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Efficacy of Petrifilm™ *E. coli* Count Plates for *E. coli* and Coliform Enumeration

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ABSTRACT

The Petrifilm *E. coli* Count plate (PEC) method was compared to the AOAC MPN method to determine the efficacy of the PEC method to detect *E. coli* and coliforms in 115 inoculated cheese samples, 94 vegetables samples, and in 100 naturally contaminated poultry samples. The PEC method was compared to two other coliform plate count methods. The 24 h PEC method is as good as or better than the AOAC MPN method for the detection of E. coli. In addition, qualitative results suggest that the PEC method may be more sensitive than the 9 tube MPN method for the detection of very low numbers of E. coli. Comparable coliform results were obtained.

The determination of *Escherichia coli* can be used to assess the sanitary quality of food and water. Improved *E. coli* testing methods have substantially shortened the time required to obtain an *E. coli* count (*9,11*). The use of a fluorgenic compound, 4-methylumbelliferyl-*b*-D-glucuronide (MUG), to determine B-glucuronidase activity produced by *E. coli* was first reported by Buehler et al. (*3*). The presence of beta-glucuronidase enzyme activity is associated almost exclusively with *E. coli* among gram-negative enteric microorganisms (*7*). The feasibility of detecting beta-glucuronidase enzyme from *E. coli* in foods has been demonstrated in numerous laboratories (*2*). The use of MUG to detect beta-glucuronidase in coliform selective broth and solid media has allowed the 24 h presumptive detection of *E. coli*, and may eventually replace the much longer confirmed MPN procedure (*5*).

Petrifilm™ *E. coli* Count plates contain a beta-glucuronidase-specific indicator dye that precipitates a permanent blue halo around *E. coli* colonies, in addition to coliform selective agents found in violet red bile nutrients. Non-*E. coli* coliforms appear as red colonies with gas bubbles and *E. coli* appear as blue colonies with gas bubbles.

The objective of this study was twofold: (*1*) to compare the efficacy of Petrifilm *E. coli* Count plates to the AOAC *E. coli* MPN procedure, and (*2*) to compare the efficacy of Petrifilm *E. coli* Count plates to the MPN, violet red bile agar (VRBA), and Petrifilm Coliform Count plate procedures for the enumeration of coliforms in cheese, vegetables, and poultry.

We have employed an alternate method to statistically compare the plate count method to the MPN method. The traditional criteria for the equivalency of two methods (i.e., slope approx. 1.0, intercept approx. 0.0, and correlation coefficient > 0.9) cannot produce meaningful comparisons when one method (MPN) employs a statistical approximation with large confidence ranges. As an alternative equivalency criterion, we propose to compare each direct plate count to the lower and upper limits of the MPN 95% confidence interval. Any direct plate count which falls outside this confidence interval would be considered to be different in a meaningful way from the MPN count. By applying a sign test to the results we can determine if either method has a tendency to produce higher counts.

MATERIALS AND METHODS

Experimental design

Two methodologies were used for the enumeration of *E. coli* and four methodologies for the enumeration of coliforms. Samples were diluted 1:10 with phosphate buffer and homogenized. The homogenates were plated in duplicate on Petrifilm *E. coli* Count plates, Petrifilm Coliform Count plates, and once on violet red bile agar (VRBA) plates. The homogenates were also analyzed by using confirmed coliform and *E. coli* MPN procedures, except that Lauryl Sulfate Trypone-MUG was used in place of LST Broth. *E. coli* and coliform colonies from naturally contaminated poultry samples plated on Petrifilm *E. coli* Count plates were confirmed by IMVIC pattern (*10*) and/or Micro ID (Organon Teknika).

Samples

A total of 309 samples were obtained from local supermarkets. The product categories were cheese, frozen vegetables, and raw poultry.

The cheese types obtained for analysis were munchee, colby, cheddar, monterrey jack, and brick. The five vegetable types were carrots, corn, broccoli, green beans, and peas. Each of these products had very low or no coliforms and *E. coli*. Five

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lots of each type of cheese were divided and inoculated at four different inoculation levels (see below). Similarly, five lots of each vegetable were also divided for inoculation at four different levels. The poultry samples included ground turkey, turkey wings, and chicken. None of the poultry samples were inoculated since most contained coliforms and *E. coli*.

Preparation of cultures for inoculation

Nineteen *E. coli* food isolates and 15 coliform food isolates were used as inoculating organisms. Different combinations of single *E. coli* and coliform strains were used to inoculate each product. Isolates selected for inoculation were grown overnight in Trypticase Soy Broth at 35°C. The cell number of each culture was estimated by direct microscopic count. Dilutions were prepared with phosphate buffer for inoculation of sample slurries. The test organisms included 19 *E. coli*, 5 *Klebsiella oxytoca*, 3 *Enterobacter aerogenes*, 3 *E. cloacae*, 3 *E. agglomerans*, and 1 *K. planticola* isolate.

Inoculation of cheese and vegetables

Cheese and vegetable samples were divided into 50-g portions. A 200-ml portion of phosphate buffer was added to each and a homogenate was prepared by stomaching the mixture. The homogenates were inoculated at four levels and mixed thoroughly. The levels were: (*1*) 15 *E. coli*/g + 15 non-*E. coli* coliform/g (*2*) 60 *E. coli*/g + 60 non-*E. coli* coliform/g; (*3*) 330 *E. coli*/g + 330 non-*E. coli* coliform/g; and (*4*) 30 *E. coli*/g + 660 non-*E. coli* coliform/g.

Microbiological analysis of cheese, vegetables, and poultry

The inoculated cheese samples were divided into two equal portions of 125 g. To one portion, 125 ml of phosphate buffer was added to prepare a full 1:10 dilution of the product, mixed, and the dilution plated onto the Petrifilm *E. coli* and Petrifilm Coliform Count plates in duplicate, and was also used to prepare a 3-tube MPN series with LST-MUG. To the second portion, 125 ml of 4% Sodium Citrate was added, blended, and the dilution plated with Violet Red Bile Agar (VRBA) and a VRBA overlay.

The inoculated vegetables were analyzed by the method described for the cheese, except the citrate buffer was omitted (*15*).

Homogenates of 25 g poultry and 225 ml of phosphate buffer were prepared. One-ml portions of the homogenate, and 1:10, 1:100, and 1:1000 dilutions were plated on duplicate Petrifilm Coliform Count plates and Petrifilm *E. coli* plates and used to inoculate a 3 tube LST-MUG MPN series.

Petrifilm plates were incubated at 32 ± 1°C for cheese and 35 ± 1°C for vegetables and poultry for 24 ± 2 h. Coliform colonies were enumerated according to the instructions issued with the Petrifilm Coliform Count plates. Red colonies associated with gas bubbles were counted as coliforms and blue colonies with gas bubbles were counted as *E. coli*. Petrifilm *E. coli* plates were reincubated for an additional 24 ± 2 h at the appropriate temperature and *E. coli* colonies were counted again. VRBA plates were incubated at 32 ± 1°C for cheese and 35 ± 1°C for vegetables and poultry for 24 ± 2 h. The AOAC incubation conditions were followed for all MPN tubes.

Data analysis

Linear regression analysis was used to compare the coliform counts/g between the Petrifilm *E. coli* plate, Petrifilm Coliform Count plate, and VRBA. The least-squares regression line and 95% confidence limits were calculated. Slopes and intercepts were calculated and tested for equivalence to the line of equality of methods (slope = 1, intercept = 0). All coliform counts/g were first converted to $\log_{10}$ counts/g to more nearly match the underlying normality assumption.

Because of the lack of precision of MPN counts, conventional comparisons between Petrifilm methods and MPN are not appropriate. Therefore, we compared the PEC count to the lower and upper limits of the MPN 95% confidence interval. Any PEC count which fell outside of this confidence interval would be considered different in a meaningful way from the MPN count. Sign tests were used to compare the number of PEC counts above the confidence interval to the number below to test for trends.

McNemar's test, with correction, (*13*) was used to compare the data re-coded into a positive/negative format.

RESULTS

Statistical comparison of E. coli *enumeration data*

Petrifilm E. coli *Count plates vs. AOAC MPN*

The results of the 94 test samples of inoculated vegetables are presented in Table 1. The Petrifilm *E. coli* Count plate (PEC) counts were within the MPN 95% confidence range for 73% of the 24 h counts and 71% of the 48 h counts. The PEC counts were above the range for 22% of the 24 h counts and 24% of the 48 h counts, and

TABLE 1. *Quantitative comparison of Petrifilm and MPN methods for* E. coli.

Sample	Number	Incubation Time	PEC = MPN	PEC > MPN	PEC < MPN
Vegetables	94	24 h	69 (73%)	21 (22%)[a]	4 (5%)[a]
Cheese	115	24 h	69 (60%)	17 (15%)	29 (25%)
Poultry	100	24 h	66 (66%)	19 (19%)	15 (15%)
TOTAL	309		204 (66%)	57 (18%)	48 (16%)
Vegetables	94	48 h	67 (71%)	23 (24%)[a]	4 (5%)[a]
Cheese	115	48 h	76 (66%)	25 (22%)	14 (12%)
Poultry	100	48 h	68 (68%)	19 (19%)	13 (13%)
TOTAL	309		211 (68%)	67 (22%)[a]	31 (10%)[a]

[a]Significantly more PEC samples were above the MPN confidence range than below.

below the range for 5% of the 24 h counts and 5% of the 48 h counts. For both 24 and 48 h, significantly more PEC samples were above the range than below.

PEC counts of 115 samples of inoculated cheeses (Table 1) were within the MPN 95% confidence range for 60% of the 24 h counts and 66% of the 48 h counts. The PEC counts were above the range for 15% of the 24 h counts and 22% of the 48 h counts, and below the range for 25% of the 24 h counts and 12% of the 48 h counts. Neither of the above vs below comparisons were significant, suggesting no trend for one method to yield higher counts.

PEC counts of 110 naturally contaminated poultry samples were within the MPN 95% confidence range for 66% of the 24 h counts and 68% of the 48 h counts. The PEC counts were above the range for 19% of the 24 h counts and 19% of the 48 h counts, and below the range for 15% of the 24 h counts and 13% of the 48 h counts. Neither of the above vs below comparisons were significant, again suggesting no trend for one method to produce higher counts.

A visual representation of the 48 h data is presented in Figure 1. For all 309 samples tested, the PEC counts were within the MPN 95% confidence range for 66% of the 24 h counts and 68% of the 48 h counts. The PEC counts were above the range for 18% of the 24 h counts and 22% of the 48 h counts, and below the range for 16% of the 24 h counts and 10% of the 48 h counts.

For the 48 h data, significantly more PEC counts were above the confidence range than below (22% vs 10%), suggesting the PEC counts tend to be higher than MPN counts for all samples combined. For the 24 h data, no trend was detected for one method to produce higher counts.

Statistical comparison of coliform enumeration data

When PEC coliform counts were compared with confirmed MPN counts, the PEC coliform counts were typically in agreement with the MPN coliform counts whether the samples were naturally contaminated or artificially inoculated. As shown in Table 2, the PEC coliform counts were within the 95% confidence range for 77-85% of the samples. The PEC counts were above the range for 4-13% of the samples and below the range for 10-11% of the samples. A visual presentation of the data is given in Fig. 2. While a high percentage of PEC cheese sample counts fell within the MPN confidence range (85%), significantly more counts fell below (11%) than above (4%) the MPN confidence range. For vegetables, poultry, and all combined samples, neither of the above vs below comparisons were significant, suggesting no trend for one method to produce higher counts.

Petrifilm E. coli *Count plates vs. violet red bile agar plates and Petrifilm Coliform Count plates.*

The results for Petrifilm *E. coli* Count plates and VRBA plates are presented in Fig. 3 with the regression line and 95% confidence limits. Figure 4 illustrates the comparison of PEC and PVRB plates. Petrifilm Coliform Count plates compared in a similar way to VRBA plates are shown in Fig. 5. Table 3 lists the slopes, intercepts, and correlation coefficients for the comparisons. PEC plates compared favorably to Petrifilm Coliform Count plates with a correlation of 0.96 and a slope of 0.96, and less favorable to VRBA plates with a correlation of 0.85 and a slope of 0.83. This difference may result from the nature of coli-

TABLE 2. *Quantitative comparison of Petrifilm and MPN methods for coliforms.*

Sample	Number	PEC = MPN	PEC > MPN	PEC < MPN
Vegetables	94	78 (83%)	6 (6%)	10 (11%)
Cheese	110	93 (85%)	4 (4%)[a]	13 (11%)[a]
Poultry	100	77 (77%)	13 (13%)	10 (10%)
TOTAL	304	248 (82%)	23 (8%)	33 (10%)

[a]Significantly more PEC samples were below the MPN confidence range than above.

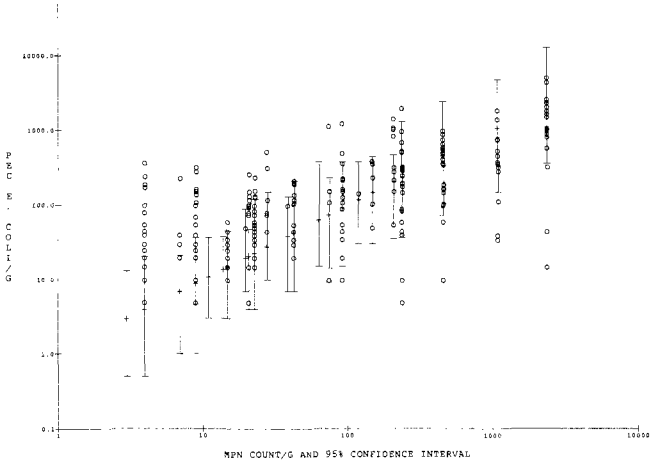

Figure 1. *Relationship between PEC* E. coli *counts/g and most probable number (MPN)* E. coli *counts/g plotted on MPN 95% confidence intervals.*

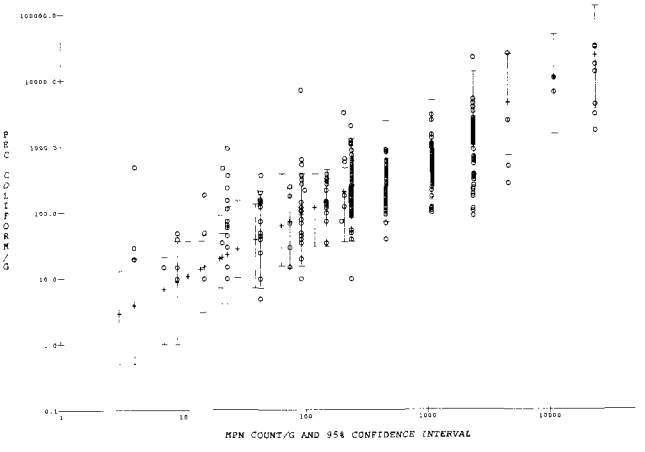

Figure 2. *Relationship between PEC E. coli counts/g and most probable number (MPN) coliform counts/g plotted on MPN 95% confidence intervals.*

form identification on the two different media. Presumptive coliform colonies were not confirmed.

Qualitative comparison of Petrifilm E. coli *Count plates and confirmed MPN for low level* E. coli *contamination*

In order to compare the ability of PEC plates and the AOAC MPN to detect very low levels of *E. coli* contamination, the data were reevaluated in a positive/negative format. That is, any Petrifilm plate containing one or more *E. coli* colonies and/or any MPN series containing one or more positive *E. coli* confirmed tubes was considered a positive result for *E. coli* in the sample tested. Blank plates and/or a negative MPN confirmed *E. coli* series were considered negative results for *E. coli* in the sample tested. The results are presented in Table 4. Analyzed by McNemar's test, no significant difference was shown between the MPN sensitivity and the PEC sensitivity for cheese and vegetables. For naturally contaminated poultry, however, one Petrifilm plate was not as sensitive as the total MPN series. This may be the result of the different quantities of samples tested; 0.1 g for a single Petrifilm plate and 0.333 g for an MPN series. However, when two Petrifilm plates were used to test 0.2 g of sample, there was no significant difference between the sensitivities of the methods for poultry samples.

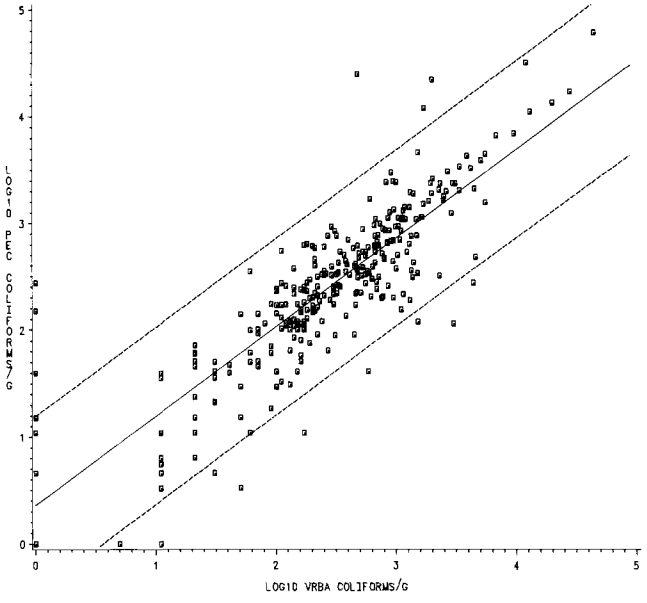

Figure 3. *Relationship of log_{10} coliform counts/g determined by Petrifilm* E. coli *Count plates to violet red bile agar (VRBA) plates indicated by linear regression (solid line) with 95% confidence limits (dashed line).*

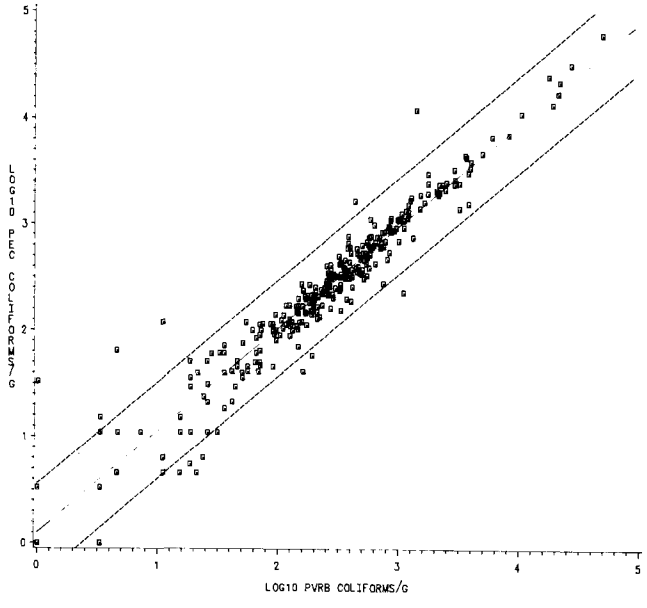

Figure 4. *Relationship of log_{10}coliform counts/g determined by Petrifilm* E. coli *counts plates to Petrifilm Coliform Count plates indicated by linear regression (solid line) with 95% confidence limits (dashed line).*

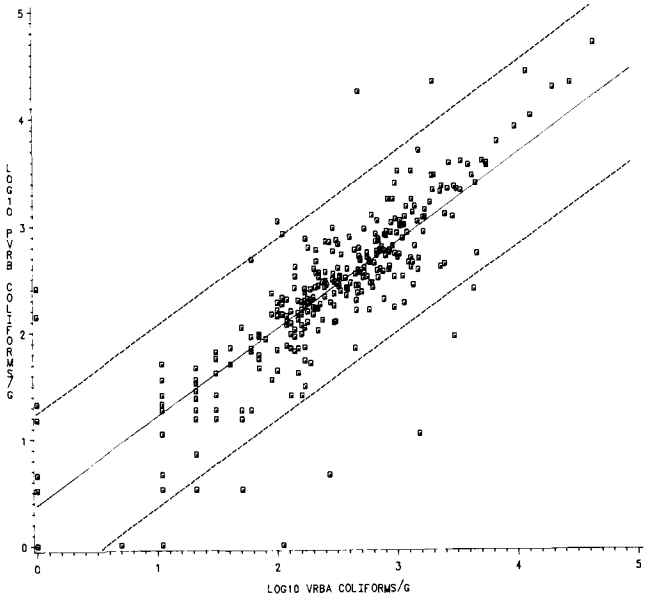

Figure 5. *Relationship of log_{10} coliform counts/g determined by Petrifilm Coliform Count plates to violet red bile agar (VRBA) plates indicated by linear regression (solid line) with 95% confidence limits (dashed line).*

Predictive value of Petrifilm E. coli *Count plates.*

Up to five blue gassing colonies were isolated from individually naturally contaminated poultry samples for confirmation. Of 97 blue gassing colonies isolates, 96 (99%) were confirmed as *E. coli* and 1 as *Salmonella choleraesuis*. Up to five red gassing colonies were isolated from individual naturally contaminated poultry samples for confirmation. Of 81 red gassing colonies, 80 (99%) were non-*E. coli* and 1 was a glucuronidase negative *E. coli*. Blue nongassing colonies may or may not be *E. coli*. These colonies need to be confirmed.

TABLE 3. *Regression analysis of plating methods for coliforms.*

PEC vs. VRBA	N	Correlation	Slope	Intercept
Vegetables	94	0.92	0.84[a]	0.41[b]
Cheese	110	0.86	0.84[a]	0.35[b]
Poultry	100	0.81	0.81[a]	0.33
All	304	0.85	0.83[a]	0.36[b]
PEC vs. PVRB	**N**	**Correlation**	**Slope**	**Intercept**
Vegetables	94	0.96	1.02	-0.06
Cheese	110	0.97	0.98	0.07
Poultry	100	0.95	0.93	0.16
All	304	0.96	0.96	0.10
PVRB vs. VRBA	**N**	**Correlation**	**Slope**	**Intercept**
Vegetables	94	0.94	0.80[a]	0.50[b]
Cheese	110	0.89	0.86[a]	0.29[b]
Poultry	100	0.79	0.80[a]	0.34
All	304	0.84	0.82[a]	0.39[b]

[a]Slope significantly different (P<0.01) from slope 1.
[b]Intercept significantly different (P<0.01) from intercept of 0.

DISCUSSION

In this study, the Petrifilm *E. coli* Count plate (PEC) method was compared to the AOAC MPN method to determine the efficacy of the PEC method to detect *E. coli* and coliforms in food samples. In addition, the PEC method was compared to two other coliform plate count methods. The traditional analysis by slope, intercept, and correlation coefficient was used for all methods except those involving MPN estimates. Use of the traditional linear regression analysis for comparative data generated by the confirmed MPN method would show a fundamental error in reasoning, and result in fallacious conclusions. For example, a linear regression analysis of the log data in Fig. 1 would demonstrate a slope of 0.694 and intercept of 0.500. Both measures would lead to the incorrect conclusion that the Petrifilm and AOAC MPN methods are different. We have proposed an alternative statistical analysis for MPN methods, whereby only a direct count data point which falls outside of the MPN 95% confidence interval can be considered different in a meaningful way for the MPN index number. We determined the number of data points that fell above and below the 95% confidence intervals and applied a statistical analysis to determine if either method tends to produce higher bacterial counts. This statistical method was chosen because of the inherent imprecision of the MPN results.

The MPN method has been described in detail in numerous publications. Woodward (*16*) indicated that the 95% confidence interval for a single determination using three tubes at each dilution, covers a range of approximately 1.27 log cycles. Experimental data by Silliker et al. (*14*) expanded the 95% confidence interval to 1.85 log cycles for peanut butter, buttermilk, and egg albumen. By increasing the number of replicates to either 5 or 10 tubes per dilution, Russek and Colwell (*12*) calculated that the average 95% confidence interval can be reduced to about an order of magnitude. Therefore, use of less than 5 tubes per dilution is inadequate for samples that require precision of less than an order of magnitude. In order to be more precise, an MPN series should employ 5 or 10 replicates per dilution. In practice however, the 3 tube MPN series is used for convenience over precision.

For *E. coli* counts in naturally contaminated poultry, approximately equal numbers of PEC counts fell above and below the MPN confidence range at both 24 and 48 h (Table 1) suggesting no trend for either method to produce higher counts. However, for inoculated vegetables, the PEC counts tended to be higher than the MPN counts at both 24 and 48 h. With inoculated cheese samples, as with poultry samples, no trend for either method to produce higher counts was observed. The positive and negative predictive values for Petrifilm *E. coli* Count plates were quite high. Of 97 blue gassing colonies isolated from naturally contaminated poultry, 96 were *E. coli*. Of 81 red gassing colonies, only 1 was a glucuronidase negative *E. coli*.

For coliform counts, the PEC method compared favorably to the confirmed MPN method with no overall trend observed for either method to produce higher counts (Table 2). While a high percentage of PEC cheese sample counts fell within the MPN confidence range (85%), significantly more counts fell below (11%) than above (4%) the MPN confidence range. Overall, the PEC coliform count method was in agreement with the confirmed MPN, VRBA, and Petrifilm Coliform Count methods for inoculated cheese and vegetables, and naturally contaminated poultry.

Reevaluation of the *E. coli* data in a positive/negative format indicated that the PEC method, using a single PEC plate, was as sensitive as the 9 tube AOAC MPN method for inoculated vegetables and cheese. For naturally contaminated poultry, two PEC plates were required to match the sensitivity of the 9 tube AOAC MPN method. Since

TABLE 4. *Qualitative comparison of plate and MPN methods for* E. coli.

		# Positive for *E. coli* (McNemar's Test)			
Product	# Samples	MPN	PEC Plate 1	PEC Plate 2	PEC 1 + 2
Vegetable	94	83	85 (0.12)	80 (0.44)	85 (0.12)
Cheese	115	106	101 (1.23)[a]	103 (0.30)	105 (0.00)
Poultry	100	74	59 (6.32)	52 (15.7)	65 (2.56)

[a]$X^2 = \frac{(a-b-1)^2}{a+b}$

where (a) is the number positive by method 1 and negative by method 2, and (b) is the number positive by method 2 and negative by method 1. X^2 values > 3.84 are significant.

the MPN method assays 0.333 g of sample whereas one Petrifilm plate assays 0.1 g and two plates assay 0.2 g, the results are indicative of a lack of sensitivity of the MPN at very low *E. coli* levels. It has always been assumed that the sensitivity of the 9 tube MPN series was <3.0 *E. coli*/g based on a total of 0.333 g of sample tested. Our data indicate that the MPN method may not always reach its theoretical limit of sensitivity. The limits of sensitivity may depend upon the character of the sample tested. For example, it is known that MPN assays are susceptible to bacterial interference (*1,4,6*). Raw poultry samples usually contain more gram-negative background flora than powdered milk. In addition, differential growth rates of non-*E. coli* coliforms and other indigenous gram-negatives may mask the presence of low levels of *E. coli*. Petrifilm plates, while not influenced by growth rate variations, may come closer than MPN methods to estimating the true *E. coli* count for each sample tested.

We have determined that the Petrifilm *E. coli* Count plate method is as good as or better than the AOAC MPN method for the detection of *E. coli* in inoculated cheese, vegetables, and naturally contaminated poultry in 24 h. In addition, the Petrifilm *E. coli* Count plated method was equivalent to the confirmed MPN, VRBA, and the Petrifilm Coliform Count methods for the detection of coliforms in the same food groups.

REFERENCES

1. Allen, M. J., and E. E. Geldreich. 1975. Bacteriological criteria for ground-water quality. Ground Water 13:1-7.
2. Andrews, W. H., C. R. Wilson, and P. L. Pollma. 1987. Glucuronidase assay in a rapid MPN determination for recovery of *Escherichia coli* from selected foods. J. Assoc. Off. Anal, Chem. 70:31-39.
3. Buehler, H. J., P. A. Katzman, and E. A. Doisy. 1951. Studies on *b*-glucuronidase from *E. coli*. Proc. Soc. Exp. Biol. Med. 76:672-676.
4. Geldrich, E. E., H. D. Nash, D. J. Reasoner, and R. H. Taylor. 1972. The necessity of controlling bacterial populations in potable waters: community water supply. J. Am. Water Works Assoc. 64:596-602.
5. Hartman, P. A. 1988. The Mug (glucuronidase) Test for *Escherichia coli* in Food and Water. Fifth International Symposium on Rapid Methods and Automation in Microbiology and Immunology. Eds. A. Turano Springer - Verlag, Berlin - Heidleberg - New York - Tokyo.
6. Hutchison, D., R. H. Weaver, and M. Scheraga. 1943. The incidence and significance of microorganisms antagonistic to *Escherichia coli* in water. J. Bacteriol. 45:29.
7. Kilian, M., and P. Bulow. 1976. Rapid diagnosis of *Enterobacteriaciae*. I. Detection of bacterial glycosidases. Acta. Path. Microbiol. Scand. Sect. B, 84:245-251.
8. Mehlman, I. J., H. A. Andrews, and B. A. Wentz. 1984. Coliform bacteria, pp. 5.01-5.07. *In* Bacteriological Analytical Manual, 6th ed., Association of Official Analytical Chemists, Washington, D. C.
9. Poelma, P. L., C. R. Wilson, and W. H. Andrews. 1987. Rapid fluorogenic enumeration of *Escherichia coli* selected, naturally contaminated high moisture foods. J. Assoc. Off. Anal. Chem. 70:991-993.
10. Powers, E. M., and T. G. Lott. 1977. Simplified 48-Hour IMVIC Test: An Agar Plate Method. Appl. Environ. Microbiol., 34:274-279.
11. Rippey, S. R., L. A. Chandler, and W. D. Watkins. 1987. Fluorometric method for enumeration of *Escherichia coli* in molluskan shellfish. J. Food Prot., 50:685-690.
12. Russek, E., and R. R. Colwell. 1983. Computation of Most Probable Numbers. Appl. Environ. Microbiol., 45:1646-1650.
13. Siegel, S. 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, NY, NY.
14. Silliker, J. H., D. A. Gasis, and A. May. 1979. ICMSF Methods Studies XI. Collaborative/Comparative Studies on Determination of Coliforms Using the Most Probable Number Procedure. J. Food Prot., 42:638-644.
15. Standard Methods for the Examination of Dairy Products. 1985. 15th Ed. pp. 211-214. American Public Health Association, Washington, D. C.
16. Woodward, R. L. 1957. How Probable is the Most Probable Number? J. Amer. Water Works Assoc., 49:1060-1068.