FOOD BIOLOGICAL CONTAMINANTS

3M™ Petrifilm™ Staph Express Count Plate Method for the Enumeration of *Staphylococcus aureus* in Selected Types of Meat, Seafood, and Poultry: 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. Four foods—cooked, diced chicken; cured ham; smoked salmon; and pepperoni—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 4 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 may be used to identify *S. aureus* from all suspect colonies, as some *S. aureus* may be stressed or otherwise appear atypical on the plates. 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 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.

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 cooked and diced chicken, cured ham, smoked salmon, and pepperoni. 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

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Table 1. *Staphylococcus aureus* used to inoculate test samples

ATCC	Product
8095	Pepperoni
13565	Cured ham
8095	Smoked salmon
49476	Cooked, diced chicken

were stored at –70°C in laboratory medium containing 15% sterile glycerol. *Enterococcus faecalis* (ATCC 14506) was inoculated to achieve background competitive flora.

Working cultures were maintained on tryptic soy agar (TSA) slants, and the inocula were cultivated in trypticase soy broth (TSB) incubated at 35°C. Stationary-phase cultures stored overnight at 4°C were diluted with Butterfield's phosphate-buffered dilution water (BPD) prior to inoculation of the test samples.

Inoculation of Foods

The test samples were prepared by inoculation with a wet suspension of cells. 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. Each portion was homogeneously mixed with a KitchenAid™ mixer for 15 min.

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 (cooked, diced chicken, and smoked salmon) for 1 week or refrigerated (cured ham and pepperoni) for 2–3 days 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. Chicken and salmon were sent on dry ice and were stored frozen (<–15°C) upon arrival. Ham and pepperoni were packed with ice packs to maintain a temperature of <7°C during transport. Upon arrival, the samples were stored in a refrigerator (2–8°C). One food type was shipped for each week of the study.

Microbiological Analyses

Twelve laboratories analyzed the samples for *S. aureus* using the Petrifilm plate method, as well as the AOAC method. Eight samples for each of the 4 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. The BPA plates were incubated at 35–37°C for 45–48 h.

The BPA plates were incubated at 35–37°C for 45–48 h. 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 plates were planted in 0.2 mL Brain Heart Infusion (BHI). BHI cultures were inoculated 18–24 h at 35 ± 1°C. A 0.5 mL 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. Indefinite values, such as <1, were treated as missing and excluded from statistical analysis.

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 assuming that the transformed numbers would be normally distributed and of homogenous variance. 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 2003.11
Enumeration of *Staphylococcus aureus* in Selected Meat, Seafood, and Poultry
3M™ Petrifilm™ Staph Express Count Plate Method
First Action 2003

(Applicable to the enumeration of *S. aureus* in cooked and diced chicken, ham, salmon, and pepperoni.)

Caution: Autoclave materials after use.

See Table **2003.11** 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.

Table 2003.11. Interlaboratory study results of 3M™ Petrifilm™ Staph Express Count plate and Baird-Parker agar for detection of *S. aureus* in foods

		Petrifilm Staph Express Count plate								Baird-Parker agar							
Food	Level	*N*[a]	Mean[b]	s_r	RSD_r, %	r	s_R	RSD_R, %	R	*N*[a]	Mean[b]	s_r	RSD_r, %	r	s_R	RSD_R, %	R
Chicken	Low	3	2.82	0.03[c]	1.90	0.15	0.15	5.38	0.42	10	2.75	0.17	6.30	0.48	0.32	11.80	0.91
	Med[d]	10	3.86	0.08[c]	2.22	0.24	0.13	3.32	0.36	11	3.62	0.16	4.46	0.45	0.50	13.91	1.40
	Med+[e]	9	4.89	0.07[c]	1.35	0.18	0.10	1.95	0.27	9	4.80	0.15	3.13	0.42	0.27	5.54	0.74
Ham	Low	10	2.86	0.09	3.23	0.26	0.25	8.70	0.70	9	2.93	0.07	2.33	0.19	0.32	10.85	0.89
	Med	9	3.88	0.05[c]	1.30	0.14	0.24	6.04	0.66	10	3.82	0.16	4.16	0.44	0.47	12.37	1.32
	Med+	10	4.87	0.10	2.13	0.29	0.22	4.62	0.63	10	4.83	0.10	2.02	0.27	0.43	8.97	1.21
Salmon	Low	8	2.55	0.10	3.92	0.28	0.17	6.56	0.47	10	2.51	0.13	5.34	0.38	0.20	7.84	0.55
	Med	7	3.46	0.14	3.98	0.38	0.18	5.22	0.50	12	3.46	0.12	3.40	0.33	0.32	9.18	0.89
	Med+	8	4.45	0.13	2.88	0.36	0.32	7.20	0.90	12	4.29	0.12	2.78	0.33	0.42	9.80	1.18
Pepperoni	Low	NA[f]															
	Med	9	1.81	0.07[c]	3.83	0.19	0.21	11.47	0.58	11	1.93	0.19	9.96	0.54	0.30	15.43	0.83
	Med+	8	3.71	0.04	1.00	0.10	0.18	4.93	0.51	11	3.68	0.08	2.29	0.24	0.35	9.45	0.97

[a] Number of laboratories used in the analysis after the outlier tests and removal of those with *S. aureus* in the uninoculated control samples.
[b] $\log_{10}$ *S. aureus* count/g.
[c] Significantly better repeatability ($p < 0.05$).
[d] Med = Medium level of inoculation with *S. aureus*.
[e] Med+ = Medium level of inoculation with *S. aureus* plus inoculation with background organism (*E. faecalis*).
[f] NA = No *S. aureus* colonies were detected as the inoculation level was missed.

(**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 in 1 L water. Prepare and store in glass, screw-cap bottle. 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 ca 175 μL 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. Sample Preparation

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 16 000–18 000 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 colonies 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 the 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.

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Results and Discussion

For each food, samples were obtained from different retail 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. All 12 laboratories completed the analyses of the 4 foods. The *S. aureus* counts for the test samples are presented in base 10 logarithmic form in Tables 2–5. Interlaboratory study results (mean $\log_{10}$ counts, s_r, RSD_r, r, s_R, RSD_R, R) are presented in Table **2003.11**.

Of the 251 red-violet colonies or colonies associated with a pink zone on the Petrifilm Staph Express Count plate, or plate and disk, 248 were coagulase-positive *S. aureus*. Of the 289 suspect colonies on BPA, 288 were coagulase-positive *S. aureus*.

Cooked, Diced Chicken

One laboratory (Laboratory 10) reported *S. aureus* in its uninoculated control sample; therefore, its data were removed from the statistical analysis for that particular method.

The Cochran and Grubbs tests were applied to the data to determine outliers. Outliers were detected at each inoculation level for the Petrifilm Staph Express Count plate method and for the BPA method at the medium plus background flora level of inoculation. These values were removed from the statistical 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 the low, medium, and medium plus background flora levels of inoculation.

The repeatability variance of the Petrifilm Staph Express Count plate method was better than that of the BPA method for the low, medium, and medium plus background flora levels of inoculation.

Cured Ham

Because of inconsistencies in the data from Laboratory 2, these results were not figured into the calculations. Two laboratories (Laboratories 5 and 1) reported *S. aureus* in their uninoculated control samples. The data for the particular

Table 2. *S. aureus* $\log_{10}$ counts for chicken by 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	3.00	2.95	3.15	3.10	3.94	3.84	4.10	3.99	5.04	4.95	5.01	4.68
2	<1.00	<1.00	<1.00	<1.00	2.88[c]	1.78[c]	2.54	2.15	3.08[c]	2.89[c]	3.08	2.92	3.85[c]	3.83[c]	3.69[c]	3.99[c]
3	<1.00	<1.00	<1.00	<1.00	2.60	2.65	2.72	2.79	3.94	3.95	3.88	3.90	4.77	4.98	4.70	5.12
4	<1.00	<1.00	<1.00	<1.00	3.05	2.91	3.00	3.09	4.03	3.89	3.90	4.12	4.94	TNTC[d]	5.03	5.07
5	<1.00	<1.00	<1.00	<1.00	2.00[c]	2.40[c]	2.08	2.43	2.95[c]	2.30[c]	2.79	2.30	3.23[c]	3.20[c]	2.90[c]	3.18[c]
6	<1.00	<1.00	<1.00	<1.00	2.86	2.81	2.70	2.66	3.76	3.80	3.88	3.86	4.79	4.81	4.60	4.78
7	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	3.77	3.91	3.49	3.85	4.99	4.94	4.67	4.70
8	<1.00	<1.00	<1.00	<1.00	2.91	2.85	2.72	2.30	3.73	3.63	3.08	3.40	4.74	4.75	4.57	4.30
9	<1.00	<1.00	<1.00	<1.00	2.85	2.91	2.97	2.90	3.90	3.99	4.00	3.92	4.95	4.95	5.16	5.05
10	<1.00	<1.00	<1.00	1.00	2.63	2.70	2.58	2.72	3.98	3.83	3.80	3.79	4.92	4.90	4.68	4.94
11	<1.00	<1.00	<1.00	<1.00	2.85	2.96	3.09	2.69	3.79	3.94	4.00	3.98	4.91	4.79	5.01	5.00
12	<1.00	<1.00	<1.00	<1.00	2.61	2.60	2.63	2.96	3.56	3.76	3.57	3.66	4.72[c]	4.08[c]	4.49	4.46

[a] $\log_{10}$ *S. aureus* count/g. Staph Express = Petrifilm Staph Express Count plate.
[b] A and B indicate duplicate test portions.
[c] Outliers not used in statistical analyses.
[d] TNTC = Too numerous to count.

Table 3. *S. aureus* $\log_{10}$ counts for ham by 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	2.04	2.94	2.84	3.09	3.17	3.98	3.94	4.08	4.01	4.91	4.97	4.98	5.10
2	—[c]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	<1.00	<1.00	<1.00	<1.00	3.01	3.17	3.16	3.15	3.97	4.06	4.15	4.03	5.05	5.10	5.16	5.16
4	<1.00	<1.00	<1.00	<1.00	2.96	3.00	3.11	3.20	4.02	4.14	4.21	4.16	5.05	5.07	5.08	5.17
5	<1.00	1.60	<1.00	<1.00	2.04	1.95	1.30[d]	1.48[d]	2.88	2.89	2.77	2.91	4.10	4.25	4.10	4.13
6	<1.00	<1.00	<1.00	<1.00	2.63	2.69	2.87	2.76	3.62	3.62	3.58	3.61	4.58	4.79	4.58	4.52
7	<1.00	<1.00	<1.00	<1.00	3.08	2.90	3.08	3.11	4.09	4.09	4.14	4.17	5.08	4.94	5.04	4.92
8	<1.00	<1.00	<1.00	<1.00	2.82	2.63	2.65	2.54	3.52[d]	3.99[d]	3.36	3.87	4.60	4.46	4.38	4.11
9	<1.00	<1.00	<1.00	<1.00	3.26	3.20	3.26	3.18	4.09	4.12	4.24	4.21	5.09	5.03	5.10	5.12
10	<1.00	<1.00	<1.00	<1.00	2.68	2.45	2.88	2.68	3.73	3.68	3.96	3.89	4.63	4.85	4.98	5.10
11	<1.00	<1.00	<1.00	<1.00	3.00	3.00	3.18	3.20	3.93	3.89	4.32	4.10	5.03	5.00	5.33	5.42
12	<1.00	<1.00	<1.00	<1.00	2.53	2.46	2.36	2.30	3.40	3.53	3.57	3.18	4.72	4.45	4.69	4.43

[a] $\log_{10}$ *S. aureus* count/g. Staph Express = Petrifilm Staph Express Count plate.
[b] A and B indicate duplicate test portions.
[c] — = Inconsistencies in data; data not used in analysis.
[d] Outliers not used in statistical analyses.

Table 4. *S. aureus* log_{10} counts for salmon by 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	2.64	2.61	2.67	3.68	3.41	3.66	3.67	4.52	4.38	4.61	4.40
2	2.20	2.28	<1.00	<1.00	2.34	2.34	1.60[c]	1.30[c]	2.57	2.62	2.83	2.62	3.59	3.56	3.57	3.68
3	<1.00	<1.00	<1.00	<1.00	2.74	2.53	2.60	2.56	3.53	3.62	3.70	3.61	4.45	4.45	4.43	4.57
4	<1.00	<1.00	<1.00	<1.00	2.89	2.75	2.73	2.60	3.59	3.73	3.87	3.58	4.49	4.43	4.40	4.43
5	<1.00	<1.00	<1.00	<1.00	2.38	2.43	1.85[c]	1.78[c]	3.41	3.18	3.36	3.08	4.09	4.27	3.73	4.02
6	2.26	1.95	<1.00	<1.00	2.57	2.18	2.56	2.11	3.26	3.23	3.26	3.11	4.20	4.13	3.60	3.88
7	2.08	2.79	<1.00	<1.00	2.63	2.49	2.63	2.57	3.59	3.36	3.52	3.62	4.61	4.59	4.56	4.81
8	<1.00	<1.00	<1.00	<1.00	2.57	2.60	2.41	2.18	4.32[c]	4.34[c]	3.70	3.95	4.91	5.31	5.02	5.06
9	<1.00	<1.00	<1.00	<1.00	2.46	2.26	2.51	2.34	3.26	3.51	3.54	3.58	4.32	4.32	4.28	4.45
10	<1.00	1.84	<1.00	<1.00	2.43	1.48	2.49	2.40	3.36	3.40	3.40	3.34	4.20	4.18	4.23	4.15
11	<1.00	<1.00	<1.00	<1.00	2.53	2.72	2.81	2.78	3.58	3.41	3.72	3.59	4.43	4.61	4.49	4.51
12	<1.00	<1.00	<1.00	<1.00	2.43	2.41	2.40	2.20	3.18	3.32	3.36	3.40	4.03	4.15	4.01	4.05

[a] Log_{10} *S. aureus* count/g. Staph Express = Petrifilm Staph Express Count plate.
[b] A and B indicate duplicate test portions.
[c] Outliers not used in statistical analyses.

Table 5. *S. aureus* log_{10} counts for pepperoni by 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	<1.00	<1.00	<1.00	<1.00	1.60	1.70	2.28	2.30	2.72[c]	2.78[c]	3.92	3.91
2	<1.00	1.48	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	1.30	1.60	<1.00	1.70	3.15	3.08	2.85	3.08
3	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	1.78	1.95	2.18	1.95	3.81	3.74	4.12	3.96
4	<1.00	<1.00	<1.00	<1.00	<1.00	1.00	<1.00	<1.00	<1.00	1.60	1.60	1.00	3.53	3.59	3.41	3.40
5	1.00	<1.00	1.00	<1.00	<1.00	1.00	<1.00	1.00	1.85	1.30	1.30	1.30	3.75	3.70	3.54	3.64
6	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	1.00	1.78	1.70	1.95	1.85	3.43	3.46	3.34	3.23
7	<1.00	<1.00	<1.00	<1.00	<1.00	1.00	<1.00	<1.00	2.04	2.11	1.90	1.95	3.85	3.90	3.96	3.93
8	<1.00	<1.00	<1.00	<1.00	1.00	<1.00	<1.00	1.00	<1.00	1.60	2.04	1.70	3.80	3.89	3.75	3.88
9	<1.00	<1.00	<1.00	<1.00	1.30	<1.00	<1.00	<1.00	1.90	1.85	1.95	2.04	3.54	3.57	3.70	3.52
10	<1.00	<1.00	<1.00	<1.00	1.60	<1.00	<1.00	<1.00	2.11	2.23	1.78	2.08	3.94	3.97	3.73	3.81
11	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	2.08	1.70	1.70	2.15	2.32	3.65	3.66	4.02	4.10
12	<1.00	1.00	<1.00	<1.00	1.00	1.00	1.48	<1.00	1.78	1.00	2.11	1.85	3.45	3.43	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 not used in statistical analyses.

methods from these laboratories were removed from the statistical analysis.

An outlier (from Laboratory 5) was detected by the Single Grubbs test for the BPA method for the low level of inoculation. In addition, an outlier was detected by the Cochran test for the Petrifilm Staph Express Count plate method at the medium level of inoculation. After removal of these values, no additional outliers were detected.

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 the low, medium, and medium plus background flora levels of inoculation.

The repeatability variance of the Petrifilm Staph Express Count plate method was significantly better than that of the BPA method at the medium 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 low and medium plus background flora levels of inoculation.

Smoked Salmon

Some laboratories (Laboratories 2, 6, 7, and 10) reported *S. aureus* in their uninoculated control samples. The data from these laboratories were removed from the statistical analysis.

Outliers were detected by the Double Grubbs test for the BPA method at the low level of inoculation. The aberrant values were removed, the tests were repeated, and no additional outliers were found. Outliers were detected by the Single Grubbs test for the Petrifilm Staph Express Count plate method at the medium level of inoculation. The aberrant value was removed, 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 the low, medium, and medium plus background flora levels 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 low, medium, and medium plus background levels of inoculation.

Pepperoni

Very few counts of *S. aureus* were reported for both methods at the low level of inoculation, suggesting that the target level was not achieved. As a result, performance estimates were only calculated at the medium and medium plus background flora levels of inoculation.

Some laboratories reported *S. aureus* in their uninoculated control samples. The data from these laboratories were removed from the statistical analysis.

An outlier was detected by the Single Grubbs test for the Petrifilm Staph Express Count plate method at the medium plus background level of inoculation. The aberrant value was removed, 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 the medium and medium plus background flora levels of inoculation.

The repeatability variance of the Petrifilm Staph Express Count plate method was significantly better than 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 the method were similar to those of the 72 h, 3 plate BPA method for analysis of selected types of meat, poultry, and seafood. Because the results were not statistically different and because 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 cooked and diced chicken, cured ham, smoked salmon, and pepperoni.

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