FOOD BIOLOGICAL CONTAMINANTS

3M™ Petrifilm™ Staph Express Count Plate Method for the Enumeration of Staphylococcus aureus in Selected Dairy Foods: Collaborative Study

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The 3M™ Petrifilm™ Staph Express Count plate method was compared with AOAC Official Method 975.55 for the enumeration of Staphylococcus aureus in selected foods. Five foods—ice cream, raw milk, yogurt, whey powder, and cheese—were analyzed for S. aureus by 12 collaborating laboratories. For each food tested, the collaborators received 8 blind test samples consisting of a control sample, a low inoculation level, a medium inoculation level, and a medium inoculation level with background flora, each in duplicate. The mean $\log_{10}$ counts for the methods were comparable for all 5 foods. The repeatability and reproducibility variances of the 24 h Petrifilm Staph Express Count plate method were similar to those of the 72 h standard method.

Accurate and rapid detection of Staphylococcus aureus is of significant interest to the food industry. Enumeration of this bacterium is often used to assess food quality and safety. The 3M™ Petrifilm™ Staph Express Count plate and disk system is used to enumerate S. aureus in foods. The Petrifilm Staph Express Count plate contains a plating medium and a water-soluble gelling agent optimized for the growth of staphylococcal bacteria yet inhibitory to the growth of most nonstaphylococcal bacteria.

Test portions are added at a volume of 1.0 mL per plate. Pressure applied to a plastic spreader placed on the top film spreads the test portion over a growth area of approximately 30 cm^2. The gelling agent is allowed to solidify, and the plates are then incubated for 24 2 h at 35 1 C or 37 1 C (temperature based on validated references). Red-violet colonies on the plate are S. aureus. When only red-violet colonies are present, count the colonies; the test is complete.

If background flora are encountered in testing, the Petrifilm Staph Express disk is used to identify S. aureus from all suspect colonies, as some S. aureus may be stressed or otherwise appear atypical on the plate. The Petrifilm Staph Express disk is used whenever colonies other than red-violet are present on the plate; for example, black colonies or blue-green colonies. The Petrifilm Staph Express disk contains a dye and deoxyribonucleic acid. S. aureus produces deoxyribonuclease (DNase), which reacts with the dye to form pink zones. The disk is inserted into the plate, and the plate and disk are incubated for a minimum of 1 h and a maximum of 3 h at 35 1 C or 37 1 C. S. aureus (and occasionally, S. hyicus and S. intermedius, which can produce enterotoxins) generate a pink zone. Count the pink zones as S. aureus, regardless of the size of the zone.

A comparative study (Horter and Lindberg, unpublished) demonstrated that the Petrifilm Staph Express Count plate is as effective as the classical method for the identification and enumeration of S. aureus. This report describes a collaborative study comparing the Petrifilm Staph Express Count plate method with the AOAC plate method (Official Method 975.55) for enumerating S. aureus in foods.

Collaborative Study

Test Foods

The food types selected for the study were ice cream, raw milk, yogurt, whey powder, and cheese. Foods were obtained from local retail stores.

Test Organisms

The S. aureus organisms were obtained from the American Type Culture Collection (ATCC; Manassas, VA; Table 1) and were stored at –80 C in laboratory medium containing 15%

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Table 1. Staphylococcus aureus used to inoculate test samples

ATCC	Product
51740	Strawberry ice cream
27664	Raw milk
25923	Vanilla yogurt
8095	Whey powder
13565	Mozzarella cheese

sterile glycerol. Enterococcus faecalis (ATCC 14506) was inoculated to achieve background competitive flora.

Working cultures used for all foods except for whey powder were maintained on brain heart infusion (BHI) slants, and the inocula were cultivated on tryptic soy agar plates incubated at 35 C. A 1.0 McFarland solution was prepared and diluted with phosphate-buffered dilution water prior to inoculation of the test samples. For whey powder, the S. aureus organisms were lyophilized and diluted in whey powder to achieve the target levels.

Inoculation of Foods

The test samples were prepared by inoculation with a wet suspension of cells for ice cream, raw milk, yogurt, and cheese. Approximately 6000 g of each product was ground to obtain a homogeneous mixture. Each product was divided into four 1500 g portions. One portion was inoculated with the S. aureus strain in the range of 100–1000 cells/g and no inoculation of a background flora organism. The second portion was inoculated with the S. aureus strain in the range of 1001–10 000 cells/g and no inoculation of a background flora organism. The third portion was inoculated with the S. aureus strain in the range of 1001–10 000 cells/g and with the background organism in the range of 10 001–100 000 cells/g. The fourth portion served as an uninoculated control. A lyophilized culture was diluted to the same levels used for the other foods.

Preparation of Test Samples

Inoculated and uninoculated test samples were divided into subsamples containing approximately 55 g. For each product analyzed, duplicate subsamples of the uninoculated control and of each of the 3 inoculated portions were sent to each collaborator. Samples were packaged in leak-proof containers, identified by test sample code. The sample code was a singular, multidigit randomized number. Samples were frozen (ice cream) for 1 week or refrigerated (milk, yogurt, cheese) for 2–3 days prior to analysis. The whey powder was stored at ambient temperature for 1 week prior to analysis.

Shipment of Test Samples

Samples were shipped by overnight carrier to arrive the day before initiation of analysis. Shipments were made according to Dangerous Goods Regulations as defined by International Air Transport Association. Ice cream was sent on dry ice and stored frozen (<–15°C) upon arrival. Milk, yogurt, and cheese were packed with ice packs to maintain a temperature of <7 C during transport. Upon arrival, the samples were stored in the refrigerator (2–8 C). The whey powder was shipped and stored at ambient temperature upon arrival. One food type was shipped for each week of the study.

Microbiological Analyses

Twelve laboratories analyzed the samples for S. aureus using both the Petrifilm plate method and the AOAC method. Eight samples, for each of the 5 foods tested, were analyzed by the 2 methods. The collaborators received blind samples and were instructed to dilute all test portions and plate the 10^{-1}, 10^{-2}, 10^{-3} dilutions onto each of the Petrifilm and Baird-Parker agar (BPA) plates. The Petrifilm plates were incubated at 35 1 C for 24 2 h. When only red-violet colonies were present, the colonies were counted as S. aureus; the test was complete.The BPA plates were incubated at 35–37 C for 45–48 h and then enumerated.

If background flora (black, or blue-green colonies) were encountered in testing, the Petrifilm Staph Express disk was used to identify S. aureus from all suspect colonies. After the disk was inserted into the plate, the plate and disk were then incubated for a minimum of 1 h and a maximum of 3 h at 35 1 C. The pink zones were counted as S. aureus, regardless of the size of the zone.

For both methods, one suspect colony from a plate at each inoculation level was selected for a coagulase test. Colonies from the Petrifilm plates were streaked to BPA. These BPA plates were incubated overnight at 35 1 C. Colonies from these BPA plates and those selected straight from the BPA method plates were transferred into 0.2 mL BHI. BHI cultures were incubated 18–24 h at 35 1 C. A 0.5 mL volume of rabbit plasma preserved with ethylene diamine tetraacetic acid was then added to each tube of BHI, and the tubes were re-incubated at 35 1 C for an additional 6 h.

Statistical Analysis of Data

The collaborators recorded the red-violet colony counts or the pink zone counts for the Petrifilm Staph Express Count plates, or plates and disks, and the colony counts for the BPA plates. The presence or absence of clot formation in the coagulase test was recorded for all methods. Petrifilm plates or plates and disks with 1–150 colonies or pink zones, respectively, and BPA plates with 20–200 colonies were selected for analysis. If none of the plates had the minimum number of colonies or zones, the exact count on the least dilute test was selected for analysis. If the plates were too crowded to estimate counts, the count was reported as too numerous to count.

Procedures described by AOAC INTERNATIONAL were used to calculate colony forming units (CFU)/g [Official Methods of Analysis (2002) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD]. The base 10 logarithms of colony counts or pink zone counts were used for statistical analysis under the assumption that the transformed numbers would be normally distributed and of homogenous variance. Indefinite data values (e.g., <1.0) were treated as missing values in

the analyses. Repeatability (s_r) and reproducibility (s_R) standard deviations, relative standard deviations of repeatability (RSD_r) and reproducibility (RSD_R), and repeatability and reproducibility values (r and R, respectively) were calculated according to AOAC procedures once outliers were determined by the Cochran and Grubbs tests [Youden, W.J., & Steiner, E.H. (1975) Statistical Manual of the AOAC, AOAC, Arlington, VA]. Repeatability variances were compared using an F-ratio test [Snedecor, G.W., & Cochran, W.G. (1980) Statistical Methods, 7th Ed., Iowa State University Press, Ames, IA]. Mean $\log_{10}$ counts were compared by analysis of variance [Snedecor, G.W., & Cochran, W.G. (1980) Statistical Methods, 7th Ed., Iowa State University Press, Ames, IA]. In all statistical tests, a resulting value of $p < 0.05$ was taken to indicate a significant difference.

AOAC Official Method 2003.08
Enumeration of Staphylococcus aureus in Selected Dairy Foods
3M™ Petrifilm™ Staph Express Count Plate Method
First Action 2003

(Applicable to the enumeration of S. aureus in ice cream, raw milk, yogurt, whey powder and cheese.)

Caution: Autoclave materials after use.

See Table 2003.08 for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

The Petrifilm Staph Express Count plate is a sample-ready culture medium system which contains a cold-water-soluble gelling agent. The chromogenic, modified Baird-Parker medium in the plate is selective and differential for S. aureus. Diluted test portions are added at a volume of 1.0 mL per plate. The gelling agent is allowed to solidify after inoculation, and the plate is then incubated for 24 2 h at 35 1 C or 37 1 C. Red-violet colonies on the plate are S. aureus. When only red-violet colonies are present, count the colonies; the test is complete.

If background flora are encountered in testing, the Petrifilm Staph Express disk is used to identify S. aureus from all suspect colonies. Use the Petrifilm Staph Express disk whenever colonies other than red-violet are present on the plate; for example, black colonies or blue-green colonies. The Petrifilm Staph Express disk contains a dye and deoxyribonucleic acid. S. aureus produces deoxyribonuclease (DNase), which reacts with the dye to form pink zones. The disk is inserted into the plate, and the plate and disk are then incubated for a minimum of 1 h and a maximum of 3 h at 35 1 C or 37 1 C. S. aureus (and occasionally, S. hyicus and S. intermedius, which can produce enterotoxins) produce a pink zone. Count the pink zones as S. aureus, regardless of the size of the zone.

B. Apparatus and Reagents

(a) 3M Petrifilm Staph Express Count plate.—Plates, available from 3M Microbiology (St. Paul, MN), contain chromogenic modified Baird-Parker medium and a cold-water-soluble gelling agent.

(b) 3M Petrifilm Staph Express disk.—Disks, available from 3M Microbiology, contain toluidine blue-O and DNA.

(c) Plastic spreader.—Spreader with handle, available from 3M Microbiology, has a smooth flat side and is designed to spread the test suspension evenly over plate growth area.

(d) Pipets.—Calibrated 1.0 and 10.0 mL serological pipets with 0.1 mL graduations. 3M™ Electronic Pipettor and tips, or equivalent, may be used to deliver 1.0 mL.

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

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

(g) Phosphate-buffered dilution water.—(1) Stock solution.—Dissolve 34 g KH_2PO_4 in 500 mL H_2O, adjust to pH 7.2 with approximately 175 L 1M NaOH, and dilute to 1 L. Store in refrigerator. (2) Diluent.—Dilute 1.25 mL stock solution to 1 L with H_2O. Prepare dilution blanks with this solution, dispensing enough to allow for losses during autoclaving. Autoclave 15 min at 121 C.

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

(i) Incubator.—Maintaining 35 1 C or 37 1 C.

(j) Balance.—2000 0.1 g capacity.

(k) pH indicator strips.—To measure a range of 6.0–8.0.

C. Preparation of Test Suspensions

Use balance, B(j), with capacity of 2 kg and sensitivity of 0.1 g to aseptically weigh 50 g test portion into blender jar, B(h). Add 450 mL dilution water, B(g), and blend at 16 000 to 18 000 rpm for 2 min to homogenize. If entire test sample is <50 g, weigh portion of test sample and add dilution water to make a 1:10 dilution. As required, adjust pH of diluted test portion to 6.0–8.0 with 1M NaOH, B(f), (ca 0.1 mL/g test portion). Do not use diluents containing citrate, bisulfite, or thiosulfate as they can inhibit growth. Prepare all decimal dilutions with 90 mL dilution water plus 10 mL from the previous dilution. Pipets, B(d), must accurately deliver the required volume. Do not use pipets to deliver <10% of their total volume. For example, to deliver 1 mL, do not use pipet >10 mL; to deliver 0.1 mL, do not use pipet >1 mL. Mix all dilutions by shaking 25 times through 30 cm arc in 7 s.

D. Analysis

Place Petrifilm Staph Express Count plate, B(a), on flat surface. Lift top film and inoculate 1 mL portion of test suspension onto center of bottom film. Carefully roll top film down onto inoculum. Distribute test suspension over 30 cm^2 growth area with downward pressure on handle of plastic spreader, B(c). Leave plate undisturbed to permit gelling agent to solidify. Incubate plates at 35 1 C or 37 1 C for 24 2 h. In incubator, B(i), place plate in horizontal position in stacks not exceeding 20 units. Count plates with colony counter, B(e). Observe colony colors. If no colonies or only red-violet colonies are present after 24 2 h, count red-violet colo-

Table 2003.08. Interlaboratory study results of 3M™ Petrifilm™ Staph Express Count plate method and Baird-Parker agar method for detection of S. aureus in foods

		Petrifilm Staph Express Count plate or plate and disk								Baird-Parker agar							
Food	Level[a]	N[b]	Mean[c]	s_r	RSD_r, %	r	s_R	RSD_R, %	R	N[b]	Mean[c]	s_r	RSD_r, %	r	s_R	RSD_R, %	R
Ice cream	Low	11	1.57	0.32	20.24	0.89	0.32	20.56	0.90	11	1.61	0.33	20.30	0.91	0.34	20.81	0.94
	Med	10	2.58	0.06	2.49	0.18	0.09	3.46	0.25	11	2.54	0.08	3.20	0.23	0.18	7.11	0.51
	Med+	9	2.59[d]	0.10	3.88	0.28	0.10	3.88	0.28	10	2.66	0.09	3.26	0.24	0.12	4.41	0.33
Raw milk	Low	9	2.57	0.12	4.54	0.33	0.18	6.97	0.50	7	2.63	0.12	4.69	0.34	0.18	6.92	0.51
	Med	9	3.54	0.11	3.00	0.30	0.13	3.54	0.35	8	3.50	0.12	3.44	0.34	0.21	6.03	0.59
	Med+	10	3.50	0.30	8.62	0.85	0.30	8.72	0.86	10	3.42	0.12[e]	3.53	0.34	0.38	10.97	1.05
Cheese	Low	8	2.77	0.06[e]	2.10	0.16	0.07	2.51	0.19	10	2.53	0.16	6.48	0.46	0.30	12.09	0.86
	Med	7	3.74	0.09	2.32	0.24	0.12	3.09	0.32	7	3.58	0.15	4.23	0.42	0.23	6.34	0.64
	Med+	8	3.75	0.05[e]	1.23	0.13	0.08	2.12	0.22	8	3.66	0.11	3.05	0.31	0.20	3.05	0.55
Whey powder	Low	9	2.58	0.07	2.63	0.19	0.18	6.91	0.50	7	3.55	0.03[e]	0.74	0.07	0.33	9.37	0.93
	Med	10	3.44	0.08	2.40	0.23	0.29	8.55	0.82	9	3.48	0.14	3.86	0.38	0.31	8.84	0.88
	Med+	9	3.53	0.16	4.50	0.44	0.20	5.71	0.57	10	3.53	0.19	5.49	0.54	0.33	9.22	0.91
Yogurt	Low	10	3.11	0.05[e]	1.49	0.13	0.22	7.02	0.61	9	2.99	0.11	3.80	0.32	0.44	14.65	1.23
	Med	10	4.02	0.09	2.11	0.24	0.39	9.74	1.10	10	3.86	0.16	4.03	0.44	0.51	13.18	1.42
	Med+	11	4.03	0.17	4.22	0.48	0.48	11.78	1.33	10	3.94	0.30	7.75	0.86	0.49	12.36	1.36

[a] Inoculation levels include a low level, medium level, and medium with a background organism.
[b] Number of laboratories used in the analysis after the outlier tests.
[c] Log_{10} S. aureus count/g.
[d] Significantly different ($p < 0.05$).
[e] Significantly better repeatability ($p < 0.05$).

nies on the plate as S. aureus; the test is complete. If any colony colors other than red-violet are present, use a Petrifilm Staph Express disk, B(b).

Insert Petrifilm Staph Express disk into plate. Apply pressure by sliding a gloved finger firmly across entire disk area (including edges) to ensure uniform contact of disk with gel and to eliminate any air bubbles. Incubate plates and disks, in stacks of no more than 20 units, for at least 60 min and no longer than 3 h at 35 1 C or 37 1 C. Enumerate pink zones as S. aureus whether or not colonies are present. Pink zones are usually associated with S. aureus but may indicate S. hyicus or S. intermedius. Colonies not associated with a pink zone are not S. aureus and should not be counted.

Safety note: The test kit itself does not contain any pathogenic components but the enriched test suspension may contain S. aureus. Therefore, it is recommended that all test samples be discarded according to standard laboratory hazardous waste procedures.

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Results and Discussion

For each food, samples were obtained from different sources. Background counts were monitored. The S. aureus counts of the foods were too low to allow them to be used as naturally contaminated samples. Therefore, samples were artificially inoculated.

Twelve laboratories participated in the study. Because the protocol was not followed by one laboratory, the data from that laboratory are not listed in the tables and were not included in the analysis. The S. aureus counts for the test samples from the remaining laboratories are presented in logarithmic form in Tables 2–6. Interlaboratory study results (mean log_{10} counts, s_r, RSD_r, r, s_R, RSD_R, R) are presented in Table 2003.08.

Of the 468 red-violet colonies or colonies associated with a pink zone on the Petrifilm Staph Express Count plate, or plate and disk, 442 were coagulase-positive S. aureus. Of the 545 suspect colonies on BPA, 500 were coagulase-positive S. aureus.

Ice Cream

The Cochran and Grubbs tests to determine outliers were applied to the data. The Single Grubbs test identified an outlier for the Petrifilm Staph Express Count plate method at the medium and the medium plus background flora levels of inoculation. The value responsible for activating the test for each level was removed, and the outlier tests were repeated. No additional outliers were found.

The mean log_{10} S. aureus counts by the Petrifilm Staph Express Count plate method were not significantly different from

Table 2. S. aureus log_{10} counts for ice cream by the 3M™ Petrifilm™ Staph Express Count plate method and Baird-Parker agar method[a]

	Uninoculated level				Low level				Medium level				Medium level and background			
	Staph Express		Baird-Parker		Staph Express		Baird-Parker		Staph Express		Baird-Parker		Staph Express		Baird-Parker	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	<1.00	<1.00	1.30	1.78	1.95	1.70	2.54	2.52	2.62	2.69	2.76	2.67	2.99	2.73
2	<1.00	<1.00	<1.00	<1.00	1.00	1.70	1.00	1.70	2.56	2.53	2.66	2.74	2.53	2.70	2.74	2.57
3	<1.00	<1.00	<1.00	<1.00	1.48	1.60	1.85	2.15	2.53	2.62	2.58	2.48	2.57	2.62	2.73	2.67
4	<1.00	<1.00	<1.00	<1.00	1.70	1.00	1.00	1.85	2.43	2.57	2.58	2.58	2.51	2.57	2.69	2.53
5	<1.00	<1.00	<1.00	<1.00	1.48	1.00	1.78	1.30	2.56	2.48	2.40	2.18	2.58	2.41	2.51	2.51
6	<1.00	<1.00	<1.00	<1.00	2.15	1.70	1.30	1.00	2.64	2.56	2.61	2.49	1.85[c]	<1.00	2.56	2.53
7	<1.00	<1.00	<1.00	<1.00	1.30	1.78	1.30	2.00	2.69	2.65	2.66	2.74	2.57	2.67	2.72	2.76
8	<1.00	<1.00	<1.00	<1.00	2.00	1.60	1.70	1.85	2.66	2.57	2.64	2.80	2.54	2.72	2.72	2.66
9	<1.00	<1.00	<1.00	<1.00	1.70	1.30	1.90	1.70	2.58	2.45	2.28	2.40	2.40	2.64	2.68	2.57
10	<1.00	<1.00	<1.00	<1.00	1.48	1.60	1.48	1.60	2.18[c]	<1.00	2.18	<1.00	<1.00	<1.00	<1.00	<1.00
11	<1.00	<1.00	<1.00	<1.00	1.95	1.95	1.48	1.78	2.80	2.68	2.69	2.65	2.63	2.53	2.66	2.58

[a] Log_{10} S. aureus count/g. Staph Express = Petrifilm Staph Express Count plate.
[b] A and B indicate duplicate test portions.
[c] Outliers.

Table 3. S. aureus log_{10} counts for raw milk by the 3M™ Petrifilm™ Staph Express Count plate method and Baird-Parker agar method[a]

	Uninoculated level				Low level				Medium level				Medium level and background			
	Staph Express		Baird-Parker		Staph Express		Baird-Parker		Staph Express		Baird-Parker		Staph Express		Baird-Parker	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	<1.00	<1.00	2.51	2.69	2.34	2.26	3.41	3.54	3.20	3.32	2.90	3.66	3.28	2.95
2	<1.00	<1.00	<1.00	<1.00	2.59	2.57	2.61	2.66	2.48[c]	2.78[c]	2.30[c]	2.30[c]	2.48	3.57	2.85	2.78
3	<1.00	<1.00	<1.00	<1.00	2.67[c]	1.30[c]	2.74	<1.00	3.72	3.58	3.83	3.61	3.66	3.69	3.68	3.54
4	<1.00	<1.00	<1.00	<1.00	2.40	2.34	<1.00	2.86	3.30	3.36	2.60[c]	3.72[c]	3.32	3.41	3.80	3.48
5	<1.00	<1.00	<1.00	<1.00	2.52	2.46	2.57	2.34	3.49	3.48	3.34	3.64	3.53	3.48	3.46	3.49
6	<1.00	<1.00	<1.00	<1.00	2.49	2.08	2.74	2.40	<1.00	2.30[c]	2.70[c]	2.95[c]	2.30[c]	2.60[c]	2.00[c]	2.78[c]
7	<1.00	<1.00	<1.00	<1.00	2.62	2.59	2.72	2.68	3.68	3.38	3.61	3.67	3.63	3.52	3.66	3.73
8	<1.00	<1.00	<1.00	<1.00	2.70	2.67	2.76	2.67	3.63	3.67	3.54	3.67	3.60	3.59	3.64	3.67
9	<1.00	<1.00	<1.00	<1.00	1.48[c]	1.30[c]	<1.00	<1.00	3.61	3.52	3.45	3.61	3.72	3.72	3.66	3.74
10	<1.00	<1.00	<1.00	<1.00	2.78	2.60	1.00[c]	1.90[c]	3.67	3.43	3.08	3.23	3.52	3.67	2.90	2.70
11	<1.00	<1.00	<1.00	<1.00	2.80	2.76	2.61	2.77	3.64	3.57	3.62	3.52	3.74	3.63	3.64	3.69

[a] Log_{10} S. aureus count/g. Staph Express = Petrifilm Staph Express Count plate.
[b] A and B indicate duplicate test portions.
[c] Outliers.

Table 4. S. aureus $\log_{10}$ counts for mozzarella cheese by the 3M™ Petrifilm™ Staph Express Count plate method and Baird-Parker agar method[a]

	Uninoculated level				Low level				Medium level				Medium level and background			
	Staph Express		Baird-Parker		Staph Express		Baird-Parker		Staph Express		Baird-Parker		Staph Express		Baird-Parker	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	<1.00	<1.00	2.65	2.73	2.68	2.79	3.59	3.56	3.52	3.36	3.80	3.68	3.52	3.46
2	<1.00	<1.00	<1.00	<1.00	2.71	2.73	2.57	2.36	<1.00	3.73	<1.00	3.48	3.72	3.72	3.54	3.28
3	<1.00	<1.00	<1.00	<1.00	2.81	2.78	2.38	2.04	3.85	3.82	3.48	3.08	3.81	3.74	3.52	3.45
4	<1.00	<1.00	<1.00	<1.00	1.95[c]	2.23[c]	2.00	2.00	2.43[c]	2.51[c]	2.26[c]	2.36[c]	2.59[c]	2.40[c]	2.38[c]	2.34[c]
5	<1.00	<1.00	<1.00	<1.00	2.72	2.73	2.76	2.67	3.74	3.54	3.51	3.64	3.69	3.59	3.75	3.52
6	<1.00	<1.00	<1.00	<1.00	<1.00	1.48[c]	2.56	2.36	2.95[c]	3.00[c]	2.78[c]	2.70[c]	2.95[c]	<1.00	2.70[c]	2.78[c]
7	—[d]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	<1.00	<1.00	<1.00	<1.00	2.79	2.85	2.81	2.84	3.74	3.82	3.73	3.76	3.88	3.83	3.89	3.85
9	<1.00	<1.00	<1.00	<1.00	2.81	2.72	2.76	2.79	3.80	3.71	3.79	3.84	3.70	3.74	3.78	3.81
10	<1.00	<1.00	<1.00	<1.00	2.91	2.80	2.60	2.04	3.76	3.97	3.30	3.62	3.70	3.69	3.85	3.60
11	<1.00	<1.00	<1.00	<1.00	2.72	2.87	2.76	2.79	3.74	3.81	3.76	3.87	3.83	3.85	3.88	3.81

[a] $\log_{10}$ S. aureus count/g. Staph Express = Petrifilm Staph Express Count plate.
[b] A and B indicate duplicate test portions.
[c] Outliers.
[d] — = Laboratory did not participate in testing.

Table 5. S. aureus $\log_{10}$ counts for whey powder by the 3M™ Petrifilm™ Staph Express Count plate method and Baird-Parker agar method[a]

	Uninoculated level				Low level				Medium level				Medium level and background			
	Staph Express		Baird-Parker		Staph Express		Baird-Parker		Staph Express		Baird-Parker		Staph Express		Baird-Parker	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	<1.00	<1.00	2.52	2.36	2.26	2.08	3.03	3.26	3.04[c]	3.26[c]	3.43	3.43	3.18	2.90
2	<1.00	<1.00	<1.00	<1.00	2.40	2.48	2.89	2.60	3.15	3.20	3.48[c]	2.95[c]	3.46	3.38	3.66	3.36
3	<1.00	<1.00	<1.00	<1.00	2.81[c]	2.40[c]	2.69	2.49	3.62	3.52	3.51	3.48	3.88	3.43	3.68	3.26
4	<1.00	<1.00	<1.00	<1.00	<1.00	2.48	<1.00	2.04	3.30	3.30	3.18	3.20	3.15	3.18	3.34	3.04
5	—[d]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	<1.00	<1.00	<1.00	<1.00	2.34	2.28	2.28	2.20	2.90	2.90	2.78	<1.00	2.38[c]	2.72[c]	3.42	3.05
7	<1.00	<1.00	<1.00	<1.00	2.81	2.76	2.96	2.86	3.70	3.60	3.85	3.78	3.81	3.60	3.96	3.83
8	<1.00	<1.00	<1.00	<1.00	2.72	2.83	2.83	2.77	3.72	3.79	3.88	3.85	3.41	3.80	3.51	3.86
9	<1.00	<1.00	<1.00	<1.00	2.70	2.72	2.83	2.83	3.67	3.59	3.78	3.73	3.68	3.59	3.83	3.81
10	<1.00	<1.00	<1.00	<1.00	2.73	2.60	2.00	2.30	3.74	3.64	3.85	3.85	3.49	3.66	3.70	3.90
11	<1.00	<1.00	<1.00	<1.00	2.67	2.59	2.57	2.66	3.68	3.48	3.64	3.64	3.65	3.54	3.68	3.70

[a] $\log_{10}$ S. aureus count/g. Staph Express = Petrifilm Staph Express Count plate.
[b] A and B indicate duplicate test portions.
[c] Outliers.
[d] — = Laboratory did not participate in testing.

Table 6. S. aureus log_{10} counts for yogurt by the 3M™ Petrifilm™ Staph Express Count plate method and Baird-Parker agar method[a]

	Uninoculated level				Low level				Medium level				Medium level and background			
	Staph Express		Baird-Parker		Staph Express		Baird-Parker		Staph Express		Baird-Parker		Staph Express		Baird-Parker	
Lab	A[b]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	<1.00	<1.00	3.28	3.14	2.94	2.76	4.25	4.28	3.96	3.59	4.31	4.37	3.76	4.15
2	<1.00	<1.00	<1.00	<1.00	3.10	3.08	3.04	3.05	4.28	4.11	3.87	4.13	4.11	4.18	3.90	3.43
3	<1.00	<1.00	<1.00	<1.00	3.16	3.15	2.26	2.00	4.30[c]	3.78[c]	3.56	3.41	3.03	3.65	3.15	4.03
4	<1.00	<1.00	<1.00	<1.00	2.65	2.59	2.59	2.46	3.69	3.65	3.53	3.57	3.53	3.64	3.41	3.56
5	<1.00	<1.00	1.48	<1.00	3.00	3.10	3.17	3.19	3.88	3.96	4.11	4.12	4.11	4.18	4.15	4.13
6	<1.00	<1.00	<1.00	<1.00	2.91	2.96	3.21	3.06	3.23	3.08	2.85	3.11	3.15	3.20	3.63	3.30
7	<1.00	<1.00	<1.00	<1.00	3.15	3.18	3.21	3.27	4.13	4.11	4.22	4.27	4.04	4.36	4.20	4.45
8	<1.00	<1.00	<1.00	<1.00	3.20	3.25	3.12	3.18	4.15	4.26	4.12	4.24	4.11	4.24	4.20	4.32
9	<1.00	<1.00	<1.00	<1.00	3.40	3.46	3.41	3.68	4.43	4.48	4.64	4.60	4.46	4.71	4.61	4.77
10	<1.00	<1.00	<1.00	<1.00	2.30[c]	2.60[c]	2.30[c]	3.28[c]	3.95	3.70	3.60	3.30	4.20	4.15	3.30	3.95
11	<1.00	<1.00	<1.00	<1.00	3.23	3.23	3.23	3.34	4.38	4.30	4.15	4.43	4.38	4.58	4.23	4.48

[a] Log_{10} S. aureus count/g. Staph Express = Petrifilm Staph Express Count plate.
[b] A and B indicate duplicate test portions.
[c] Outliers.

those by the BPA method for the low and medium levels of inoculation. The mean log_{10} S. aureus counts by the Petrifilm Staph Express Count plate method were significantly different from those by the BPA method for the medium plus background flora level of inoculation. On average, counts from the BPA method were greater in value than those from the Petrifilm Staph Express Count plate method.

The repeatability variance of the Petrifilm Staph Express Count plate method was not significantly different from that of the BPA method for all levels of inoculation.

Raw Milk

Outliers were detected for the Petrifilm Staph Express Count plate method and the BPA method at all levels of inoculation. These values were removed from the analysis.

The mean log_{10} S. aureus counts by the Petrifilm Staph Express Count plate method were not significantly different from those by the BPA method for all levels of inoculation.

The repeatability variance of the Petrifilm Staph Express Count plate method was not significantly different from that of the BPA method for the low and medium levels of inoculation. The repeatability variance of the BPA method was better than that of the Petrifilm Staph Express Count plate method for the medium plus background flora level of inoculation.

Mozzarella Cheese

The Cochran test identified an outlier for the Petrifilm Staph Express Count plate method at the low level of inoculation. The aberrant value was removed, the tests were repeated, and an additional outlier was found by the Single Grubbs test. Once again, the outlier was removed from the analysis. No additional outliers were found. At the medium and medium plus background flora levels of inoculation, outliers were detected by the Double Grubbs test for both the Petrifilm Staph Express Count plate method and the BPA method. These values were removed from the analysis.

The mean log_{10} S. aureus counts by the Petrifilm Staph Express Count plate method were not significantly different from those by the BPA method at all levels of inoculation.

The repeatability variance of the Petrifilm Staph Express Count plate method was better than that of the BPA method at the low level and the medium plus background level of inoculation. The repeatability variance of the Petrifilm Staph Express Count plate method was not significantly different from that of the BPA method at the medium level of inoculation.

Whey Powder

Outliers were identified for the Petrifilm Staph Express Count plate method at the low and medium plus background flora levels of inoculation. The values were removed from the analysis, and the tests were repeated. No additional outliers were detected. An outlier was also detected for the BPA method at the medium level of inoculation. The value was removed from the analysis, and the tests were repeated. Another outlier was identified. This value was also deleted, the tests were repeated, and no additional outliers were found.

The mean log_{10} S. aureus counts by the Petrifilm Staph Express Count plate method were not significantly different from those by the BPA method for all levels of inoculation.

The repeatability variance of the BPA method was better than that of the Petrifilm Staph Express Count plate method at the low level of inoculation. The repeatability variance of the

Petrifilm Staph Express Count plate method was not significantly different from that of the BPA method at the other levels of inoculation.

Vanilla Yogurt

One laboratory (Laboratory 5) reported S. aureus in its uninoculated control sample; therefore, its data were removed from the statistical analysis for that particular method.

An outlier was identified for the Petrifilm Staph Express Count plate method at the low and medium levels of inoculation. An outlier was also detected for the BPA method at the low level of inoculation. The values were removed from the analysis.

The mean $\log_{10}$ S. aureus counts by the Petrifilm Staph Express Count plate method were not significantly different from those by the BPA method for all levels of inoculation.

The repeatability variance of the Petrifilm Staph Express Count plate method was better than that of the BPA method at the low level of inoculation. The repeatability variance of the Petrifilm Staph Express Count plate method was not significantly different from that of the BPA method at the medium and medium plus background flora levels of inoculation.

Recommendations

The mean $\log_{10}$ counts of the 24 h Petrifilm Staph Express Count plate method and the repeatability and reproducibility variances of that method were similar to those of the 72 h, 3 plate BPA method for analysis of selected dairy foods. Additionally, the participating laboratories praised the speed and ease of use of the Petrifilm Staph Express Count plate method. Because the results were not statistically different and favorable comments were received from the collaborators, it is recommended that the Petrifilm Staph Express Count plate method be adopted Official First Action for the enumeration of S. aureus in selected dairy foods.

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