two methods was satisfactory. The laser counter would save a considerable amount of time, because it will count spiral plates at the rate of 1 to 4 s/plate, whereas the manual counts of spiral plates requires an average of about 1 min/plate.

Even without the laser counter, the SPLPC would effect a considerable saving of time and materials if used instead of the SPC and, in addition, the SPLPC requires much less bench space (important in a dairy laboratory) than the SPC. For example, in examining 50 samples of milk by the SPC at 10^{-2} and 10^{-3} dilutions, 50 dilution blanks and 100 plates must be set up and identified with the samples and/or the dilutions plated. Each of the 50 samples must be diluted 10^{-2}, and two dilutions (10^{-3} and 10^{-2}) must be plated from the 10^{-2} dilution, the dilution and plating steps necessitating the use of 100 pipettes. The plates must then be poured with agar that is mixed with the dilution, and the agar must be allowed to solidify before the plates can be inverted and stacked. The time required for a better-than-average analyst to set up and plate 50 samples of milk by the SPC is estimated to be approximately 3 h, and the bench space required for an efficient operation is approximately 25 linear feet (7.6 m).

Most of these steps, materials, and space requirements are avoided when the SPLPC is used. The plates are prepoured; no dilution blanks or pipettes are used. So an analyst familiar with the spiral plating machine can examine 50 samples of milk in about 1 h. Space requirements are much less (about a fifth of that required by an analyst examining the samples by the SPC), because the spiral plates may be stacked before inoculating and immediately after.

We believe that the results of this study show that the SPLPC could be substituted for the SPC in the examination of milk with a count range of approximately 500 to 300,000/ml without any appreciable loss of accuracy and with a considerable saving of time and money.

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