FOOD BIOLOGICAL CONTAMINANTS

Dry Rehydratable Film Method for Rapid Enumeration of Coliforms in Foods (3M™ Petrifilm™ Rapid Coliform Count Plate): Collaborative Study

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A rehydratable dry-film plating method for coliforms in foods, the 3M™ Petrifilm™ Rapid Coliform Count plate method, was compared with the U.S. Food and Drug Administration's *Bacteriological Analytical Manual* method for nondairy foods and the American Public Health Association's *Standard Methods for the Examination of Dairy Products* (SMEDP) method for dairy foods. Six food types, vanilla ice cream, cheddar cheese, fresh refrigerated uncooked pasta, wheat flour, prepared frozen macaroni and cheese, and frozen hash browns, were analyzed for coliforms by 11 collaborating laboratories. For each food product tested, the collaborators received 8 blind samples consisting of a control sample and 3 levels of inoculated sample, each in duplicate. The mean log counts for the methods were comparable. The repeatability and reproducibility variances of the Petrifilm Rapid Coliform Count method at 14 and 24 h were not significantly different from those of the standard methods.

T he 3M™ Petrifilm™ Rapid Coliform Count (RCC) plate, which is used for enumerating coliforms, is designed to have a shortened incubation period. The Petrifilm RCC plate is a ready-made culture medium system that contains modified violet red bile (VRB) nutrients, a cold-water-soluble gelling agent, a pH indicator, and a tetrazolium indicator that facilitates colony enumeration.

Samples are added at a volume of 1.0 mL per plate. Pressure applied to a plastic spreader placed on the topfilm spreads the sample over a growth area of approximately 20 cm^2. The gelling agent is allowed to solidify and the plates are then incubated up to 24 h at 35 ± 1 C. Coliforms produce red colonies on the plates by reduction of the indicator and produce acid and gas by fermentation of lactose. The gas is trapped by the film and appears as one or more small bubbles associated with the colony.

In a comprehensive precollaborative comparative study (McIntyre, unpublished) the Petrifilm RCC plate provided results similar to those of the U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (BAM; 1) method for nondairy foods and the American Public Health Association's Standard Methods for the Examination of Dairy Products (SMEDP; 2) method for dairy foods for enumeration of coliforms. This collaborative study compared the Petrifilm RCC method at 14 and 24 h with the standard methods for enumerating coliforms in foods.

Collaborative Study

Test Foods

The food types selected for the study were vanilla ice cream, cheddar cheese, fresh refrigerated uncooked pasta, wheat flour, prepared frozen macaroni and cheese, and frozen hash browns. Foods were obtained from local grocery stores.

Test Organisms

The organisms were obtained from the American Type Culture Collection (ATCC) or the University of Minnesota (Table 1) and were stored at –70°C in laboratory medium containing 15% sterile glycerol.

Working cultures were maintained on eosin methylene blue (EMB) agar plates, and inocula were cultivated in nutrient broth and incubated at 35 ± 1°C. Stationary-phase cultures

Table 1.   Food products and coliform organisms

Food product	Coliform organism	Organism source
Vanilla ice cream	*Escherichia coli* ATCC 11775	ATCC-type strain
Cheddar cheese	*Enterobacter cloacae* U45[a]	Clinical
Fresh refrigerated uncooked pasta	*Klebsiella oxytoca* U33[a]	Clinical
Wheat flour	*Enterobacter aerogenes* ATCC 13048	ATCC-type strain
Prepared frozen macaroni and cheese	*Klebsiella pneumoniae* ATCC 13883	ATCC-type strain
Frozen hash browns	*Citrobacter freundii* ATCC 8090	ATCC-type strain

[a] University of Minnesota isolate.

in nutrient broth were diluted with Butterfield's phosphate-buffered dilution water (BPD) prior to inoculation of test samples.

Inoculation of Foods

Several one-half gallon containers of vanilla ice cream were thawed to softness, aseptically combined, and mixed. The product was then divided into 4 portions; the first 3 portions were inoculated and the fourth served as an uninoculated control. The inoculum was added directly to the samples and then mixed manually. The inoculum levels differed by powers of 10 and were in the range of 10–100, 101–1000, and 1001–10 000 cells/mL for the low, medium, and high inocula, respectively. The vanilla ice cream was allowed to refreeze following the inoculation.

Approximately 4200 g cheddar cheese was obtained from several retail packages. The cheddar cheeses were combined and ground in a Sunbeam food processor and shaken manually to achieve uniformity after inoculation. The sample was then divided into 4 portions and inoculated according to the method described for vanilla ice cream.

Approximately 4200 g fresh refrigerated uncooked pasta was obtained and blended thoroughly. The fresh refrigerated uncooked pasta was divided into 4 portions and inoculated according to the method described for vanilla ice cream.

Approximately 4200 g wheat flour was obtained and blended thoroughly. The flour was divided into 4 portions and inoculated with a dry inoculum at the same levels as described for vanilla ice cream.

Approximately 4200 g prepared frozen macaroni and cheese and 4200 g frozen hash browns were obtained and thawed. Each product was blended thoroughly, divided into 4 portions, and inoculated according to the method described for vanilla ice cream. Each sample was refrozen after inoculation.

The inoculated dry samples were allowed to stabilize at room temperature 2 weeks prior to analysis. The inoculated frozen samples were allowed to stabilize overnight; the inoculated refrigerated samples were stored overnight at 2–8°C and shipped the following day.

Preparation of Samples

Inoculated and uninoculated samples were divided into ca 50 g samples. Each 50 g sample was divided in half to form duplicate 25 g subsamples. For analysis, duplicate subsamples of the uninoculated control and 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, multi-digit random number.

Shipment of Samples

Samples were shipped by overnight carrier to arrive the day before initiation of analysis. The cheddar cheese and fresh refrigerated uncooked pasta were packed with ice packs to maintain a temperature of <7°C during shipment. The vanilla ice cream, prepared frozen macaroni and cheese, and frozen hash browns were shipped frozen on dry ice. The wheat flour was shipped at ambient temperature. Upon arrival, samples were stored at the specified conditions until they were analyzed the following day. One food type was shipped for each week of the study.

Microbiological Analysis

Each collaborator received 8 samples of each food type to be tested. Collaborators were instructed to analyze each sample by the Petrifilm RCC and VRB agar methods or the 3-tube most probable number (MPN) method (1). The VRB agar plates were incubated at 32 ± 1°C for 24 ± 2 h, and the Petrifilm plates at 35 ± 1°C for 14 and 24 h. The lauryl tryptose broth (LST) tubes were incubated at 35 ± 1°C and examined at 24 ± 2 h and 48 ± 2 h.

Coliforms growing on the Petrifilm plates were counted at 14 h and again at 24 h. At 14 and 24 h, confirmed colonies on the Petrifilm plate were counted. Confirmed colonies were red colonies associated with one or more gas bubbles and with or without yellow acid zones. For dairy foods, the VRB agar plates were examined, and colonies that were brick red with a pink halo of precipitated bile salt were counted and confirmed. For nondairy foods, the LST tubes were examined for gas and confirmed.

For the VRB agar method, 2 suspect colonies per sample were selected from each VRB agar plate. The colonies were each stabbed with a sterile inoculating needle and transferred to brilliant green lactose bile broth (BGLB). The tubes were incubated at 32 ± 1°C and examined for gas production at 24 and 48 ± 2 h. Typical colonies that produced gas in BGLB were considered confirmed results.

For the MPN method, all gassing LST tubes were confirmed by gently agitating each gassing LST tube and transferring a loopful of suspension to a tube of BGLB broth. These tubes were incubated at $35 \pm 1°C$ and examined for gas production at 24 and 48 ± 2 h. Gassing LST tubes that also produced gas in BGLB were considered confirmed coliform results. Because of information obtained during the precollaborative study (McIntyre, unpublished), colonies associated with gas on the Petrifilm RCC plate were reported as confirmed coliforms.

Statistical Analysis of Data

Collaborators recorded the colony counts for Petrifilm plates and VRB agar plates as well as data for MPN analyses on data sheets. Petrifilm and agar plates with 10–150 colonies were selected. If no plate had at least 10 colonies, the exact count on the least dilute sample was recorded. If the plates were too crowded to estimate counts, the count was reported as too numerous to count.

Procedures described by Peeler and Maturin in BAM (3) were used to calculate colony forming units per gram (CFU/g). The base 10 logarithms of colony counts and MPN indexes were used for statistical analysis under the assumption that the transformed numbers would be normally distributed and of homogenous variance. Repeatability (s_r) and reproducibility (s_R) standard deviations and relative standard deviations of repeatability (RSD_r) and reproducibility (RSD_R) were calculated according to AOAC INTERNATIONAL procedures after outliers were determined by the Cochran and Grubbs tests (4). Repeatability variances were compared using an F-ratio test (5). Mean log counts were compared by analysis of variance (5). In all statistical tests, a resulting value of $p < 0.05$ was taken to indicate a significant difference.

AOAC Official Method 2000.15
Rapid Enumeration of Coliforms in Foods
Dry Rehydratable Film Method,
Petrifilm™ Rapid Coliform Count Plate
First Action 2000

(Applicable to determination of confirmed coliforms at 14 and 24 h in foods. Not applicable to hash brown potatoes.)

See Tables **2000.15 A** and **B** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Method uses bacterial culture plates of dry medium and cold-water-soluble gel. Undiluted or diluted test suspensions are added to plates at a volume of 1.0 mL per plate. Pressure, when applied to plastic spreader placed on overlay film, spreads the suspension evenly over a 20 cm^2 growth area. Gelling agent is allowed to solidify, and plates are incubated and then counted. A serological pipet or automatic pipet can be used for suspension addition for bacterial count analysis.

B. Apparatus and Reagents

(**a**) *Petrifilm Rapid Coliform Count (RCC) plates*.—Plates (available from 3M Microbiology Products, St. Paul, MN 55144) contain modified violet red bile (VRB) nutrients, cold-water-soluble gelling agent, tetrazolium indicator dye, and pH indicator.

(**b**) *Plastic spreader*.—Provided with Petrifilm plates, has a recessed side and a smooth flat side, designed to spread suspension evenly over plate growth area.

(**c**) *Pipets*.—1.0 and 10.0 mL serological pipets with 0.1 mL graduations. Pipets must accurately deliver required volume. Do not use <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. (Calibrated 3M™ Electronic Pippetor, or equivalent, may be used to deliver 1.0 mL.)

(**d**) *Colony counter*.—Quebec Dark-Field Colony Counter (VWR Scientific Products, Willard, OH), or equivalent.

(**e**) *Sterile sodium hydroxide solution.*.—1M. Dissolve 40 g NaOH in 1 L water. Autoclave 15 min at 121°C.

(**f**) *Dilution water*.—Prepare stock solution by dissolving 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.

(**g**) *Blender or stomacher*.—Waring blender or equivalent, or Seward Ltd. (98 Great North Rd, London N2 OGN UK, +44(0)20 8365 4100) 400 stomacher, or equivalent.

C. Preparation of Test Portion

Prepare test sample as in **966.23B** (*see* 17.2.01). Specified dilutions are for maximum sensitivity. Higher dilutions may be plated as needed. Do not use diluents containing citrate or thiosulfate. Mix all dilutions by shaking 25x through 30 cm arc in 7 s. Blend or stomach solids 2 min to homogenize.

(**a**) *Whole milk, 2% milk, 1% milk, skim milk, and raw milk*.—Plate 1 mL undiluted or diluted product on dry-film coliform count plate, **B(a)**. Incubated colony count on undiluted plate is count/g.

(**b**) *Ice cream and mixes, chocolate milk*.—Make 1:10 dilution of product [11 g/99 mL dilution water, **B(f)**]. Plate 1 mL on dry-film coliform count plate, **B(a)**. Incubate. Multiply colony count by dilution to obtain count/g.

(**c**) *Butter and margarine*.—Proceed as in (**b**) with diluent prewarmed to 40–45°C. Do not use citrate buffer to homogenize product.

(**d**) *Sour cream, yogurt, and frozen yogurt*.—Proceed as in (**b**). After dilution adjust pH to 6.5–7.5 with 1M NaOH, **B(e)**, (ca 0.1 mL/g product).

(**e**) *Cheddar cheese, cottage cheese, instant nonfat dry milk, whey powder, and related products*.—Proceed as in (**b**). Do not use citrate buffer to homogenize product.

(**f**) *Nondairy foods*.—Weigh 50 g test portion in sterile blender jar. Add 450 mL diluent and blend for 2 min in high speed blender jar at 16 000–18 000 rpm. As required, adjust pH of suspension to 6.5–7.5 with 1M NaOH, **B(e)**, (ca 0.1 mL/g suspension). If entire test sample contains <50 g, weigh a portion of test sample and add sterile diluent to make 1:10 dilution. Prepare all decimal dilutions with 90 mL sterile diluent plus 10 mL previous dilution unless otherwise specified.

Table 2000.15A.   Results of interlaboratory study of the Petrifilm RCC method at 14 and 24 h for detection of coliforms in foods

Food type	Level[a]	n[b]	Mean[c]	s_r	RSD$_r$, %	s_R	RSD$_R$, %
			14 H				
Cheddar cheese	Low	10 (0)	1.86	0.27	14.52	0.32	17.20
	Medium	10 (0)	2.87	0.13	4.53	0.20	6.97
	High	10 (0)	3.90	0.08	2.05	0.19	4.87
Vanilla ice cream	Low	9 (0)	2.78	0.06	2.16	0.15	5.40
	Medium	10 (0)	3.67	0.11	3.00	0.19	5.18
	High	11 (0)	4.72	0.10	2.12	0.19	4.03
Flour	Uninoc.	11 (0)	3.26	0.27[d]	8.28	0.44	13.50
	Low	11 (0)	2.92	0.29[e]	9.93	0.34	11.64
	Medium	11 (0)	3.15	0.45	14.29	0.45	14.29
	High	11 (0)	3.49	0.21[d]	6.02	0.35	10.03
Macaroni and cheese	Low	9 (0)	2.21	0.16[e]	7.24	0.37	16.74
	Medium	10 (0)	3.17	0.19[e]	5.99	0.36	11.36
	High	10 (0)	4.32	0.12[e]	2.78	0.29	6.71
Fresh refrigerated uncooked pasta	Low	10 (0)	2.34	0.15[d]	6.41	0.37	15.81
	Medium	10 (0)	3.33	0.14[e]	4.20	0.36	10.81
	High	10 (0)	4.26	0.30	7.04	0.43	10.09
			24 H				
Cheddar cheese	Low	11 (0)	1.89	0.26	13.76	0.31	16.40
	Medium	11 (0)	2.89	0.13	4.50	0.21	7.27
	High	11 (0)	3.93	0.09	2.29	0.19	4.83
Vanilla ice cream	Low	9 (0)	2.84	0.05	1.76	0.14	4.93
	Medium	11 (0)	3.70	0.11	2.97	0.19	5.14
	High	11 (0)	4.78	0.10	2.09	0.16	3.35
Flour	Uninoc.	11 (0)	3.33	0.26[d]	7.81	0.41	12.31
	Low	11 (0)	2.97	0.27[e]	9.09	0.30	10.10
	Medium	9 (2)	3.22	0.38	11.80	0.38	11.80
	High	11 (0)	3.52	0.16[d]	4.55	0.32	9.09
Macaroni and cheese	Low	10 (0)	2.20	0.24	10.91	0.39	17.73
	Medium	10 (0)	3.24	0.17[d]	5.25	0.30	9.26
	High	10 (0)	4.37	0.11[e]	2.52	0.26	5.95
Hash brown potatoes	Low	11	NG[f]	NG	NG	NG	NG
	Medium	11	NG	NG	NG	NG	NG
	High	11	NG	NG	NG	NG	NG
Fresh refrigerated uncooked pasta	Low	10 (0)	2.36	0.16[d]	6.78	0.37	15.68
	Medium	10 (0)	3.34	0.13[d]	3.89	0.35	10.48
	High	10 (0)	4.27	0.30	7.03	0.42	9.84

[a] Low = 10–100 cfu/mL, medium = 100–1000 cfu/mL, high = 1000–10 000 cfu/mL.

[b] Number of laboratories with complete data; number of outliers appears in parentheses.

[c] Log coliform count/g.

[d] Significantly better repeatability ($p < 0.01$).

[e] Significantly better repeatability ($p < 0.05$).

[f] NG = colonies with yellow acid zones were present; however, no gassing colonies were detected.

Table 2000.15B. Results of interlaboratory study of standard method for detection of coliforms in foods

Food type	Level[a]	n[b]	Mean[c]	s_r	RSD$_r$, %	s_R	RSD$_R$, %
Cheddar cheese	Low	11 (0)	1.90	0.29	15.26	0.30	15.79
	Medium	11 (0)	2.95	0.13	4.41	0.21	7.12
	High	11 (0)	3.99	0.12	3.01	0.20	5.01
Vanilla ice cream	Low	9 (2)	3.03	0.07	2.31	0.08	2.64
	Medium	9 (2)	3.83	0.07	1.83	0.11	2.87
	High	11 (0)	4.81	0.08	1.66	0.24	4.99
Flour	Uninoc.	11 (0)	1.97	0.67	34.01	0.95	48.22
	Low	10 (0)	1.72	0.54	31.40	0.69	40.12
	Medium	11 (0)	2.51	0.48	19.12	0.65	25.90
	High	11 (0)	2.78	0.57	20.50	0.90	32.37
Macaroni and cheese	Low	10 (0)	2.27	0.30	13.22	0.55	24.23
	Medium	10 (0)	3.20	0.41	12.81	0.65	20.31
	High	9 (1)	4.04	0.22	5.45	0.35	8.66
Hash brown potatoes	Low	10 (0)	2.06	0.41	19.90	0.69	33.50
	Medium	10 (0)	3.23	0.25	7.74	0.53	16.41
	High	10 (0)	4.00	0.96	24.00	1.09	27.25
Fresh refrigerated uncooked pasta	Low	10 (0)	2.15	0.55	25.58	0.62	28.84
	Medium	10 (0)	3.38	0.38	11.24	0.68	20.12
	High	10 (0)	4.37	0.46	10.53	0.46	10.53

[a] Low = 10–100 cfu/mL, medium = 100–1000 cfu/mL, high = 1000–10 000 cfu/mL.

[b] Number of laboratories with complete data; number of outliers appears in parentheses.

[c] Log coliform count/g.

D. Analysis

Place dry Petrifilm RCC plate, **B**(**a**), on flat surface. Lift top film and inoculate 1 mL suspension onto center of bottom film. Carefully roll top film down onto inoculum. Distribute inoculum over 20 cm^2 growth area with downward pressure on center of plastic spreader device (flat side down). Leave plate undisturbed to permit gelling agent to solidify. Incubate plates up to 24 h at $35 \pm 1°C$. In incubator, place plates in horizontal position or in Petrifilm plate rack, clear side up, in stacks not exceeding 20 units. Count plates within 1 h after incubation period is completed.

Petrifilm RCC plates can be counted on a standard colony counter or other illuminated magnifier. Do not count colonies on foam dam because they are removed from selective influence of the medium. Do not count artifact bubbles that may be present. Confirmed coliforms will appear as red colonies associated with gas after 8–24 h of incubation. They are red colonies associated with one or more gas bubbles and with or without yellow acid zones. Plates with 10–150 colonies are to be selected. If no plate has at least 10 red colonies with gas, record the exact count on the least dilute inoculum. If all plates have counts >150, determine the estimated count by counting the number of colonies in one or more representative squares, determining average number per square, and then multiplying the average number by 20 (circular growth area is ca 20 cm^2).

If the plates are too crowded to estimate counts, report the count as too numerous to count.

Ref.: *J. AOAC Int*. **85**, 58–60(2002)

Results

For each food type, samples were obtained from different sources. Background counts were monitored. Coliform counts of foods were too low for use as naturally contaminated samples. Hence, samples were artificially inoculated. Eleven laboratories participated in the study; all completed analyses of the 6 foods. The coliform counts for the test samples are presented in logarithmic form in Tables 2–13; duplicate test samples are denoted by 'A' and 'B' in the Tables. Interlaboratory study results (mean log counts, s_r, RSD$_r$, s_R, and RSD$_R$) are presented in Tables 14–16.

Cheddar Cheese

The Cochran and Grubbs tests to determine outliers were applied to the data. No outliers were detected.

The mean log coliform counts by the VRB agar method were not significantly different from the mean log confirmed coliform counts read at 14 h and those read at 24 h by the Petrifilm RCC method for low and medium levels of contamination.

Table 2.   Coliform log counts at the control and low inoculation levels for cheddar cheese by the Petrifilm RCC and the VRBA methods[a]

	Control						Low					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		VRBA		14 h confirm		24 h confirm		VRBA	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.114	1.903	2.230	1.954	2.000	1.903
2	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	1.845	2.255	1.778	2.255	2.000	2.342
3	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	1.602	1.000	1.699	1.000	2.041	1.477
4	NR	NR	NR	NR	<1.000	<1.000	1.602	1.602	1.602	1.602	1.845	1.778
5	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.041	1.602	2.079	1.845	1.477	1.954
6	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.114	2.041	2.114	2.114	2.301	2.176
7	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	1.778	1.903	1.778	1.903	2.000	1.000
8	NR	NR	<1.000	<1.000	<1.000	<1.000	NR	NR	1.903	1.845	2.041	2.041
9	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.079	1.477	2.079	1.477	1.903	1.699
10	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.146	1.602	2.146	1.699	1.954	1.699
11	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.301	2.176	2.301	2.176	2.114	2.079

[a] Log coliform counts/g, 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.
[b] The duplicate test samples are denoted by 'A' and 'B.' NR = data not reported.

Table 3.   Coliform log counts at the medium and high inoculation levels for cheddar cheese by the Petrifilm RCC and the VRBA methods[a]

	Medium						High					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		VRBA		14 h confirm		24 h confirm		VRBA	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	3.000	2.763	3.104	2.903	3.093	3.061	3.919	3.732	3.982	3.778	4.143	3.886
2	2.820	3.049	2.813	3.090	3.079	3.301	3.898	3.991	3.903	4.068	4.079	3.875
3	2.602	2.462	2.602	2.462	2.748	2.732	3.362	3.505	3.362	3.544	3.519	3.633
4	3.000	2.857	3.000	2.869	3.000	2.973	3.881	3.869	3.892	3.875	4.021	4.033
5	2.851	2.748	2.881	2.763	2.914	2.690	3.892	3.778	3.903	3.799	4.072	3.724
6	2.857	2.968	2.949	3.097	3.086	3.124	4.025	4.100	4.086	4.124	4.204	4.107
7	2.826	2.477	2.851	2.491	2.898	2.556	3.978	3.908	4.004	3.959	4.053	4.013
8	NR	NR	2.708	2.732	2.851	3.029	NR	NR	4.049	3.919	4.104	3.964
9	2.903	2.826	2.934	2.792	2.799	2.531	3.978	4.079	4.041	4.079	4.176	3.949
10	3.124	2.982	3.146	2.987	3.137	2.898	3.934	3.914	3.982	3.940	3.881	3.826
11	3.146	3.204	3.176	3.230	3.146	3.204	4.176	4.000	4.204	4.000	4.301	4.204

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.
[b] The duplicate test samples are denoted by 'A' and 'B.' NR = data not reported.

Table 4. Coliform log counts at the control and low inoculation levels for vanilla ice cream by the Petrifilm RCC and the VRBA methods[a]

	Control						Low					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		VRBA		14 h confirm		24 h confirm		VRBA	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.716	2.806	2.826	2.892	3.017	3.045
2	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.556	2.447	2.556	2.447	2.732	2.785
3	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.857	2.863	2.863	2.892	2.940	3.114
4	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.851	2.964	2.851	2.964	3.124	3.033
5	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.708	2.663	2.708	2.690	2.898	2.959
6	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.968	2.964	2.987	2.996	3.173	3.079
7	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.716	2.748	2.724	2.756	2.954	3.093
8	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.477	<1.000	2.699	2.806	3.061	2.929
9	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.964	2.813	2.996	2.929	2.748	2.826
10	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.892	2.813	2.898	2.813	3.041	3.037
11	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	2.886	2.799	2.898	2.799	3.076	2.978

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.
[b] The duplicate test samples are denoted by 'A' and 'B.'

Table 5. Coliform log counts at the medium and high inoculation levels for vanilla ice cream by the Petrifilm RCC and the VRBA methods[a]

	Medium						High					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		VRBA		14 h confirm		24 h confirm		VRBA	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	3.580	3.462	3.643	3.602	3.940	3.996	4.653	4.716	4.748	4.785	4.903	4.978
2	3.415	3.279	3.415	3.279	3.176	3.204	4.398	4.643	4.415	4.653	4.204	4.342
3	3.875	3.792	3.875	3.813	3.833	3.732	4.833	4.833	4.845	4.929	4.826	4.875
4	3.778	3.756	3.778	3.756	3.792	3.934	4.934	4.778	4.934	4.778	4.968	4.778
5	3.681	3.633	3.681	3.633	3.643	3.681	4.663	4.602	4.681	4.613	4.785	4.672
6	3.740	4.045	3.756	4.068	4.146	4.124	4.851	5.127	4.869	5.130	5.207	5.152
7	3.362	3.556	3.362	3.556	3.892	3.875	4.778	4.763	4.778	4.763	4.954	4.919
8	<1.000	<1.000	3.748	3.633	3.672	3.813	4.398	4.342	4.623	4.591	4.699	4.748
9	3.724	3.785	3.771	3.903	3.949	4.013	4.763	4.531	5.029	4.863	4.940	4.908
10	3.813	3.763	3.813	3.771	3.839	3.771	4.845	4.820	4.857	4.820	4.505	4.690
11	3.462	3.716	3.477	3.724	3.732	3.898	4.690	4.806	4.708	4.813	4.771	4.949

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.
[b] The duplicate test samples are denoted by 'A' and 'B.'

Table 6.   Coliform log counts at the control and low inoculation levels for flour by the Petrifilm RCC and the MPN methods[a]

	Control						Low					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		MPN		14 h confirm		24 h confirm		MPN	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	2.845	2.903	3.279	3.000	0.602	1.634	2.903	2.954	3.000	3.146	2.380	1.968
2	2.851	3.544	2.845	3.531	2.634	3.176	2.477	3.079	2.477	3.079	2.968	2.845
3	3.362	3.146	3.398	3.146	3.1761	2.634	2.929	2.973	2.934	3.017	1.968	2.176
4	3.505	3.279	3.544	3.279	3.380	3.322	<2.000	3.279	2.477	3.322	1.633	2.322
5	2.785	2.079	2.785	2.255	2.322	<0.477	2.643	2.380	2.716	2.568	<0.477	<0.477
6	3.114	3.301	3.204	3.301	1.362	0.602	3.342	3.230	3.255	3.230	0.954	0.954
7	3.699	4.114	3.699	4.176	0.954	2.845	3.301	3.000	3.301	3.000	1.322	1.634
8	3.079	2.903	3.146	3.079	1.362	2.380	2.903	2.602	3.114	3.079	1.362	0.954
9	3.699	3.505	3.785	3.556	1.634	1.041	2.477	2.301	2.602	2.301	2.176	0.954
10	3.748	3.322	3.748	3.342	<0.477	0.602	2.903	2.903	2.903	3.000	1.602	1.176
11	3.633	3.415	3.690	3.415	2.380	<0.477	2.301	3.301	2.845	3.342	0.602	2.380

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.
[b] The duplicate test samples are denoted by 'A' and 'B.'

Table 7.   Coliform log counts at the medium and high inoculation levels for flour by the Petrifilm RCC and the MPN methods[a]

	Medium						High					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		MPN		14 h confirm		24 h confirm		MPN	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	3.663	2.778	3.724	3.342	2.380	2.176	3.322	3.568	3.544	3.690	2.176	2.633
2	3.230	2.778	3.176	2.778	3.380	3.968	3.230	2.845	3.230	2.845	2.968	3.968
3	3.146	3.505	3.146	3.505	2.176	3.380	3.663	3.699	3.699	3.699	3.380	3.380
4	3.415	2.477	3.415	2.602	2.380	2.633	3.531	3.708	3.531	3.740	2.380	3.176
5	2.544	2.591	2.580	2.602	2.362	1.633	3.045	2.724	3.079	2.748	2.845	2.633
6	3.176	3.447	3.176	3.431	1.968	2.380	3.681	4.155	3.580	3.785	1.362	1.968
7	NR	3.477	<3.000	4.279	2.176	1.634	4.114	3.477	4.041	3.699	2.968	3.176
8	3.000	3.845	3.114	3.505	2.380	1.362	3.491	3.431	3.531	3.613	1.362	1.362
9	3.114	3.322	3.146	3.447	2.633	2.968	3.806	3.681	3.940	3.681	4.380	3.634
10	3.079	3.041	3.114	3.041	1.362	2.380	3.477	3.568	3.531	3.568	2.380	4.362
11	2.602	3.672	2.602	3.672	2.633	2.633	3.380	3.279	3.415	3.322	2.633	1.968

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.
[b] The duplicate test samples are denoted by 'A' and 'B.' NR = data not reported.

Table 8. Coliform log counts at the control and low inoculation levels for prepared frozen macaroni and cheese by the Petrifilm RCC and the MPN methods[a]

	Control						Low					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		MPN		14 h confirm		24 h confirm		MPN	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	LA	<1.000	LA	<1.000	LA	<0.477	<1.000	<1.000	1.477	2.041	2.633	2.633
2	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.342	2.623	2.322	2.623	2.633	2.380
3	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.041	<1.000	2.204	1.477	2.380	1.633
4	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.322	2.362	2.342	2.362	2.380	2.633
5	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	1.602	1.845	1.903	2.000	1.041	1.968
6	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.301	2.279	2.342	2.322	2.380	2.380
7	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	1.778	1.477	1.845	1.602	1.322	1.176
8	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.857	2.531	2.863	2.531	2.875	2.380
9	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.415	2.342	2.531	2.398	2.633	2.633
10	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP
11	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.204	2.491	2.230	2.491	2.633	2.633

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.
[b] The duplicate test samples are denoted by 'A' and 'B.' LA = laboratory accident; DP = laboratory did not participate.

Table 9. Coliform log counts at the medium and high inoculation levels for prepared frozen macaroni and cheese by the Petrifilm RCC and the MPN methods[a]

	Medium						High					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		MPN		14 h confirm		24 h confirm		MPN	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	2.544	3.000	2.845	3.255	3.380	2.380	4.114	4.041	4.104	4.301	4.176	4.380
2	3.613	3.580	3.623	3.591	3.633	3.633	4.580	4.681	4.580	4.690	>5.041	5.041
3	2.839	2.982	2.892	3.041	2.633	2.633	4.025	3.991	4.079	4.107	3.968	4.380
4	3.176	3.380	3.176	3.380	3.380	2.968	4.556	4.322	4.556	4.322	4.380	4.633
5	2.447	2.785	2.653	2.908	2.633	1.633	3.892	3.881	3.964	3.940	2.301	1.845
6	3.079	3.505	3.114	3.519	3.968	3.633	4.176	4.362	4.204	4.362	4.380	3.968
7	2.851	3.037	2.898	3.053	2.380	3.176	4.049	3.964	4.114	3.987	3.875	3.633
8	3.643	3.342	3.663	3.342	3.968	3.633	4.505	4.833	4.568	4.851	4.380	4.663
9	3.447	3.398	3.477	3.415	3.633	3.968	4.415	4.544	4.447	4.544	4.380	4.663
10	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP
11	3.491	3.342	3.491	3.380	3.176	3.633	4.230	4.447	4.301	4.462	4.663	>5.041

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.
[b] The duplicate test samples are denoted by 'A' and 'B.' DP = laboratory did not participate.

Table 10.   Coliform log counts at the control and low inoculation levels for hash brown potatoes by the Petrifilm RCC and the MPN methods[a]

	Control						Low					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		MPN		14 h confirm		24 h confirm		MPN	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	<1.000	<1.000	<1.000	<1.000	1.968	2.633
2	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP
3	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	<1.000	<1.000	<1.000	<1.000	1.968	1.633
4	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	<1.000	<1.000	<1.000	<1.000	2.380	2.380
5	NR	NR	<1.000	<1.000	<0.477	<0.477	NR	NR	2.000	2.079	0.477	0.602
6	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	<1.000	<1.000	<1.000	<1.000	1.968	1.362
7	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	<1.000	<1.000	<1.000	<1.000	1.968	1.176
8	NR	NR	<1.000	<1.000	<0.477	<0.477	<1.000	<1.000	1.301	<1.000	2.380	2.968
9	NR	NR	<1.000	<1.000	<0.477	<0.477	NR	NR	<1.000	<1.000	2.633	1.968
10	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	<1.000	<1.000	<1.000	<1.000	1.633	2.633
11	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	<1.000	<1.000	<1.000	<1.000	2.633	2.380

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.
[b] The duplicate test samples are denoted by 'A' and 'B.' NR = data not reported. DP = laboratory did not participate.

Table 11.   Coliform log counts at the medium and high inoculation levels for hash brown potatoes by the Petrifilm RCC and the MPN methods[a]

	Medium						High					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		MPN		14 h confirm		24 h confirm		MPN	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	<2.000	<2.000	<2.000	<2.000	3.968	4.380	<3.000	<3.000	<3.000	<3.000	4.663	3.380
2	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP
3	<2.000	<2.000	<2.000	<2.000	2.968	2.634	<3.000	<3.000	<3.000	<3.000	4.380	3.968
4	<2.000	<2.000	<2.000	<2.000	3.634	3.380	<3.000	<3.000	<3.000	<3.000	4.663	4.663
5	NR	NR	2.531	1.903	2.322	2.634	NR	NR	3.532	3.826	4.663	4.663
6	<2.000	<2.000	<2.000	<2.000	3.176	2.380	<3.000	<3.000	<3.000	<3.000	2.380	2.380
7	<2.000	<2.000	<2.000	<2.000	2.968	2.968	<3.000	<3.000	<3.000	<3.000	3.875	3.633
8	NR	<2.000	2.845	3.255	3.380	3.041	<3.000	<3.000	4.000	4.176	4.663	4.380
9	NR	NR	<2.000	<2.000	3.634	3.380	NR	NR	<3.000	<3.000	0.602	4.663
10	<1.000	<1.000	<1.000	<1.000	3.176	3.362	<2.000	<2.000	<2.000	<2.000	4.663	4.380
11	<2.000	<2.000	<2.000	<2.000	3.634	3.634	<3.000	<3.000	<3.000	<3.000	4.663	4.663

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.
[b] The duplicate test samples are denoted by 'A' and 'B.' NR = data not reported. DP = laboratory did not participate.

Table 12. Coliform log counts at the control and low inoculation levels for fresh refrigerated uncooked pasta by the Petrifilm RCC and the MPN methods[a]

	Control						Low					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		MPN		14 h confirm		24 h confirm		MPN	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.431	2.633	2.431	2.690	2.633	2.380
2	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP
3	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.672	2.643	2.672	2.653	2.968	2.380
4	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.732	2.491	2.732	2.491	2.380	2.968
5	NR	NR	<1.000	<1.000	<0.477	<0.477	1.602	1.954	1.602	2.041	1.968	1.968
6	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.748	2.778	2.771	2.799	1.380	2.845
7	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.362	2.322	2.362	2.322	1.968	2.380
8	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	1.903	2.079	2.000	2.079	1.362	1.633
9	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.000	2.146	2.000	2.146	0.845	2.380
10	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.826	2.556	2.833	2.568	2.380	3.041
11	<1.000	<1.000	<1.000	<1.000	<0.477	<0.477	2.176	1.845	2.204	1.845	1.845	1.380

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.

[b] The duplicate test samples are denoted by 'A' and 'B.' NR = data not reported. DP = laboratory did not participate.

Table 13. Coliform log counts at the medium and high inoculation levels for fresh refrigerated uncooked pasta by the Petrifilm RCC and the MPN methods[a]

	Medium						High					
	Petrifilm RCC plate method				Standard method		Petrifilm RCC plate method				Standard method	
	14 h confirm		24 h confirm		MPN		14 h confirm		24 h confirm		MPN	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B
1	3.531	3.544	3.531	3.556	3.591	4.362	4.255	4.643	4.342	4.633	4.380	5.041
2	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP
3	3.623	3.491	3.633	3.505	2.380	3.380	4.633	4.279	4.633	4.279	4.663	3.968
4	3.732	3.462	3.732	3.462	3.968	3.875	4.531	4.398	4.531	4.398	4.663	5.041
5	2.991	3.009	2.996	3.013	3.380	3.380	4.613	3.778	4.613	3.778	5.041	3.968
6	3.623	3.778	3.643	3.785	3.380	3.633	4.672	4.568	4.681	4.580	4.380	4.663
7	3.362	3.176	3.362	3.176	2.968	3.633	4.041	4.477	4.079	4.505	4.176	3.633
8	3.041	2.613	3.079	2.681	2.176	1.633	3.778	3.716	3.778	3.724	3.380	3.968
9	2.881	2.964	2.881	2.991	3.633	2.968	3.903	3.914	3.959	3.940	4.380	4.380
10	3.663	3.845	3.663	3.857	4.079	3.968	4.748	4.813	4.756	4.826	>5.041	4.663
11	3.152	3.124	3.182	3.149	3.633	3.591	4.111	3.301	4.127	3.322	4.380	3.968

[a] Log coliform counts/g; 14 h confirm = Petrifilm RCC plate confirmed counts read at 14 h; 24 h confirm = Petrifilm RCC plate confirmed counts read at 24 h.

[b] The duplicate test samples are denoted by 'A' and 'B.' DP = laboratory did not participate.

Table 14.   Interlaboratory study results of the Petrifilm RCC method at 14 h for detection of coliforms in foods

Food type	Level	n^a	Mean^b	s_r	RSD$_r$, %	s_R	RSD$_R$, %
Cheddar cheese	Low	10 (0)	1.86	0.27	14.52	0.32	17.20
	Medium	10 (0)	2.87	0.13	4.53	0.20	6.97
	High	10 (0)	3.90	0.08	2.05	0.19	4.87
Vanilla ice cream	Low	9 (0)	2.78	0.06	2.16	0.15	5.40
	Medium	10 (0)	3.67	0.11	3.00	0.19	5.18
	High	11 (0)	4.72	0.10	2.12	0.19	4.03
Flour	Uninoc.	11 (0)	3.26	0.27^c	8.28	0.44	13.50
	Low	11 (0)	2.92	0.29^d	9.93	0.34	11.64
	Medium	11 (0)	3.15	0.45	14.29	0.45	14.29
	High	11 (0)	3.49	0.21^c	6.02	0.35	10.03
Macaroni and cheese	Low	9 (0)	2.21	0.16^d	7.24	0.37	16.74
	Medium	10 (0)	3.17	0.19^d	5.99	0.36	11.36
	High	10 (0)	4.32	0.12^d	2.78	0.29	6.71
Hash brown potatoes	Low	11	NG^e	NG	NG	NG	NG
	Medium	11	NG	NG	NG	NG	NG
	High	11	NG	NG	NG	NG	NG
Fresh refrigerated uncooked pasta	Low	10 (0)	2.34	0.15^c	6.41	0.37	15.81
	Medium	10 (0)	3.33	0.14^c	4.20	0.36	10.81
	High	10 (0)	4.26	0.30	7.04	0.43	10.09

[a]   Number of laboratories with complete data; number of outliers appears in parentheses.

[b]   Log coliform count/g.

[c]   Significantly better repeatability ($p < 0.01$).

[d]   Significantly better repeatability ($p < 0.05$).

[e]   NG = Colonies with yellow acid zones were present; however, no gassing colonies were detected.

The repeatability variance of the Petrifilm RCC procedures was not significantly different from that for the VRB agar method for low, medium, and high levels of inoculation.

Vanilla Ice Cream

Low level of inoculation.—The Cochran and Grubbs tests to determine outliers were applied to the data. Neither the Cochran nor the single Grubbs test detected any laboratories with values that did not fit well with the rest of the data. However, the pair-value Grubbs test was positive for the VRB agar method. The 2 values responsible for activating the test (from Laboratories 2 and 9) were removed and the outlier tests were repeated. No additional outliers were found. The results are reported with the removal of the 2 aberrant values in order to follow the AOAC harmonized outlier removal procedure.

The mean log coliform counts by the VRB agar method were statistically different from the mean log confirmed coliform counts by the Petrifilm RCC method read at both 14 and 24 h.

The repeatability variances of the Petrifilm RCC procedures were not significantly different from the repeatability variance of the VRB agar method.

Medium level of inoculation.—Outliers were detected by the pair-value Grubbs test. These values (from Laboratories 2

and 6) were removed. The results are reported with the removal of the 2 values.

The mean log coliform counts by the VRB agar method were statistically different from the mean log confirmed coliform counts by the Petrifilm RCC method read at both 14 and 24 h.

The repeatability variances of the Petrifilm RCC procedures measuring confirmed coliforms at 14 and 24 h were not significantly different from the repeatability variance of the VRB agar method.

High level of inoculation.—No outliers were detected by the tests.

The mean log coliform counts by the VRB agar method were not significantly different from the mean log confirmed coliform counts read at 14 h and the mean log confirmed coliform counts read at 24 h by the Petrifilm RCC method.

The repeatability variances of the Petrifilm RCC procedures measuring confirmed coliforms at 14 and 24 h were not significantly different from the repeatability variance of the VRB agar method.

Wheat Flour

Uninoculated level of contamination.—No outliers were detected by the tests.

Table 15. Interlaboratory study results of the Petrifilm RCC method at 24 h for detection of coliforms in foods

Food type	Level	n^a	Mean[b]	s_r	RSD_r, %	s_R	RSD_R, %
Cheddar cheese	Low	11 (0)	1.89	0.26	13.76	0.31	16.40
	Medium	11 (0)	2.89	0.13	4.50	0.21	7.27
	High	11 (0)	3.93	0.09	2.29	0.19	4.83
Vanilla ice cream	Low	9 (0)	2.84	0.05	1.76	0.14	4.93
	Medium	11 (0)	3.70	0.11	2.97	0.19	5.14
	High	11 (0)	4.78	0.10	2.09	0.16	3.35
Flour	Uninoc.	11 (0)	3.33	0.26^c	7.81	0.41	12.31
	Low	11 (0)	2.97	0.27^d	9.09	0.30	10.10
	Medium	9 (2)	3.22	0.38	11.80	0.38	11.80
	High	11 (0)	3.52	0.16^c	4.55	0.32	9.09
Macaroni and cheese	Low	10 (0)	2.20	0.24	10.91	0.39	17.73
	Medium	10 (0)	3.24	0.17^c	5.25	0.30	9.26
	High	10 (0)	4.37	0.11^d	2.52	0.26	5.95
Hash brown potatoes	Low	11	NG^e	NG	NG	NG	NG
	Medium	11	NG	NG	NG	NG	NG
	High	11	NG	NG	NG	NG	NG
Fresh refrigerated uncooked pasta	Low	10 (0)	2.36	0.16^c	6.78	0.37	15.68
	Medium	10 (0)	3.34	0.13^c	3.89	0.35	10.48
	High	10 (0)	4.27	0.30	7.03	0.42	9.84

[a] Number of laboratories with complete data; number of outliers appears in parentheses.

[b] Log coliform count/g.

[c] Significantly better repeatability ($p < 0.01$).

[d] Significantly better repeatability ($p < 0.05$).

[e] NG = Colonies with yellow acid zones were present; however, no gassing colonies were detected.

The mean log coliform counts by the MPN procedure were significantly different from the mean log confirmed coliform counts read at 14 h and those read at 24 h by the Petrifilm RCC method. The mean log coliform counts by the Petrifilm RCC method were greater in value than those obtained by the MPN procedure.

The repeatability variances of the Petrifilm RCC procedures were significantly better ($p < 0.01$) than those for the MPN method for the uninoculated level.

Low level of inoculation.—No outliers were detected.

The mean log coliform counts by the MPN procedure were significantly different from the mean log confirmed coliform counts read at 14 h and those read at 24 h by the Petrifilm RCC method. The mean log coliform counts by the Petrifilm RCC method were greater in value than those obtained by the MPN procedure.

The repeatability variances of the Petrifilm RCC procedure using confirmed counts read at 14 h and 24 h were significantly better ($p < 0.05$) than that for the MPN method.

Medium level of inoculation.—Outliers were detected by the pair-value Grubbs test for the Petrifilm RCC method recording confirmed counts at 24 h. These values were removed, the outlier tests were rerun, and no additional values were detected that did not fit well with the rest of the data in the set.

The mean log coliform counts by the MPN procedure were significantly different from those read at 14 h and 24 h by the Petrifilm RCC method. The mean log coliform counts by the Petrifilm RCC method were greater in value than those obtained by the MPN procedure.

The repeatability variances of the Petrifilm RCC procedures using confirmed counts read at 14 h and 24 h were not significantly different from that for the MPN method.

High level of inoculation.—No outliers were detected.

The mean log coliform counts by the MPN procedure were significantly different from those read at 14 h and 24 h by the Petrifilm RCC method. The mean log coliform counts by the Petrifilm RCC method were greater in value than those obtained by the MPN procedure.

The repeatability variances of the Petrifilm RCC procedure using confirmed coliform counts read at 14 h and 24 h were significantly better ($p < 0.01$) than that for the MPN method.

Prepared Frozen Macaroni and Cheese

At the low level of inoculation, outliers were detected using the pair-value Grubbs test for the MPN method. These values were removed and the tests were rerun. Further outliers were detected for the MPN method. Because the overall fraction of

Table 16.   Interlaboratory study results of the standard method for the detection of coliforms in foods

Food type	Level	n^a	Mean^b	s_r	RSD$_r$, %	s_R	RSD$_R$, %
Cheddar cheese	Low	11 (0)	1.90	0.29	15.26	0.30	15.79
	Medium	11 (0)	2.95	0.13	4.41	0.21	7.12
	High	11 (0)	3.99	0.12	3.01	0.20	5.01
Vanilla ice cream	Low	9 (2)	3.03	0.07	2.31	0.08	2.64
	Medium	9 (2)	3.83	0.07	1.83	0.11	2.87
	High	11 (0)	4.81	0.08	1.66	0.24	4.99
Flour	Uninoc.	11 (0)	1.97	0.67	34.01	0.95	48.22
	Low	10 (0)	1.72	0.54	31.40	0.69	40.12
	Medium	11 (0)	2.51	0.48	19.12	0.65	25.90
	High	11 (0)	2.78	0.57	20.50	0.90	32.37
Macaroni and cheese	Low	10 (0)	2.27	0.30	13.22	0.55	24.23
	Medium	10 (0)	3.20	0.41	12.81	0.65	20.31
	High	9 (1)	4.04	0.22	5.45	0.35	8.66
Hash brown potatoes	Low	10 (0)	2.06	0.41	19.90	0.69	33.50
	Medium	10 (0)	3.23	0.25	7.74	0.53	16.41
	High	10 (0)	4.00	0.96	24.00	1.09	27.25
Fresh refrigerated uncooked pasta	Low	10 (0)	2.15	0.55	25.58	0.62	28.84
	Medium	10 (0)	3.38	0.38	11.24	0.68	20.12
	High	10 (0)	4.37	0.46	10.53	0.46	10.53

[a] Number of laboratories with complete data; number of outliers appears in parentheses.

[b] Log coliform count/g.

laboratories would exceed 2/9, precision estimates were calculated using all the data at the low level of inoculation.

An outlier for the MPN method was also detected using the single Grubbs test at the high level of inoculation. This value was removed and the precision estimates were calculated.

The mean log coliform counts by the MPN procedure were not significantly different from those read at 14 h and 24 h by the Petrifilm RCC method for all levels of inoculation.

The repeatability variances of the Petrifilm RCC procedures were significantly better or not significantly different from the repeatability variances of the MPN method for all levels of inoculation.

Hash Brown Potatoes

At all levels of inoculation, colonies with yellow acid zones were present using the Petrifilm RCC plate; however, because no gassing colonies were detected, these procedures could not be compared with the standard method.

Fresh Refrigerated Uncooked Pasta

No outliers were present at any levels of inoculation.

The mean log coliform counts by the MPN procedure were not significantly different from those read at 14 h and 24 h by the Petrifilm RCC method for all levels of inoculation.

The repeatability variance of the Petrifilm RCC procedures using the confirmed counts at 14 h and 24 h was significantly

better ($p < 0.01$) than the repeatability variance of the MPN method at the low and medium levels of inoculation. The repeatability variance of the Petrifilm RCC procedures using the confirmed counts at 14 h and 24 h was not significantly different from that of the MPN method at the high level of inoculation.

Discussion

The superior repeatability of the Petrifilm method is evident in the confirmed counts with approximately 46% cases being better than that of the standard method.

The mean log counts of the Petrifilm RCC methods were significantly higher than those by the MPN procedure for flour. In the precollaborative study, this difference was also detected. The Petrifilm methods appear to be more sensitive than the MPN method.

For some unknown reason, gas production was suppressed in the Petrifilm method for hash brown potatoes. However, in the precollaborative study (Tables 17 and 18; McIntyre, unpublished), this suppression was not seen, and no differences, on average, were detected between the Petrifilm RCC method and the standard method. The repeatability of the Petrifilm RCC method was not significantly different from that of the standard method.

Table 17. Results from precollaborative study of analysis for coliforms by Petrifilm RCC plate method read at 14 and 24 h, the VRBA method, and the MPN method. All results are expressed in log CFU/g.

Food type	log RCC 14 h	log RCC 24 h	log VRBA	log MPN
Frozen hash brown potatoes	2.40241	2.50935	2.326606	2.380211
Frozen hash brown potatoes	3.311625	3.321726	3.182575	3.968483
Frozen hash brown potatoes	4.160073	4.16111	4.066178	4.176091
Frozen hash brown potatoes	2.321726	2.341974	2.317742	2.380211
Frozen hash brown potatoes	3.332321	3.37097	3.188288	4.041393
Frozen hash brown potatoes	4.16111	4.16111	4.175124	5.041393
Frozen hash brown potatoes	2.332321	2.361317	2.329482	1.968483
Frozen hash brown potatoes	3.309547	3.317742	3.227422	3.380211
Frozen hash brown potatoes	4.249155	4.259257	4.310588	3.968483
Frozen hash brown potatoes	2.321726	2.342423	2.370576	3.041393
Frozen hash brown potatoes	3.234174	3.26891	3.141651	4.041393
Frozen hash brown potatoes	4.218081	4.237836	4.15507	3.633468
Frozen hash brown potatoes	2.241437	2.254601	2.343318	1.968483
Frozen hash brown potatoes	3.175124	3.20412	3.154815	3.662758
Frozen hash brown potatoes	4.26317	4.276334	4.1519	4.662758
Frozen hash brown potatoes	2.446881	2.477121	2.405116	2.662758
Frozen hash brown potatoes	3.223579	3.238561	3.30103	3.662758
Frozen hash brown potatoes	4.392309	4.426545	4.298848	4.380211
Frozen hash brown potatoes	2.130036	2.176091	2.190106	2.380211
Frozen hash brown potatoes	3.300486	3.321726	3.51142	2.968483
Frozen hash brown potatoes	4.112655	4.143946	4.213516	3.968483
Frozen hash brown potatoes	2.26317	2.346423	2.326606	2.380211
Frozen hash brown potatoes	3.361317	3.414652	3.376792	2.113943
Frozen hash brown potatoes	4.322219	4.37097	4.254601	4.662758
Frozen hash brown potatoes	2.426545	2.468257	2.39794	2.662758
Frozen hash brown potatoes	3.16111	3.16111	3.247077	3.380211
Frozen hash brown potatoes	4.322219	4.322219	4.366197	4.380211
Frozen hash brown potatoes	2.16111	2.190106	2.112655	2.176091
Frozen hash brown potatoes	2.966135	3.03439	3.088132	3.176091
Frozen hash brown potatoes	3.989079	4.104945	4.146084	3.968483

In general, there was minimal response regarding the methods themselves from the collaborators. Specifically, the samples of wheat flour at the 1:10 dilution were difficult to interpret for both the Petrifilm RCC method and the reference method.

Recommendation

The repeatability and reproducibility precision estimates of the Petrifilm RCC plate method at 14 and 24 h were not significantly different from those for the reference methods (the VRB agar method for dairy and the MPN method for nondairy). Therefore, it is recommended that the Petrifilm Rapid Coliform Count method be adopted Official First Ac-

tion for enumeration of presumptive coliforms at 14 h and confirmed coliforms at 14 and 24 h in food.

Table 18.   Quantitative comparison of Petrifilm Rapid Coliform Count plate at the 14 and 24 h incubation time period and MPN methods for coliforms in hash brown potatoes

	Method		
Incubation time, h	RCC = MPN	RCC < MPN	RCC > MPN
14	28	1	0
24	29	0	0

References

(1)   U.S. Food and Drug Administration (1995) *Bacteriological Analytical Manual*, 8th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, pp 4.01–4.29

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