MICROBIOLOGICAL METHODS

3M™ Petrifilm™ Enterobacteriaceae Count Plate Method for Enumeration of Enterobacteriaceae in Selected Foods: Collaborative Study[1]

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The practice of detecting and enumerating all oxidase-negative, glucose-fermenting-Gram-negative rods (i.e., the family Enterobacteriaceae) is used to indicate unsanitary or inadequate food processing conditions. The objective of this interlaboratory collaborative study was to evaluate and compare the methods described in Standard Methods for the Examination of Dairy Products (SMEDP) and the Compendium of Methods for the Microbiological Examination of Foods (Compendium) with a commercial product, the 3M™ Petrifilm™ Enterobacteriaceae Count Plate, for the recovery of Enterobacteriaceae in foods. Six foods—cheddar cheese, milk, flour, frozen prepared meals, frozen broccoli, and nut pieces—were analyzed for Enterobacteriaceae by 12 collaborating laboratories. For each food tested, the collaborators received 8 blind test portions consisting of a control test portion and 3 levels of inoculated test portion, each in duplicate. Each test portion was tested by the Petrifilm Enterobacteriaceae Count Plate method as well as the SMEDP or Compendium methods. The precision estimates (repeatability or within-laboratory variation, and reproducibility or between-laboratory variation) were calculated with standard statistical techniques.

Determination of coliforms has traditionally been used in the food industry as an indicator of unsanitary conditions or inadequate processing. This method identifies coliforms by their ability to ferment lactose with the production of gas and acid; however, it does not determine the presence of all members of the Enterobacteriaceae group. The practice of detecting and enumerating all fermentative Gram-negative organisms (i.e., the family Enterobacteriaceae) has become more common in the food industry, especially in European countries. It is thought that enumeration of a wider, better-defined group of organisms, such as Enterobacteriaceae, rather than a more specific group like the coliforms, will provide a more thorough assessment of the food (1). Enterobacteriaceae are enumerated by counting Gram-negative rods which ferment glucose. Organisms are enumerated regardless of their ability to produce gas. Traditional methods for enumerating Enterobacteriaceae use violet red bile agar with glucose (VRBG; 2).

The 3M™ Petrifilm™ Enterobacteriaceae (EB) Count Plate is designed for the enumeration of Enterobacteriaceae in foods. The product consists of a medium optimized for the growth of Enterobacteriaceae, yet inhibitory to the growth of Gram-positive bacteria. A pH indicator, a dye to enhance the visualization of growth, and a cold-water-soluble gelling agent are included in the plate. Diluted test portions are added at a volume of 1.0 mL per plate. Pressure applied to a plastic spreader placed on the overlay film spreads the test portion over a growth area of approximately 20 cm^2. The gelling agent is allowed to solidify and the plates are then incubated.

A comparative study (3) demonstrated that the Petrifilm EB plate provided results similar to those of the reference method for enumeration of Enterobacteriaceae. This report describes a collaborative study comparing the Petrifilm EB Count Plate method with the reference methods described in Standard Methods for the Examination of Dairy Products (SMEDP; 4) and the Compendium of Methods for the Microbiological Examination of Foods (Compendium; 2). In addition, ISO standard 7402 (5) was consulted for guidance on the enumeration of Enterobacteriaceae.

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[1] Poster presentation at the 1997 AOAC Annual Meeting.
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Table 1. Foods and Enterobacteriaceae

Foods	Organism
Cheddar cheese	Escherichia coli ATCC 11775
Milk	Proteus vulgaris ATCC 8427
Flour	Klebsiella oxytoca U33
Frozen prepared meals	Klebsiella pneumoniae ATCC 13883
Frozen broccoli	Edwardsiella tarda ATCC 15947
Nut pieces	Citrobacter freundii ATCC 8090

Collaborative Study

Test Foods

The foods selected for inclusion in this study are listed in Table 1. Single production lots were obtained from local retail outlets. Background counts of Enterobacteriaceae in the products obtained for the study were monitored and recorded.

Test Organisms

Six different species from the Enterobacteriaceae family were used in the study. Each was used to inoculate a different food type (Table 1). The organisms were stored at –70 C in laboratory medium containing 15% sterile glycerol. Working cultures were maintained on eosin methylene blue agar plates and the inoculum was cultivated in nutrient broth (NB) and incubated at 37 C. Stationary phase cultures in NB were diluted with dilution water for inoculation of the test products.

Inoculation of Foods

The presence of Enterobacteriaceae in these food products indicates post-process contamination. Therefore, the test portions were prepared by inoculation with a wet suspension of cells for high-moisture foods and a dry inoculum for dry food products.

Approximately 4200 g cheddar cheese was obtained from several retail packages. The cheese was combined through grinding and rolling to achieve uniformness, and then divided into 4 portions; the first 3 portions were inoculated (low, medium, or high inoculum) and the 4th portion served as an uninoculated control. The inoculum was added to the test portions with intermittent grinding. The inoculation levels differed by powers of 10 and were in the range of 10–100 cells/g for the low, 101–1000 cells/g for the medium, and 1001–10 000 cells/g for the high inocula.

Approximately 4200 mL pasteurized milk were aseptically combined and mixed. The product was divided into 4 portions and inoculated at the levels described for cheese.

Approximately 4200 g flour and 4200 g nut pieces were obtained and blended thoroughly. Each product was then divided into 4 portions and inoculated according to the method described for cheese.

Approximately 4200 g frozen meals and 4200 g frozen broccoli were obtained and thawed. Each product was then mixed thoroughly and divided into 4 portions and inoculated according to the method described for cheese.

Preparation of Samples

Inoculated and uninoculated test portions were divided into approximately 60 g samples, which were each divided in half to form duplicate subsamples. For each product to be analyzed, duplicate subsamples of the uninoculated control and duplicate subsamples of each of the 3 inoculation levels were prepared for each collaborator. Samples were packaged in leak-proof containers, identified by product date, analysis date, and sample code. The sample code was a singular, multidigited, randomized number.

Shipment of Samples

Samples were shipped by overnight carrier to arrive the day before initiation of analysis. The cheese, milk, frozen prepared meals, and frozen broccoli were packed with ice packs to maintain a temperature of <7 C during shipment. The flour and nut pieces were shipped at ambient temperature. Upon arrival, the samples were stored properly until they were analyzed the following day. One product type was shipped during each week of the study.

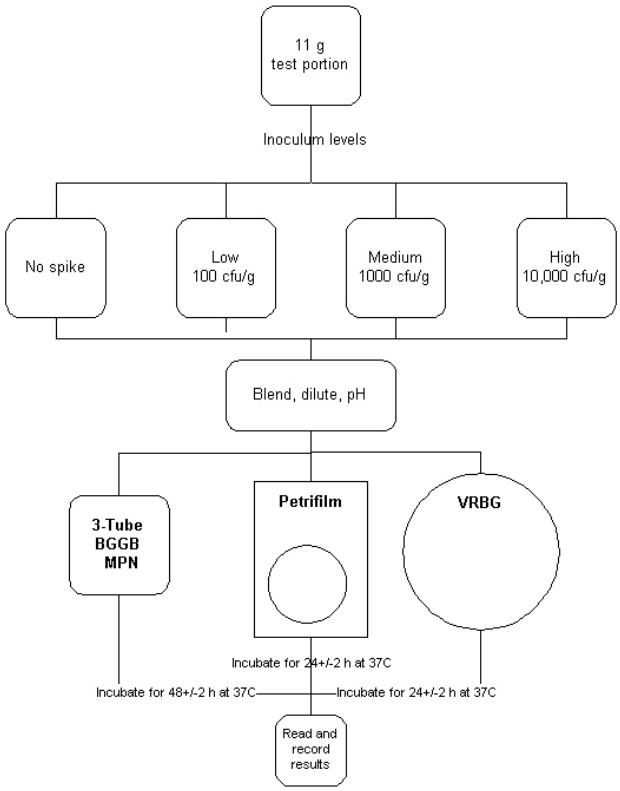

Figure 1. Flow diagram for Petrifilm, VRBG, and BGGB methods.

Microbiological Analysis

Twelve laboratories analyzed the samples for Enterobacteriaceae using the Petrifilm EB Count Plate method, as well as the SMEDP and Compendium methods (Figure 1). Eight samples for each of the 6 foods tested were analyzed by the Petrifilm EB Count Plate method, the VRBG plate method, and the 3-tube MPN method using brilliant green glucose bile (BGGB) broth (5). Because collaborators received blind samples, they were instructed to dilute all samples and plate the 10^{-1}, 10^{-2}, and 10^{-3} dilutions onto each of the Petrifilm and VRBG plates; the 3-tube MPNs in BGGB were performed on 5 dilutions (10^{-1}–10^{-5}). The plates were incubated at 37 ± 1 C for 24 ± 2 h; the BGGB tubes were incubated at 37 ± 1 C for 48 ± 2 h.

All red colonies producing gas, regardless of acid production, were counted on the Petrifilm plates. All nongassing colonies with yellow acid zones were also counted on the Petrifilm plates. Counts were recorded at 24 2 h. Deep-red colonies, with or without an associated halo of precipitated bile salts, were counted after 24 2 h on VRBG. Growth results were recorded for the BGGB tubes after 48 ± 2 h of incubation at 37 ± 1 C.

Statistical Analysis of Data

The collaborators recorded data for Petrifilm plate, VRBG agar, and MPN analyses on data log sheets provided by the Study Director. The data sheets were returned by the collaborators to the Study Director for data analysis.

Petrifilm EB plates with 10–150 colonies were selected for use in data analysis. If no plate had at least 10 qualifying colonies, the exact raw count on the least dilute sample was recorded. If all the plates had counts >150, estimates were made on the plates by counting the number of colonies in one or more representative squares, determining the average number per square, and multiplying the average number by 20. If the plates were too crowded for estimated counts, the count was reported as too numerous to count (TNTC).

VRBG plates with 15–150 colonies were selected for use in data analysis. If no plate had at least 15 deep-red colonies, the exact raw count on the least dilute sample was recorded. If all the plates had counts >150, an estimated count was recorded, if possible; otherwise, the count was reported as TNTC.

The base 10 logarithms of the colony counts and MPN indices were used for statistical analyses under the assumption that the transformed numbers would be normally distributed and of homogeneous variance. Indefinite data values (e.g., <1.0, >5.0) and other noncountable or inestimable values (e.g., TNTC) were treated as missing values in the analyses. Repeatability and reproducibility precision estimates were calculated according to AOAC procedures once outliers were determined by the Cochran and Grubbs tests (6). Repeatability variances were compared by an F-ratio test (7). Mean $\log_{10}$ counts were compared by analysis of variance (7). For most statistical tests, a resulting value of $p < 0.05$ was taken to indicate a significant difference. However, for some tests, a significant difference was indicated by $p < 0.01$. For other tests, the actual level of significance was given.

AOAC Official Method 2003.01
Enumeration of Enterobacteriaceae in Selected Foods

Petrifilm™ Enterobacteriaceae Count Plate Method
First Action 2003

(Applicable to enumeration of Enterobacteriaceae organisms in cheddar cheese, milk, flour, frozen prepared meals, frozen broccoli, and nut pieces.)

See Tables 2003.01A and 2003.01B for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Method uses bacterial culture plates of dry medium with pH indicator and cold-water-soluble gel. Undiluted or diluted test portions are added to plates at 1.0 mL per plate. Pressure applied to plastic spreader placed on overlay film spreads the test portion evenly over 20 cm^2 growth area. Gelling agent is allowed to solidify, and plates are incubated for 24 ± 2 h at 37 ± 1 C and then counted.

B. Apparatus and Reagents

(a) Petrifilm Enterobacteriaceae (EB) Count Plates.—Plates, available from 3M Microbiology Products (3M Center, Bldg 260, St. Paul, MN 55144), contain VRBG nutrients, pH indicator, cold-water-soluble gelling agent, and tetrazolium indicator dye.

(b) Plastic spreader.—Provided with Petrifilm plates; has smooth flat side designed to spread test portion evenly over plate growth area.

(c) Pipets.—1.0 and 10.0 mL serological pipets with 0.1 mL graduations. (Calibrated 3M™ Electronic Pipettor and tips, or equivalent, may be used to deliver 1.0 mL.) Pipets must accurately deliver required volume. Do not use pipets to deliver <10% of their total volume. For example, to deliver 1 mL, do not use pipet >10 mL; to deliver 0.1 mL, do not use pipet >1 mL.

(d) Colony counter.—Standard apparatus, Quebec Model, available from many suppliers, or one providing equivalent magnification and visibility.

(e) Dilution water.—Prepare stock solution as follows: Dissolve 34 g KH_2PO_4 in 500 mL water, adjust to pH 7.2 with 1M NaOH (ca 175 mL), and dilute to 1 L with water. Prepare buffered water for dilutions by diluting 1.25 mL stock solution to 1 L with boiled and cooled water. Autoclave 15 min at 121 C.

(f) Sterile sodium hydroxide solution.—1M. Dissolve 40 g NaOH in water and dilute to 1 L water. Autoclave 15 min at 121 C.

(g) Blender.—High-speed (16 000–18 000 rpm) with sterile jar.

(h) Incubator.—Maintaining 37 1 C.

(i) Balance.—2000 ± 0.1 g capacity.

Table 2003.01A. Repeatability performance of Petrifilm Enterobacteriaceae Count Plate, VRBG, and MPN methods for detection of Enterobacteriaceae in foods

		Petrifilm Enterobacteriaceae Count Plate					VRBG					MPN				
Food	Level	n[a]	Mean[b]	s_r	RSD_r, %	r	n[a]	Mean[b]	s_r	RSD_r, %	r	n[a]	Mean[b]	s_r	RSD_r, %	r
Cheese	Uninoculated	0					0					9	2.09	0.35	16.75	0.98
	Low	12	3.02[c]	0.18[d]	5.96	0.51	12	3.03	0.06[e]	1.94	0.17	11	3.12	0.31	9.79	0.86
	Medium	12	4.07[c]	0.14[d]	3.33	0.38	12	4.06	0.11	2.69	0.31	12	4.23	0.38	8.89	1.05
	High	10	4.91[c]	0.05[d,f]	0.92	0.13	12	4.93	0.13	2.59	0.36	9	4.82	0.26	5.31	0.72
Milk	Uninoculated	0					0					7	1.67	0.31	18.60	0.87
	Low	11	2.49[c,g]	0.06[d,f]	2.57	0.18	12	2.27	0.50	22.19	1.42	12	1.93	0.12	6.00	0.32
	Medium	12	3.34[c]	0.10[d,f]	3.00	0.28	12	3.27	0.21	6.46	0.60	12	2.65	0.42	15.79	1.17
	High	11	4.34[c]	0.10[d,f]	2.37	0.29	11	4.19	0.20	4.69	0.55	11	3.24	0.63	19.34	1.75
Flour	Uninoculated	12	2.70	0.25[d]	9.33	0.71	12	2.98	0.28	9.23	0.78	12	2.44	0.47	19.20	1.31
	Low	12	2.73[c]	0.32	11.78	0.91	12	2.95	0.30	10.28	0.86	12	2.24	0.40	18.02	1.13
	Medium	11	2.83[c]	0.38	13.31	1.06	12	3.06	0.28	9.15	0.79	12	2.20	0.51	23.36	1.44
	High	12	2.89[g]	0.26[d]	8.90	0.72	12	3.16	0.28	8.84	0.79	12	2.87	0.52	18.13	1.46
Prepared meals	Uninoculated	0					0					9	1.47	0.34	23.04	0.95
	Low	11	2.61[c]	0.13[d]	4.81	0.35	11	2.52	0.14	5.69	0.40	9	2.42	0.42	17.43	1.18
	Medium	11	3.51[g]	0.08[d,f]	2.21	0.22	12	3.30	0.14	4.35	0.40	12	3.34	0.38	11.42	1.07
	High	11	4.47[g]	0.08[d,f]	1.74	0.22	12	3.98	0.21	5.24	0.59	8	4.30	0.52	12.21	1.47
Frozen broccoli	Uninoculated	0					0					11	1.32	0.34	26.06	0.96
	Low	10	1.60	0.32	19.91	0.90	5	1.36	0.32	23.62	0.91	11	1.58	0.36	23.02	1.02
	Medium	12	2.66[c,g]	0.14[d,f]	5.15	0.39	11	2.02	0.29	14.28	0.81	11	2.11	0.45	21.18	1.25
	High	11	3.44[c,g]	0.29	8.49	0.82	10	2.84	0.55	19.38	1.55	10	2.71	0.48	17.73	1.34
Nuts	Uninoculated	10	2.74	0.21	7.53	0.58	10	2.89	0.24	8.45	0.69	9	2.67	0.34	12.76	0.95
	Low	10	3.50	0.29[d]	8.40	0.83	9	3.64	0.44	12.06	1.24	9	3.48	0.53	15.31	1.49
	Medium	7	4.09	0.50	12.10	1.40	7	4.13	0.47	11.38	1.32	5	3.82	0.68	17.89	1.91
	High	7	4.20	0.29[d]	6.92	0.82	7	4.14	0.49	11.86	1.39	7	3.83	0.85	22.13	2.37

[a] Number of laboratories with positive results.
[b] Log_{10} Enterobacteriaceae count/g.
[c] Significantly different from MPN method ($p < 0.05$).
[d] Significantly better repeatability than for the MPN method.
[e] Significantly better repeatability than for the Petrifilm plate method.
[f] Significantly better repeatability than for the VRBG method.
[g] Significantly different from VRBG method ($p < 0.05$).

Table 2003.01B. Reproducibility performance of Petrifilm Enterobacteriaceae Count Plate, VRBG, and MPN methods for detection of Enterobacteriaceae in foods

		Petrifilm Enterobacteriaceae Count Plate					VRBG					MPN				
Food	Level	n[a]	Mean[b]	s_r	RSD_r, %	r	n[a]	Mean[b]	s_r	RSD_r, %	r	n[a]	Mean[b]	s_r	RSD_r, %	r
Cheese	Control	0					0					9	2.09	0.98	46.73	2.73
	Low	12	3.02	0.20	6.69	0.57	12	3.03	0.10	3.43	0.29	11	3.12	0.34	10.80	0.94
	Medium	12	4.07	0.14	3.44	0.39	12	4.06	0.12	2.93	0.34	12	4.23	0.38	8.98	1.06
	High	10	4.91	0.24	4.89	0.68	12	4.93	0.29	5.84	0.81	9	4.82	0.27	5.60	0.76
Milk	Control	0					0					7	1.67	0.58	34.61	1.62
	Low	11	2.49	0.44	17.67	1.24	12	2.27	0.50	22.19	1.42	11	1.93	0.53	27.60	1.49
	Medium	12	3.34	0.18	5.27	0.50	12	3.27	0.39	11.87	1.09	11	2.65	0.73	27.73	2.05
	High	10	4.34	0.18	4.17	0.51	10	4.19	0.32	7.57	0.89	9	3.24	0.93	28.77	2.61
Flour	Control	12	2.70	0.33	12.15	0.92	12	2.98	0.37	12.34	1.04	12	2.44	0.47	19.20	1.31
	Low	12	2.73	0.37	13.46	1.04	12	2.95	0.44	14.99	1.25	12	2.24	0.45	20.21	1.27
	Medium	11	2.83	0.41	14.35	1.15	12	3.06	0.42	13.70	1.18	12	2.20	0.63	28.72	1.77
	High	12	2.89	0.29	10.10	0.82	12	3.16	0.37	11.74	1.04	12	2.87	0.65	22.52	1.81
Prepared meals	Control	0					0					9	1.47	0.34	23.04	0.95
	Low	11	2.61	0.17	6.43	0.47	11	2.52	0.28	11.23	0.80	9	2.42	0.42	17.43	1.18
	Medium	11	3.51	0.10	2.72	0.27	12	3.30	0.28	8.39	0.78	12	3.34	0.64	19.30	1.80
	High	11	4.47	0.21	4.71	0.59	12	3.98	0.37	9.22	1.03	8	4.30	0.52	12.21	1.47
Frozen broccoli	Control	0					0					11	1.32	0.51	38.43	1.42
	Low	10	1.60	0.43	26.99	1.22	5	1.36	0.35	26.03	1.00	11	1.58	0.48	30.27	1.34
	Medium	12	2.66	0.40	14.98	1.12	11	2.02	0.61	30.30	1.72	11	2.11	0.78	36.72	2.17
	High	11	3.44	0.55	16.14	1.56	10	2.84	0.68	23.87	1.91	10	2.71	0.87	32.07	2.43
Nuts	Control	10	2.74	0.48	17.47	1.35	10	2.89	0.42	14.70	1.20	9	2.67	0.34	12.76	0.95
	Low	10	3.50	0.68	19.33	1.91	9	3.64	0.71	19.57	2.01	9	3.48	0.65	18.58	1.81
	Medium	7	4.09	0.66	16.20	1.87	7	4.13	0.68	16.50	1.92	5	3.82	0.69	18.02	1.93
	High	7	4.20	0.47	11.18	1.32	7	4.14	0.55	13.37	1.56	7	3.83	1.09	28.40	3.05

[a] Number of laboratories with positive results.
[b] Log_{10} Enterobacteriaceae count/g.

C. Sample Preparation

Aseptically weigh 11.0 g unthawed test portion into blender jar, B(g). Add 99 mL dilution water, B(e), and blend at 16 000–18 000 rpm for 2 min to homogenize. If entire test sample is <50 g, weigh portion of test sample and add dilution water to make 1:10 dilution. Adjust pH of diluted test portion to 6.5–7.5 with 1M NaOH, B(f), ca 0.1 mL/g test portion. Do not use diluents containing citrate, bisulfite, or thiosulfate as they can inhibit growth. Prepare all decimal dilutions with 90 mL dilution water plus 10 mL from previous dilution. Mix all dilutions by shaking 25 times through 30 cm arc in 7 s.

D. Analysis

Place dry Petrifilm EB plate, B(a), on flat surface. Lift top film and inoculate 1 mL test suspension onto center of film base. Carefully roll top film down onto inoculum. Distribute test portion over 20 cm^2 growth area with downward pressure on center of plastic spreader device, B(b), flat side down. Leave plate undisturbed for minimum of 1 min to permit gel to solidify. Incubate plates for 24 ± 2 h at 37 ± 1 C. In incubator, place plates in horizontal position, clear side up, in stacks not exceeding 20 plates. Count plates promptly (within 2 h) after incubation period.

Count Petrifilm EB plates on standard colony counter, B(d), or other illuminated magnifier. Count all red colonies producing gas regardless of acid production. Also count all nongassing colonies with yellow acid zones. Select plates with 10–150 colonies. If no plate has at least 10 qualifying colonies, record the exact raw count on the least dilute test suspension. If all the plates have counts >150, make estimates on plates by counting the number of colonies in one or more representative squares, determining the average number per square, and multiplying the average number by 20. If plates are too crowded for estimated counts, report the count as too numerous to count (TNTC). Do not count colonies on the foam dam because they are removed from the selective influence of the medium. Do not count artifact bubbles that may be present.

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Results

Twelve laboratories participated in the study. The Enterobacteriaceae counts for the test portions are presented in logarithmic form in Tables 2–13. Repeatability and reproducibility standard deviations (s_r and s_R), relative standard deviations (RSD_r and RSD_R), and repeatability and reproducibility values (r and R) of the methods are presented in Tables 2003.01A and 2003.01B.

Cheese

The MPN log_{10} counts at the low level of inoculation from Laboratory 7 were identified by the Cochran and/or Grubbs test(s) as outliers and were eliminated from the analysis. The Petrifilm plate log_{10} counts at the high level of inoculation from Laboratories 5 and 6 were also identified by the Cochran and/or Grubbs test(s) as outliers and were eliminated from the analysis. At the high level of inoculation, the MPN log_{10} counts from Laboratory 1 were identified as outliers and were eliminated from the analysis.

Table 2. Enterobacteriaceae log_{10} counts at control and low inoculation levels for cheese by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Control						Low					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	<1.000	<1.000	<1.000	<1.000	0.602	0.602	2.903	2.903	2.908	2.987	2.968	2.633
2	<1.000	<1.000	<1.000	<1.000	2.968	2.633	3.049	2.996	3.114	2.929	3.380	2.968
3	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.886	2.954	3.068	3.090	2.591	3.176
4	<1.000	<1.000	<1.000	<1.000	1.968	1.633	2.924	2.869	2.857	2.898	3.380	2.968
5	<1.000	<1.000	<1.000	<1.000	2.380	3.380	2.954	3.053	2.892	2.978	2.968	3.380
6	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.954	2.903	3.033	3.149	3.041	3.176
7	4.477	<1.000	4.301	<1.000	>5.041	4.380	3.845	3.000	3.188	3.124	4.380	4.380
8	<1.000	<1.000	<1.000	<1.000	<0.447	<0.447	3.090	2.973	2.964	3.064	3.380	2.968
9	<1.000	<1.000	<1.000	<1.000	3.380	2.380	2.898	2.785	2.982	2.940	2.968	2.380
10	<1.000	<1.000	<1.000	1.000	2.380	2.380	3.146	3.204	3.146	3.146	3.380	3.041
11	<1.000	<1.000	<1.000	<1.000	1.633	1.633	2.991	3.061	3.025	2.996	3.633	2.968
12	<1.000	<1.000	<1.000	<1.000	<0.447	<0.447	3.104	3.124	3.204	3.146	3.633	3.633

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number.

[b] A and B = Duplicate test portions.

Table 3. Enterobacteriaceae log_{10} counts at medium and high inoculation levels for cheese by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Medium						High					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	4.076	3.944	3.875	4.029	4.380	3.968	4.903	5.000	5.041	5.053	5.041	4.176
2	3.954	4.104	4.176	4.097	4.380	3.875	4.929	4.982	5.423	5.064	5.041	5.041
3	4.107	4.068	3.968	4.111	4.380	3.380	4.991	5.041	5.146	5.137	4.663	4.663
4	3.973	3.991	3.929	3.944	4.663	3.968	4.924	4.968	4.857	4.892	>5.041	4.380
5	3.845	4.045	3.869	4.104	3.633	4.380	5.146	4.690	4.732	5.149	4.663	5.041
6	4.146	4.342	4.114	4.322	3.968	4.663	4.924	5.114	4.949	5.041	>5.041	>5.041
7	3.699	4.176	3.903	4.204	4.380	4.380	4.301	4.204	4.124	4.230	>5.041	>5.041
8	4.041	4.079	4.029	3.944	4.663	4.380	5.079	5.021	4.799	4.898	5.041	5.041
9	4.146	4.176	4.053	4.072	3.968	4.380	4.898	4.869	4.886	4.681	4.663	4.663
10	4.380	4.176	4.086	4.029	4.380	4.380	5.000	5.045	5.134	5.037	5.041	4.663
11	4.021	4.140	4.079	4.064	4.176	4.380	4.914	4.991	4.919	4.959	4.663	5.041
12	3.973	4.158	4.114	4.301	3.968	4.380	5.100	5.049	5.164	5.100	5.041	5.041

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number.

[b] A and B = Duplicate test portions.

Table 4. Enterobacteriaceae log_{10} counts at control and low inoculation levels for milk by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Control						Low					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.518	2.462	2.255	2.204	1.968	1.968
2	<1.000	<1.000	<1.000	2.146	<0.477	0.602	2.230	2.322	1.301	2.792	0.954	1.176
3	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.362	2.556	1.954	2.602	2.380	2.380
4	<1.000	<1.000	<1.000	<1.000	2.380	2.380	2.505	2.447	2.415	2.146	2.380	2.380
5	<1.000	<1.000	1.903	1.000	2.380	2.968	2.778	2.740	2.544	2.544	2.380	2.633
6	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.462	2.477	2.613	2.380	2.380	2.380
7	3.000	<1.000	3.763	1.903	2.380	1.362	2.716	2.672	2.505	2.771	>5.041	1.362
8	<1.000	<1.000	<1.000	<1.000	1.633	<0.477	2.362	2.477	2.322	1.954	2.176	1.968
9	<1.000	<1.000	<1.000	<1.000	>5.041	1.362	2.415	2.380	2.255	2.785	1.362	1.362
10	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.342	1.778	1.301	2.176	1.633	1.362
11	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.342	2.447	2.881	1.477	1.362	1.362
12	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.602	2.505	2.000	2.301	2.633	2.380

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number.

[b] A and B = Duplicate test portions.

Table 5. Enterobacteriaceae $\log_{10}$ counts at medium and high inoculation levels for milk by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Medium						High					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	3.342	3.279	3.146	3.255	2.633	2.845	4.342	4.342	4.079	4.146	3.968	3.176
2	3.114	2.954	3.653	4.061	2.176	2.380	4.079	4.204	4.000	4.672	2.633	3.633
3	3.342	3.477	3.322	3.477	2.380	2.380	4.415	4.544	4.342	4.113	2.380	3.968
4	3.230	3.322	2.732	3.057	3.380	2.380	4.362	4.380	4.110	4.060	2.380	3.380
5	3.491	3.491	3.362	3.643	3.176	3.380	4.518	4.415	4.361	4.568	>5.041	4.176
6	3.176	3.322	3.447	3.415	2.380	2.633	4.279	4.505	4.301	4.462	2.968	3.380
7	3.556	3.708	3.531	3.934	>5.041	3.380	4.756	TNTC	5.079	TNTC	2.380	>53041
8	3.204	3.518	2.903	3.230	3.633	3.633	4.398	4.342	4.041	4.113	4.176	5.041
9	3.462	3.653	3.415	3.643	1.362	2.633	4.663	5.688	4.279	5.692	>5.041	3.968
10	3.114	3.230	3.176	3.000	1.968	2.380	4.009	4.204	3.663	4.041	3.380	2.380
11	2.903	3.204	2.518	2.982	1.362	1.361	4.176	4.041	3.869	3.812	1.361	1.362
12	3.301	3.322	2.653	2.996	3.968	3.041	4.518	4.279	4.143	3.944	3.968	3.968

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number. TNTC = Too numerous to count.

[b] A and B = Duplicate test portions.

Table 6. Enterobacteriaceae $\log_{10}$ counts at control and low inoculation levels for flour by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Control						Low					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	2.771	2.851	3.602	3.176	2.968	2.968	2.886	2.964	3.415	3.398	3.322	2.633
2	2.845	2.699	3.090	2.929	2.380	2.380	2.875	2.903	2.724	2.869	2.380	1.968
3	3.279	2.919	2.996	2.934	1.968	1.968	3.362	3.255	3.415	3.519	1.968	2.380
4	2.041	2.000	3.519	2.892	2.380	2.380	1.699	2.732	3.623	2.568	2.380	2.380
5	2.833	2.623	3.362	3.982	2.968	2.845	2.477	2.653	3.580	3.322	2.633	1.968
6	2.204	2.740	3.124	2.477	2.633	1.633	2.996	2.204	3.068	3.265	1.362	1.633
7	3.146	2.279	2.903	2.845	2.633	2.380	2.954	3.146	3.061	2.771	2.380	2.380
8	2.944	3.127	2.845	2.991	1.968	2.845	2.519	2.833	2.398	2.279	2.176	1.633
9	2.613	2.716	2.602	2.845	1.633	2.633	2.643	2.898	2.681	2.875	1.633	2.633
10	2.716	2.908	2.633	2.875	2.663	3.041	2.447	3.146	2.398	3.288	2.380	2.380
11	2.785	2.342	2.771	2.255	2.845	1.362	2.519	2.431	2.398	2.230	1.845	2.380
12	2.663	2.778	2.954	2.944	2.633	2.380	2.653	2.431	2.869	2.833	1.968	2.968

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number.

[b] A and B = Duplicate test portions.

Table 7. Enterobacteriaceae log_{10} counts at medium and high inoculation levels for flour by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Medium						High					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	2.732	2.875	3.672	3.114	2.633	2.380	2.708	3.093	3.633	3.580	3.176	3.380
2	2.380	2.973	2.732	3.188	1.633	3.380	2.968	3.068	3.041	3.322	3.380	3.633
3	3.176	3.000	3.146	3.255	2.633	1.968	3.000	3.041	3.301	3.380	2.322	2.380
4	2.398	2.903	3.146	3.204	2.380	2.845	2.799	2.204	3.380	3.079	2.380	2.968
5	LA	2.568	3.491	3.398	3.380	2.968	3.114	2.699	3.708	3.602	3.968	2.968
6	3.013	1.845	3.146	3.531	2.380	1.362	2.792	3.100	3.176	3.398	2.380	3.380
7	3.643	3.301	3.708	3.000	1.362	1.362	3.447	3.301	3.279	3.342	3.176	3.380
8	2.996	2.580	2.447	2.301	2.176	1.176	3.362	2.778	3.255	2.778	2.633	2.380
9	2.978	2.908	3.121	2.785	2.176	1.633	2.863	2.672	3.068	2.322	1.968	1.968
10	3.602	2.778	3.544	2.903	2.380	2.380	2.699	2.792	2.771	2.863	2.380	3.041
11	2.415	2.447	2.176	2.491	1.633	1.633	2.380	2.964	2.342	3.146	2.633	4.380
12	2.623	2.973	2.903	3.124	2.633	2.380	2.820	2.591	3.193	2.763	2.633	1.968

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number. LA = Laboratory accident.

[b] A and B = Duplicate test portions.

Table 8. Enterobacteriaceae log_{10} counts at control and low inoculation levels for frozen meals by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Control						Low					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.978	2.929	3.025	3.057	2.633	3.633
2	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.699	2.447	2.255	2.342	2.633	1.591
3	<1.000	<1.000	<1.000	<1.000	2.380	1.362	2.544	2.643	2.602	2.447	2.633	2.633
4	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.591	2.672	2.447	2.491	2.380	2.380
5	<1.000	<1.000	<1.000	<1.000	2.380	1.362	2.633	2.491	2.653	2.699	2.380	2.968
6	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.591	2.176	2.415	2.114	2.633	1.633
7	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.462	2.477	2.653	2.653	3.380	5.041
8	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	LA	2.839	LA	2.869	LA	2.380
9	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.690	2.477	2.556	2.301	2.380	1.968
10	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.602	2.663	1.778	2.255	2.380	2.633
11	<1.000	<1.000	<1.000	<1.000	1.362	1.362	2.568	2.643	2.505	2.613	2.380	3.041
12	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.633	2.505	2.568	2.716	2.322	2.633

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number. LA = Laboratory accident.

[b] A and B = Duplicate test portions.

Table 9. Enterobacteriaceae log_{10} counts at medium and high inoculation levels for frozen meals by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Medium						High					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	3.959	3.881	3.875	3.851	3.633	4.380	4.806	4.820	3.839	3.681	>5.041	4.663
2	3.398	3.580	3.076	3.220	2.380	1.362	4.447	4.544	3.869	3.813	1.362	3.591
3	3.580	3.519	2.903	3.176	3.968	3.633	4.491	4.699	3.799	4.152	4.380	4.663
4	3.699	3.591	3.146	3.398	3.875	3.633	4.519	4.431	4.021	4.170	4.380	3.380
5	3.398	3.556	3.531	3.380	3.380	3.380	4.362	4.580	4.415	4.362	4.176	4.380
6	3.362	3.322	3.079	3.146	3.380	2.380	4.114	4.041	3.833	3.881	3.875	3.591
7	3.477	3.447	3.204	3.447	3.968	3.633	4.362	5.332	4.114	4.544	4.380	>5.041
8	3.568	3.556	3.301	3.672	3.633	3.380	4.477	4.531	3.903	4.342	3.968	3.968
9	3.591	3.462	3.079	2.778	2.968	2.633	4.114	4.176	3.041	3.708	3.380	3.176
10	3.431	3.447	3.176	3.146	3.380	3.380	4.431	4.544	3.531	3.568	4.663	4.663
11	3.623	3.431	3.230	3.204	3.663	3.380	4.568	4.580	3.813	4.000	5.041	5.041
12	3.568	3.519	3.613	3.477	3.041	3.633	4.568	4.544	4.491	4.519	4.663	4.663

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number.

[b] A and B = Duplicate test portions.

Table 10. Enterobacteriaceae log_{10} counts at control and low inoculation levels for frozen broccoli by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Control						Low					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	1.954	<1.000	2.431	1.477	1.633	1.362	2.380	1.845	2.881	2.415	2.633	1.968
2	<1.000	1.301	<1.000	<1.000	0.602	0.954	1.477	1.000	1.000	<1.000	0.954	0.954
3	<1.000	<1.000	1.602	<1.000	2.602	1.633	<1.000	1.699	1.477	1.000	1.633	2.176
4	<1.000	<1.000	<1.000	<1.000	0.602	1.633	1.903	1.845	1.301	<1.000	<0.477	1.633
5	1.301	1.000	1.000	1.301	1.362	0.954	1.000	1.699	1.000	1.301	1.633	1.845
6	1.699	<1.000	1.903	1.301	1.362	1.362	<1.000	1.477	1.845	1.602	1.362	1.362
7	<1.000	3.944	<1.000	3.000	0.602	>5.041	1.778	1.301	1.477	1.000	1.362	1.633
8	<1.000	1.301	<1.000	<1.000	1.633	1.633	1.602	1.000	<1.000	<1.000	1.968	1.633
9	1.477	2.000	1.778	1.477	1.362	1.633	1.954	2.431	1.602	2.255	1.362	1.968
10	<1.000	<1.000	<1.000	<1.000	0.602	0.602	1.301	1.602	1.301	<1.000	0.954	0.954
11	1.301	1.000	1.000	1.000	1.968	1.633	1.000	1.000	1.301	<1.000	1.845	0.602
12	<1.000	<1.000	<1.000	<1.000	1.362	1.176	1.778	2.114	<1.000	1.000	1.968	1.845

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number.

[b] A and B = Duplicate test portions.

Table 11. Enterobacteriaceae log_{10} counts at medium and high inoculation levels for frozen broccoli by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Medium						High					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	3.663	3.568	3.362	3.041	2.633	3.380	4.643	4.204	4.255	3.699	4.663	3.380
2	2.531	2.740	1.602	1.301	1.362	0.602	3.301	3.505	2.176	2.633	1.633	1.968
3	2.431	2.580	1.301	1.301	2.380	2.633	LA	3.568	LA	2.544	LA	2.875
4	2.881	3.009	2.041	1.845	1.845	2.968	3.519	3.342	2.699	2.748	3.176	2.633
5	2.415	2.633	2.342	2.079	1.968	2.380	3.342	3.643	2.477	3.681	3.633	2.968
6	2.580	2.602	2.771	2.690	1.362	1.362	3.740	3.230	3.000	3.041	1.362	2.380
7	2.820	2.380	2.633	2.204	2.380	3.380	3.447	2.602	3.875	2.000	>5.041	2.633
8	2.477	2.301	1.602	1.000	2.968	2.380	2.643	2.380	<1.000	1.301	3.380	2.633
9	2.491	2.724	1.602	2.000	1.968	1.362	3.491	3.898	3.176	3.380	2.968	2.380
10	2.114	2.114	1.477	2.000	0.954	0.954	3.204	3.279	2.556	2.041	1.362	0.954
11	2.342	2.342	2.580	1.845	5.602	2.380	3.029	2.732	2.839	2.362	2.633	2.633
12	2.914	3.093	1.778	1.903	2.380	2.633	3.903	4.362	2.898	3.061	3.633	3.633

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number. LA = Laboratory accident.

[b] A and B = Duplicate test portions.

Table 12. Enterobacteriaceae log_{10} counts at control and low inoculation levels for nut pieces by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Control						Low					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	2.778	3.301	4.079	3.301	2.633	2.633	3.230	3.681	3.204	3.591	3.633	3.968
2	—	—	—	—	—	—	—	—	—	—	—	—
3	2.681	2.279	2.431	2.204	2.380	2.633	3.204	3.415	2.845	3.826	3.380	4.663
4	1.602	1.845	2.447	2.934	2.845	>5.041	2.322	3.415	3.204	4.049	2.591	>5.041
5	2.898	2.881	3.158	3.000	2.633	2.633	3.415	3.556	3.279	3.613	3.176	2.968
6	2.813	2.756	2.771	2.903	3.380	2.380	5.152	4.505	5.310	4.964	4.380	4.380
7	3.301	3.204	3.204	3.230	2.968	2.380	4.415	3.602	4.230	3.602	4.380	3.380
8	—	—	—	—	—	—	—	—	—	—	—	—
9	3.079	3.531	3.114	2.857	2.968	2.633	3.799	2.778	3.833	3.176	3.380	3.380
10	2.519	2.301	2.716	2.672	2.380	2.380	3.491	4.143	3.362	4.170	2.633	3.380
11	2.699	2.716	2.623	2.362	2.380	2.380	2.973	3.045	2.991	3.033	3.633	2.380
12	2.826	2.724	3.079	2.699	2.633	3.380	2.949	2.851	3.104	2.934	3.380	2.968

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number. — = Test portions did not arrive in time; laboratory did not participate.

[b] A and B = Duplicate test portions.

Table 13. Enterobacteriaceae $\log_{10}$ counts at medium and high inoculation levels for nut pieces by Petrifilm EB Count Plate, VRBG agar, and MPN methods[a]

	Medium						High					
	PEB		VRBG		MPN		PEB		VRBG		MPN	
Lab	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
1	4.863	5.255	4.839	5.190	>5.041	>5.041	TNTC	4.204	TNTC	4.114	>5.041	>5.041
2	—	—	—	—	—	—	—	—	—	—	—	—
3	LA	4.146	LA	4.033	LA	4.663	3.716	3.869	3.839	4.068	4.322	5.041
4	2.929	TNTC	2.875	TNTC	2.968	>5.041	4.415	3.778	4.699	3.301	>5.041	>5.041
5	3.940	3.342	3.991	3.322	4.380	3.176	4.959	4.301	5.164	4.079	4.380	3.968
6	3.602	4.785	3.903	4.886	3.380	3.380	4.914	4.690	4.978	4.851	4.380	4.380
7	4.591	4.869	5.068	4.839	>5.041	5.041	5.149	TNTC	4.813	TNTC	>5.041	>5.041
8	—	—	—	—	—	—	—	—	—	—	—	—
9	3.903	4.833	3.845	4.949	3.968	>5.041	3.613	3.785	3.643	3.447	3.176	0.602
10	TNTC	3.982	TNTC	3.756	3.968	2.968	3.869	TNTC	3.732	TNTC	3.380	4.380
11	3.748	3.908	3.763	3.826	3.968	2.968	3.908	3.792	3.892	3.699	3.968	3.633
12	3.785	3.041	3.778	3.255	4.663	3.968	4.477	4.000	4.230	3.903	4.663	3.380

[a] Log_{10} Enterobacteriaceae count/g. PEB = Petrifilm Enterobacteriaceae Count Plate. VRBG = Violet red bile agar fortified with glucose. MPN = Most probable number. TNTC = Too numerous to count. — = Laboratory did not participate. LA = Laboratory accident.

[b] A and B = Duplicate test portions.

The mean $\log_{10}$ counts by the VRBG method were not significantly different from those by the Petrifilm plate procedure for all levels of inoculation. The mean $\log_{10}$ counts by the MPN procedure were significantly different from those of the Petrifilm plate method for all levels of inoculation with p-values of 0.04, 0.03, and 0.02, respectively.

The repeatability variance of the Petrifilm plate method was significantly better ($p < 0.01$) than for the VRBG method at the high inoculation level and was not significantly different from the VRBG method for the medium inoculation level. The repeatability variance of the VRBG method was significantly better ($p < 0.01$) than for the Petrifilm plate method for the low level of inoculation. The repeatability variance of the Petrifilm plate method was significantly better ($p < 0.05$) than for the MPN procedure for the low level of inoculation. For the medium and high levels of inoculation, the repeatability variance of the Petrifilm plate method was significantly better ($p < 0.01$) than for the MPN method.

Milk

At the low level of inoculation, the Petrifilm plate $\log_{10}$ counts from Laboratory 10 were considered to be outliers, i.e., they did not fit well with the rest of the data and were removed from the calculations of mean $\log_{10}$ counts, repeatability standard deviations, and reproducibility standard deviations. Both the Petrifilm plate $\log_{10}$ counts and the VRBG method $\log_{10}$ counts at the high level of inoculation from Laboratory 9 were outliers and were removed before the mean $\log_{10}$ counts and the repeatability and reproducibility variances were calculated.

The mean $\log_{10}$ count of the Petrifilm plate method was not significantly different from that of the VRBG method at the medium level of inoculation. The mean $\log_{10}$ counts of the Petrifilm plate method were significantly different from those of the VRBG method and MPN procedure for all other levels of inoculation. The Petrifilm plate mean $\log_{10}$ counts were higher than those of the other methods.

The repeatability variance of the Petrifilm plate method was significantly better ($p < 0.01$) than for the VRBG method for the low and medium levels of inoculation and was significantly better ($p < 0.05$) for the high level of inoculation. For the low level of inoculation, the Petrifilm plate method's repeatability variance was significantly better ($p < 0.05$) than for the MPN method. For the medium and high levels of inoculation, the repeatability variance of the Petrifilm plate method was significantly better ($p < 0.01$) than for the MPN method.

Flour

A statistical difference between the mean $\log_{10}$ counts from the Petrifilm plate procedure and those of VRBG method at the low level of inoculation did not exist. There was no statistical difference between the mean $\log_{10}$ counts from the Petrifilm plate procedure and those of the MPN method at the high level of inoculation. For the low and medium levels of inoculation, the Petrifilm plate $\log_{10}$ counts were higher, on average, than those of the MPN method. For the medium and high levels of inoculation, the VRBG $\log_{10}$ counts were higher, on average, than those of the Petrifilm plate method.

The repeatability variance of the Petrifilm method was significantly better ($p < 0.05$) than for the MPN procedure for the uninoculated and high levels of inoculation.

Prepared Meals

Outliers were found in the MPN method at the low level of inoculation for Laboratories 1 and 7, in the Petrifilm plate method at the medium level of inoculation for Laboratory 1, in the Petrifilm plate method at the high level of inoculation for Laboratory 7, and in the MPN method for Laboratories 2 and 4. These values were removed before the mean $\log_{10}$ counts and the repeatability and reproducibility variances were calculated.

The mean $\log_{10}$ counts by the Petrifilm plate procedure were not significantly different from those of the VRBG method at the low level of inoculation. The $\log_{10}$ counts of the Petrifilm plate procedure were higher than those of the VRBG method, on average, at the medium and high levels of inoculation. The mean $\log_{10}$ counts by the Petrifilm plate procedure were not significantly different from those of the MPN method at the medium and high levels of inoculation. The $\log_{10}$ counts of the Petrifilm plate method were higher, on average, than those of the MPN method at the low level of inoculation.

The repeatability variance of the Petrifilm plate method was significantly better ($p < 0.05$ and $p < 0.01$, respectively) than that of the VRBG method for the medium and high levels of inoculation. The repeatability variance of the Petrifilm plate method was significantly better ($p < 0.01$) than that of the MPN method for all levels of inoculation.

Frozen Broccoli

The $\log_{10}$ counts at the low level of inoculation from the VRBG method from Laboratory 1 were identified by the Cochran and/or Grubbs test(s) as outliers and were eliminated from the analysis.

On average, the $\log_{10}$ counts by the Petrifilm plate procedure were higher than those of the VRBG and MPN methods at all levels of inoculation except the low level where there was no statistical difference.

The repeatability variance of the Petrifilm plate method was significantly better ($p < 0.05$) than that of the VRBG method for the medium and high levels of inoculation and was significantly better ($p < 0.01$) than that of the MPN method for the medium level of inoculation.

Nuts

The $\log_{10}$ counts at the low level of inoculation from the VRBG method from Laboratory 5 were identified by the Cochran and/or Grubbs test(s) as outliers and were eliminated from the analysis.

A statistical difference between the mean $\log_{10}$ counts of the Petrifilm plate method and those of the VRBG and MPN procedures did not exist.

The repeatability variance of the Petrifilm plate method was significantly better ($p < 0.05$) than that of the MPN procedure for the low level of inoculation. It was significantly better ($p < 0.01$) than that of the MPN procedure for the high level of test portions.

Recommendations

The mean $\log_{10}$ counts and the repeatability/reproducibility precision estimates of the Petrifilm EB Count plate method were similar to those for the VRBG method and better than those of the MPN method. Therefore, it is recommended that the Petrifilm EB Count plate method be adopted First Action for the enumeration of Enterobacteriaceae in cheddar cheese, pasteurized liquid milk, flour, frozen prepared meals, frozen broccoli, and nut pieces.

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