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Performance Comparison of the BioSys Optical Assay and the Violet Red Bile Agar Method for Detecting Coliforms in Food Products

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ABSTRACT

Coliform counts in a variety of foods, including dairy products (raw milk, pasteurized milk, yogurt, butter, and ice cream), meats (pork sausage, ground beef, and raw chicken), raw eggs, and chocolate, were performed by the rapid automated BioSys optical assay and the conventional method with violet red bile agar (VRBA). The standard deviation (SD) among five replicate counts for the optical assay was similar to or better than that obtained with VRBA plates for all foods tested. The average SD for all foods tested was 0.21 for the optical assay and 0.30 for the VRBA plates. At very low concentrations of coliforms (1 to 10 CFU/ml for liquid products and 10 to 100 CFU/g for solid samples), the average SDs were 0.26 and 0.47, respectively. The optical assay was less susceptible to interference by noncoliform organisms. In naturally contaminated samples, bacteria such as *Serratia liquefaciens, Pantoea* spp., *Vibrio fluvialis, Aeromonas hydrophilia*, and *Pseudomonas* spp. formed typical colonies in VRBA, resulting in false-positive results or a need to verify colonies in brilliant green lactose broth. The optical assay appeared to be more selective than the VRBA conventional method, detecting fewer noncoliforms. There was close agreement in test results between the two methods, as indicated by correlation coefficients of 0.92 to 0.99 obtained for the regression analysis of the two methods. In most cases both methods distinguished accurately between positive samples containing coliforms and negative controls. All products tested using the automated BioSys Optical Assay for coliforms yielded results more quickly (typically 10 to 12 h) than did those tested with the conventional VRBA method (24 to 72 h with confirmation).

The BioSys Optical System measures microbial growth by monitoring pH and other biochemical reactions that generate a color change as microorganisms in the broth grow and metabolize. Samples are inoculated into ready-to-use vials that contain a specific broth and an agar barrier layer at the bottom that separates the sample-containing broth from the optical measurement area. A color change in the agar parallels a color change in the broth without letting sample particles or turbidity influence measurements *(11, 12).*

The light from light-emitting diodes passes though the agar to a photo diode on the opposite side of the vial that reads the color change as microbial growth occurs. This measurement is taken every 6 min, and as soon as a color change is detected, the time of detection is recorded. Detection times (DTs) are inversely related to the number of organisms in the sample. The coliform assay is based on the fermentation of lactose by coliforms, resulting in the formation of acid. The coliform vial medium has a base of peptone, yeast extract with lactose as the carbon source, selective agents such as bile salts, sodium lauryl sulfate, and other inhibitors of gram-positive bacteria, and bromocresol purple as the pH indicator. Acidification of the medium due to lactose utilization changes the pH and therefore the color of the medium.

The BioSys coliform AOAC study *(4)* revealed that coliforms belonging to all four genera (*Escherichia, Citrobacter, Enterobacter,* and *Klebsiella*) are detected by this assay, for complete inclusivity. The test system excluded all noncoliforms except for two strains of *Salmonella.* The optical assay produced consistent results even when there were variations in sample volume, broth volume in the vial, incubation temperature, and sample temperature.

In many food products, coliforms are routinely monitored to assess the sanitary conditions under which the product was produced. Various methods have been investigated for detecting coliforms *(1, 2, 6, 7, 13).* The most commonly used standard method medium for the detection of coliforms is violet bile red agar (VRBA) *(3).*

In several studies, most of them dealing with a single product, close correlation between the VRBA plate count method and the optical assay have been demonstrated *(5, 8–10).* However, in none of these studies were the relative accuracy, repeatability, and selectivity of these two methods evaluated. The purpose of this study was to compare the reproducibility of the VRBA method with that of the optical assay and to determine the accuracy of results obtained for a large variety of foods. Another objective was to evaluate the selectivity of the VRBA plate count method compared with that of the medium used in the optical assay vials.

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MATERIALS AND METHODS

Products tested. The following products were tested in this study: pasteurized milk (whole milk, Meijers, Grand Rapids, Mich.), ice cream (Kroger Deluxe French Vanilla, Kroger, Cincinnati, Ohio), unsalted butter (Land O'Lakes, Arden Hills, Minn.), yogurt (strawberry, Dannon, Minster, Ohio), semisweet baking chocolate (Nestle, Solon, Ohio), raw eggs, sausage roll (Bob Evans Original, Bob Evans, Columbus, Ohio), ground beef (85% lean, Kroger), and whole chicken (Perdue broiler, Perdue, Horsham, Pa.).

Inoculated products. Because it was difficult to obtain naturally contaminated pasteurized milk, butter, yogurt, ice cream, and chocolate, these products were inoculated with coliforms isolated from dairy products. The milk inoculum used contained a mixture of coliforms including species of *Escherichia, Citrobacter, Klebsiella,* and *Enterobacter.* The samples also contained noncoliform bacteria such as *Bacillus, Serratia, Alcaligenes,* and *Acinetobacter.* The total coliform count in this inoculum was assessed by counting the inoculum sample on VRBA plates, and the total aerobic bacterial count was assessed on plate count agar. The milk inoculum was maintained in frozen milk until use, when it was thawed and decimally diluted in Butterfield's phosphate buffer. The appropriate dilution was then added to the food samples. For eggs, a coliform mixture (*Klebsiella* and *Enterobacter* species) isolated from contaminated eggs was used. The coliform count of the mixture was determined, and the mixture was maintained frozen in scrambled raw egg until use.

The products were inoculated with target concentrations of 0 to 100,000 CFU/g or ml. The inoculated product was held refrigerated for 24 to 48 h to allow the coliforms to adjust to the product prior to testing.

Naturally contaminated products. Store-bought whole raw chicken, ground beef, and sausage contained some coliforms. To achieve a higher level of contamination, the products were left at room temperature for 4 to 7 h to obtain three samples with three different levels of contamination (low, medium, and high). These samples were used to assess the accuracies of the methods at different contamination levels. The objective was to use natural products in this study because they better reflect real-life situations. However, because products were store bought, it was not always possible to attain the desired levels of contamination.

Study overview. In this study, the results of the BioSys optical assay for coliforms were compared with those of the *Bacteriological Analytical Methods (3)* solid medium method (VRBA) for enumeration of coliforms. Various foods containing coliforms concentrations ranging from 0 to 100,000 CFU/g were tested. From each product at each level of inoculation, five replicate samples were created. Each replicate sample was analyzed by the optical assay and the VRBA plate count method.

Sample preparation for dairy products. Eleven grams of dairy product diluted with 99 ml of Butterfield's phosphate buffer (pH 7.2) was added to the bag and stomached for 2 min. For yogurt, the pH of the solution (sample plus buffer) was adjusted with 1 N NaOH to 6.7 ± 0.2. Ten sample bags were prepared for each level of inoculation; one group of 10 bags remained uninoculated and the other four groups of 10 bags were inoculated with the appropriate amount of organisms. Five samples from each level of inoculation were tested by the conventional method and five samples were tested by the optical assay.

Sample preparation for chocolate. Bulk chocolate (semisweet baking) was subdivided into four 300-g portions. One portion remained uninoculated, and the other three portions were inoculated with different concentrations of coliforms by adding 5 ml of the appropriate dilution of the frozen inoculated dairy product to the 300-g sample of melted chocolate. The samples and inoculum were then thoroughly mixed. After solidification, the four portions of chocolate were held for 48 h in the refrigerator before processing. From each level of inoculated chocolate, 10 50-g samples were created, diluted in 450 ml of warm Butterfield's phosphate buffer, and blended for 2 min. Five samples from each level of inoculation were tested by the conventional method and five were tested by the optical assay.

Sample preparation for liquid eggs. A bulk mixture of liquid eggs was subdivided into four portions. One portion remained uninoculated, and three portions were inoculated with different levels of inoculum from the contaminated egg. After inoculation, the four portions were held for 48 h in the refrigerator before processing. From each level of inoculation, ten 50-g samples were created, diluted in 450 ml of Butterfield's phosphate buffer, and thoroughly mixed by swirling. Five samples from each level of inoculation were tested by the conventional method and five were tested by the optical assay.

Sample preparation for whole chickens. Three separate whole chickens from different lots were left in bags, and 400 ml of Butterfield's phosphate buffer was added to each bag containing the chicken. The chicken was massaged with the buffer solution, thoroughly mixing the diluent. From each 400 ml of chicken diluent, 10 samples were created. Five samples were tested by the conventional method and five were tested by the optical assay.

Sample preparation for ground beef and sausage. Three different lots of each ground product were used for the study. Each 50-g product sample was blended with 450 ml of Butterfield's phosphate buffer for 2 min in a blender. Each blended product was divided into 10 samples. Five samples were tested by the conventional method and five were tested by the optical assay.

Sample split. One technician prepared the samples, and a second technician performed the assays on ''blind'' coded samples. All test samples were fully randomized and labeled with a code so that the technician performing the assays was unaware of the concentration of bacteria present.

Calibrations. A calibration curve of 40 to 80 paired points (DTs and CFU per gram) were generated by testing product samples with a range of counts from 1 to 10,000,000 CFU/ml as determined by plating the samples in VRBA. The calibration curves were used to translate DTs of the optical system to CFU per milliliter or CFU per gram.

Conventional method. Samples were evaluated for coliforms using the BAM conventional method *(3).* Plate counts were performed on serial dilutions prepared from the 1:10 dilution of the samples using Butterfield's phosphate buffer. VRBA cooled to 45 ± 2°C was added to each dilution plate, mixed thoroughly, and allowed to solidify. After an overlay of VRBA was added, the plates were incubated for 18 to 24 h at 32°C for dairy products and at 35°C for other food products. For plates with 25 to 250 colonies, colonies were counted. A few colonies were taken from plates with 25 to 250 colonies and transferred to separate tubes of brilliant green lactose broth (BGLB). These tubes were then incubated at 35°C and examined at 24 and 48 h for gas production. For lower dilution levels (1 to 10 CFU/g and 10 to 100 CFU/g), the colony counts were done on the dilution exhibiting the highest number of colonies. Some plates contained less than 25 colonies.

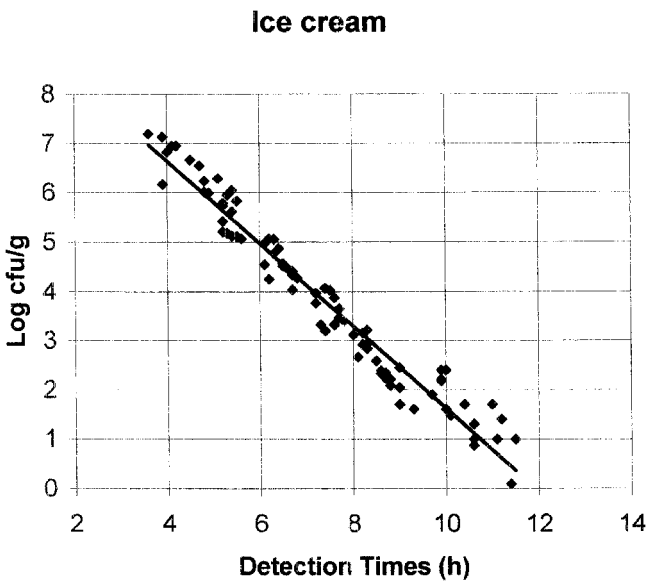

FIGURE 1. *Relationship between detection times and log CFU for coliforms in ice cream.*

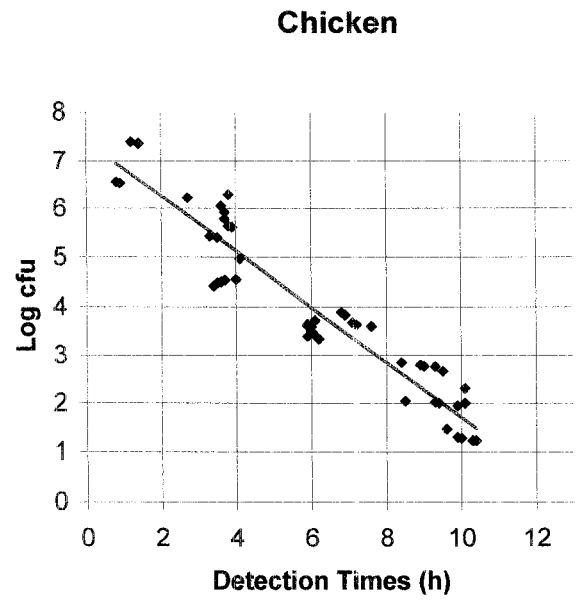

FIGURE 2. *Relationship between detection times and log CFU for coliforms in chicken rinse.*

At least 10 representative coliform colonies from VRBA plates were confirmed using BGLB tubes.

Aerobic plate counts were performed by plating the appropriate decimal dilutions in plate count agar and incubating the plates at 32°C for 48 h. After incubation, colonies were counted for plates containing 25 to 250 colonies.

BioSys optical assay. Five milliliters of the 1:10 sample suspension or 5.0 ml of the pasteurized milk sample was added to the coliform vial (BioSys, Inc., Ann Arbor, Mich.) containing 5 ml of coliform medium. The vial content was mixed by inverting the vial 10 times. The caps were slightly opened to allow air to enter into the vial. The vials were inserted into the BioSys instrument and monitored for 14 h at 35°C. The system automatically monitored the samples and reported results. All positive vials for which DTs were recorded were confirmed for the presence of coliforms using the conventional method *(3)* confirmation tests except that five drops of each sample from positive vials were inoculated into a tube of BGLB instead of an inoculum from a colony.

Statistical methods. The relationship between the optical assay and VRBA conventional coliform counts can be described by the line equation log(CFU/g) = $a + b$DT. It can be presented as $Y = a + bX$, where Y is the value of the log(CFU/g) and X is the DT in hours. The two parameters a and b are calculated as follows:

$$a = \bar{Y} - b\bar{X} \quad \text{and}$$

$$b = \frac{n\sum xy - \left(\sum x\right)\left(\sum y\right)}{n\sum x^2 - \left(\sum x\right)^2}$$

The correlation coefficient was used to determine the relationship between the optical assay counts and VRBA plate counts. The closer the correlation coefficient is to 1.0, the closer the relationship between the two methods. The regression coefficient was calculated using the following equation:

$$\rho_{x,y} = \frac{\mathrm{Cov}(X, Y)}{\sigma_x \cdot \sigma_y}$$

where

$$-1 \le \rho_{xy} \le 1 \quad \text{and}$$

$$\mathrm{Cov}(X, Y) = \frac{1}{n}\sum_{j=1}^{n}(x_j - \mu_x)(y_j - \mu_y)$$

The standard deviation (SD) is an indication of the spread of the points around the mean, and it was calculated using the following equation:

$$\sqrt{\frac{n\sum x^2 - \left(\sum x\right)^2}{n(n-1)}}$$

where n is the number of multiple plates used (in this study, n was always equal to 5) and x is the CFU per gram or CFU per milliliter value.

RESULTS

Calibration curves and DTs. A calibration curve was generated for each food product relating DTs to log CFU per gram or CFU per milliliter. Ice cream (Fig. 1) and chicken (Fig. 2) data illustrate typical calibration curves. Similar curves were obtained for the other food products.

Table 1 summarizes the calibration data obtained for all products. High correlation coefficients for the relationship of DT to log CFU per gram were obtained for all products. Using the line equations attained, one can calculate the time it will take to detect a single coliform, if present, in each product (Table 1, last column). For most products tested, coliform concentrations >10 CFU/g will be detected in 9 to 11 h (DT) with the optical assay. Chocolate appears to be more inhibitory to coliforms, resulting in a delayed DT of 14 h for >1 CFU/g. The time to detection for all products was much shorter than the 24 h required for the VRBA plates.

Reproducibility results for pasteurized milk. The results of the five sample pairs for each level of inoculation tested by the conventional method and the optical assay are shown in Table 2. For each sample, the total aerobic flora

TABLE 1. *Relationship between optical detection times and CFU for various food products*

Product	No. of points used in regression analysis	Line equation obtained by linear regression	Regression coefficient	Detection time for low numbers (h)[a]
Pasteurized milk	59	log CFU = 9.184 − 0.969 × DT	−0.99	9.5
Yogurt	59	log CFU = 9.589 − 0.693 × DT	−0.97	12.4
Ice cream	87	log CFU = 9.980 − 0.836 × DT	−0.98	10.7
Butter	63	log CFU = 10.624 − 0.922 × DT	−0.97	10.4
Chocolate	50	log CFU = 9.219 − 0.576 × DT	−0.99	14.3
Raw eggs	49	log CFU = 9.804 − 0.796 × DT	−0.98	11.1
Chicken rinse	51	log CFU = 7.406 − 0.570 × DT	−0.95	11.2
Ground beef	50	log CFU = 9.441 − 0.622 × DT	−0.95	13.6
Sausage	54	log CFU = 9.658 − 0.794 × DT	−0.92	10.9

[a] Calculated time to detection of >10 CFU/g for solid samples and >1 CFU/ml for liquid samples.

was also counted on standard plate count agar to determine the ratio of interfering bacteria to coliforms. The ratio of normal flora to coliforms was approximately 20:1 (27:1 to 17:1). All organisms selected from both the VRBA plates and the optical assay vials produced gas in BGLB within 48 h. As expected, neither method detected coliforms in the clean pasteurized milk (Table 2). For both methods, the counts obtained for the five samples at each concentration level were similar, in most cases with overlapping results, showing close agreement between the two methods.

For example, at the inoculation level of 100 to 1,000 CFU/ml, the average count was 120 CFU/ml for the VRBA plate method and 176 CFU/ml for the optical assay. These results are within 1 SD of each other, thereby showing close agreement. The optical assay detected the presence of coliforms in all five replicate samples, whereas one of the five VRBA plates failed to detect these coliforms. The average SD was 0.007 for the optical assay and 0.170 for the plate. The most significant difference in SD was at the low level of 1 to 10 CFU/ml, where the optical assay had much closer results among the five replicates (Table 2).

Reproducibility results for artificially inoculated products. Because the products in this group (butter, yogurt, ice cream, chocolate, and eggs) were inoculated and all the inocula contained coliforms, all organisms selected from both the plates and the vials produced gas in BGLB within 48 h. Most of the products in this group had low total aerobic counts; therefore, some samples were inoculated with 4.6 to 5.9 × 10^5 CFU/g of naturally occurring organisms, including *Bacillus*, *Acinetobacter*, *Alcaligenes*, *Aeromonas*, and *Serratia*. Neither the VRBA plates nor the optical assay was affected by these organisms, even at high inoculum levels.

TABLE 2. *Reproducibility obtained with five replicates of VRBA plate counts and counts by the BioSys optical assay for pasteurized milk*

VRBA			Optical assay		
Count range for the five plates (CFU/ml)			Count range for the five vials (CFU/ml)[a]		
Minimum	Maximum	SD	Minimum	Maximum	SD
0	0	NA	0	0	NA
<1	5	0.31	3	5	0.11
13	22	0.09	27	34	0.05
106	142	0.11	129	252	0.05

[a] CFU per milliliter calculated from the line equation in Table 1.

Table 3 summarizes results of the five sample pairs for each product at each level of inoculation tested by the VRBA method and the optical assay. For all products utilizing either of the two methods, the counts obtained for the five replicate samples at each concentration were similar, in most cases within ±2 SD of each other.

The optical assay was more sensitive at low levels of coliforms in some of the products. With butter counts, for example, two of the five VRBA plates in the low dilution (1 to 10 CFU) did not have colonies present on them, whereas all five vials for the optical assay at the same level detected the presence of coliforms.

The average SD in all cases was lower (equal in one case) for the optical assay than for the VRBA plates, especially for the low-count samples where greater differences between the VRBA method and the optical assay were observed. At the low concentrations (1 to 10 CFU/g), the average SD was 0.48 for the VRBA method and 0.23 for the optical assay. Because coliforms are normally found in low numbers in food products, this finding was important.

Reproducibility results for naturally contaminated samples. Chicken rinse prepared from three store-bought chickens was used in the study; one chicken was used immediately, and the other two were temperature abused (one for 4 h at room temperature and one overnight at room temperature), returned to the refrigerator, and tested the next morning.

All optical assay vials contained coliforms, as indicated by gas formation from lactose in BGLB within 24 h. Many colonies isolated from the VRBA plates were negative in BGLB, indicating that the organisms could not ferment lactose to form gas. On average, only 3 or 4 of 10 colonies were positive in BGLB (range, 1 of 10 to 5 of 10). To preclude the possibility that the results were due to a bad batch of VRBA, the liquid from the three chicken rinses was tested again on VRBA from two different manufactur-

TABLE 3. *Reproducibility obtained with five replicates of VRBA plate counts and counts by the BioSys optical assay for artificially inoculated products*

	VRBA			Optical assay		
	Count range for the five plates (CFU/g)			Count range for the five vials (CFU/g)[a]		
Product	Minimum	Maximum	SD	Minimum	Maximum	SD
Yogurt	1.00E+01	1.00E+02	0.39	1.60E+01	3.00E+01	0.11
	2.00E+02	7.00E+02	0.26	2.05E+02	1.00E+03	0.27
	3.00E+03	5.80E+03	0.11	1.90E+03	3.10E+03	0.08
Average			0.25			0.15
Butter	<1.0E+01	6.00E+01	0.93	2.00E+00	2.10E+01	0.43
	1.10E+02	2.70E+02	0.16	3.100E+01	2.12E+02	0.34
	7.00E+02	1.17E+03	0.16	7.57E+02	1.43E+03	0.13
Average			0.42			0.30
Ice cream	1.00E+01	8.00E+01	0.34	1.30E+01	1.09E+02	0.37
	1.10E+02	3.80E+02	0.22	2.95E+02	7.65E+02	0.16
	3.00E+03	5.80E+03	0.10	2.90E+03	6.30E+03	0.12
Average			0.22			0.22
Chocolate	1.00E+01	9.00E+01	0.37	2.10E+01	6.20E+01	0.19
	1.10E+02	3.80E+02	0.31	1.21E+02	3.90E+02	0.36
	8.00E+02	5.80E+03	0.09	8.70E+02	1.90E+03	0.14
Average			0.26			0.23
Raw eggs	1.00E+01	6.00E+01	0.36	2.80E+01	4.00E+01	0.07
	2.80E+02	3.90E+02	0.06	4.37E+02	9.09E+02	0.14
	1.94E+03	2.53E+03	0.06	1.57E+03	3.28E+03	0.14
Average			0.16			0.12

[a] CFU per milliliter calculated from the line equation in Table 1.

ers: Difco, Becton Dickinson (Sparks, Md.) and Alpha Biosciences (Baltimore, Md.). The results from the two batches of VRBA were similar; the majority of typical colonies were negative for coliforms in BGLB from the two manufacturers.

These results illustrate the importance of confirming typical colonies from chicken rinse on VRBA with BGLB. The organisms forming typical colonies in VRBA without gas in BGLB were identified as *Serratia liquefaciens*, *Pantoea* spp., *Vibrio fluvialis*, *Aeromonas hydrophilia*, and *Pseudomonas* spp. using the API 20E kit (bioMérieux, Hazelwood, Mo.).

The fact that so many colonies were typical in VRBA, negative in BGLB, and identified as noncoliforms made it difficult to obtain reliable results from the conventional method. The VRBA plate count results need to be corrected by the percentage of BGLB coliform-positive colonies *(3)*. The counts obtained by the two methods for the three samples were similar (Table 4), in most cases with overlapping results, showing close agreement (within the experimental error) between the two methods.

The SD for both methods was higher for the naturally contaminated samples than for the inoculated samples, probably because of the more diverse flora. The SD of the optical assay was lower than that for the conventional method.

Reproducibility results for ground beef. Three different lots of ground beef were obtained from the same supermarket; one lot was fresh and tested immediately, a second lot was on the shelf of the supermarket for several days, and the third lot was 1 day old but was temperature abused overnight at room temperature. All samples were returned to the refrigerator and tested the next morning.

All optical assay vials contained coliforms, as indicated by gas formation in BGLB within 24 h. For ground beef, similar to the situation encountered with the chicken rinse, most ''typical'' colonies isolated from conventional VRBA plates were negative in BGLB. Only between 1 of 5 and 1 of 10 colonies were BGLB positive. VRBA-positive, BGLB-negative organisms were isolated and identified using API 20E kits. Most were identified as *Serratia* spp. (particularly *S. liquefaciens*) and *Pantoea* spp. The low number of BGLB-positive tubes made it difficult to obtain reliable counts of true coliforms on the plates. These findings illustrate the importance of using the confirmation step (BGLB) when utilizing VRBA for coliform counts.

The count ranges and SDs are shown in Table 4. The average SD was 0.32 for the optical assay and 0.38 for the conventional method; this difference is not significant. For both methods, the counts obtained for the five samples at each concentration were similar, in most cases with overlapping results, showing close agreement between the two methods.

Reproducibility results for sausage. Three store-bought sausage rolls from three different lots were used in the study. One was used immediately, whereas the others

TABLE 4. *Reproducibility obtained with five replicates of VRBA plate counts and counts by the BioSys optical assay for naturally contaminated products*

	VRBA			Optical assay		
	Count range for the five plates (CFU/g)			Count range for the five vials (CFU/g)[a]		
Product	Minimum	Maximum	SD	Minimum	Maximum	SD
Chicken rinse	2.20E+03	4.40E+03	0.13	7.45E+03	1.10E+04	0.07
	4.00E+03	7.80E+03	0.13	1.19E+03	3.39E+03	0.18
	9.20E+04	8.27E+05	0.37	1.17E+05	1.98E+05	0.10
Average			0.21			0.12
Ground beef	1.00E+01	1.50E+02	0.46	1.96E+01	3.97E+02	0.53
	1.61E+04	1.06E+05	0.46	2.92E+04	1.06E+05	0.23
	5.90E+04	2.09E+05	0.22	5.17E+04	1.88E+05	0.20
Average			0.38			0.32
Sausage	2.00E+01	4.90E+02	0.97	1.70E+01	1.09E+02	0.43
	9.00E+01	3.00E+03	0.67	5.20E+01	4.62E+02	0.36
	1.90E+04	7.80E+04	0.25	2.62E+04	1.36E+05	0.27
Average			0.63			0.35

[a] CFU per milliliter calculated from the line equation in Table 1.

were temperature abused (one for 4 h at room temperature and one overnight at room temperature) and then stored in the refrigerator overnight. All optical assay vials contained coliforms, as indicated by gas formation in BGLB within 24 h. In this product, almost all VRBA colonies tested were confirmed as coliforms in BGLB, producing gas in 48 h.

For both methods, the SD for sausage was higher than that for other products tested, possibly because of the uneven distribution of organisms in the product, resulting in widely ranging results (Table 4) and high SDs for both methods. The average SD was 0.35 for the optical assay and 0.63 for the VRBA method, showing better reproducibility for the optical assay. The range of counts obtained by the two methods for the three samples was similar (Table 4), with overlapping results, showing close agreement between the two methods.

Reproducibility results for additional food samples. Further investigation of the prevalence of noncoliforms forming ''typical'' colonies on VRBA was undertaken. In additional products tested (cookie dough, bean sprouts, mushrooms, lettuce, and mussels), more than 80% of the colonies on VRBA were positive in BGLB; however, a few colonies were negative in BGLB and were identified mainly as *Serratia*, *Pantoea*, and *Erwinggella*. From the additional products tested, only in raw milk were 100% of the colonies BGLB positive, indicating the importance of colony confirmation.

DISCUSSION

Several studies have been conducted comparing the BioSys optical assay with the conventional VRBA plate count method for the enumeration of coliforms in various food products *(5, 8–10).* In all of these studies, a calibration curve was obtained showing close agreement between results of the optical assay and those of the conventional method, as obtained in the current study; however, in none of the previous studies was the reproducibility of the optical assay compared with the conventional VRBA method calculated.

We demonstrated that when using VRBA for naturally contaminated products, it is essential to verify that the colonies obtained are indeed coliforms. In some of the food products tested, only 10 to 20% of the colonies were positive for coliforms in BGLB. However, with the optical assay all vials tested were positive in BGLB, thereby making such verification unnecessary.

The time required to obtain results with the optical assay was much shorter than that required by the VRBA plate method. Products with coliforms above the specified concentration were detected in half the time (10 to 12 h) required by the VRBA method (22 to 24 h). The required verification step takes an additional 48 h, making the total time for the plate assay 72 h.

Both methods distinguished well between samples that did not contain coliforms and samples that contained the target organism; however, the optical assay was more sensitive in detecting very low concentrations of coliforms. In the few samples containing very low numbers of organisms (pasteurized milk and butter), the optical assay was capable of detecting the presence of coliforms in all five replicates, whereas VRBA plates did not detect coliforms in several of the samples.

Comparing data for all five replicates of each sample, the average SD of the optical assay was lower (equal in one case) than that for the conventional method, indicating better repeatability for the optical assay, especially at very low concentrations (1 to 10 CFU/ml for liquid products and 10 to 100 CFU/g for solid samples) of coliforms; the average SD was 0.26 for the optical assay and 0.47 for VRBA plates. The SDs for both methods were higher for the naturally contaminated samples than for the inoculated samples, probably because of the more diverse flora.

The optical assay for coliforms was not sensitive to the presence of high numbers of other organisms, resulting in accurate results regardless of the presence of other contaminants. In naturally contaminated samples, the conventional method was prone to the formation of coliform-like colonies by noncoliform organisms. This finding illustrates the importance of the confirmation of VRBA coliform colonies in BGLB. The noncoliforms *S. liquefaciens*, *Pantoea* spp., *V. fluvialis*, *A. hydrophilia*, and *Pseudomonas* spp. in the samples grew as typical coliform colonies in VRBA. The optical assay appears to be more selective than the VRBA conventional method, detecting fewer noncoliforms.

REFERENCES

1. Cheng, S. S., P. M. Gray, G. J. Woo, and D. H. Kang. 2003. A new alginate-based method for determining coliforms in milk. *J. Food Prot.* 66:2146–2150.
2. Clark, J. A., and A. H. el-Shaarawi. 1993. Evaluation of commercial presence-absence test kit for detection of total coliforms, *Escherichia coli*, and other indicator bacteria. *Appl. Environ. Microbiol.* 59:380–388.
3. Feng, P., S. D. Weagant, and M. A. Grant. 1998. *Escherichia coli* and coliform bacteria, chap. 4. *In* U.S. FDA bacteriological analytical manual, 8th ed., Revision A. AOAC International, Gaithersburg, Md.
4. Firstenberg-Eden, R. F. 2003. BioSys/MicroFoss COLIFORM Test granted PTM status BAM analytical methods. *Lab. Manag. AOAC Int.* May/June 2003:56–58.
5. Firstenberg-Eden, R. F., D. Foti, S. T. McDougal, and J. Baker. 2002. Optical instrument for the rapid detection of microorganisms in dairy products. *Int. Dairy J.* 12:225–232.
6. Madden, R. H., and A. Gilmour. 1995. Impedance as an alternative to MPN enumeration of coliforms in pasteurized milk. *Lett. Appl. Microbiol.* 21:387–388.
7. Matner, R. R., T. L. Fox, D. E. McIver, and M. S. Curiale. 1990. Efficacy of petrifilm *E. coli* and coliform enumeration. *J. Food Prot.* 53:145–150.
8. Odumeru, J. A., and J. Belvedere. 2002. Validation of the MicroFoss system for enumeration of total viable organisms, coliforms, and *E. coli* in ground beef. *J. Microbiol. Methods* 50:33–38.
9. Russell, S. M. 2001. Evaluation of the BioSys Optical Assay for rapidly enumerating populations of aerobic bacteria, coliforms, and *Escherichia coli* from broiler chicken carcasses. *J. Appl. Poult. Res.* 10:141–149.
10. Russell, S. M. 2001. Evaluation of the BioSys Optical Assay for rapidly enumerating populations of aerobic bacteria, coliforms, and *Escherichia coli* from ground pork. *J. Food Prot.* 64:669–673.
11. Russell, S. M., and J. M. Bailey. 2000. Plug'N Play HACCP. *WATT Poult. USA* June 2000:30–36.
12. Shelef, L. A., and R. F. Firstenberg-Eden. 1997. Novel selective and nonselective optical detection of microorganisms. *Lett. Appl. Microbiol.* 25:202–206.
13. Towsend, D. E., R. L. Irving, and A. Naqui. 1998. Comparison of the Simplate coliform and *Escherichia coli* test with petrifilm, three tube MPN, and VRBA+MUG methods for enumerating coliforms and *E. coli* in food. *J. Food Prot.* 61:444–449.