FOOD BIOLOGICAL CONTAMINANTS

Evaluation of Peel Plate™ EC for Determination of *E. coli* and Coliform or Total Coliform in Dairy Products

AOAC *Performance Tested Method*℠ 061501

Abstract

Peel Plate™ EC is a low-profile plastic, 47 mm culture dish with an adhesive top that contains a dried medium with Gram-negative selective agents and with enzyme substrate indicators for β-galactosidase (coliform) and β-glucuronidase (*Escherichia coli*). The method provides a conventional quantitative coliform (red) and *E. coli* (blue/purple/black) count with simple rehydration and incubation for 24 ± 2 h at 35 ± 1°C, while providing a total coliform result, sum of *E. coli,* and coliform without color differential in dairy products at 32 ± 1°C for 24 ± 2 h. Dairy matrixes claimed and supported with total coliform data are whole milk, skim milk, chocolate milk (2% fat), heavy cream (35% fat), pasteurized whole goat milk, ultra-high-temperature pasteurized milk, powdered milk, lactose-reduced milk, strawberry milk, shredded cheddar cheese, raw cow milk, raw goat milk, raw sheep milk, sour cream, condensed milk, eggnog, vanilla ice cream, condensed whey, yogurt, and cottage cheese. Matrixes claimed for *E. coli* and total coliform detection are raw ground beef, mixed cellulose 0.45 µm filtered bottled water, environmental sponge of stainless steel, raw ground turkey, dry dog food, liquid whole pasteurized eggs, milk chocolate, leafy green (mixed greens) rinse/flume water, irrigation water, poultry carcass rinse, and large animal carcass sponge. The method has been independently evaluated for total coliform in whole milk, skim milk, chocolate milk, and heavy cream. The method was also independently evaluated for *E. coli* and coliform in ground beef, filtered bottled water, and sponge rinse from stainless steel surfaces. In inclusivity and exclusivity studies, the method detected 57 of 58 different strains of coliform and *E. coli* at 32 ± 1°C and 35 ± 1°C in and excluded 31 of 32 different noncoliform strains consisting of Gram-negative and Gram-positive bacteria. In the matrix study, each matrix was assessed separately at each contamination level in comparison to an appropriate reference method. Colony counts were determined for each level and then $\log_{10}$ transformed. The transformed data were evaluated for repeatability, log-mean comparison between methods with 95% confidence interval, and r^2. A 95% confidence interval range of −0.5 to 0.5 on the mean difference was used as the acceptance criterion to establish significant statistical difference between methods. The evaluations demonstrate that the Peel Plate EC method provides no statistical differences across most of the matrixes. The coliform r^2 values were greater than 0.9 except in the case of skim milk (r^2 = 0.77 and 0.69), sheep milk (0.84), and chocolate (0.81). In the case of skim milk, the three highest concentrations were significantly biased low compared with the reference method, whereas in the case of chocolate, the highest concentration was significantly biased high. The *E. coli* r^2 values were greater than 0.9 except in the case of hog rinse (0.89), flume water (0.82), and chocolate (0.77). The lower values were generally from only a 1 log difference between highest and lowest concentrations except in the case of chocolate, in which the highest concentration was biased high compared with the reference method. Within-method repeatability of Peel Plate EC was similar to the reference method, with relative SDs generally less than 5% when $\log_{10}$ means were ≥1.5. QC data support that the Peel Plate EC is stable for 1 year when refrigerated. Incubation temperature ranges, 30–36°C, and times, 22–26 and 48 h for yogurt, were not significantly different in paired *t*-test comparison. The method is selective without the need for confirmation, although confirmation of coliform and *E. coli* was performed as part of the validation work.

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The method was independently tested, evaluated, and certified by the AOAC Research Institute as a *Performance Tested Method*℠. *See* http://www.aoac.org/testkits/steps.html for information on certification.

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Scope of Method

(a) *Target organisms.*—Generic *Escherichia coli* and coliform or total coliform, the sum of both types.

(b) *Matrixes.*—(*1*) Total coliform dairy products; pasteurized whole milk, skim milk, 2% chocolate milk, 35% cream, pasteurized whole goat milk, ultra-high-temperature (UHT) pasteurized milk, nonfat dried milk, lactose-reduced milk, strawberry milk, shredded cheddar cheese, yogurt, cottage cheese, raw cow milk, raw goat milk, raw sheep milk, sour cream, condensed milk, eggnog, condensed whey, and vanilla ice cream.

(*2*) *E. coli* and coliform in nondairy products; raw ground beef, raw ground turkey, liquid whole pasteurized eggs, U.S. Department of Agriculture, Agriculture Research Service (USDA ARS), carcass rinses of chicken and USDA ARS 300 cm^2 surface sponge of hog carcass (1), dry dog food, milk chocolate, bottled water (mineral, no gas), leafy green (mixed greens) neutralized flume water, irrigation water, and environmental sponge of stainless steel.

(c) *Summary of validated performance claims.*—(*1*) *Dairy products.*—Performance not statistically different [95% confidence interval (CI)], with the exception of the three highest skim milk levels, on mean, the difference between Peel Plate™ EC and the reference method was within the range of –0.5 to 05 (2, 3) from that of the U.S. Food and Drug Administration, National Conference of Interstate Milk Shipments (FDA NCIMS) Reference 2400a forms cultural procedures and Violet Red Bile Agar (VRBA) pour plate method (4, 5).

(*2*) *Ground beef, ground turkey, liquid whole pasteurized egg, carcass rinses for E. coli and coliform.*—Performance not statistically different from that of the USDA Food Safety and Inspection Service *Microbiology Laboratory Guidebook* 3.01, Quantitative Analysis of Bacteria in Foods as Sanitary Indicators with the FDA *Bacteriological Analytical Manual* (FDA/BAM), Chapter 4, Enumeration of *Escherichia coli* and the Coliform Bacteria, Section I.G, reference methods (1, 6).

(*3*) *Dry dog food and milk chocolate.*—Performance not statistically different from that of the FDA/BAM Chapter 4, I.G, reference method.

(*4*) *Bottled water and leafy green (mixed greens) neutralized flume water.*—Performance not statistically different from that of the FDA/BAM Chapter 4, Section III, D., reference method.

(*5*) *Irrigation water.*—Performance not statistically different from that of the U.S. Environmental Protection Agency (EPA) Method 1604 (7).

(*6*) *Surface sponge of stainless steel.*—Performance not statistically different from that of the International Organization for Standardization 18593:2004, Microbiology of food and animal feeding stuffs–Horizontal methods for sampling techniques from surfaces using contact plates and swabs (8) with FDA/BAM Chapter 4, Section I.G, reference methods.

Definitions

(a) *Repeatability (s_r).*—The SD of replicates for each analyte at each concentration of each matrix for each method.

(b) *Log_{10} mean difference between candidate and reference methods.*—Mean difference between candidate and reference method log_{10} transformed results with lower and upper CI for each analyte at each concentration of each matrix. Differences between methods are considered significant when the CI falls outside (–0.5 to 0.5).

(c) r^2.—Square of the correlation coefficient of log-log linear regression of studied concentrations.

(d) *Paired t-test.*—*P* value for a two-tailed *t*-test, *P* value <0.05 indicates significance at the 95% confidence level.

Principle

Peel Plate EC is a Gram-negative–selective medium used to support and color differentiate, at 35 ± 1°C for 24 ± 2 h, the growth of coliform and *E. coli* in test samples. Peel Plate EC contains the enzyme substrates salmon-gal (6-chloro-3-indolyl-B-D-galactopyranoside) used to detect β-galactosidase produced by coliform and x-glucuronide (5-bromo-4-chloro-3-indolyl-B-D-glucuronide) used to detect β-glucuronidase produced by a majority of generic *E. coli*. Peel Plate EC also contains gelling and wicking agents that absorb and self-wick the sample. At 32°C incubation with dairy products, observed colonies on Peel Plate EC are scored as total coliform because *E. coli* does not reliably color differentiate.

General Information

Coliform and *E. coli* are routinely tested in food manufacture as a sanitary process indicator. *E. coli* is a subset of coliform bacteria and traditionally considered a fecal indicator. Coliform and *E. coli* in food or on food manufacturing surfaces can signal a higher risk of pathogens and a breakdown in sanitary practices. For example, in milk production, cow udders should be cleaned of dirt and fecal matter before a milking machine is applied. The Pasteurized Milk Ordinance specifies less than 100 coliform/mL in raw milk and less than 10 coliform/mL or per gram in pasteurized dairy products (9). *E. coli* per milliliter is used as a running process control indicator for low pathogen risk in meat production facilities (10). *E. coli* and coliform are also used in managing bacterial risks in water municipalities under the Total Coliform Rule (11). Because water is such an important aspect in agricultural practices, *E. coli* and coliform standards are proposed in food safety modernization regulations affecting produce manufacture (12). Because *E. coli* and coliform are used so frequently by the food industry, there is a need for simple, low-cost, ready-to-use methods for testing. Peel Plate EC is a simple method to detect and quantify coliform and *E. coli* in foods and water that is being studied and validated in this work.

Materials and Methods

Test Kit Information

(a) *Kit name*.—Peel Plate EC.

(b) *Catalog No*.—PP-EC-100K, 100 Peel Plate EC tests.

(c) *Ordering information*.—Charm Sciences, Inc., 659 Andover St, Lawrence, MA 01843; Tel.: 978-687-9200, Fax: 978-687-9216; e-mail: info@charm.com, http://www.charm.com.

Test Kit Components

(a) Two foil bags containing 50 Peel Plate ECs, each with blue indicator desiccants. Additional supplies and reagents required depending on application.

(b) *Butterfield's phosphate-buffered dilution water (BPBDW)*.—Buffer KH_2PO_4 (34 g to 500 mL distilled or reverse osmosis water; adjust pH to 7.2 with 1 N NaOH; bring final volume to 1 L with distilled or reverse osmosis water. Add 99 mL to dilution bottles and sterilize 15 min at 121°C. Store in refrigerator. Or purchase (e.g., Weber Scientific, Hamilton, NJ, item No. 3127-14 or alternative manufacturer equivalent).

(c) *Buffered peptone water*.—Peptone 10 g, sodium chloride 5 g, disodium phosphate 3.5 g, monopotassium phosphate 1.5 g, and distilled water 1 L. Add 99 mL to dilution bottles and sterilize 15 min at 121°C. Store in refrigerator. Final pH 7.2 ± 0.2.

(d) *1 mL pipet tips*.

(e) *Sodium bisulfite dilution buffer (SBDB)*–0.2 g sodium bisulfite dissolved in 99 mL BPBDW.

Apparatus

(a) *Pipettor*.—1 mL.

(b) *Incubator*.—32 ± 1°C or 35 ± 1°C, depending on test matrix.

(c) *Light box for back illuminating and counting plates*.

(d) *Magnifying glass*.— 2× or 4× for examining plates.

(e) *Stomacher*.—Seward 400 paddle type or equivalent.

Safety Precautions

(a) Perform tests with clean washed and gloved hands, assuming potential pathogenic bacteria.

(b) Microbiological cultures and reagents should be collected into biohazardous bags and autoclaved. Dispose of according to local, state, and federal regulations.

General Preparation

(a) Observe Good Laboratory Practices for microbial testing. Avoid specimen contamination.

(b) Test on a level surface in a clean area that is free of dust and blowing air.

(c) Avoid hand contact with test samples and Peel Plate EC medium.

(d) Log serially dilute sample into Butterfield's or microbiologically suitable water to obtain the countable range 1–154 coliform/mL or test multiple dilutions to attain the countable range.

Sample Preparation

Dairy:

(a) White milk dairy samples (raw milk and pasteurized whole, lower fat percentage, and skim) may be tested directly or serially diluted to a countable range (1–154 CFU/mL).

(*1*) To serially dilute, add 11 mL into 99 mL dilution buffer. Other automated dilution pipets and dilution schemes are acceptable.

(b) Neat chocolate milk should be diluted 1 part to 1 part buffer (1:2 dilution) and 1 mL plated onto two plates. Chocolate milk may also be serially diluted into a countable range (1–154 CFU/mL).

(c) Add 11 g of solid dairy (ice cream, sour cream, heavy cream, condensed whey, etc.) to 99 mL of dilution buffer that has been mixed 25 times in an arc of 1 ft in 7 s. Perform additional dilutions as needed to reach countable range (1–154 CFU/mL).

(d) For fermented solid dairy (cottage cheese, yogurt, shredded cheese, etc.) containing active lactic acid bacteria (laboratory culture) prepare a selective diluent (SBDB) by dissolving 0.2 g sodium bisulfite into 99 mL dilution blank. Add 11 g product in 99 mL SBDB and homogenize or stomach for 1 min.

(e) For milk powders and evaporated/condensed, reconstitute with water to normal milk solid content and let any undissolved solids settle. Test liquid fraction as with dairy.

Foods (ground meats, liquid eggs, dried dog food, chocolate):

(a) Add 50 g of food (ground meat, ground dried dog food, or 30°C liquefied chocolate) to 450 mL of dilution buffer, shake (25 times in arc 1 ft for 7 s), and let settle 1 min to test sample.

(b) For eggs, add 100 g to 900 mL of microbiologically suitable dilution blank, shake (25 times in arc 1 ft for 7 s), and let settle 1 min to test sample.

(c) Continue to dilute 10 mL of prior dilution in 90 mL of dilution blank to reach countable range (1–154 CFU/mL).

Waters:

(a) Water may be tested directly to achieve a detection limit of 1 coliform/mL. If water is chlorine disinfected, it should be neutralized with sodium thiosulfate (1 tablet containing 10 mg/100 mL water sample) before testing.

(b) For a detection limit of 1 coliform/100 mL, a 100 mL water sample may be filtered through a 0.45 µm mixed cellulose filter and applied to a Peel Plate EC rehydrated with 1.5 mL sterile water.

Method Procedure

(a) Place Peel Plate onto a level surface. Apply pressure with fingers to the rear rectangular platform to keep the plate flat.

(b) Lift the cover vertically upwards, completely exposing the dried media culture disc. Leave the cover adhered to the back of the plate.

(c) While holding the cover up, keeping the plate flat on the surface, vertically dispense 1.0 mL of sample or sample dilution to the center of the exposed Peel Plate disc. Expel pipet contents rapidly with even force within 2–3 s. The sample will self-wick to the edges of the disc.

(*1*) In case of testing filtered water, rehydrate the center of the disc with 1.5 mL sterile water and apply filter of filtered water sample, with filtered side up.

(d) Reapply the adhesive cover without wrinkling. Press cover around edges of plate to ensure proper seal.

(e) Incubate plates with adhesive cover down, clear side up.

(*1*) Incubate at 32 ± 1°C for 24 ± 2 h for milk and dairy products, except yogurt (48 ± 3 h).

(*2*) Incubate at 35 ± 1°C for 24 ± 2 h for water, environmental, and meat samples.

(*3*) Plates can stack by aligning the two pillars. Stacking up to 20 will not affect plate heat transfer.

Interpretation and Test Result Report

(a) At the end of the incubation period, observe plates for colonies viewed through the clear side of the Peel Plate EC. Each colored spot, blue/purple (*E. coli*) and red (coliform), represents 1 CFU. The sum of spots is reported as the total coliform CFU per milliliter of the diluted sample.

(*1*) At 35°C, the sum of blue spots *is E. coli* per milliliter and the sum of red spots is coliform per milliliter in the diluted sample.

(b) Multiply CFU per milliliter by dilution to calculate CFU per milliliter (or CFU per gram) of original sample.

(*1*) In the case of neat chocolate milk, add the sum of the two plates of the 1:2 diluted product for a CFU per milliliter of neat product.

(*2*) In the case of yogurt, score dark red and blue/purple colonies in the reddish background of the plate.

(c) In case of spreading bacteria, score 1 CFU for each count dark spot within the spread growth as a single colony. Blended colonies are scored as a single CFU.

(d) Counts of 1–154 CFU/mL (or g) are considered countable, whereas counts outside that range are considered estimates. Samples with results outside of countable range (>154 CFU/mL or g) can be diluted and retested.

Confirmation

The Peel Plate EC method uses selective medium and enzyme substrates to detect coliform and generic *E. coli* without the need for confirmation steps. Although it is not necessary, it may be desired to confirm colonies into a traditional selective medium. The cover may be lifted and colonies picked into Brilliant Green Lactose Broth (BGLB) broth to confirm coliform. BGLB will acidify and gas in 48 ± 3 h at 35 ± 1°C when coliform is present. *E. coli* may be confirmed by picking colonies into LC-MUG broth and observing for fluorescence after 48 ± 3 h at 35 ± 1°C. Appropriate optional confirmation procedures are described in FDA/BAM Chapter 4 (6).

Validation Study

This validation study was conducted under the AOAC Research Institute *Performance Tested Method*^{SM} program and the AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (13). Method developer studies were conducted at Charm Sciences, Inc., Lawrence, MA, and included matrix studies for all claimed matrixes, product consistency and stability studies, and robustness testing. The independent laboratory study was conducted by Q Laboratories, Inc., Cincinnati, OH, and included the inclusivity/exclusivity studies and matrix studies for seven of the claimed food/and or surface matrixes. Additionally, collaborative dairy matrixes followed NCIMS Laboratory Committee protocol of using at least three external laboratories and five fortified study concentrations. The four validated dairy matrixes were prepared by Q Laboratories and tested as well as sent to four additional testing laboratories: Milk Regulatory Consultants, Russellville, MO; Eurofin-DQCI, Mound View, MN; Dairygold, Tukwila, WA; and Charm Sciences, Inc., Lawrence, MA.

Inclusivity and Exclusivity Studies

The inclusivity and exclusivity evaluation was conducted at Q Laboratories. All test materials required for the Peel Plate EC method were provided by Charm Sciences, Inc.

Methodology.—In these studies, 58 different coliform inclusivity isolates (including 17 *E. coli* strains) and 32 nontarget aerobic bacteria exclusivity isolates were evaluated using the Peel Plate EC method. All inclusivity isolates were cultured in lauryl tryptose broth (LST) at 35 ± 1°C for 24 ± 2 h. All exclusivity isolates were cultured in the nonselective medium, brain heart infusion broth, at temperatures and conditions optimal for organism growth. All isolates were serially diluted in Butterfield's phosphate buffer to obtain a level in which the countable range (1–154 CFU/plate) of the Peel Plate EC method was expected. The Peel Plate EC method followed the manufacturer's directions. All plating was performed in duplicate for each isolate. The duplicate plates were stacked and incubated at two temperatures: 35 ± 1°C and 32 ± 1°C for 24 ± 2 h. Following incubation, the plates were enumerated by counting all red colonies (coliform) and blue/purple/green colonies (*E. coli*), according to the manufacturer's instructions.

Results and discussion.—Tables 1 and 2 present the inclusivity bacterial strains and exclusivity study strains. Of the 58 coliform inclusivity isolates evaluated at both 32 and 35°C, 57 were correctly detected, including all 17 *E. coli* strains. The one inclusivity strain that was incorrectly excluded by the method was *Escherichia blattae* American Type Culture Collection (ATCC, Manassas, VA) 29907. In addition to this, six *E. coli* isolates produced red colonies instead of the typical blue/purple/green colonies. This may be because they were weak producers or did not produce glucuronidase enzyme, which is produced by the majority of generic *E. coli* strains. At least two of these strains, O157:H7 (ATCC 43895) and O145 National Collection of Type Cultures (United Kingdom) 10279, are Shiga type *E. coli* known not to produce the enzyme. Of the 32 exclusivity strains evaluated, 31 were correctly excluded. The strain that was detected as coliform was *Shigella sonnei* (ATCC 9290). The only difference in coloration observed between isolates incubated at 35°C versus 32°C was with *E. coli* ATCC 11229. At 32°C, the isolate produced a blue-colored colony, but at 35°C the isolate produced a purple-colored colony. No differences were observed in the coloration of the remaining isolates between the two temperatures; however, some differences were observed between the colony counts enumerated on plates incubated at 32 and 35°C for the same isolates.

Method Comparison Studies and Independent Laboratory Studies

Peel Plate EC: Dairy Matrixes

Dairy matrixes were evaluated in Peel Plate EC at 32 ± 1°C for 24 ± 2 h, with the exception of yogurt for 48 ± 3 h, in

Table 1. Inclusivity organisms

Genus/species	Source	ID Mo.	32°C Peel Plate EC, CFU/plate	Colony color(s)	35°C Peel Plate EC, CFU/plate	Colony color(s)
Citrobacter amalonaticus	ATCC[a]	25405	8	Red	38	Red
Citrobacter braakii	ATCC	43162	138	Red	142	Red
Citrobacter farmeri	ATCC	51633	21	Red	64	Red
Citrobacter freundii	ATCC	8090	67	Red	99	Red
C. freundii	NCTC[b]	9750	63	Red	107	Red
C. freundii	ATCC	43864	111	Red	133	Red
C. freundii	Q Laboratories[c]	QL11007-10	128	Red	146	Red
Citrobacter koseri	ATCC	27156	113	Red	119	Red
C. koseri	ATCC	BAA-895	56	Red	76	Red
Citrobacter youngae	ATCC	11102	106	Red	137	Red
Cronobacter muytjensii	ATCC	51329	121	Red	131	Red
Cronobacter sakazakii	Q Laboratories	QL11007-9	81	Red	95	Red
Enterobacter aerogenes	ATCC	13048	117	Red	123	Red
E. aerogenes	ATCC	35029	126	Red	132	Red
E. aerogenes	ATCC	51697	102	Red	98	Red
Enterobacter amnigenus	ATCC	51816	122	Red	149	Red
Enterobacter cancerogenus	Q Laboratories	QL11010-1	52	Red	56	Red
Enterobacter cloacae	ATCC	13047	131	Red	139	Red
E. cloacae	NBRC[d]	13535	52	Red	51	Red
E. cloacae	NBRC	13536	110	Red	118	Red
E. cloacae	ATCC	23355	70	Red	84	Red
Enterobacter gergoviae	ATCC	33028	88	Red	102	Red
Escherichia blattae	ATCC	29907	0	NA[e]	0	NA
Escherichia coli	ATCC	26	15	Purple	15	Purple
E. coli	ATCC	4157	15	Purple	17	Blue
E. coli	ATCC	8677	61	Purple	63	Purple
E. coli	ATCC	8739	80	Red	45	Red
E. coli	ATCC	9637	83	Purple	58	Purple
E. coli O145	NCTC[b]	10279	109	Red	113	Red
E. coli	ATCC	10536	46	Red	61	Red
E. coli	ATCC	11229	64	Blue	76	Purple
E. coli	ATCC	13706	50	Blue	53	Blue
E. coli	NBRC	15034	128	Blue	134	Blue
E. coli	ATCC	25922	126	Blue	127	Blue
E. coli	ATCC	35218	131	Purple	148	Purple
E. coli	ATCC	35421	86	Blue	97	Blue
E. coli O157:H7	ATCC	43895	117	Red	125	Red
E. coli	ATCC	51813	78	Red	83	Red
E. coli	Q Laboratories	QL11007-8	93	Purple	90	Purple
E. coli	Q Laboratories	QL11010-2	50	Red	46	Red
Escherichia fergusonii	ATCC	35469	13	Red	52	Red
E. fergusonii	ATCC	35470	16	Red	33	Red
Escherichia hermannii	ATCC	33650	32	Red	43	Red
E. hermannii	ATCC	33651	88	Red	106	Red
Escherichia vulneris	ATCC	29943	37	Red	52	Red
Hafnia alvei	ATCC	51815	139	Red	141	Red
Klebsiella oxytoca	ATCC	43165	107	Red	112	Red
Klebsiella pneumoniae	ATCC	49334	68	Red	62	Red
K. pneumoniae	ATCC[1]	700324	76	Red	78	Red
K. pneumoniae	ATCC[1]	10031	4	Red	11	Red
K. pneumoniae subsp. *ozaenae* Type 4	ATCC	11296	17	Red	35	Red
K. pneumoniae	ATCC	13882	54	Red	65	Red

Table 1. (*continued*)

Genus/species	Source	ID Mo.	32°C Peel Plate EC, CFU/plate	Colony color(s)	35°C Peel Plate EC, CFU/plate	Colony color(s)
K. pneumoniae	ATCC	13883	113	Red	120	Red
K. pneumoniae subsp. *pneumonia*	ATCC[1]	4352	52	Red	67	Red
K. pneumoniae subsp. *pneumonia*	Q Laboratories	QL11007-7	120	Red	128	Red
Kluyvera intermedia	ATCC	33110	29	Red	2	Red
Pantoea agglomerans	ATCC	19552	7	Red	36	Red
Rahnella aquatilis	ATCC	55046	82	Red	90	Red

[a] ATCC = American Type Culture Collection.

[b] NCTC = National Collection of Type Cultures.

[c] Q Laboratories, Inc., Culture Collection.

[d] NBRC = National Biological Resources Center (Japanese Culture Collection).

[e] NA = Not available.

comparison to VRBA 32 ± 1°C for 24 ± 2 h for total coliform, the sum of coliform and *E. coli*. *E. coli* color differentiation in dairy products was erratic and varied by dairy matrix type, so it was determined to not claim *E. coli* differentiation from coliform in dairy products and use the combined colonies regardless of color as a total coliform determination.

Sample preparation.—In all dairy matrix studies, cocktails of assorted heat-stressed (50°C for 10 min) coliform and *E. coli* strains were fortified into product and allowed to acclimate for 48 h at 2–8°C. The acclimated material was quantified using the VRBA method and then used for creating fortification levels. Whole milk, skim, chocolate, and heavy cream test samples were prepared by Q Laboratories and sent to three NCIMS testing laboratories (Milk Regulatory Consultants, Eurofin-DQCI, and Dairygold) and to the Charm Sciences laboratory for testing as part of the NCIMS validation. Five fortified concentrations targeting below and above the NCIMS Pasteurized Milk Ordinance action level, 10 coliform/mL or g product and ranging 2 logs, were evaluated per the NCIMS requirement. Additional dairy matrixes prepared in the manufacturer's laboratory used three to five concentrations to meet AOAC Research Institute Performance Tested Method validation requirement for claimed matrixes. Neat and/or 10^{-1} dilutions were used to evaluate detection in the countable ranges of 1–154 CFU/mL dilution.

Reference method for dairy.—Five replicate test portions from each contamination level were assayed in duplicate according to a modification of the FDA/NCIMS Form 2400a guidelines. Neat samples were shaken 25 times in 7 s with a 1 ft movement. Within 3 min of agitation for whole, skim, and chocolate milk test matrixes, 1:10 dilutions were prepared by adding 11 mL (or 11 g) of neat sample into 99 mL of BPBDW and shaken 25 times in 7 s with a 1 ft movement. Within 3 min of agitation and after bubble settling, 1 mL neat and 1:10 sample dilutions were plated into the Petri dishes. For heavy cream and other dairy products for AOAC Research Institute validation of the method, 1 mL of the 1:10 dilution was plated in duplicate. Approximately 10 mL of tempered (44–46°C) VRBA was poured into the Petri dishes, swirled, and allowed to solidify. The plates were inverted and incubated at 32 ± 1°C for 24 ± 2 h. Following incubation, typical colonies (≥0.5 mm colonies surrounded by a zone of precipitated bile acids) were enumerated. Duplicate plates in the countable range of 1–150 colonies were averaged and reported as coliform count/mL (or count/g).

Peel Plate EC method for dairy.—Five replicate test portions from each contamination level were assayed in duplicate. Whole and skim milk samples were evaluated at neat and 1:10 dilution levels in duplicate. Neat chocolate and strawberry milk were diluted 1:1 and two 1 mL portions plated per sample. Other dairy products were diluted 1:10 and 1 mL plated. As noted in the method description, cultured dairy products containing active laboratory cultures (e.g., cottage cheese, shredded cheese, yogurt) were added and homogenized in SBDB before testing. The Peel Plate EC covers were lifted to fully expose the dried media culture disc. Test portions were shaken 25 times in 7 s with a 1 ft movement. The 1:10 dilutions were prepared by adding 11 mL of neat sample into 99 mL of BPBDW and shaken 25 times in 7 s with a 1 ft movement. Within 3 min of agitation, 1 mL sample aliquots were dispensed onto the center of the disc. The covers were reapplied and sealed over the disc. Peel Plate EC plates were inverted, stacked 20 high, and incubated with the cover down at 32 ± 1°C for 24 ± 2 h or 48 ± 3 h for yogurt.

Confirmation methods.—Although the Peel Plate EC coliform detection is specific, both Peel Plate EC and VBRA results had 10% colonies picked for coliform confirmation. To confirm coliform, up to 10 colonies from each sample were picked and transferred to BGLB and incubated at 35 ± 1°C for 48 ± 3 h. After incubation, the BGLB tubes were examined for gas production, indicating a positive reaction and presence of coliforms. To confirm *E. coli,* a loopful of broth from each positive BGLB tube was transferred to *E. coli* medium with 4-methylumbelliferyl-β-D-glucuronide (EC-MUG) and incubated at 35 ± 1°C for 48 ± 3 h. After incubation, EC-MUG tubes were examined for fluorescence under UV light. Fluorescing EC-MUG tubes indicated the presence of *E. coli* and were confirmed by transferring a loopful of broth from a positive EC-MUG tube to tryptic soy agar and incubating the TSA at 35 ± 1°C for 24 ± 2 h. Growth from TSA was used for final confirmation of *E. coli* by VITEK® 2 GN following AOAC *Official Method* **2011.17**.

Dairy matrix results and discussion.—Analyses of all matrixes were conducted for each contamination level. Reported are confirmed coliforms by the BGLB method. Logarithmic transformations of the total coliform counts (CFU per gram or CFU per milliliter) and paired statistical analysis were performed. The difference of means and their 95% CIs for each contamination level were determined. A $\log_{10}$ mean difference value less than the standard alpha value of 0.5 with

Table 2. Exclusivity organisms

Genus/species	Source	ID No.	32°C Peel Plate EC, CFU/plate	Colony color(s)	35°C Peel Plate EC, CFU/plate	Colony color(s)
Acinetobacter baumannii	ATCC[a]	19606	0	NA[b]	0	NA
Acinetobacter calcoaceticus	ATCC	23055	0	NA	0	NA
Aeromonas caviae	ATCC	15468	0	NA	0	NA
Aeromonas hydrophila	ATCC	49140	0	NA	0	NA
Alcaligenes faecalis	ATCC	8750	0	NA	0	NA
Bacillus amyloliquefaciens	ATCC	23842	0	NA	0	NA
Bacillus cereus	ATCC	11778	0	NA	0	NA
Bacillus pumilis	ATCC	700814	0	NA	0	NA
Bacillus subtilis	ATCC	6051	0	NA	0	NA
Corynebacterium jeikeium	ATCC	43734	0	NA	0	NA
Edwardsiella tarda	ATCC	15947	0	NA	0	NA
Lactobacillus casei	ATCC	11578	0	NA	0	NA
Lactobacillus lactis	ATCC	4797	0	NA	0	NA
L. lactis	ATCC	11454	0	NA	0	NA
Micrococcus luteus	ATCC	10240	0	NA	0	NA
Morganella morganii	ATCC	25829	0	NA	0	NA
Proteus hauseri	ATCC	13315	0	NA	0	NA
Proteus mirabilis	ATCC	7002	0	NA	0	NA
Proteus vulgaris	ATCC	6380	0	NA	0	NA
Providencia rettgeri	ATCC	14505	0	NA	0	NA
Providencia stuartii	Q Laboratories[c]	QL11007-5	0	NA	0	N/A
Pseudomonas aeruginosa	ATCC	27853	0	NA	0	NA
Pseudomonas alcaligenes	ATCC	14909	0	NA	0	NA
Pseudomonas fluorescens	ATCC	13525	0	NA	0	NA
Salmonella enterica subspecies *enterica* serovar Enteritidis	ATCC	13076	0	NA	0	NA
Serratia marcescens	ATCC	14756	0	NA	0	NA
Shigella flexneri	ATCC	9199	0	NA	0	NA
Shigella sonnei	ATCC	9290	53	Red	68	Red
Staphylococcus aureus	ATCC	6538	0	NA	0	N/A
Streptococcus pyogenes	ATCC	9696	0	NA	0	NA
Staphylococcus warneri	ATCC	49454	0	NA	0	NA
Yersinia enterocolitica	ATCC	49397	0	NA	0	NA

[a] ATCC = American Type Culture Collection.

[b] NA = Not available.

[c] Q Laboratories, Inc., Culture Collection.

CIs within –0.5 and 0.5 indicated no statistical difference between the Peel Plate EC and VRBA methods. Results are reported in Table 3. Additional NCIMS laboratory participant's data are reported and compared in Annex Tables 1–4, along with graphical presentation of all log means for whole milk, skim, chocolate and light cream in Annex Figures 1–4. The dairy matrixes studied—whole milk, chocolate milk, heavy cream, UHT whole milk, lactose-reduced milk, strawberry milk, sour cream, vanilla ice cream, egg nog, raw cow milk, raw goat milk, raw sheep milk, powdered milk, and pasteurized goat milk—showed no significant differences with the reference method VRBA except in a few of the lowest concentrations where spike levels caused a high SD and an upper confidence limit (UCL) greater than 0.5 log. Only the skim matrix showed significant differences at the middle, middle-high, and high spike levels. These differences were observed by all laboratories using the shared samples and were more pronounced in the neat results than in the 10^{-1} dilution. It is not clear why only the higher spike levels showed the difference, but the low and middle-low did not. The target bacterial high spike level (>20 000) compared with the observed high spike level (approximately 6000) shows that the bacteria were additionally stressed in the skim matrix during the 48 h acclimation period. It appears that the reference method better resuscitated the stressed organisms compared with Peel Plate EC. A matrix influence and stress on the bacteria are also partially supported by UCL of skim powder milk lower than $\log_{10}$ 0.5. Additional experiments to try to replicate this hypothesized matrix effect were not successful. In two additional experiments using the same and different *E. coli*/coliform strains, the mean $\log_{10}$ differences between the comparative and reference methods did not show significant differences with UCL and lower confidence limit within $\log_{10}$ (–0.5 and 0.5). This would suggest that the observed differences in the matrix samples were a sample preparation anomaly.

Table 3. Peel Plate EC method for total coliform versus VRB method with dairy matrixes

Matrix	Fortified microorganisms ATCC No. (% injury)	Contamination level	Candidate method			Reference method				95% CI[e]		r^{2h}
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r	Mean diff.[d]	LCL[f]	UCL[g]	
Whole milk	*Enterobacter amnigenus* 51816 (66%) *Escherichia coli* 8739 (71%)	None	<0.1	NA[i]	NA	<0.1	NA	NA	NA	NA	NA	
		1	0.898	0.124	13.8	0.924	0.122	13.2	−0.026	−0.151	0.099	0.99
		2	1.414	0.066	4.7	1.442	0.114	7.9	−0.028	−0.121	0.065	
		3	1.547	0.064	4.1	1.641	0.086	5.2	−0.093	−0.182	−0.005	
		4	1.628	0.072	4.4	1.749	0.082	4.6	−0.121	−0.216	−0.026	
		5	2.384	0.086	3.6	2.528	0.071	2.8	−0.144	−0.224	−0.064	
Whole milk[j]	*E. amnigenus* 51816 (66%) *E. coli* 8739 (71%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	
		1	0.754	0.173	22.9	0.818	0.325	39.7	−0.065	−0.387	0.258	0.99
		2	1.437	0.100	7.0	1.564	0.088	5.6	−0.127	−0.206	−0.047	
		3	1.528	0.065	4.3	1.698	0.055	3.2	−0.170	−0.214	−0.127	
		4	1.713	0.062	3.6	1.845	0.029	1.6	−0.132	−0.181	−0.083	
		5	2.370	0.064	2.7	2.601	0.068	2.6	−0.231	−0.309	−0.153	
Chocolate milk	*Citrobacter freundii* 8090 (53%) *E. coli* 11229 (55%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	
		1	0.779	0.139	18	0.897	0.107	12	−0.175	−0.211	−0.138	0.97
		2	1.135	0.136	12.0	1.452	0.067	4.6	−0.317	−0.44	−0.194	
		3	1.462	0.138	9.4	1.76	0.075	4.3	−0.298	−0.391	−0.206	
		4	1.599	0.102	6.4	1.877	0.057	3.0	−0.278	−0.355	−0.2	
		5	1.947	0.04	2.1	2.121	0.039	1.8	−0.175	−0.211	−0.138	
Chocolate milk[j]	*C. freundii* 8090 (53%) *E. coli* 11229 (55%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	
		1	0.832	0.121	14.5	1.037	0.109	10.5	−0.214	−0.343	0.086	0.93
		2	1.001	0.383	38.3	1.467	0.078	5.3	−0.466	−0.77	−0.161	
		3	1.591	0.119	7.5	1.851	0.04	2.2	−0.261	−0.331	−0.191	
		4	1.475	0.16	10.8	1.921	0.034	1.8	−0.446	−0.553	−0.337	
		5	1.717	0.076	4.4	2.13	0.02	0.9	−0.412	−0.47	−0.354	
Skim milk	*Enterobacter cloacae* 13047 (61%) *E. coli* 51813 (50%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.69
		1	0.458	0.19	41.5	0.352	0.249	70.7	0.107	−0.054	0.267	
		2	1.089	0.088	8.1	1.063	0.088	8.3	0.026	−0.056	0.108	
		3	0.491	0.251	51.1	1.551	0.048	3.1	−1.06	−1.238	−0.883	
		4	1.72	0.177	10.3	2.07	0.201	9.7	−0.351	−0.532	−0.17	
		5	2.014	0.136	6.8	2.441	0.077	3.2	−0.428	−0.547	−0.308	
Skim milk[j]	*E. cloacae* 13047 (61%) *E. coli* 51813 (50%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.77
		1	0.456	0.555	121.7	0.474	0.535	112.9	−0.018	−0.512	0.477	
		2	1.046	0.138	13.2	1.22	0.104	8.5	−0.174	−0.336	−0.013	
		3	0.452	0.267	59.1	1.413	0.055	3.9	−0.961	−1.15	−0.772	
		4	1.648	0.233	14.1	2.055	0.92	44.8	−0.407	−0.555	−0.26	
		5	1.869	0.113	6.0	2.383	0.061	2.6	−0.515	−0.588	−0.441	
Heavy cream	*Enterobacter aerogenes* 13048 (54%) *E. coli* 25922(56%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1
		Low	1.36	0.556	40.9	1.274	0.508	39.9	0.087	−0.179	0.352	
		Medium	1.917	0.161	8.4	1.81	0.153	8.5	0.107	0.011	0.202	
		High	2.141	0.132	6.2	2.045	0.104	5.1	0.095	−0.038	0.229	
Heavy cream[j]	*E. aerogenes* 13048 (54%) *E. coli* 25922 (56%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.97
		Low	1.272	0.173	13.6	1.218	0.478	39.2	0.053	−0.349	0.456	
		Medium	1.802	0.104	5.8	1.612	0.182	11.3	0.19	0.032	0.348	
		High	2.112	0.107	5.1	2.061	0.085	4.1	0.051	−0.052	0.155	
Powder skim milk	*E. coli* 25922 (40%) *C. freundii* 8090 (39%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1
		Low	0.67	0.28	41.3	1.08	0.10	9.0	−0.41	−0.60	−0.22	
		Medium	1.37	0.07	5.1	1.77	0.06	3.2	−0.40	−0.48	−0.33	
		High	1.58	0.08	5.4	2.04	0.04	2.2	−0.45	−0.52	−0.38	

Table 3. (*continued*)

Matrix	Fortified microorganisms ATCC No. (% injury)	Contamination level	Candidate method			Reference method			Mean diff.[d]	95% CI[e]		r[2h]
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[f]	UCL[g]	
Past. whole goat milk	*E. coli* 25922 (29%) *C. freundii* 8090 (68%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.9
		Low	1.42	0.05	3.2	1.34	0.11	8.1	0.08	0.01	0.16	
		Medium	1.32	0.14	10.2	1.48	0.05	3.7	−0.16	−0.27	−0.06	
		High	2.02	0.04	1.9	2.07	0.06	2.9	−0.05	−0.11	0.00	
Raw cow milk	*E. coli* 25922 (0%) *C. freundii* 8090 (0%)	Natural	−0.35	0.57	164	−0.63	0.54	94.7	0.28	−0.23	0.8	0.99
		Low	0.71	0.60	85.3	0.23	0.24	105.4	0.48	0.05	0.90	
		Medium	1.62	0.29	18.2	1.53	0.35	22.6	0.09	−0.27	0.44	
		High	2.08	0.10	4.7	2.01	0.13	6.3	0.06	−0.05	0.18	
Raw goat milk	*E. coli* 25922 (0%) *C. freundii* 8090 (0%)	Natural	0.26	0.5	196	0.44	0.53	120	−0.28	−0.70	0.34	0.93
		Low	0.47	0.24	52.0	0.37	0.51	139.9	0.10	−0.34	0.54	
		Medium	0.64	0.19	29.3	0.71	0.27	37.8	−0.07	−0.34	0.19	
		High	1.12	0.12	10.3	1.05	0.08	7.6	0.07	0.00	0.14	
Raw sheep milk	*E. coli* 25922 (30%) *C. freundii* 8090 (58%)	Natural	−0.58	0.53	92	−0.11	0.64	569	−0.47	−1.18	0.24	0.84
		Low	1.22	0.46	37.6	1.36	0.20	15.0	−0.14	−0.49	0.22	
		Medium	1.55	0.26	16.7	1.36	0.52	38.3	0.19	−0.16	0.55	
		High	2.04	0.08	4.1	1.56	0.56	35.9	0.49	0.05	0.93	
Vanilla ice cream	*E. coli* 25922 (27%) *C. freundii* 8090 (39%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.97
		Low	2.13	0.15	6.8	2.38	0.12	5.2	−0.25	−0.42	−0.08	
		Medium	2.42	0.06	2.5	2.69	0.05	1.9	−0.27	−0.32	−0.21	
		High	2.51	0.08	3.2	2.88	0.06	2.0	−0.37	−0.45	−0.29	
Sour cream	*E. coli* 25922 (40%) *C. freundii* 8090 (34%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1
		Low	−0.17	0.59	−349.2	−0.16	0.61	−386.3	−0.01	−0.69	0.66	
		Medium	1.41	0.08	5.9	1.35	0.09	6.9	0.06	−0.04	0.16	
		High	1.65	0.07	4.3	1.65	0.06	3.4	0.00	−0.06	0.07	
Lactose reduced milk	*E. coli* 25922 (37%) *C. freundii* 8090 (71%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.98
		Low	1.44	0.11	7.9	1.27	0.10	8.1	0.17	0.04	0.30	
		Medium	1.56	0.09	5.7	1.59	0.08	5.2	−0.03	−0.09	0.03	
		High	2.00	0.10	4.9	2.19	0.04	1.6	−0.19	−0.26	−0.11	
UHT- whole milk	*E. coli* 25922 (32%) *C. freundii* 8090 (43%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1
		Low	1.28	0.09	6.9	1.24	0.11	8.5	0.04	−0.06	0.13	
		Medium	1.82	0.05	2.7	1.91	0.04	1.9	−0.09	−0.12	−0.06	
		High	2.04	0.03	1.5	2.15	0.04	1.9	−0.11	−0.16	−0.07	
Egg nog	*E. coli* 25922 (24%) *C. freundii* 8090 (35%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	
		Low	1.58	0.25	15.8	1.65	0.19	11.4	−0.07	−0.29	0.16	
		Medium	1.80	0.18	9.8	1.98	0.17	8.6	−0.18	−0.40	0.04	0.98
		High	2.36	0.09	4.0	2.45	0.11	4.5	−0.09	−0.20	0.03	
Strawberry milk	*E. coli* 25922 (25%) *C. freundii* 8090 (27%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.99
		Low	1.54	0.09	5.9	1.76	0.06	3.2	−0.23	−0.30	−0.15	
		Medium	2.03	0.03	1.6	2.23	0.02	1.0	−0.20	−0.22	−0.17	
		High	2.39	0.19	7.8	2.47	0.11	4.5	−0.08	−0.23	0.06	
Condensed milk	*E. coli* 25922 (23%) *C. freundii* 8090 (94%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1
		Low	0.52	0.68	130.8	0.65	0.57	87.1	−0.14	−0.77	0.50	
		Medium	1.90	0.24	12.4	1.95	0.13	6.5	−0.05	−0.25	0.16	
		High	2.57	0.07	2.6	2.69	0.07	2.6	−0.13	−0.21	−0.05	
Cottage cheese	*E. coli* 25922 (12%) *E. cloacae* 13047 (26%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.98
		Low	1.96	0.22	11.1	2.10	0.13	6.1	−0.14	−0.33	0.06	
		Midium	2.86	0.05	1.6	2.82	0.03	1.2	0.04	0.01	0.08	
		High	3.07	0.06	1.9	3.18	0.04	1.4	−0.11	−0.17	−0.06	

Table 3. (*continued*)

Matrix	Fortified microorganisms ATCC No. (% injury)	Contamination level	Candidate method			Reference method				95% CI[e]		r^{2h}
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r	Mean diff.[d]	LCL[f]	UCL[g]	
Condensed whey (20%)	*E. coli* 25922 (12%) *E. cloacae* 13047 (20%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.99
		Low	2.02	0.11	5.4	2.08	0.21	9.9	−0.07	−0.19	0.06	
		Midium	2.41	0.04	1.8	2.43	0.11	4.6	−0.02	−0.10	0.06	
		High	2.81	0.04	1.3	2.89	0.05	1.7	−0.08	−0.12	−0.04	
Shredded cheese	*E. coli* 25922 (12%) *E. cloacae* 13047 (26%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.99
		Low	1.57	0.27	17.1	1.80	0.11	6.1	−0.23	−0.42	−0.04	
		Midium	2.42	0.09	3.9	2.52	0.12	4.6	−0.10	−0.18	−0.02	
		High	2.74	0.11	3.9	2.91	0.07	2.3	−0.18	−0.28	−0.07	
Yogurt	*E. coli* 25922 (12%) *E. cloacae* 13047 (26%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.94
		Low	2.10	0.12	5.6	2.30	0.14	6.1	−0.20	−0.32	−0.09	
		Midium	2.47	0.09	3.8	2.54	0.12	4.6	−0.07	−0.19	0.05	
		High	2.79	0.06	2.3	3.05	0.09	2.8	−0.26	−0.30	−0.21	

[a] Mean of five replicate portions, plated in duplicate, after logarithmic transformation: Log_{10} [CFU/g + (0.1)f].

[b] Repeatability SD.

[c] Relative SD for repeatability.

[d] Mean difference between the candidate and reference methods.

[e] Confidence interval.

[f] 95% lower confidence limit for difference of means.

[g] 95% upper confidence limit for difference of means.

[h] Square of correlation coefficient.

[i] NA = Not applicable.

[j] Independent laboratory–performed.

In the case of cultured dairy products containing the laboratory cultures, the homogenized products without the additional selective SBDB cause a strong red color interference in the Peel Plate EC test. It is speculated that the protein curds are protecting these laboratory cultures from the selective chemicals of Peel Plate EC and thus the laboratory cultures are expressing β-galactosidase enzyme during growth. With the SBDB, the Peel Plate EC method is able to selectively identify coliform from any remaining laboratory culture interference, if any. Thus the SBDB in sample preparation of the cultured products is critical. There were no significant differences observed with shredded cheese and cottage cheese made in SBDB compared with the reference method prepared in BPBDW. There were no significant differences between methods observed with yogurt except that yogurt required additional incubation, a total of 48 ± 3 h, to distinguish coliform colonies from the reddish laboratory culture background. The dairy matrixes data support that Peel Plate EC total coliform results without a confirmatory step are not significantly different from VRBA/BGLB confirmation of total coliform results.

Peel Plate EC: Nondairy Matrixes

Ground meat, liquid eggs, dried dog food, chocolate, surface sponges of stainless steel and large animal hides, bottled water, flume water, and irrigation water matrixes were evaluated in Peel Plate EC at 35 ± 1°C for 24 ± 2 h in comparison to FDA/BAM, USDA MLG, and EPA methods as described in the following method paragraphs.

Sample preparation.—Ground beef, ground turkey, and pasteurized liquid eggs were purchased at local grocery stores. Sample matrixes were split into control and low, medium, and high fortification levels and inoculated with various freshly cultured, or heat-stressed, coliform and *E. coli* strains indicated in Tables 4 and 5. Five replicate test portions from each contamination level for the ground meats and eggs were assayed in a paired analysis following the Peel Plate EC method and USDA MLG 3.01, through section 3.5, and FDA/BAM Chapter 4, Section I.G., solid medium method MUG reference method. Prepared samples were assayed by the Peel Plate EC and harmonized reference methods at the 1:10 and subsequent serial dilution levels to get countable ranges of 1–154 CFU/mL.

Following the USDA MLG 3.01 guidelines, 450 mL of sterile buffered peptone water were added to 50 ± 0.1 g test portions of ground meat in sterile stomacher bags. For liquid eggs, 900 mL BPBDW was added to 100 mL of the test portion to make a 1:10 dilution. The samples were homogenized for 2 min. Subsequent dilutions were prepared by adding 10 mL of the prior dilution into 90 mL of BPBDW to achieve a countable range of 1–154 CFU/mL.

Hog rinse sponge samples representing 300 cm^2/25 mL were sent frozen from an abattoir from Missouri. These were thawed and pooled for subsequent fortification and testing. Whole chicken carcasses were purchased from a local grocer and 400 mL BPBDW per carcass added in a plastic bag and shaken for 2 min to get a test solution for testing and fortification. Samples were split into control and low, medium, and high fortification levels and inoculated with freshly cultured coliform and *E. coli* strains indicated in Tables 4 and 5. Five replicate test portions from each contamination level were assayed in a paired

Table 4. Peel Plate EC method for total coliform versus VRB method with 35°C matrixes

Matrix	Fortified microorganisms ATCC No. (% injury)	Contamination level	Candidate method			Reference method			Mean diff.[d]	95% CI[e]		r^{2h}
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[f]	UCL[g]	
Ground beef (80% lean)	*Citrobacter freundii* 8090 (0%) *Escherichia coli* 25922 (0%)	None	<0.1	NA[i]	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	1.63	0.25	15.6	1.56	0.35	22.2	0.06	−0.24	0.37	
		Mid	2.98	0.09	3.1	3.06	0.10	3.4	−0.07	−0.10	−0.04	
		High	3.97	0.05	1.2	4.01	0.05	1.1	−0.04	−0.09	0.01	
Ground beef (77% lean)[j]	*Enterobacter aerogenes* 35029 (0%) *E. coli* 11229 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	1.71	0.18	6.3	1.68	0.14	8.2	0.02	−0.15	0.20	
		Mid	2.14	0.05	2.2	2.22	0.06	2.8	−0.08	−0.12	−0.04	
		High	3.17	0.034	1.2	3.20	0.02	0.7	−0.03	0.07	0.02	
Ground turkey	*Enterobacter cloacae* 13047 (0%) *E. coli* 11775 (0%)	Natural	−0.42	0.62	146	−0.26	0.66	257	−0.17	−0.40	0.07	1.0
		Low	2.19	0.12	5.4	2.15	0.07	3.4	0.04	−0.05	0.13	
		Mid	3.13	0.05	1.5	3.14	0.05	1.6	−0.02	−0.07	0.03	
		High	4.13	0.03	0.8	4.16	0.05	1.1	−0.03	−0.07	0.02	
Bottled water	*Klebsiella pneumoniae* 13883 (0%) *E. coli* 11775 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	0.52	0.27	51.6	0.64	0.18	28.1	−0.12	−0.40	0.16	
		Mid	1.24	0.13	10.6	1.32	0.11	8.6	−0.08	−0.20	0.04	
		High	1.55	0.05	3.5	1.62	0.07	4.5	−0.07	−0.13	−0.01	
Bottled water[j]	*K. pneumoniae* 13882 (0%) *E. coli* 35218 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	0.95	0.05	5.1	0.79	0.12	14.6	0.16	0.02	0.28	
		Mid	1.74	0.05	2.8	1.76	0.03	1.6	−0.02	−0.08	0.04	
		High	2.03	0.03	1.6	2.06	0.04	1.7	−0.03	−0.08	0.02	
Stainless steel Surface sponge	*E. cloacae* 13047 (0%) *E. coli* 25922 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	0.80	0.15	18.9	0.72	0.15	20.6	0.07	−0.04	0.18	
		Mid	2.79	0.21	7.5	2.61	0.18	6.8	0.17	0.10	0.24	
		High	3.97	0.13	3.3	3.88	0.11	2.8	0.09	0.01	0.18	
Stainless steel surface sponge[j]	*Citrobacter freundii* 8090 (0%) *E. coli* 25922 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.99
		Low	2.03	0.18	8.8	2.17	0.08	3.9	−0.14	−0.34	−0.06	
		Mid	3.57	0.07	2.0	3.52	0.08	2.1	0.05	0.02	0.09	
		High	4.24	0.08	1.9	4.38	0.09	2.1	−0.14	−0.33	−0.04	
Irrigation water	*Cronobacter sakazakii* 29544 (0%) *E. coli* 25922 (0%)	Natural	0.74	0.16	21.5	0.58	0.12	20.9	0.17	0.03	0.31	0.99
		Low	0.87	0.12	14.3	0.66	0.21	31.0	0.20	0.05	0.35	
		Mid	1.12	0.06	5.1	1.03	0.14	13.6	0.09	−0.03	0.22	
		High	1.34	0.15	10.9	1.25	0.06	4.8	0.09	−0.03	0.21	
Liquid eggs	*K. pneumoniae* 13883 (40%) *E. coli* 25922 (11%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	3.08	0.04	1.5	2.99	0.07	2.4	0.09	0.01	0.16	
		Mid	3.86	0.07	1.7	3.66	0.09	2.5	0.20	0.13	0.26	
		High	4.84	0.05	1.1	4.47	0.12	2.6	0.37	0.30	0.45	
Dried dog food	*C. freundii* 8090 (46%) *E. coli* 25922 (7%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.97
		Low	2.94	0.03	0.9	2.99	0.07	2.4	−0.04	−0.09	0.00	
		Mid	3.93	0.07	1.9	3.95	0.08	2.1	−0.02	−0.07	0.03	
		High	4.98	0.04	0.7	4.98	0.05	1.1	0.00	−0.04	0.05	
Chocolate	*C. freundii* 8090 (0%) *E. coli* 25922 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.81
		Low	1.91	0.22	11.3	1.90	0.21	10.8	0.02	−0.11	0.14	
		Mid	2.94	0.09	3.1	3.00	0.13	4.3	−0.06	−0.11	−0.01	
		High	3.85	0.08	2.1	3.04	0.18	5.8	0.80	0.69	0.92	
Flume water	*K. pneumoniae* 13883 (0%) *E. coli* 25922 (0%)	Natural2	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	2.03	0.17	8.3	1.97	0.06	2.9	0.05	−0.07	0.18	
		Natural1	0.25	0.09	35.0	0.23	0.05	23.2	0.02	−0.05	0.09	
		Mid	2.10	0.27	12.7	1.97	0.04	2.0	0.14	−0.06	0.34	
		High	2.25	0.09	3.9	2.23	0.05	2.4	0.02	−0.05	0.09	

Table 4. (*continued*)

Matrix	Fortified microorganisms ATCC No. (% injury)	Contamination level	Candidate method			Reference method			Mean diff.[d]	95% CI[e]		r^{2h}
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[f]	UCL[g]	
Chicken rinse	*E. cloacae* 13047 (0%) *E. coli* 11775 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.95
		Low	1.44	0.09	6.6	1.48	0.09	6.3	−0.04	−0.14	0.06	
		Mid	2.11	0.07	3.2	2.07	0.15	7.3	0.05	−0.08	0.17	
		High	2.15	0.02	1.0	1.96	0.07	3.7	0.19	0.13	0.25	
Hog carcass rinse	*C. freundii* 8090 (0%) *E. coli* 25922 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	1.30	0.10	7.4	1.46	0.10	6.9	−0.16	−0.26	−0.06	
		Mid	1.45	0.13	8.7	1.65	0.05	2.8	−0.20	−0.31	−0.10	
		High	1.73	0.09	5.3	2.02	0.04	2.2	−0.28	−0.35	−0.22	

[a] Mean of five replicate portions, plated in duplicate, after logarithmic transformation: Log_{10} [CFU/g + (0.1)f].

[b] Repeatability SD.

[c] Relative SD for repeatability.

[d] Mean difference between the candidate and reference methods.

[e] Confidence interval.

[f] 95% lower confidence limit for difference of means.

[g] 95% upper confidence limit for difference of means.

[h] Square of correlation coefficient.

[i] NA = Not applicable

[j] Independent laboratory-performed.

analysis following the Peel Plate EC method and FDA/BAM Chapter 4, I.G., reference method. The serial dilutions were prepared by adding 10 mL from the stomacher bag into 90 mL of BPBDW or prior dilution level to reach countable ranges of coliform.

Dried dog food and chocolate chips were purchased at a local grocer. Dog food was ground <40 mesh. Chocolate chips were melted and held at 35 ± 1°C. Sample matrixes were split into control and low, medium, and high fortification levels and inoculated with various freshly cultured, or heat stressed, coliform and *E. coli* strains indicated in Tables 4 and 5. Five replicate test portions from each contamination level for the ground dog food and chocolate were assayed in a paired analysis following the Peel Plate EC method and FDA/BAM Chapter 4, Section I.G. Prepared samples were assayed by the Peel Plate EC and reference method at the 1:10 and subsequent serial dilution levels to get countable ranges of 1–154 CFU/mL.

Bottled water was purchased from local grocer. A produce manufacturer in California collected and shipped mixed-green flume water that was thiosulfate neutralized before testing. The manufacturer also collected irrigation water from a mixed source, ground/surface, and shipped. Two-liter samples were split into control and low, medium, and high fortification levels and inoculated with freshly cultured coliform and *E. coli* strains indicated in Tables 4 and 5. Ten replicate 100 mL test portions from each contamination level were assayed by both the Peel Plate EC method and the reference method, FDA/BAM Chapter 4, Section III, D, in the case of bottled and flume water and EPA Method 1604 in the case of irrigation water. For the comparison, 100 mL test portions were filtered through sterile, gridded, 47 mm diameter, 0.45 µm pore size mixed-cellulose membrane filters and applied to each method.

Stainless steel coupons were prepared and sampled following ISO 18593. Fresh cultures of coliform and *E. coli,* as indicated in Tables 4 and 5, were applied to the surface and allowed to dry overnight (16–24 h). Five replicate test portions from each contamination level were assayed in a paired analysis following the Peel Plate EC method and harmonized FDA/BAM Chapter 4, I.G., reference method. Stainless steel environment samples were assayed by the Peel Plate EC and harmonized reference methods from the initial preparation and a 1:10 dilution. Sampling sponges were moistened with 10 mL of peptone water and used to sample 100 cm^2 stainless steel surfaces. Sponges were transferred to sterile stomacher bags and 25 mL of peptone water was added to the sampling sponge bag and homogenized by stomaching for 1 min. The resulting mixture was serial diluted 10 mL in 90 mL BPBDW as necessary to get into countable range.

FDA/BAM Chapter 4, I.G. reference method.—Following the FDA/BAM Chapter 4, I.G., reference method, 1 mL aliquots of sample preparation were plated into sterile Petri dishes and approximately 10 mL of tempered VRBA was added to the plates, swirled, and allowed to solidify. After the agar solidified, approximately 10 mL of tempered VRBA with MUG was overlaid onto the agar. After the agar solidified, the plates were inverted and incubated at 35 ± 1°C for 18–24 h. Following incubation, typical colonies (≥0.5 mm colonies surrounded by a zone of precipitated bile acids) were enumerated. Duplicate plates in the countable range of 1–154 colonies were averaged and reported as coliform count/mL.

To determine the presence of *E. coli*, plates were placed under long-wave UV light and observed for bluish fluorescence around the colonies. To confirm *E. coli,* up to 10 colonies from each sample were picked and transferred to EC-MUG and incubated at 35 ± 1°C for 48 ± 3 h. After incubation, EC-MUG tubes were examined for fluorescence under UV light. Fluorescing EC-MUG tubes indicated the presence of *E. coli*. *E. coli* was confirmed by transferring a loopful of broth from a positive EC-MUG tube to TSA and incubating at 35 ± 1°C for 24 ± 2 h. Growth from TSA was used for final confirmation of *E. coli* by VITEK 2 GN following AOAC *Official Method* **2011.17**.

Table 5. Peel Plate EC method for *E. coli* versus LST-MUG/MicroID method with 35°C matrixes

Matrix	Fortified microorganisms ATCC No. (% injury)	Contamination level	Candidate method			Reference method				95% CI[e]		r^{2h}
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r	Mean diff.[d]	LCL[f]	UCL[g]	
Ground beef (70% lean)	*Citrobacter freundii* 8090 (0%) *Escherichia coli* 25922 (0%)	None	<0.1	NA[i]	NA	<0.1	NA	NA	NA	NA	NA	0.98
		Low	1.53	0.26	16.8	1.27	0.53	41.8	0.26	−0.14	0.67	
		Mid	2.57	0.21	8.2	2.79	0.21	7.7	−0.23	−0.41	−0.04	
		High	3.65	0.16	4.4	3.74	0.13	3.4	−0.09	−0.25	0.07	
Ground beef (46% lean)[j]	*Enterobacter aerogenes* 35029 (0%) *E. coli* 11229 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	1.71	0.18	6.3	1.68	0.14	8.2	0.02	−0.15	0.20	
		Mid	2.14	0.05	2.2	2.22	0.06	2.8	−0.08	−0.12	−0.04	
		High	3.17	0.034	1.2	3.20	0.02	0.7	−0.03	0.07	0.02	
Ground turkey	*Enterobacter cloacae* 13047 (0%) *E. coli* 11775 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.99
		Low	1.77	0.21	11.6	1.82	0.09	4.9	−0.04	−0.17	0.08	
		Mid	2.71	0.12	4.3	2.54	0.58	22.6	0.17	−0.31	0.64	
		High	3.77	0.19	5.0	3.80	0.18	4.7	−0.03	−0.24	0.18	
Bottled water	*Klebsiella pneumoniae* 13883 (0%) *E. coli* 11775 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	0.52	0.27	51.6	0.62	0.20	32.4	−0.10	−0.39	0.18	
		Mid	1.24	0.13	10.6	1.32	0.11	8.6	−0.08	−0.20	0.04	
		High	1.54	0.05	3.5	1.61	0.08	5.2	−0.07	−0.14	0.01	
Bottled water[j]	*K. pneumoniae* 13882 (0%) *E. coli* 35218 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	0.75	0.11	14.8	0.79	0.12	14.6	−0.04	−0.13	0.05	
		Mid	1.52	0.06	4.1	1.45	0.03	1.8	0.07	−0.03	0.17	
		High	1.79	0.04	2.4	1.77	0.03	1.8	0.02	−0.06	−0.09	
Stainless steel Surface sponge	*E. cloacae* 13047 (0%) *E. coli* 25922 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	0.10	0.59	611	0.46	0.35	76.0	−0.36	−0.91	0.19	
		Mid	1.74	0.66	37.9	1.58	0.76	48.1	0.15	−0.37	0.68	
		High	2.34	0.55	23.7	2.21	0.44	19.9	0.13	−0.33	0.59	
Stainless steel Surface sponge[j]	*C. freundii* 8090 (0%) *E. coli* 25922 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	0.48	0.34	71.3	0.66	0.14	20.5	−0.17	−0.58	0.23	
		Mid	2.17	0.13	5.9	2.21	0.08	3.8	−0.04	−0.13	0.06	
		High	2.82	0.08	3.0	2.83	0.07	2.5	−0.01	−0.18	0.15	
Irrigation water	*Cronobacter sakazakii* 29544 (0%) *E. coli* 25922 (0%)	Natural	−0.75	0.54	72.9	−0.69	0.50	73.2	−0.06	−0.56	0.44	0.98
		Low	−0.24	0.53	218.0	−0.21	0.70	329.0	−0.03	−0.72	0.66	
		Mid	0.38	0.20	52.1	0.35	0.29	82.3	0.03	−0.23	0.29	
		High	0.54	0.17	31.1	0.72	0.16	22.7	−0.18	−0.29	−0.07	
Liquid eggs	*K. pneumoniae* 13883 (40%) *E. coli* 25922 (11%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.99
		Low	1.93	0.52	26.8	1.90	0.64	33.7	0.03	−0.42	0.48	
		Mid	3.03	0.13	4.2	2.93	0.15	5.0	0.10	−0.02	0.22	
		High	4.10	0.10	2.5	3.68	0.23	6.2	0.42	0.27	0.57	
Dried dog food	*C. freundii* 8090 (46%) *E. coli* 25922 (7%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	3.01	0.20	6.7	2.92	0.24	8.2	0.08	0.00	0.17	
		Mid	4.08	0.09	2.3	4.01	0.15	3.7	0.06	−0.09	0.21	
		High	5.04	0.12	2.4	5.09	0.13	2.6	−0.06	−0.19	0.08	
Chocolate	*C. freundii* 8090 (0%) *E. coli* 25922 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.77
		Low	1.79	0.24	13.6	1.90	0.21	10.8	−0.11	−0.26	0.04	
		Mid	2.84	0.09	3.3	2.97	0.16	5.2	−0.13	−0.21	−0.06	
		High	3.73	0.10	2.7	2.95	0.20	6.8	0.78	0.68	0.89	
Flume water	*K. pneumoniae* 13883 (0%) *E. coli* 25922 (0%)	Natural 2	<0.1	NA	NA	<0.1	NA	NA	NA	NA	<0.1	0.82
		Low	0.75	0.17	22.2	0.91	0.13	14.0	−0.16	−0.26	−0.05	
		Natural 1	<0.1	NA	NA	<0.1	NA	NA	NA	NA	<0.1	
		Mid	1.33	0.07	5.5	1.84	0.10	5.5	−0.51	−0.60	−0.42	
		High	1.62	0.08	4.8	1.73	0.14	7.9	−0.11	−0.21	−0.02	

Table 5. (*continued*)

Matrix	Fortified microorganisms ATCC No. (% injury)	Contamination level	Candidate method			Reference method			Mean diff.[d]	95% CI[e]		r^{2h}
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[f]	UCL[g]	
Chicken rinse	*E. cloacae* 13047 (0%) *E. coli* 11775 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.99
		Low	1.10	0.11	9.6	0.57	0.58	102	0.53	0.15	0.91	
		Mid	1.78	0.10	5.6	1.70	0.15	8.9	0.08	−0.07	0.23	
		High	1.79	0.04	2.0	1.60	0.07	4.6	0.19	0.12	0.26	
Hog carcass rinse	*C. freundii* 8090 (0%) *E. coli* 25922 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.89
		Low	0.78	0.13	16.9	1.01	0.18	17.5	−0.23	−0.41	−0.05	
		Mid	0.94	0.16	16.7	1.40	0.05	3.3	−0.45	−0.58	−0.33	
		High	1.57	0.12	7.4	1.76	0.04	2.5	−0.20	−0.29	−0.11	

[a] Mean of five replicate portions, plated in duplicate, after logarithmic transformation: Log_{10} [CFU/g + (0.1)f].

[b] Repeatability SD.

[c] Relative SD for repeatability.

[d] Mean difference between the candidate and reference methods.

[e] Confidence interval.

[f] 95% lower confidence limit for difference of means.

[g] 95% upper confidence limit for difference of means.

[h] Square of correlation coefficient.

[i] NA = Not applicable.

[j] Independent laboratory-performed.

To confirm coliform, up to 10 colonies from each sample were picked and transferred to BGLB and incubated at 35 ± 0.5°C for 48 ± 3 h. Following incubation, the BGLB tubes were examined for gas production.

FDA/BAM Chapter 4, Section III, D, and EPA 1604 reference methods.—Following the filter procedure, the membrane filter was transferred to M-Endo or 1604 medium and incubated at 35 ± 0.5°C for 22–24 h. Following incubation, pink to dark red colonies with a green metallic sheen were enumerated on M-Endo, and white fluorescent and blue colonies were enumerated for Method 1604. For coliform confirmation, 5–10 colonies were picked and transferred to LST tubes and incubated at 35 ± 0.5°C for 48 ± 3 h. LST tubes were examined for gas production. Gas-positive LST tubes were subcultured into BGLB by transferring a loopful of LST into BGLB and incubating at 35 ± 0.5°C for 48 ± 3 h. Following incubation, BGLB tubes were observed for gas production. Gas-positive BGLB tubes indicate the presence of coliform. To confirm the presence of *E. coli,* 5–10 colonies were picked and transferred to EC-MUG broth and incubated at 35 ± 0.5°C for 48 ± 3 h. After 48 ± 3 h of incubation, EC-MUG tubes were examined for fluorescence under UV light. Fluorescing EC-MUG tubes indicated the presence of *E. coli*. *E. coli* was confirmed by transferring a loopful of broth from a positive EC-MUG tube to TSA and incubating at 35 ± 1°C for 24 ± 2 h. Growth from TSA was used for final confirmation of *E. coli* by VITEK 2 GN following AOAC *Official Method* **2011.17**.

Peel Plate EC method.—Five replicate test portions from each inoculation level were assayed in duplicate. The Peel Plate covers were lifted to fully expose the dried media culture disc. For the serial dilutions, 1:10 or 1:100 (or appropriate dilutions) 1 mL sample aliquots were dispensed onto the center of the disc. In the case of bottled water, irrigation water, and flume water, 100 mL samples were vacuum filtered through mixed cellulose acetate 0.45 µm filters. Peel Plate ECs with covers lifted were rehydrated with 1.5 mL sterile water and filtered samples carefully applied to the media culture disc to avoid forming a bubble under the filter. The covers were reapplied and sealed over the disc. Peel Plate EC plates were stacked 20 high and incubated with the cover down at 35 ± 1°C for 24 ± 2 h. Following 24 h of incubation, all red, purple, black, blue, or green colonies were enumerated and reported as the total coliform CFU/mL. *E. coli* typically appear dark blue, purple, or greenish in color. Non-*E. coli* coliforms appear red and can typically be differentiated from *E. coli* using the Peel Plate EC method. In irrigation water, light pink colonies and clear growing colonies on filter were excluded as noncoliform. After 24 h of incubation, *E. coli* and coliform could be differentiated on Peel Plate EC plates.

To confirm coliform, up to 10 colonies from each sample were picked and transferred to BGLB and incubated at 35 ± 0.5°C for 48 ± 3 h. After incubation, the BGLB tubes were examined for gas production, indicating a positive reaction and presence of coliforms. To confirm *E. coli,* up to 10 colonies from each sample were picked and transferred to EC-MUG and incubated at 35 ± 1°C for 48 ± 3 h. After incubation, EC-MUG tubes were examined for fluorescence under UV light. Fluorescing EC-MUG tubes indicated the presence of *E. coli*. *E. coli* was confirmed by transferring a loopful of broth from a positive EC-MUG tube to TSA and incubating the TSA at 35 ± 1°C for 24 ± 2 h. Growth from TSA was used for final confirmation of *E. coli* by VITEK 2 GN following AOAC *Official Method* **2011.17**.

Matrix studies results and discussion.—Analyses of all matrixes were conducted for each contamination level. Reported

are confirmed coliforms count by the LST/BGLB method and *E. coli* by EC-MUG and a biochemical identification (MicroID, Remel Microbiology Products Thermo Scientific Products, Lenexa, KS) of cultured isolates. Logarithmic transformations of the total coliform counts (CFU per gram or CFU per milliliter) and paired statistical analysis were performed. All data are reported and no outliers were removed. The difference of log-means and their 95% CIs for each contamination level were determined. A $\log_{10}$ mean difference value less than the standard alpha value of 0.5 with CIs within –0.5 to 0.5 indicated no statistical difference between the Peel Plate EC method and the reference methods. Results of total coliform matrix experiments and fortification levels are reported in Table 4. Results of differentiated *E. coli* determined in each matrix are reported in Table 5.

Results of ground beef, ground turkey, liquid eggs, dried dog food, and chocolate in comparison to FDA/BAM VRBA with VRBA-MUG overlay demonstrate no significant differences at any of the concentrations or matrixes for either total coliform or *E. coli* determination with the exception of chocolate at the highest spike level, in which Peel Plate EC had a significantly higher recovery of both *E. coli* and coliform in comparison to the reference method.

Independent laboratory results using ground beef matched the manufacturer data. The replication of the comparative method was similar to the reference method in all studies. The *E. coli* comparisons were limited by the reference method quantification limit of about 20 per plate before MUG diffusion made plates too numerous to count. Therefore, the SDs of the *E. coli* determinations are larger because of needing to use the next-level serial dilution and lower counting plate.

In the case of chocolate, natural fluorescence in the matrix made *E. coli* discrimination from coliform in the reference method too difficult to determine. Thus *E. coli* was calculated from total coliform counts based on the percentage of BGLB/LST-MUG positive pick confirmation. It is unclear why the reference method underestimated the coliform and therefore *E. coli* at the highest spike level and largest 10^{-2} dilution. The Peel Plate EC method, however, did accurately detect the theoretical fortification level.

Results of bottled water and flume water in comparison to *m*-Endo agar demonstrate no significant difference at any of the study concentrations in coliform or *E. coli* determinations, except for the *E. coli* determination of the medium concentration of flume water. Bottled water results by the independent laboratory are similar to the manufacturer data, and results and SDs are consistent with the reference method. Flume water was performed as two separate studies. In the first study, there was a high natural coliform contamination identified as *Enterobacter agglomerans*. Fortified coliform at medium and high levels were not significantly higher than the natural coliform contamination. None of the determinations of coliform is significantly different between the methods. In the second study, there was no natural contamination, and the low coliform and *E. coli* concentrations reported were fortified in this second sample.

E. coli determination from *m*-Endo reference method is based on visual greenish sheen and confirmation picks and biochemical identification. The low and high concentrations in the two different flume water samples are not significantly different. The medium spike level made with the high natural contamination sample had a significantly higher *m*-Endo sheen result compared with the blue colonies on Peel Plate EC. All picks from the middle fortification level were confirmed as *E. coli,* so the *m*-Endo reference results are based on the percent confirmation rather than a precise count. Based on known spike levels, the *m*-Endo determination is an overestimate based on the chance of picking an *E. coli* compared with a coliform during the confirmation steps.

Results of the irrigation water in comparison to EPA 1604 MI show no significant difference at the different spike levels in either coliform or *E. coli* determination. Samples were highly diluted spike levels and results are reported as CFU/mL, reflecting the negative value of log means. The use of natural water and a filter resulted in some pink-pigmented, heterotrophic bacterial growth on the Peel Plate. These pink colonies could be distinguished from red coliform colonies in the Peel Plate by a trained eye and were not scored as coliform. Pink-picked colonies in BGLB did not confirm as coliform, indicating they should not be scored as coliform. Interfering pigmented bacteria could be reduced by rehydrating the Peel Plate EC with 1.5 mL of 5 µg/mL cefsulodin instead of water, as was performed in this study. Although not significantly different, the coliform determination of the Peel Plate had a slightly positive bias in comparison to the EPA 1604 method. There was no significant difference in *E. coli* determination between methods. Natural *E. coli* contamination was observed in the sample. These natural *E. coli* resulted in an unidentifiable biochemical profile in some isolates by both methods, but the enzymatic profile of the isolates had signature *E. coli* attributes, β-glucuronidase activity with indole positive isolated from both methods. It is possible that these *E. coli* were not completely separated from contaminating coliform, resulting in a mixed biochemical identification.

Results of the carcass rinse samples in comparison to FDA/BAM VRBA with VRBA-MUG overlay show no significant difference from reference method at the different spike levels in coliform and *E. coli* determinations. Chicken rinse used at mid and high concentrations contained 2–3 log/mL of an acidifying aerobe that inhibited MUG production and *E. coli* determination in the reference method, but did not interfere with Peel Plate determination of *E. coli*. In the hog rinse, the reference method VRBA-MUG positives at the higher levels could not be determined because the density of *E. coli* was at a level that the MUG indicator bled through the entire plates. In both matrixes, *E. coli* determinations from the reference method were calculated as a percentage of coliform that were picked to BGLB and confirmed LST-MUG positive with biochemical identification. This could explain the slight negative bias of the Peel Plate EC *E. coli* determination compared with reference at mid and high levels of the hog carcass rinse.

Results of the stainless steel sponge samples in comparison to FDA/BAM VRBA with VRBA-MUG overlay show no significant difference from the reference method at the different spike levels in coliform and *E. coli* determinations. There was an insignificant or slightly positive bias in the manufacturer laboratory at the three coliform levels, whereas there were insignificant or slight negative biases in the recoveries in the

independent laboratory. There were no differences in *E. coli* recovery by either method.

Product Robustness, Consistency (Lot-to-Lot), and Stability Studies

A robustness study using a Youden multivariate design was performed using perturbations of the critical steps of the Peel Plate EC method (14). The steps and perturbations evaluated were pipetting, 1.0 mL ± 5%; temperature of incubation, low 30°C and high 36°C; and time of incubation, low 22 h and high 26 h. The multivariate design assays were performed in whole milk fortified with *E. coli* ATCC 700609, and 10 replicate tests were performed under each assay condition. Each perturbation condition was compared with the control condition in a paired *t*-test analysis. Results of the robustness analysis are reported in Table 6.

Assay temperature showed no significant difference by *t*-test or paired-log *t*-test confidence levels >0.5. A shorter assay time did show a significant difference by *t*-test, but results are counterintuitive with higher CFU per milliliter at the shorter time. Using the paired-log *t*-test confidence limits, there is no significant difference between the high and low incubation times. Pipet volume did show a significant difference by *t*-test and a significant low bias by paired *t*-analysis; however, the low bias using the >0.5 log specification is not considered significantly different.

In a separate study, the rate of moisture loss from an exposed unsealed test strip and the effect of moisture loss on a test were determined. In control experiments with sealed strips, there is less than a 1% loss of weight. Moisture loss studies were performed in BPBDW fortified with *E. coli* ATCC 29522 and *Citrobacter freundii* ATCC 8090; three replicate tests were performed under each moisture loss stress. Plates had diluted samples added and were sealed (control) and left exposed in a 32°C incubator for 5, 10, 15, and 45 min to achieve water losses of 5, 10, 15, and 25%. Averages and SDs were calculated. There were 32–46% increased counts from evaporating 25% moisture and 22–24% increased counts with a 15% loss. There was less than a 5% difference from a 5% loss and 2–55% difference with a 10% loss. In log difference terms, there is not a significant difference from the plates before testing with either the coliform or *E. coli* strains.

Studies to compare lot-to-lot variation with accelerated shelf stability at room temperature for 40 days were performed and submitted to assure a replicable manufacturing process with proper QA parameters and at least a 1 year of refrigerated shelf life.

Discussion

Results of the Peel Plate EC evaluations in dairy products, whole milk, chocolate milk, heavy cream, skim powder, sour cream, egg nog, ice cream, evaporated/condensed milk, UHT milk, condensed whey, yogurt, cottage cheese, and shredded cheese show no significant differences in total coliform determination with reference method VRBA with BGLB confirmation. The exception seen with the skim milk matrix and the three highest spike levels was not replicated in additional experiments to determine if there was repeatable matrix interference. The results appear to be an anomalous matrix effect on the stressed bacteria used in preparing that study set. *E. coli* color differentiation from coliform was not consistent in all dairy matrixes; for example, heavy cream experiments showed good differentiation, but ice cream using the same *E. coli* strain showed no color differentiation. This lack of consistency could be a nonoptimal temperature, 32°C, as well as an additive (e.g., sugar, interference). *E. coli* differentiation is not claimed in dairy matrixes at 32°C. Cultured dairy products with live laboratory cultures can produce an interfering background red color from curd protection of the laboratory culture from the Peel Plate EC–selective agents. This interference can be reduced or eliminated by preparation of the sample 1:10 dilution in 0.2% SBDB. Cottage cheese, yogurt, and shredded cheese products were evaluated in the Peel Plate EC method with SBDB and compared with the reference method without SBDB. Yogurt was incubated 48 ± 3 h to distinguish coliform colonies from background. No significant difference in results with these cultured products was observed.

Results of Peel Plate EC matrixes for coliform and *E. coli* in various foods and water samples at 35°C are not significantly different from the reference methods. Coliform determinations compared with VRBA/BGLB confirmation in various foods, ground meats, dog food, liquid eggs, chocolate, stainless sponge, hog carcass sponge, and chicken rinse show no significant difference, with the exception of the highest chocolate level, which was biased high relative to the reference method. Although confirmation of Peel Plate EC coliform positives was performed, the principle of detection and the >98% confirmation results indicate that the confirmation is not necessary when using the Peel Plate EC method. *E. coli* determinations by Peel Plate EC are also not significantly different in these foods from the

Table 6. Multivariate evaluation of Peel Plate EC assay perturbations

Assay perturbation	High and low conditions	Mean, CFU/mL	SD	CV, %	Probability of difference, %	Paired *t*-test log difference[a]	LCL[b]	UCL[c]
Temperature	30°C	73	17.5	24	30	0.31	−0.76	−0.14
	36°C	68	14.7	22	36			
Pipet volume	950 µL	64	10.6	16	99	−0.07	−0.11	−0.03
	1050 µL	75	9.4	13	99			
Assay time	22 h	76	15.9	21	99	0.064	−0.02	0.15
	26 h	65	11.2	17	13			

[a] Mean log CFU/mL difference between the low and high pairs; n = 10 pairs.

[b] 95% lower confidence limit for difference of means.

[c] 95% upper confidence limit for difference of means.

reference method (VRBA/VRBA-MUG overlay with BGLB/LST-MUG confirmation), with the exception of the highest chocolate level that was biased high relative to the reference method. In fact, the Peel Plate method is easier, more robust, and extends to a greater dynamic range per plate compared with the reference methods. In the chicken rinse, acidifying background bacteria caused no fluorescence of *E. coli* in the reference method. In chocolate, the matrix interfered with fluorescence determinations. At several spike levels, the fluorescent colonies grew into each other, making quantification of more than 15–20 *E. coli*/plate impossible. Although the blue/purple color of *E. coli* in these matrixes is definitive for *E. coli* detection, not all *E. coli* strains produce the enzyme glucuronidase; therefore, the absence of the blue color is not necessarily an absence of *E. coli* when red coliform colonies are also present.

Coliform and *E. coli* determinations in various water samples, bottled, irrigation, and flume, compared with reference methods *m*-Endo LES agar and EPA Method 1604, show no significant difference. *E. coli* determination from the reference method *m*-Endo LES was less quantitative than the comparative method because color discrimination from coliform was less subjective. Peel Plate EC results in natural waters did show some noncoliform background flora producing a pink color that was distinguishable from coliform by a trained eye. With surface water applications, it could be prudent to include an antibiotic inhibitor (e.g., cefsulodin) in the method rehydrating water to reduce background flora and simplify interpretation.

Conclusions

The dairy matrixes data support that Peel Plate EC method total coliform results, the sum of all colored colonies, at 32 ± 1°C for 24 ± 2 h without a confirmatory step are not significantly different from the standard method for coliform in dairy products, VRBA/BGLB confirmation. Color differentiation of coliform from *E. coli* is not claimed for dairy products. Cultured dairy products containing laboratory cultures cause background color and require make up in a 0.2% sodium bisulfite diluent. In the case of yogurt testing, 48 ± 3 h incubation is required.

The food and water matrixes data and the inclusivity/exclusivity challenges support that the Peel Plate EC method at 35 ± 1°C for 24 ± 2 h will quantify coliform and color distinguish *E. coli* in a manner not significantly different from the reference methods. Ground meats, liquid eggs, and carcass rinses were evaluated with harmonized UDSA MLG 3.01 and FDA/BAM Chapter 4 methods. Dog food and chocolate were evaluated compared with FDA/BAM Chapter 4 methods. Flume water and bottled water were compared with methods for water in FDA/BAM Chapter 4. Irrigation water, a mixed surface water and ground water source, was compared with EPA Method 1604.

Data supplied support a quality controlled manufacture with a 1 year refrigerated shelf life of the Peel Plate EC test. Volume of pipetting is a critical step in performance during the specified 22–26 h incubation.

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