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Efficacy of Petrifilm™ VRB for Enumerating Coliforms and *Escherichia coli* from Frozen Raw Beef

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ABSTRACT

Petrifilm™ violet red bile (PVRB) compared favorably to the most probable number method (MPN) and violet red bile agar (VRBA) methods for enumerating coliforms from frozen raw ground beef. When comparing PVRB and VRBA incubated at 35°C, coliform enumeration displayed a linear relationship (correlation coefficient of 0.932). However, by analyzing 64 ground beef samples, PVRB enumerated 41% more coliforms/g than did VRBA. Two distinct colony types were observed on PVRB: *(a)* type I (butterfly in appearence) with a colony diameter equal to or greater than 1 mm and gas bubbles 2-4 mm in diameter touching the associated colony; and *(b)* type II with a colony diameter less than 1 mm in diameter and gas bubbles of the associated colony not necessarily touching the colony but within a colony diameter. The disparity between PVRB and VRBA for enumerating coliforms was attributed to non-coliforms representing approximately 50% of the type II coliform colonies. At 35°C, 83.7% of the type I colonies were *Escherichia coli*, whereas only 10.9%, of the type II colonies were *E. coli*. By elevating the incubation temperature from 35°C to 44.5°C, over 90% of the colonies in the counting dilution were type I of which 99.2% were *E. coli*. At 44.5°C, 39.4% of the type II colonies were *E. coli*; however, this colony type represented only 9.5% of the total colonies on PVRB. Therefore, a reliable method for enumerating *E. coli* from raw meat was developed by counting only the type I colonies on PVRB incubated at 44.5°C.

Enumeration of coliforms and *Escherichia coli* from a wide variety of food products is of major importance. In selected fresh and processed meat products, the number of coliforms can range from 4.0×10^1 to 3.2×10^4/g depending on the particular meat product and how it was processed and stored *(7)*. For non-meat products such as cheese and cheese products and raw and frozen vegetables, the coliform counts can average 3.2×10^2/g and 1.2×10^4/g, respectively *(4)*. In addition, stringent specifications involving the member of coliforms have been established in drinking water *(2)* and various dairy products *(9)*.

Enumeration of coliforms, fecal coliforms, and *E. coli* from different environments has been researched extensively. Recently, a dry medium system for enumerating total bacteria (Petrifilm™ SM plates) and coliforms (Petrifilm™ VRB plates) has been introduced and briefly evaluated. Petrifilm™ SM plates are comparable to the standard plate count method for enumerating total bacteria in raw milk *(3)* and fresh ground beef *(10)*. Nelson et al. *(8)* showed that coliform counts determined with Petrifilm™ VRB (PVRB) were comparable to counts with violet red bile agar (VRBA) when examining 120 raw milk samples. Furthermore, the most probable number (MPN) method using lauryl sulfate tryptose (LST) broth with a subsequent transferring to 2% brilliant green lactose bile broth (BGLB), yielded a greater coliform count than PVRB and VRBA. These authors recommended that PVRB can be used as a viable alternative for enumerating coliform bacteria from milk samples *(8)*.

The purposes of this investigation were: *(a)* to evaluate PVBR as an acceptable method for enumerating coliforms in frozen ground beef, *(b)* to determine types of microorganisms enumerated on PVRB incubated at 35° C with respect to colonial morphologies and *(c)* to determine the feasibility of using PVRB incubated at 44.5°C as a 24-h *E. coli* enumerating medium from frozen ground beef.

MATERIALS AND METHODS

Test samples and sample preparation

Frozen raw ground beef was used in all evaluations. Samples were stored at -18°C to -20°C for 1 week before testing, removed from frozen storage and allowed to thaw at room temperature before testing. An 11- or 25-g thawed sample was aseptically weighed into a sterile Stomacher 400 bag, diluted 1:10 with sterile 0.1% peptone water and macerated for 2 min by using a Stomacher (Seward medical, Techmore, Cincinnati, Ohio). From this mixture, appropriate serial dilutions were made in 0.1% sterile peptone water.

Three groups of test samples were evaluated: *(a)* seventy-four samples were used for coliform enumeration; *(b)* an additional 26 samples were used to identify specific microorganisms, based on colonial morphologies present on PVRB incubated at 35°C; *(c)* forty-seven samples were used for *E. coli* enumeration.

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Experimental design and statistical analysis

For the efficacy of enumerating coliform bacteria from frozen ground beef, three media were used: PVRB supplied by 3M (3M Center, St. Paul, MN), Difco VRBA and the media used for the standard MPN procedure *(6)*. On a per sample basis, all media were analyzed in triplicate.

For evaluating PVRB as a medium to enumerate *E. coli* from frozen ground beef, PVRB incubated at 44.5°C for 24 h was compared to the standard MPN procedure for *E. coli* enumeration *(6)*. PVRB was evaluated in duplicate.

All PVRB plates were stored at 4°C until use and inoculated with the 1:10 and 1:100 dilutions. Further inoculating procedures were according to Nelson et al. *(8)*. To prevent dehydration of the PVRB plates incubated at 44.5°C, a 9 × 12 in. pan of distilled water was placed in the bottom of the cabinet.

Standard procedures were used for culturing coliforms on VRBA *(6)* by using the same dilutions as those prepared for PVRB. The plates were inverted and incubated at 35°C for 18-24 h.

The MPN methods for enumerating coliforms consisted of incubating a 3-tube dilution series. One-milliliter volumes (using the same dilutions as for PVRB and VRBA) from the 1:100, 1:1000, and 1:10,000 dilutions were used to inoculate Difco LST tubes which were incubated at 35°C for 24 and 48 h. All positive LST tubes were subcultured in 10 ml of Difco BGLB and incubated at 35°C for 48 h. For enumeration of fecal coliforms, positive LST tubes were subcultured in Difco EC broth and incubated at 44.5°C for 24 h.

A Quebec colony counter was used to count colonies on PVRB and VRBA plates. Plates containing betweeen 15-150 colonies were selected to be counted.

Confirmation of typical coliform colony morphologies on PVRB and VRBA was accomplished by picking at least 10 colonies from plates of the counting dilution and inoculating BGLB. These tubes were incubated at 35°C for 48 h. Confirmed coliform colonies produced growth and gas after incubation. For the MPN method, growth and gas production in BGLB tubes were considered a confirmatory test and no further characterization was done. Three-tube MPN tables were used to estimate the number of coliforms/g of ground beef.

Coliform and *E. coli* counts were converted to $\log_{10}$ counts to more nearly match the underlying statistical assumptions. Standard regression methods were used to calculate least-squares regression lines and 95% confidence limits *(11)*.

Colony characterization on media

The following criteria were used to identify typical coliform colonies or reactions on each medium: *(a)* VRBA - following standard methods *(6)* characterized by dark-red colonies measuring greater than 0.5 mm with a zone of bile precipitate immediately surrounding the colony, *(b)* MPN method-followed standard methods *(6)* for characterizing typical coliform reactions, and *(c)* PVRB- dark red colonies associated with one or more gas bubbles no more than one colony diameter away.

During the confirmation of typical coliform colonies on PVRB, inconsistent reactions in the BGLB broth were observed. After analyzing 64 ground beef samples, a pattern was established which related inconsistent BGLB reactions to the size of the colony and associated gas bubble(s). The smaller the colony and associated gas bubble(s), the more often a negative reaction or no growth occurred in the BGLB after 48 h of incubation.

Investigations with the PVRB plates provided evidence that there were two distinct colony types with respect to colony and gas bubble size and the distance between the colony and its associated gas bubble(s). Red colonies with their associated gas bubbles were separated into two categories: type I - colony diameter equal to or greater than 1 mm with gas bubbles 2-4 mm in diameter touching the associated colony; and type II - colony diameter less than 1 mm with gas bubbles of the associated colony not necessarily touching the colony but within one colony diameter away from the colony. Figure 1 illustrates the type I and type II colonies observed. Further determinations were made on the microorganisms displaying the type I and type II colonies on PVRB plates.

Microbiological identification

For each sample, five each of type I and type II colonies were picked from PVRB plates, previously incubated at 35 or 44.5°C, and transferred to separate tubes of Difco tryptic soy broth (TSB) without glucose to resuscitate injured cells *(1)*. These cultures were incubated at 35°C until noticeable growth was observed (4-5 h). Each positive culture was transferred to Difco phenol red broth base containing each of the following carbohydrates at a final concentration of 1%: lactose, sucrose, glucose, sorbitol or adonitol. These cultures were incubated at 35°C up to 72 h. The following media were also inoculated for further identification: triple sugar iron agar (TSI), lysine iron agar (LIA), motility indole ornithine medium (MIO), and urea. The TSI, LIA, and MIO media were incubated at 35°C for 24 h. If a definitive identification could not be made on the basis of the above tests, Enterotube II tubes (Roche) were inoculated and incubated at 35 °C for 24 h.

RESULTS

Comparison of PVRB, VRBA and MPN methods for enumerating coliforms

Linear regression analysis was used to compare PRVB and VRBA enumeration data from frozen ground beef. Figure 2 presents the data comparing coliform counts for

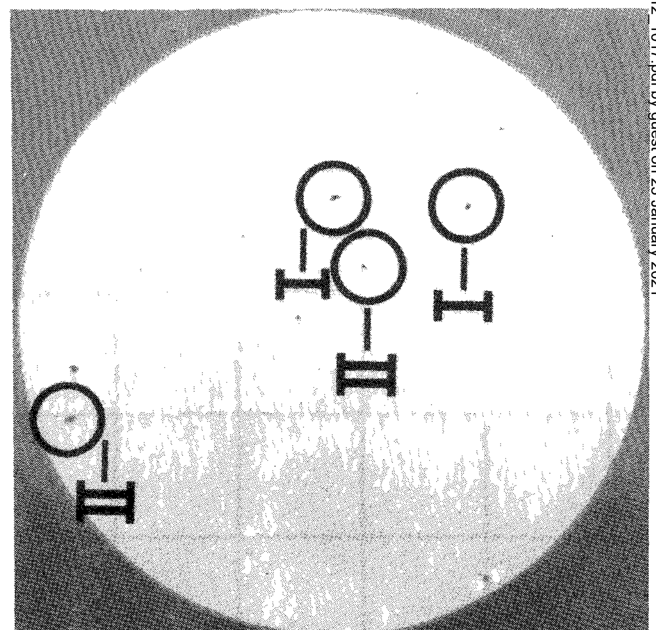

Figure 1. *Classification of colonial morphologies on Petrifilm™ violet red bile as designated by type I and type II colonies.*

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PVRB versus VRBA. The regression line had a slope of 0.749 and an intercept of 0.803. Comparing the line of equality (interrupted or dashed line with a slope of one and intercept of zero) with the regression line shows the two methods are different in enumerating coliform bacteria. However, the correlation coefficient (0.932) indicates a strong linear relationship between PVRB and VRBA.

Figures 3 and 4 illustrate scatterplot graphs comparing the mean coliform counts on PVRB or VRBA plates to the mean coliform count determined by the MPN method using 64 frozen ground beef samples. MPN mean coliform counts are bracketed by a 95% confidence interval. Mean coliform counts from PVRB or VRBA (represented by an open box) that are not contained within the brackets indicate a mean count outside the 95% confidence limit of the MPN count. Fourteen percent (9/64) of the mean PVRB counts (Fig. 3) and 33% (21/64) of the mean VRBA counts (Fig. 4) were below the bracketed 95% confidence limit for mean MPN counts. This difference of 14% versus 33% is significant ($P<0.01$ by chi-square test) indicates that significantly more mean PVRB counts were within the MPN 95% confidence limit than mean VRBA counts.

The mean and standard deviation values of coliform counts/g as determined by the PVRB, VRBA, and MPN methods for 64 frozen ground beef samples are in Table 1. The mean values for coliforms/g were 550, 389 and 1050 for PVRB, VRBA and MPN methods, respectively. All these means were significantly different from each other. As illustrated in Fig. 3 and 4, the MPN method estimated consistently more coliforms/g than the PVRB and VRBA methods. When using the criterion for a typical coliform colony as instructed by 3M, the PVRB enumerated 41% more coliforms/g than VRBA from frozen ground beef.

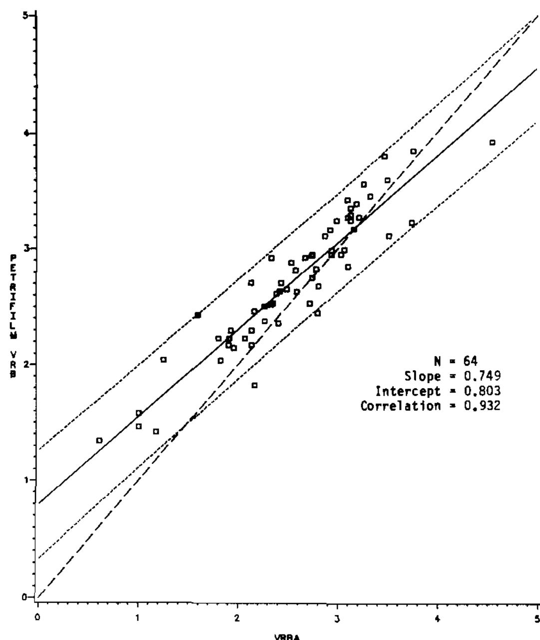

Figure 2. *Relationship of confirmed log_{10} coliform counts/g determined by Petrifilm™ VRB plates to violet red bile agar (VRBA) plates indicated by linear regression line (solid line) with 95% confidence limits (dashed lines) and line equality (broken line).*

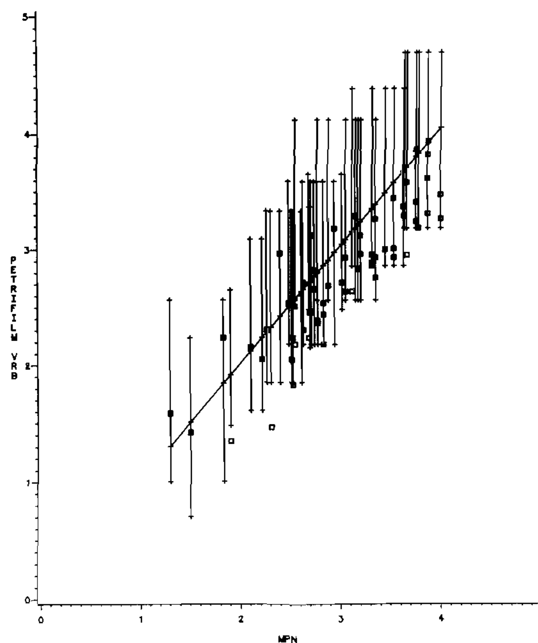

Figure 3. *Scatterplot of confirmed log_{10} coliform counts/g determined by Petrifilm™ violet red bile (PVRB) plates versus most probable number with 95% confidence limits. Open square symbols represent the mean PVRB coliform count/g for each sample.*

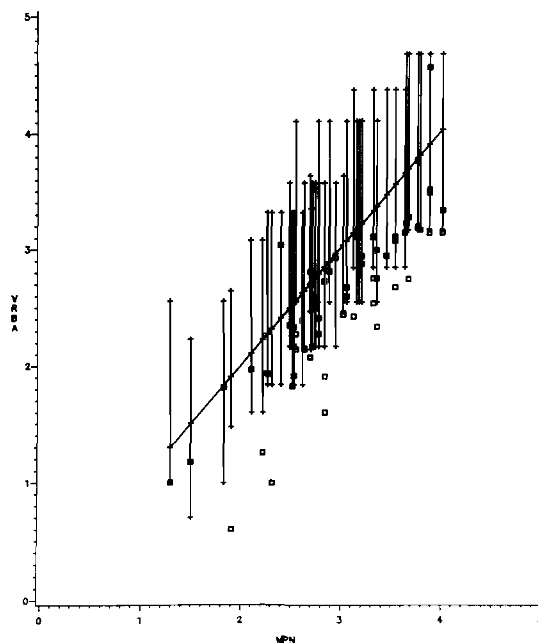

Figure 4. *Scatterplot of confirmed log_{10} coliform counts/g determined by violet red bile agar (VRBA) versus most probable number with 95% confidence limits. Open square symbols represent the mean VRBA coliform count/g for each sample.*

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TABLE 1. *Mean and standard deviation of coliform counts/g as determined by Petrifilm™ violet red bile, violet red bile agar, and the most probable number methods*[a].

Parameter	PVRB	VRBA	MPN
Mean	550b	389	1050
Standard Deviation	±38	±53	±43

[a]Analyzed 64 ground beef samples.
[b]Coliforms/g.

Characterization and enumeration of type I and II colonies on PVRB incubated at 35 and 44.5°C.

The distribution and characterization of type I and II colonies isolated from frozen raw beef by PVRB incubated at 35°C are in Table 2. By analyzing 26 raw beef samples, 73.8% of the coliform colonies counted on PVRB incubated at 35°C were type I. Of the 3751 colonies counted, 258 were identified: 99.2% type I and 48.9% type II were capable of producing acid and gas from lactose. The only lactose-negative microorganism displaying a type I colony was *Enterobacter cloacae*. *Escherichia coli* represented 83.7% of the type I colonies, whereas 10.9% of the type II colonies were characterized as *E. coli*. In addition to *E. coli, Klebsiella oxytoca* (3.9%), *Klebsiella pneumoniae* (5.4%), and *E. cloacae* (2.3%) displayed type I colonies with low but consistent frequency on PVRB incubated at 35°C. Approximately half of the bacteria displaying type II coliform colonies were lactose-negative. In addition to *E. coli*, other lactose-positive enterics enumerated as type II colonies were *E. cloacae* (14.0%), *Citrobacter freundii* (7.0%), and *Serratia liquefaciens* (4.6%). Important non-lactose fermenters displaying type II coliform colonies were *Enterobacter agglomerans* (6.9%), *E. cloacae* (12.4%), *Hafnia alvei* (7.7%), *Klebsiella ozaenae* (3.1%) and *S. liquefaciens* (15.5%).

Table 3 presents the classification and distribution of type I and II coliform colonies from PVRB incubated at 44.5°C using 21 frozen raw beef samples. The variety of enteric bacteria isolated were substantially less than from PVRB incubated at 35°C (Table 2). From a total of 2429 coliform type colonies counted on PVRB which was incubated at 44.5°C, 90.5% were type I colonies. All of the colonies identified on PVRB incubated at 44.5°C were able to ferment lactose and produce gas (data not presented). Of the 120 type I colonies from PVRB at 44.5°C, 99.2% were classified as *E. coli*, whereas 39.4% of 66 type II colonies were *E. coli*. In addition, *E. cloacae* (30.4%), *C. freudii* (13.6%) and *K pneumoniae* (6.1%) were frequently identified as type II colonies from PVRB at 44.5°C.

Comparison of PVRB incubated at 44.5°C and the MPN method for enumerating E. coli

Table 4 shows the mean and standard deviation values of *E. coli* counts/g as determined by PVRB incubated at 44.5°C for 18-24 h and the EC MPN method using 47 frozen ground beef samples. The mean values of the *E. coli*/g were 40 and 60 for type I colonies on PVRB incubated at 44.5°C and EC MPN methods, respectively. As with the coliforms (Table 1), the EC MPN method esti-

TABLE 2. *Identification of type I and type II colonies from Petrifilm violet red bile incubated at 35°C using frozen ground beef*[a].

Organisms identified	Type I[b] Lactose test + N	+ %	− N	− %	Type II[c] Lactose test + N	+ %	− N	− %
Escherichia coli	108	83.7			14	10.9	-	-
Enterobacter aerogenes	1	0.8			1	0.8	-	-
Enterobacter agglomerans	-	-			5	3.9	9	6.9
Enterobacter cloacae	3	2.3	1	0.8	18	14.0	16	12.4
Enterobacter gorgovia	-	-			5	3.9	-	-
Enterobacter sakazakii	-	-			-	-	1	0.8
Enterobacter sp	1	0.8			-	-	1	0.8
Citrobacter diversus	-	0			-	-	1	0.8
Citrobacter freundii	1	1.6			9	7.0	-	-
Hafnia alvei	-	-			-	-	10	7.7
Klebsiella oxytoca	5	3.9			2	1.5	-	-
Klebsiella ozaenae	-	-			2	1.5	4	3.1
Klebsiella pneumoniae	7	5.4			1	0.8	-	-
Morganella morganii	-	-			-	-	1	0.8
Serratia liquefaciens	1	0.8			6	4.6	20	15.5
Serratia marcescens	-	-			-	-	3	2.3
Total	128	99.2	1	0.8	63	48.9	66	51.1

[a]Twenty-six frozen ground beef samples were analyzed. Type I colonies were represented by 73.8% of the colonies on the counting dilution for PVRB incubated at 35°C. Total number of colonies counted were 3751.
[b]Type I. Colony diameter equal to or greater than 1 mm in diameter. Gas bubbles 2-4 mm in diameter touching colony.
[c]Type II colony diameter less than 1 mm in diameter with gas bubbles of the associated colony not necessarily touching the colony. The gas bubbles not in contact with the associated colony are within the colony diameter.

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mated 52% more *E. coli*/g compared to the number of *E. coli*/g enumerated by counting only type I colonies on PVRB incubated at 44.5°C for 18-24 h.

DISCUSSION

Both presence and quality of coliforms and *E. coli* as indicator of fecal contamination have been used to establish microbiological standards or guidelines for various raw and processed foods *(4)*. The more efficient the procedures are in enumerating these microorganisms, the more advantageous they are for the food or industrial microbiologist.

TABLE 3. *Classification of type I and type II colonies from Petrifilm™ violet red bile incubated at 44.5°C using frozen ground beef* [a].

Organisms	Type I[b] N	Type I[b] %	Type II[c] N	Type II[c] %
Escherichia coli	119	99.2	26	39.4
Enterobacter cloacae	-	-	20	30.4
Klebsiella pneumoniae	1	0.8	4	6.1
Klebsiella oxytoca			2	3.0
Citrobacter freundii	-	-	9	13.6
Morganella morganii			1	1.5
Citrobacter amalonaticus	-	-	1	1.5
Enterobacter sakazakii	-	-	1	1.8
Enterobacter agglomerans			1	1.5
Not identified	-	-	1	1.5
Total	120	100%	66	100%

[a]Twenty-one frozen ground beef samples were analyzed. Type I colonies represented 90.5% of the colonies on the counting dilution for PVRB incubated at 44.5°C. Total number of colonies counted were 2429.

[b]Type I colony diameter was equal to or greater than 1 mm with gas bubbles 2-4 mm in diameter touching the associated colony. All the type I colonies were lactose fermenters.

[c]Type II colony diameter was less than 1 mm in diameter with gas bubbles of the associated colony not necessarily touching the colony. The gas bubbles not in contact with the associated colony are within the colony diameter.

TABLE 4. *Mean and standard deviation of* log_{10} Escherichia coli *as determined by Petrifilm violet red bile incubated at 44.5°C compared to confirmed EC most probable number method*[a].

Parameter	VRB	MPN
Mean	40[b]	60
Standard Deviation	±3	±5

[a]Forty-seven samples of frozen ground beef were analyzed; on PVRB only type I colonies were counted as *E. coli*.

[b]*E. coli*/g.

In this study, PVRB compared favorably to the MPN and VRB methods for enumerating coliforms from frozen raw ground beef (Fig. 2, 3 and 4 and Table 1). However, several important trends were deduced from these data. As discussed by Nelson et al. *(8)* using raw milk samples, the MPN method estimated a consistently higher number of coliforms/g (lower positive predictive value) as compared to the PVRB and VRBA methods. This over estimation of the bacterial concentration when using the MPN methods has been further identified by Hall et al. *(4)*. In addition, by using raw ground beef and chicken artificially inoculated with high numbers (greater than the total bacterial count) of *E. coli*, Frampton (personal communication) found that the EC MPN method estimated a 57% higher *E. coli* count than the standard plate count medium. These studies clearly show the intrinsic shortcomings of the MPN method and the need for more MPN values per sample to reduce the significant over estimation and large standard error *(4)*. Establishing microbiological critera such as standards or guidelines that utilize the standard MPN methods for estimating bacterial concentrations must be suspect and reinvestigated using viable alternative procedures.

When comparing PVRB and VRBA incubated at 35°C, these two methods displayed a linear relationship (correlation coefficient of 0.932) for enumerating coliforms, whereas, the regression lines indicated the methods to be slightly different (Fig. 2). Since VRBA and PVRB contain a similar chemical composition with respect to inhibitory components and carbon substrates *(5,8)*, insignificant differences on enumerating coliforms would be expected. PVRB enumerated 41% more coliforms/g than VRBA from frozen raw ground beef (Table 1). By examining raw milk samples, Nelson et al. *(8)* showed that the negative predictive value for PVRB was 55%, which indicates that an "atypical" colony on PVRB was a non-coliform about half the time and a coliform the other half. These data indicate the difficulty in discerning between an "atypical" and "atypical" coliform colony on PVRB incubated at 35°C for 18-24 h.

An atypical coliform colony on PVRB is a red colony where no gas bubble is present or the gas bubble is further than one diameter from the colony *(8)*. What happened in this investigation was that some of the "atypical" coliform colonies on PVRB were counted as "typical", therefore including some non-coliform bacteria. Difficulty occurs in identifying and counting typical coliforms colonies on PVRB when: *(a)* determining if a gas bubble is a diameter away; *(b)* closing the top film after inoculation entrapping small gas bubbles; and *(c)* crowding of gas bubbles where PVRB plates contain more than 100 coliform colonies. Therefore, PVRB incubated at 35°C for enumerating coliforms from raw meat products can be inaccurate by counting atypical coliform colonies producing a substantial amount of false-positive counts.

By further examination of the typical coliform colonies

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on PVRB, two distinct colony types could be observed (Fig. 1): *(a)* type I colony diameter equal to or greater than 1 mm and gas bubbles 2-4 mm in diameter *touching* the associated colony, and *(b)* type II with a colony diameter less than 1 mm in diameter with gas bubbles of the associated colony not necessarily touching the colony but within a colony diameter. Type I colonies had a butterfly appearance making type I and type II colonies easily distinguishable. Since PVRB consistently enumerated more coliforms than VRB from the frozen ground beef (Fig. 2 and Table 1), characterization and identification of the bacterial types that produce the type I and type II colonies on PVRB became paramount for a better understanding of the selectivity of PVRB.

The disparity between PVRB and VRB incubated at 35°C for enumerating coliforms from frozen raw beef can be attributed to the presence of lactose-negative bacteria (non-coliforms) represented by approximately 50% of the type II coliform colonies on PVRB (Table 2). At 35°C, a perponderance of *E. coli* (83.7%) were identified from type I colonies, whereas, only 10.9% of the type II colonies were *E. coli*. Type I colonies represented only 73.8% of the colonies. The remaining 26.2% of the colonies were type II of which 10.9% of these were *E. coli*. Therefore, by using type I colonies on PVRB incubated at 35°C as a 24-h *E. coli* enumerating procedure, a substantial number of false-positives (type I colonies which are not *E. coli*) will be produced including *K. pneumoniae*, *K. oxyoca* and *Enterobacter* sp. In addition, a significant number of *E. coli* type II colonies would not be counted.

In 1976, Klein and Fung *(5)* showed that VRBA incubated at 44.5 ± 0.5°C for 24 h could be used to enumerate fecal coliforms from water samples. By evaluating the incubation temperature from 35 to 44.5°C, non-fecal coliforms can be eliminated *(6)*. In this study, a similar idea was investigated with PVRB incubated at 44.5°C with respect to the type I and type II coliform colonies. When PVRB was incubated at 44.5°C, over 90% of the colonies on the counting dilution were type I colonies of which 99.2% were *E. coli*. Although approximately 40% of the type II colonies were *E. coli*, this colony type represented only 10% of typical coliform colonies on PVRB incubated at 44.5°C. By counting only type I colonies on PVRB incubated at 44.5°C, over 96% of the *E. coli* present can be enumerated with less than 1.0% involving false positive colonies. In addition, the type I colonies on PVRB incubated at 44.5°C compared favorably to the EC MPN method (Table 4). Although the EC MPN method estimated 52% more *E. coli*/g than the type I colonies on PVRB incubated at 44.5°C, these data are consistent with the results of Frampton (personal communication) where the EC MPN method estimated 57% greater recovery of *E. coli*/g than a standard plate count medium from artificially inoculated raw beef and chicken. Therefore, the type I colonies on PVRB incubated at 44.5°C provide an accurate enumeration of *E. coli* from raw meat samples.

The advantages of using PVRB incubated at 44.5°C to enumerate *E. coli* are: *(a)* overnight incubation (18-24 h), *(b)* elimination of special equipment such as a water bath, *(c)* minimal laboratory preparation, and *(d)* economical use of incubator space. During this investigatiion, two observations were made concerning conditions used when PVRB is incubated at 44.5°C to enumerate *E. coli*: *(a)* the air incubator should have a temperature accuracy of ± 0.5°C *(12)*, and *(b)* a pan of water must be placed in the incubator to prevent drying of the PVRB.

Thus, using frozen ground beef, a viable 24-h *E. coli* enumerating procedure was developed involving PVRB incubated at 44.5°C and counting only type I colonies. This procedure is less cumbersome and at least 24 h faster than the conventional *E. coli* MPN method *(6)*. Before this method can be more generally applied, however, further testing must be performed with different food products both raw and cooked.

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type and concentration of insecticides. For instance, guthion at different concentrations markedly reduced mold growth and aflatoxin yield, the reduction being proportional to the guthion concentration. Actellic exhibited the same effect as guthion but to a lesser degree. Malathion at 5 ppm showed a highly significant stimulatory effect on aflatoxin production and an inhibitory effect at 10 and 20 ppm levels. It has been reported that malathion significantly inhibits production of aflatoxins at the 100 ppm concentration *(3)*. The inhibitory effect of these insecticides on G_1 and B_1 production was more pronounced in the presence of 20 ppm, and the B_1:G_1 ratio generally increased with the increase in insecticide concentration. In general, the inhibitory effect of these insecticides on *A. parasiticus* growth and aflatoxin production followed the sequence: guthion>actellic>malathion.

The chemical structures of the three insecticides are shown in Fig. 1. The formulas show that each of these insecticides has a thiophosphate ester group. The difference is mainly in the functional groups attached to the thiophosphate ester group. The extent of the inhibitory effect of the tested insecticides could be attributed to the presence of the aromatic nucleus. Malathion contains no aromatic nucleus, while actellic and guthion have heterocyclic rings, the latter has two fused heterocyclic rings. This suggests that the inhibitory effect could be increased by increasing the aromaticity since guthion with two fused rings showed the most powerful inhibitory effect towards aflatoxin production.

In conclusion, compounds in the present study possess dual functions, i.e., each compound can be used to control pests and/or weeds or to increase plant growth and yield, depending on the nature of the compound. At the recommended application rate, with the exception of IAA and treflan, all compounds suppressed mold growth and aflatoxin production.

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