FOOD BIOLOGICAL CONTAMINANTS

Validation of the Peel Plate™ AC for Detection of Total Aerobic Bacteria in Dairy and Nondairy Products

AOAC *Performance Tested Method*[SM] 071501

Abstract

Peel Plate™ AC (aerobic count) is a low-profile plastic 47 mm culture dish with adhesive top that contains a dried standard plate count medium with oxidation/reduction indicator triphenyl tetrazolium chloride (TTC) that turns red with dehydrogenase enzyme activity of growing aerobic bacteria. The method provides a conventional quantitative count with simple rehydration and incubation for 48 ± 3 h at 35 ± 1°C for most food matrixes and 32 ± 1°C for 48 ± 3 h for dairy products. Dairy matrixes claimed and supported with total aerobic count data are whole milk, skim milk, chocolate milk (2% fat), light cream (20% fat), pasteurized whole goat milk, ultra-high temperature pasteurized milk, nonfat dried milk, lactose-reduced milk, strawberry milk, raw cow milk, raw goat milk, raw sheep milk, condensed skim milk, and vanilla ice cream. Food matrixes claimed for aerobic count detection are raw ground beef, environmental sponge of stainless steel, raw ground turkey, dry dog food, liquid whole pasteurized eggs, milk chocolate, poultry carcass rinse, and large animal carcass sponge. The method has been independently evaluated for aerobic count in dairy products: whole milk, skim milk, chocolate milk, and light cream. The method was also independently evaluated for aerobic count in food matrixes: ground beef and sponge rinse from stainless steel surfaces. 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, mean comparison between methods with 95% confidence interval (CI), and r^2. A CI range of (−0.5, 0.5) on the mean difference was used as the acceptance criterion to establish significant statistical differences between methods. The evaluations demonstrate that the Peel Plate AC provides no statistical differences across most of the matrixes with $r^2 > 0.96$. In the case of skim milk, there were significant differences that may be explained by a matrix-related stress on the spiked organisms but were not repeated in subsequent experiments. Within method repeatability of Peel Plate AC was similar to reference method with relative standard deviations in the ranges of 2 to 5% when $\log_{10}$ means were ≥1.5. Quality control data support that Peel Plate AC is stable for at least 1 year refrigerated. Incubation temperature ranges 30–36°C and times 45 –51 h were not significantly different.

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

Annex Tables and Figures are available on the *J. AOAC Int*. website, http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac

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Scope of Method

(a) *Target organisms.*—Aerobic bacteria. A general group of bacteria that grow in the presence of oxygen and produce dehydrogenase activity that convert a triphenyl tetrazolium chloride (TTC) indicator to red in an oxidation/reduction reaction.

(b) *Matrixes.*—(*1*) *Dairy products*.—Pasteurized whole milk, skim milk, 2% chocolate milk, 20% cream, pasteurized whole goat milk, ultra-high temperature (UHT) pasteurized milk, nonfat dried milk, lactose-reduced milk, strawberry milk, raw cow milk, raw goat milk, raw sheep milk, condensed milk, and vanilla ice cream.

(*2*) *Nondairy products*.—Raw ground beef, raw ground turkey, liquid whole pasteurized eggs, U.S. Department of Agriculture (USDA), Agriculture Research Service (ARS) carcass rinses of chicken and USDA ARS 300 cm^2 surface-sponge of hog carcass, dry dog food, milk chocolate, 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 skim milk, on mean difference between Peel Plate™ AC (aerobic count) and

reference methods within the range of (−0.5, 0.5) (1, 2) from that of the U.S. Food and Drug Administration (FDA) National Conference of Interstate Milk Shipments (NCIMS) Reference 2400a Form cultural procedures and Standard Plate Count Agar (SPCA) pour plate method (3, 4).

(*2*) *Ground beef, ground turkey, liquid whole pasteurized egg, carcass rinses for aerobic bacteria, dry dog food, and milk chocolate.*—Performance not statistically different from that of USDA, Food Safety and Inspection Service (FSIS) Microbiology Laboratory Manual 3.01, Quantitative Analysis of Bacteria in Foods as Sanitary Indicators (USDA-FSIS *Microbiology Laboratory Guidebook*-MLG) with the FDA *Bacteriological Analytical Manual* (BAM) Chapter 3, Enumeration of Aerobic Bacteria, Conventional Plate Count Method (FDA/BAM) reference methods (5, 6).

(*3*) *Surface sponge of stainless steel.*—Performance not statistically different from that of the International Organization for Standardization (ISO) 18593:2004, Microbiology of Food and Animal Feeding Stuffs–Horizontal Methods for Sampling Techniques from Surfaces Using Contact Plates and Swabs (7) with FDA/BAM Chapter 3, Conventional Plate Count Method Reference Methods (5, 6).

Definitions

(a) *Repeatability (s_r).*—Standard deviation 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 95% CI for each analyte at each concentration of each matrix. Differences between methods are considered significant when the CI falls outside (−0.5, 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-tail *t*-test; $P < 0.05$ indicates significance at the 95% confidence level.

Principle

Peel Plate AC is a conventional plate count medium containing tryptone, yeast extract and dextrose to support aerobic bacteria respiration. The dehydrogenase enzyme indicator TTC turns aerobic bacterial colonies red at 32 ± 1°C for dairy products or 35 ± 1°C for food matrixes for 48 ± 3 h in test samples. Peel Plate AC also contains gelling and wicking agents which absorb and self-diffuse the sample.

General Information

Aerobic bacteria are routinely tested in food manufacture as a sanitary process indicator and shelf-life predictor. Aerobic bacteria on food or in food manufacture can signal a breakdown in sanitary practices and potential problems related to food spoilage. For example, in milk production, Grade A Pasteurized Milk Ordinance specifies raw milk to contain less than 100 000 colony-forming units per milliliter (CFU/mL). After pasteurization dairy products should contain less than 20 000 CFU/mL or CFU/g (8). Aerobic bacteria are used as a running process control indicator for zone containment and low pathogen risk in meat production facilities (9). Aerobic bacteria are a sanitary hygiene and process indicator proposed in food safety modernization regulations affecting produce manufacture (10). Because aerobic counts are used so frequently by the food industry, there is a need for simple, low-cost, ready-to-use methods for testing. Peel Plate AC is a simple method to detect and quantify aerobic bacteria in foods and on food and nonfood contact surfaces that is being studied and validated in this work.

Materials and Methods

Test Kit Information

(a) *Kit name*.—Peel Plate AC.

(b) *Catalog number*.—PP-AC-100K, 100 Peel Plate AC tests.

(c) *Ordering information*.—Charm Sciences, Inc. (Lawrence, MA; www.charm.com).

Test Kit Components

(a) Two foil bags containing 50 Peel Plate AC each with blue indicator desiccants.

Additional Supplies and Reagents Required Depending on Application

(a) *Butterfield's Phosphate Buffered Dilution Water* (BPBDW).—Buffer KH_2PO_4 34 g to 500 mL distilled (DI) or reverse osmosis (RO) water and adjust pH to 7.2 with 1 N NaOH and bring final volume to 1 L with DI or RO water. Add 99 mL to dilution bottles and sterilize 15 min at 121°C. Store in refrigerator. Alternatively, purchase from Weber Scientific (Hamilton, NJ; Cat. No. 3127-14).

(b) *Buffered peptone water (BPW)*. Peptone 10 g, sodium chloride 5 g, disodium phosphate 3.5 g, monopotassium phosphate 1.5 g, DI 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.

(c) 1 mL pipet tips.

Apparatus

(a) 1 mL pipettor.

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

(c) Light box for back illumination and counting plates.

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

(e) Stomacher®- Seward 400 paddle type or equivalent.

Safety Precautions

(a) Follow Good Laboratory Practices and perform tests in designated areas with washed and clean hands using appropriate protective equipment, such as gloves and/or goggles, if specified.

(b) Microbiological cultures and reagents should be collected into biohazardous bags and autoclaved. Dispose 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, and free of dust and blowing air.

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

(d) Log serially dilute sample into BPBDW or microbiologically suitable water to obtain the countable range 25–250 aerobic bacteria/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 %, and skim) may be tested directly or serially diluted in BPBDW to a countable range (25–250 CFU/mL).

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

(b) Flavored milks should be diluted 1 part to 9 part buffer (1:10 dilution), and 1 mL plated. Flavored milk may also be serially diluted into a countable range (25–250 CFU/mL).

(c) Add 11 g solid dairy (ice cream, heavy cream, etc.) to 99 mL dilution buffer. Shake mixture 25 times in an arc 1 foot for 7 s. Perform additional dilutions as needed to reach a countable range (25–250 CFU/mL).

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

(e) Cultured dairy products are not appropriate for aerobic count determination because of the active bacterial cultures inherent in the products.

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

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

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

(c) Continue to dilute 10 mL prior dilution in 90 mL dilution blank to reach a countable range (25–250 CFU/mL).

Surface rinses:

(a) For large animal hides (hog and beef):

(*1*) Wet sponge with 10 mL sterile BPW and ring/rinse without hand contact.

(*2*) With a sterile gloved hand squeeze/rinse the sponge and wipe three 100 cm^2 areas representing shoulder, flank, and rump.

(*3*) Add the sponge to 25 mL BPW or BPBDW to the plastic bag containing the dried sponge.

(*4*) Add the sponge to the bag and squeeze/rinse/extract the sponge with buffer for 1 min to get a test solution for testing.

(b) For whole chicken:

(*1*) Add 400 mL BPBDW or BPW to a plastic bag large enough to contain a bird carcass.

(*2*) Add the carcass and rock back and forth for 1 min to get a test solution for testing.

(c) For stainless steel surfaces:

(*1*) Wet the sponge swith 10 mL sterile BPW and ring/rinse without hand contact.

(*2*) Using a gloved hand, or a handle not in contact with buffer, rinse out the wetted sponge.

(*3*) Wipe the 100 cm^2 surface with a dampened sponge.

(*4*) Add 25 mL BPW or BPBDW to the plastic bag containing the dried sponge or bag only if using the sponge with a handle.

(*5*) Add the sponge back to the buffer, detaching the handle if applicable.

(*6*) Add the sponge to the bag and squeeze/rinse/extract the sponge with buffer for 1 min to get a test solution for testing.

Method Procedure

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

(b) Lift the cover vertically upward to completely expose the dried media culture disc. Keep the cover adhered to back of the plate.

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

(d) Reapply the adhesive cover without wrinkling. Press the cover around edges of the plate to ensure the plate is properly sealed.

(e) Incubate the plates with the adhesive cover face down and the clear side up.

(*1*) Incubate milk and dairy products at 32 ± 1°C for 48 ± 3 h.

(*2*) Incubate the environmental and food samples at 35 ± 1°C for 48 ± 3 h.

(*3*) The plates can be stacked 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 the plates for any colonies, which can be viewed through the clear side of the Peel Plate AC. Each red colored spot represents one aerobic CFU. The sum of the spots is reported as the aerobic CFU/mL of the diluted sample.

(b) Multiply the CFU/mL by the dilution to calculate the CFU/mL (or CFU/g) of the original sample.

(i) In the case of surface swabs or carcass rinse, multiply the CFU/mL by the volume of the rinse buffer (e.g., 25 or 400) to the calculate CFU/rinse surface area.

(c) In case of bacterial spread, score one CFU for each count, each dark spot within the spread growth is counted as a single colony. Blended colonies are scored as a single CFU.

(d) Counts of 25 to 250 CFU/mL (or CFU/g) are considered countable, whereas counts outside this range are considered estimates. Samples with results outside the countable range (>250 CFU/mL or CFU/g) can be diluted and retested.

Confirmation

The Peel Plate AC method uses nonselective medium and enzyme substrates to detect most aerobic bacteria without confirmation steps. Although it is not necessary, it may be desirable to transfer colonies into a traditional selective medium. The cover may be lifted and colonies loop or stick-transfered into selective medium. Selective bacteria confirmation procedures are described in FDA/BAM Chapter 4.

Validation Study

This validation study was conducted under the AOAC *Research Institute Performance-Tested Method*SM (RI PTM) program and the AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (11). Method developer studies were conducted at the Charm Sciences laboratory and included matrix studies for all claimed matrixes, product consistency and stability studies, and robustness testing. The independent laboratory study and matrix studies for six of the claimed dairy, food, or surface matrixes were conducted by Q Laboratories, Inc. (Cincinnati, OH). Additionally, collaborative dairy matrixes followed the 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 were tested and sent to four additional testing laboratories: Milk Regulatory Consultants (Russellville, MO), Eurofin-DQCI (Mound View, MN), Dairygold (Tukwila, WA), and Charm Sciences.

Method Comparison (Matrix) Studies and Independent Laboratory Studies

Peel Plate AC: Dairy Matrixes

Dairy matrixes were evaluated in Peel Plate AC at 32 ± 1°C for 48 ± 3 h in comparison with SPCA 32 ± 1°C for 48 ± 3 h for aerobic count.

Sample preparation.—In all dairy matrix studies, cocktails of assorted heat-stressed bacterial strains (50°C for 10 min) were fortified into the product and allowed to acclimate for 48 h at 2–8°C. The acclimated material was quantified using the SPCA method and then used to create fortification levels. Whole milk, skim milk, chocolate milk, and light 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 near the NCIMS Pasteurized Milk Ordinance action level, 20 000 CFU/mL or CFU/g product, and ranging 2 logs were evaluated per NCIMS requirement. Additional dairy matrixes prepared in the manufacturer's laboratory used 3–5 concentrations to meet AOAC RI PTM validation requirements for claimed matrixes. Neat and/or 10^{-1} dilutions were used to evaluate detection in the countable ranges of 25 to 250 CFU/mL dilution.

Reference method for dairy.—Five replicate test portions from each contamination level were assayed in duplicate according to a modification of FDA NCIMS Form 2400a guidelines. Neat samples were shaken 25 times in 7 s with 1 foot of movement. Within 3 min of agitation for whole milk, skim milk, light cream, and chocolate milk test matrixes, 1:10 dilutions were prepared by adding 11 mL (or 11 g) of neat sample into 99 mL BPBDW and shaking 25 times in 7 s with 1 foot of movement. Within 3 min of agitation and after bubble settling, 11 mL 1:10 sample dilutions were added to 99 mL BPBDW and shaken 25 times in 7 s with 1 foot of movement to produce a 1:100 sample. Each dilution was plated in duplicate into Petri dishes. Approximately 10 mL tempered (44–46°C) SPCA was poured into the Petri dishes, swirled, and allowed to solidify. The plates were inverted and incubated at 32 ± 1°C for 48 ± 3 h. Following incubation, typical white or pigmented colonies were enumerated. Duplicate plates in the countable range of 25 to 250 colonies were averaged and reported as aerobic bacteria count per milliliter (or count per gram).

Peel Plate AC method for dairy.—Five replicate test portions from each contamination level were assayed in duplicate. Dairy product samples were evaluated at 1:10 and 1:100 dilution levels in duplicate. Raw milk and other naturally contaminated products may have necessitated additional dilution levels to reach a countable range. As noted in *Materials and Methods*, cultured dairy products are not appropriate for aerobic count determination. Test portions were shaken 25 times in 7 s with 1 foot if movement. The 1:10 dilutions were prepared by adding 11 mL neat sample into 99 mL BPBDW and shaken 25 times in 7 s with 1 foot of movement. Within 3 min of agitation, the Peel Plate AC covers were lifted to fully expose the dried media culture disc and then 1 mL sample aliquots were dispensed onto the center of the disc. The covers were reapplied and sealed over the disc. Peel Plate AC plates were inverted, stacked 20 high, and incubated with their covers at 32 ± 1°C for 48 ± 3 h.

Dairy matrixes results and discussion.—Analyses of all matrixes were conducted for each contamination level. In what follows, CFU per plate for the appropriate dilution is reported. Logarithmic transformations of the total aerobic counts (CFU/mL or CFU/g) 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 α-value of 0.5 with CIs within (−0.5, 0.5) indicated no statistical difference between the Peel Plate AC and SPCA methods. Results are reported in Table 1. Additional laboratory participants of collaborated shared sample data for the NCIMS study are reported in Annex Tables 1–4, along with graphical presentation of all log means for whole milk, skim milk, chocolate milk and light cream in Annex Figures 1–4. The dairy matrixes were studied and whole milk, chocolate milk, heavy cream, UHT whole milk, lactose-reduced milk, strawberry milk, vanilla ice cream, condensed skim milk, raw cow milk, raw goat milk, raw sheep milk, powdered milk, and pasteurized goat milk showed no significant differences with the reference method SPCA except for a few of the lowest concentrations, where spike levels resulted in a high standard deviation and an upper control limit (UCL) greater than 0.5 log. Only the skim matrix showed a significant difference in the independent laboratory at the different spike levels. This difference was observed by the other laboratories using the shared samples, however, at the Charm Sciences laboratory the differences were within significant levels and within 0.5 $\log_{10}$ from the SPCA mean. The target bacterial

Table 1. Peel Plate AC method for aerobic count versus the SPCA method with dairy matrixes

Matrix	Fortified micro-organism ATCC No. (% injury)	Contamination level	Candidate method			Reference method			Mean difference[d]	95% CI[e]		r^{2h}
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[f]	UCL[g]	
Whole milk	*Pseudomonas aeruginosa* ATCC 15442 (58.3%)	None	<0.1	NA[i]	NA	<0.1	NA	NA	NA	NA	NA	1.0
		1	1.999	0.105	5.3	1.905	0.187	9.8	0.094	−0.051	0.239	
		2	2.25	0.137	6.1	2.182	0.101	4.6	0.068	−0.064	0.199	
		3	3.58	0.057	1.6	3.521	0.059	1.7	0.059	0.01	0.106	
		4	4.245	0.031	0.7	4.197	0.05	1.2	0.048	0.025	0.071	
		5	4.42	0.053	1.2	4.417	0.059	1.3	0.003	−0.032	0.037	
Whole milk[j]	*P. aeruginosa* ATCC 15442 (58.3%)	None	<0.1	NA[i]	NA	<0.1	NA	NA	NA	NA	NA	0.97
		1	1.421	0.242	17.0	1.861	0.088	4.7	−0.44	−0.613	−0.267	
		2	2.28	0.077	3.4	2.131	0.073	3.4	0.149	0.076	0.221	
		3	3.541	0.087	2.5	3.321	0.095	2.9	0.22	0.112	0.328	
		4	4.041	0.062	1.5	3.986	0.07	1.8	0.055	−0.003	0.114	
		5	4.477	0.016	0.4	4.192	0.033	0.8	0.285	0.259	0.311	
Chocolate milk	*Escherichia coli* ATCC 11229 (52.8%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.99
		1	2.538	0.06	2.4	2.437	0.127	5.2	0.101	0.017	0.185	
		2	2.659	0.073	2.7	2.623	0.047	1.8	0.036	−0.037	0.11	
		3	2.915	0.03	1.0	2.878	0.038	1.3	0.037	0.001	0.074	
		4	3.508	0.051	1.5	3.558	0.045	1.3	−0.05	−0.101	0.001	
		5	3.691	0.029	0.8	3.632	0.062	1.7	0.059	0.01	0.107	
Chocolate milk[j]	*E. coli* ATCC 11229 (52.8%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		1	2.515	0.057	2.3	2.563	0.065	2.5	−0.048	−0.103	0.008	
		2	2.652	0.071	2.7	2.699	0.044	1.6	−0.047	−0.103	0.009	
		3	2.981	0.035	1.2	2.996	0.037	1.2	−0.015	−0.058	0.027	
		4	3.539	0.064	1.8	3.549	0.077	2.2	−0.01	−0.069	0.048	
		5	3.711	0.093	2.5	3.737	0.079	2.1	−0.026	−0.094	0.041	
Skim milk	*Leuconostoc mesenteroides* ATCC 8293 (53.9%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		1	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	
		2	1.448	0.25	17.3	1.848	0.097	5.2	−0.4	−0.567	−0.233	
		3	2.302	0.116	5.0	2.596	0.111	4.3	−0.294	−0.407	−0.181	
		4	2.326	0.078	3.4	2.667	0.085	3.2	−0.341	−0.411	−0.27	
		5	3.207	0.026	0.8	3.601	0.083	2.3	−0.394	−0.453	−0.336	
Skim milk[j]	*L. mesenteroides* ATCC 8293 (53.9%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.99
		1	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	
		2	1.403	0.173	12.3	1.913	0.125	6.5	−0.51	−0.695	−0.326	
		3	2.311	0.074	3.2	2.735	0.029	1.1	−0.424	−0.479	−0.369	
		4	2.282	0.102	4.5	2.893	0.031	1.1	−0.611	−0.679	−0.543	
		5	3.23	0.13	4.0	3.645	0.061	1.7	−0.415	−0.542	−0.285	
Light (20%) cream	*Enterococcus faecium* ATCC 8459 (62.8%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		1	1.505	0.257	17.1	1.623	0.266	16.4	−0.118	−0.356	0.121	
		2	2.305	0.109	4.7	2.499	0.079	3.2	−0.194	−0.33	−0.058	
		3	3.187	0.038	1.2	3.361	0.018	0.5	−0.174	−0.2	−0.149	
		4	3.629	0.099	2.7	3.806	0.053	1.4	−0.177	−0.229	−0.125	
		5	4.252	0.057	1.3	4.481	0.023	0.5	−0.229	−0.258	−0.201	
Light (20%) cream[j]	*E. faecium* ATCC 8459 (62.8%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		1	1.562	0.263	16.8	1.753	0.13	7.4	−0.191	−0.397	0.013	
		2	2.535	0.074	2.9	2.552	0.048	1.9	−0.017	−0.083	0.049	
		3	3.487	0.051	1.5	3.498	0.027	0.8	−0.011	−0.047	0.026	
		4	3.807	0.043	1.1	3.878	0.03	0.8	−0.071	−0.102	−0.04	
		5	4.377	0.025	0.6	4.39	0.029	0.7	−0.013	−0.026	0.001	
Pasteurized whole goat milk	*Lactococcus lactis* ATCC 11424 (20%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	3.41	0.08	2.3	3.40	0.02	0.7	0.01	−0.04	0.08	

Table 1. (*continued*)

Matrix	Fortified micro-organism ATCC No. (% injury)	Contamination level	Candidate method			Reference method			Mean difference[d]	95% CI[e]		r^{2h}
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[f]	UCL[g]	
		Mid	4.50	0.09	2.0	4.42	0.07	1.6	0.08	0.00	0.17	
		High	5.38	0.03	0.5	5.32	0.02	0.3	0.06	0.04	0.09	
Raw cow milk	Natural	Natural	1.12	0.12	11	1.20	0.14	11	−0.08	−0.24	0.08	1.0
		Low	1.96	0.04	1.9	2.15	0.05	2.5	−0.19	−0.25	−0.13	
		Mid	3.01	0.04	1.5	3.29	0.04	1.2	−0.28	−0.32	−0.24	
		High	3.97	0.08	2.0	4.34	0.03	0.8	−0.37	−0.44	−0.30	
Raw goat milk	Natural	Heated	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	4.12	0.03	0.8	3.87	0.04	1.1	0.25	0.20	0.30	
		Mid	5.00	0.04	0.8	4.66	0.06	1.2	0.34	0.30	0.39	
		High	5.99	0.05	0.8	5.67	0.10	1.7	0.33	0.24	0.39	
Raw sheep milk	Natural	Natural	1.85	0.06	3.0	1.80	0.05	2.8	0.05	−0.01	0.09	1.0
		Low	2.56	0.07	2.8	2.70	0.06	2.2	−0.14	−0.18	−0.10	
		Mid	3.30	0.11	3.2	3.39	0.10	2.9	−0.09	−0.18	0.00	
		High	3.67	0.05	1.5	3.75	0.05	1.3	−0.08	−0.12	−0.03	
Vanilla ice cream	*L. lactis* subsp. *lactis* ATCC 11424 (0%)	Natural	0.40	0.23	57	0.62	0.19	31	−0.22	−0.40	−0.03	1.0
		Low	3.07	0.04	1.2	3.04	0.02	0.7	0.03	−0.01	0.07	
		Mid	4.13	0.03	0.7	4.06	0.06	1.4	0.07	0.02	0.11	
		High	5.14	0.03	0.5	5.05	0.04	0.8	0.09	0.07	0.13	
Condensed milk	*C. freundii* ATCC 8090 (99%)	Natural	1.27	0.26	20.8	1.23	0.22	17.6	0.04	−0.27	0.34	1.0
		Low	4.01	0.07	1.8	4.22	0.03	0.7	−0.21	−0.26	−0.16	
		Mid	5.02	0.04	0.8	5.17	0.03	0.6	−0.15	−0.19	−0.11	
		High	6.08	0.04	0.6	6.32	0.09	1.4	−0.24	−0.31	−0.18	
Nonfat powder milk	*Cronobacter sakazakii* ATCC 29544 (40%)	None	1.98	0.11	5.0	2.19	0.12	5.0	−0.21	−0.29	−0.14	1
		Low	3.06	0.03	1.0	3.06	0.06	1.8	0.00	−0.04	0.06	
		Mid	3.99	0.04	1.0	3.96	0.05	1.1	0.03	0.00	0.06	
		High	5.03	0.05	1.0	5.02	0.04	0.8	0.01	−0.05	0.07	
Lactose-reduced milk	*E. faecium* ATCC 8459 (99%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	0.98
		Low	2.71	0.05	1.9	2.64	0.09	3.6	0.07	0.00	0.14	
		Mid	3.70	0.03	0.9	3.65	0.05	1.2	0.05	0.01	0.09	
		High	4.75	0.05	1.0	4.68	0.06	1.2	0.07	0.01	0.13	
UHT whole milk	*Staphylococcus aureus 6538* (15%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1
		Low	2.76	0.04	1.5	2.85	0.07	2.4	−0.09	−0.15	−0.02	
		Mid	3.82	0.06	1.5	3.91	0.06	1.6	−0.09	−0.14	−0.03	
		High	4.91	0.05	1.0	4.98	0.08	1.6	−0.07	−0.11	−0.03	
Evap-orated milk	*L. mesenteroides* ATCC 8293 (25%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	4.51	0.08	1.8	4.51	0.09	2.0	0.00	−0.07	0.07	
		Mid	5.46	0.08	1.5	5.50	0.11	2.0	−0.04	−0.14	0.06	
		High	6.45	0.08	1.2	6.46	0.07	1.2	−0.01	−0.10	0.09	
Strawberry milk	*Pseudomonas fluorescens* ATCC 13525 (0%)	Natural	3.01	0.06	2.0	3.00	0.06	2.0	0.01	−0.04	0.06	1.0
		Low	3.51	0.06	1.8	3.42	0.09	2.7	0.09	0.00	0.17	
		Mid	4.29	0.01	0.3	4.12	0.04	1.0	0.17	0.15	0.20	
		High	5.59	0.11	1.9	5.20	0.11	2.2	0.39	0.30	0.48	

[a] Mean of five replicate portions, plated in duplicate, after logarithmic transformation: $\log_{10}[CFU/g + (0.1)f]$ where f=dilution factor.

[b] Repeatability standard deviation.

[c] Relative standard deviation for repeatability.

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

[e] CI = Confidence interval.

[f] 95% LCL for difference of means.

[g] 95% UCL for difference of means

[h] Square of correlation coefficient.

[i] NA = Not applicable.

[j] Independent-laboratory performed.

high spike level (>20 000) compared with the observed high spike level (ca 6000) shows that bacteria were additionally stressed in the skim matrix during the acclimation period of 48 h. It appears that the reference method better resuscitated the stressed organisms compared with Peel Plate AC, and this same result was observed with a second method, AOAC **983.01**, used by the laboratories on these samples. Additional experiments to replicate this hypothesized matrix effect were not successful. In two additional experiments (shown in Table 2) using the same and different bacterial strains, the mean $\log_{10}$ differences between the comparative and reference methods did not show significant differences with UCL and lower control limit (LCL) within $\log_{10}$ (−0.05 and 0.5). This would suggest that the observed differences in the matrix samples were a sample preparation anomaly.

The dairy matrixes data support that Peel Plate AC aerobic bacterial count determinations are not significantly different from SPCA aerobic plate count results.

Peel Plate AC: Food and Contact Surface Matrixes

Ground meat, liquid eggs, dried dog food, chocolate, surface sponges of stainless steel, chicken rinse, and large animal hides matrixes were evaluated in Peel Plate AC at 35 ± 1°C for 48 ± 3 h in comparison with FDA/BAM, USDA MLG, and ISO methods as described in the following paragraphs.

Sample preparation.—Ground beef, ground turkey, and pasteurized liquid eggs were purchased at local grocery stores. Sample matrixes were evaluated for natural aerobic count. Samples were split into control and low, medium, and high CFU/mL levels and inoculated, when necessary, with various freshly cultured or heat-stressed bacterial strains as indicated in Table 3. Five replicate test portions from each contamination level for the ground meats and eggs were assayed in duplicate. A paired analysis following the Peel Plate AC method and USDA MLG Sections 3.01–3.5 and FDA/BAM Chapter 3 colony count procedures. Prepared samples were assayed by Peel Plate AC and harmonized reference methods at 1:10 and subsequent serial dilution levels to get countable ranges of 25 to 250 CFU/mL.

Following USDA MLG Section 3.01 guidelines, 450 mL sterile BPW 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 test portion for 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 BPBDW to achieve a countable range of 25 to 250 CFU/mL.

Hog rinse sponge samples representing 300 cm^2/25 mL were shipped frozen from an abattoir in Missouri. The samples were thawed and pooled for aerobic count determination and subsequent fortification and testing. Whole chicken carcasses were purchased from a local grocer and 400 mL BPBDW per carcass were added to 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 bacterial strains as indicated in Table 3. Five replicate test portions from each contamination level were assayed in a paired analysis following the Peel Plate AC method and FDA/BAM Chapter 3 reference method. The serial dilutions were prepared by adding 10 mL from the stomacher bag into 90 mL BPBDW, or prior dilution level, to reach countable ranges of aerobic bacteria.

Dried dog food and chocolate chips were purchased from 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 bacterial strains as indicated in Table 3. Five replicate test portions from each contamination level for the ground dog

Table 2. Peel Plate AC method for aerobic count versus SPCA method additional skim testing

Matrix gortified micro-organisms	ATCC No. (% injury)	Contamination level	Candidate method			Reference method			Mean difference[d]	95% CI[e]		r^{2h}
			Mean[a]	s_r[b]	RSD_r[c]	Mean[a]	s_r[b]	RSD_r[c]		LCL[f]	UCL[g]	
Skim milk	*Enterobacter cloacae* ATCC 13047 (61%) and *E. coli* ATCC 51813 (50%)	Low	2.39	0.07	3.1	2.71	0.07	2.4	−0.32	−0.40	−0.24	0.97
		Mid-low	3.06	0.18	5.7	3.29	0.07	2.1	−0.23	−0.38	−0.08	
		Mid	3.10	0.02	0.7	3.09	0.05	1.7	0.01	−0.02	0.04	
		Mid-high	3.86	0.03	0.7	3.90	0.05	1.4	−0.04	−0.10	0.01	
		High	4.15	0.03	0.7	4.28	0.06	1.4	−0.13	−0.17	−0.09	
Skim milk	*L. mesenteroides* ATCC 8293 (11%)	None	2.23	0.11	4.7	2.34	0.09	3.9	−0.11	−0.20	−0.01	1.0
		Low	3.06	0.03	0.9	3.05	0.02	0.6	0.01	−0.02	0.03	
		Mid	4.05	0.03	0.8	4.03	0.04	1.0	0.02	−0.01	0.06	
		High	5.01	0.04	0.7	5.01	0.04	0.7	0.0	−0.03	0.04	

[a] Mean of five replicate portions, plated in duplicate, after logarithmic transformation: $\log_{10}$[CFU/g + (0.1)f] where f=dilution factor.

[b] Repeatability standard deviation.

[c] Relative standard deviation for repeatability.

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

[e] CI = Confidence interval.

[f] 95% LCL for difference of means.

[g] 95% UCL for difference of means

[h] Square of correlation coefficient.

Table 3. Peel Plate AC method for aerobic count versus the SPCA method with food matrixes at 35°C

Matrix	Fortified micro-organism ATCC No. (%injury)	Contamination level	Candidate method			Reference method			Mean difference[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)	Natural contamination (0%)	None	2.57	0.25	9.5	2.55	0.21	8.4	0.03	−0.06	0.09	1.0
		Low	4.20	0.11	2.6	4.13	0.11	2.6	0.07	−0.04	0.19	
		Mid	6.40	0.06	1.0	6.29	0.06	0.9	0.11	0.07	0.15	
		High	7.19	0.06	0.9	7.09	0.08	1.1	0.10	0.06	0.14	
Ground beef (77% lean)[j]	Natural contamination (0%)	None	<0.1	NA[i]	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	1.06	0.13	12.7	1.12	0.16	14.7	−0.06	−0.23	0.11	
		Mid	2.83	0.03	1.1	2.78	0.03	1.17	0.05	0.02	0.08	
		High	3.25	0.08	2.5	3.16	0.14	4.5	0.09	−0.09	0.28	
Ground turkey	Natural contamination (0%)	Natural	6.05	0.04	0.6	5.97	0.05	0.9	0.08	0.03	0.12	1.0
		Low	6.86	0.08	1.2	6.77	0.14	2.0	0.09	0.02	0.17	
		Mid	6.97	0.07	1.0	6.88	0.05	0.8	0.09	0.03	0.15	
		High	7.38	0.04	0.6	7.32	0.06	0.8	0.06	0.03	0.11	
Chicken rinse	*E. cloacae* ATCC 13047 (19%)	Natural	3.16	0.04	1.4	3.04	0.06	1.9	0.12	0.07	0.17	1.0
		Low	3.18	0.05	1.5	3.29	0.03	1.0	−0.11	−0.15	−0.07	
		Mid	4.15	0.02	0.5	4.13	0.02	0.5	0.02	0.00	0.04	
		High	5.20	0.02	0.3	5.18	0.04	0.8	0.02	−0.01	0.05	
Hog carcass rinse	*E. cloacae* ATCC 13047 (19%)	Natural	0.91	0.13	14.3	0.43	0.29	68.8	0.48	0.27	0.69	1.0
		Low	2.77	0.05	1.7	2.85	0.05	1.6	−0.08	−0.13	−0.04	
		Mid	3.83	0.05	1.3	3.91	0.04	1.0	−0.08	−0.12	−0.03	
		High	4.89	0.04	0.8	4.95	0.05	1.0	−0.06	−0.11	−0.03	
Stainless steel surface sponge	*L. mesenteroides* ATCC 8293 (0%)	Background	1.48	0.53	36	1.46	0.52	35	0.02	−0.10	0.07	0.99
		Low	2.60	0.14	5.4	2.64	0.14	5.2	−0.04	−0.13	0.06	
		Mid	3.29	0.36	10.8	3.07	0.53	17.2	0.22	−0.10	0.53	
		High	5.44	0.17	3.1	5.45	0.22	4.0	−0.01	−0.09	0.08	
Stainless steel surface sponge[j]	*S. aureus* ATCC 6538 (0%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	1.28	0.17	13.5	1.48	0.32	21.8	−0.20	−0.78	0.38	
		Mid	2.10	0.19	9.0	2.10	0.20	9.3	0.00	−0.15	0.16	
		High	3.35	0.04	1.32	3.39	0.03	1.0	−0.04	−0.06	−0.02	
Liquid eggs	*P. aeruginosa* ATCC 15442 (99.2%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	2.87	0.05	1.7	2.68	0.07	2.5	0.19	0.13	0.25	
		Mid	3.92	0.04	1.1	3.73	0.07	1.9	0.19	0.13	0.24	
		High	4.80	0.08	1.8	4.63	0.06	1.3	0.17	0.12	0.22	
Dried dog food	*E. coli* ATCC 11775 (36%)	None	<0.1	NA	NA	<0.1	NA	NA	NA	NA	NA	1.0
		Low	3.06	0.09	2.8	3.05	0.10	3.1	0.01	−0.02	0.04	
		Mid	4.12	0.11	2.6	4.11	0.14	3.4	0.01	−0.04	0.06	
		High	5.11	0.10	1.9	5.08	0.09	1.8	0.03	−0.01	0.07	
Chocolate	*E. coli* ATCC 11229 (99%)	Natural	0.82	0.17	21.1	0.97	0.19	19.4	−0.15	−0.32	0.01	1.0
		Low	2.16	0.28	13.0	2.36	0.16	7.0	−0.20	−0.36	−0.05	
		Mid	2.87	0.34	11.7	2.89	0.35	12.1	−0.02	−0.06	0.04	
		High	3.82	0.16	4.1	3.74	0.18	4.8	0.08	0.02	0.13	

[a] Mean of five replicate portions, plated in duplicate, after logarithmic transformation: $\log_{10}[CFU/g + (0.1)f]$ where f=dilution factor.

[b] Repeatability standard deviation.

[c] Relative standard deviation for repeatability.

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

[e] CI = Confidence interval.

[f] 95% LCL for difference of means.

[g] 95% UCL for difference of means.

[h] Square of correlation coefficient.

[i] NA = Not applicable.

[j] Independent-laboratory performed.

food and chocolate were assayed in a paired analysis following the Peel Plate AC method and FDA/BAM Chapter 3. Prepared samples were assayed by the Peel Plate AC and reference methods at 1:10 and subsequent serial dilution levels to get countable ranges of 25 to 250 CFU/mL.

Stainless steel coupons were prepared and sampled following ISO 18593. Fresh bacteria cultures (Table 3) were applied to the surface and allowed to dry for 3–24 h. Five replicate test portions from each contamination level were assayed in a paired analysis following the Peel Plate AC method and the harmonized FDA/BAM Chapter 3 reference method. Stainless steel environment samples were assayed by the Peel Plate AC and harmonized reference methods from the initial preparation and at 1:10 dilution. Sampling sponges were moistened with 10 mL peptone water and used to sample 100 cm^2 stainless steel surfaces. Sponges were transferred to sterile stomacher bags and 25 mL peptone water were 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 obtain the countable range.

FDA/BAM chapter 3 reference method.—Following the FDA/BAM Chapter 3 reference method, 1 mL aliquots of sample preparation were plated into sterile Petri dishes and ca 10 mL tempered SPA were added to the plates, swirled, and allowed to solidify. After the agar solidified, the plates were inverted and incubated at 35 ± 1°C for 48 ± 3 h. Following incubation, all white or pigmented colonies were counted. Duplicate plates in the countable range of 25 to 250 colonies were averaged and reported as aerobic count per milliliter.

Peel Plate AC method.—Five replicate test portions from each inoculation level were assayed in duplicate. The Peel Plate AC 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. The covers were reapplied and sealed over the disc. Peel Plate AC plates were stacked 20 high and incubated with the cover down at 35 ± 1°C for 48 ± 3 h. Following 48 h of incubation, all red colonies were enumerated and reported as the total aerobic CFU per milliliter.

Matrix studies results and discussion.—Analyses of all matrixes were conducted for each contamination level. Reported is the aerobic plate count for each dilution. Logarithmic transformations of the total calculated aerobic counts (CFU/mL or CFU/g) and paired statistical analysis were performed. All data are reported and no outliers were removed. The difference of means and their 95% CIs for each contamination level were determined. A $\log_{10}$ mean difference value less than the standard α-value of 0.5 with CIs within (−0.5, 0.5) indicated no statistical difference between Peel Plate AC and reference methods. The Results of the aerobic matrix experiments and fortification levels are reported in Table 3.

Results of ground beef, ground turkey, liquid eggs, dried dog food, carcass rinses, stainless steel sponge, and milk-chocolate in comparison with SPCA demonstrate no significant differences at any of the concentrations or matrixes.

Independent laboratory results using ground beef and stainless steel matched manufacturer data. The replication of the comparative method was similar to the reference method in all studies.

Robustness, Product 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 AC method (12). The steps and perturbations evaluated were pipetting, 1.0 mL ± 5%, temperature of incubation (a low of 30°C and high of 36°C), and time of incubation (a low of 45 h and a high of 51 h. The multivariate design assays were performed in whole milk fortified with *Escherichia coli* American Type Culture Collection (ATCC; Manassas, VA) 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 4.

Assay temperatures showed no significant difference in paired log–*t*-test confidence levels >0.5, but *t*-test showed a lower bias at the higher incubation temperature. This may reflect why dairy analysis is traditionally performed in the 32 ± 1°C incubation range. A shorter assay time did not show a significant difference by *t*-test or in paired analysis CIs. As expected, pipet volume did show a significant difference by *t*-test with a positive bias in the higher volume; however, the low bias using a >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 1% loss of weight. Moisture loss studies were

Table 4. Multivariate evaluation of Peel Plate AC assay perturbations

Assay perturbation	High and low condition	Mean CFU/mL	SD	CV %	Probability of difference, %	Paired *t*-test log difference[a]	LCL[b]	UCL[c]
Temp., °C	30	193	14.3	7	65	0.33	0.01	0.14
	36	179	18.8	10	99			
Pipet vol., µL	950	199	10	5	96	−0.12	−0.44	0.21
	1050	205	21.8	11	99			
Assay time, h	45	193	14.3	7	94	0.00	−0.04	0.04
	51	195	30.5	15	76			

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

[b] 95% LCL for difference of means.

[c] 95% UCL for difference of means.

performed in BPBDW fortified with *E. coli* ATCC 29522, *Lactococcus lactis*, ATCC 11454, and *Citrobacter freundii*, ATCC 8090 and three replicate tests were performed under each moisture loss stress. Diluted samples were added to the plates and the plates were sealed (control) and left exposed in 32°C incubator for 5, 10, 15, and 45 min to achieve water losses of 5, 10, 15, and 25%. Average and standard deviations were calculated. There were <10% differences in counts under all moisture loss levels, indicating that a moisture loss ≤25% is not of significant concern.

Studies comparing 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 quality assurance parameters and at least a 1 year refrigerated shelf-life.

Discussion

Results of the Peel Plate AC evaluations at 32 ± 1°C for 48 ± 3 h in dairy products (whole milk, chocolate milk, light cream, nonfat dried milk powder, ice cream, evaporated skim milk, condensed skim milk, UHT milk, raw cow milk, raw goat milk, raw sheep milk, and pasteurized goat milk) showed no significant differences in total aerobic count determination with reference method SPCA. The exception seen in the skim milk matrix was not replicated in additional experiments used to determine whether there was repeatable matrix interference. The results appear to be an anomalous matrix effect on the stressed bacteria used in preparing that study set.

Results of Peel Plate AC matrixes for aerobic count in various foods and rinse samples at 35°C were not significantly different from the reference methods. Aerobic count determinations compared with SPCA confirmation in various foods, ground meats, dog food, liquid eggs, milk-chocolate, stainless sponge, hog carcass sponge, and chicken rinse showed no significant difference.

Conclusions

The dairy matrix data support that the Peel Plate AC method at 32 ± 1°C for dairy at 48 ± 3 h will detect total aerobic bacteria. The results are not significantly different from the SPCA method in dairy products.

The food and surface sponge matrix data support that the Peel Plate AC method at 35 ± 1°C for 48 ± 3 h will quantify aerobic count in a manner not significantly different from the reference methods. Ground meats, liquid eggs and carcass rinses were evaluated with harmonized USDA MLG Section 3.01 and FDA/BAM Chapter 3 methods. Dog food and chocolate were evaluated according to FDA/BAM Chapter 3 methods. Stainless steel surfaces were evaluated with harmonized ISO 18593 and the FDA/BAM Chapter 3 method.

The data supplied supports a quality controlled manufacture with a 1 year refrigerated shelf-life as indicated by high temperature stress testing of the Peel Plate AC test. The volume of pipetting is a critical step in performance during the specified 45–51 h incubation.

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