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AOAC Official Method 2003.07
Enumeration of *Staphylococcus aureus* in Selected Types of Processed and Prepared Foods
3M™ Petrifilm™ Staph Express Count Plate Method
First Action 2003
Final Action 2006

(Applicable to the enumeration of *S. aureus* in frozen lasagna, custard, frozen mixed vegetables, frozen hashbrowns, and frozen batter-coated mushrooms.)

Caution: Autoclave materials after use.

See Table **2003.07** 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 55144, USA), 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. Electronic pipettor and tips, or equivalent, may be used to deliver 1.0 mL. 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.

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

(**f**) *Sodium hydroxide solution*.—Sterile 1M. Dissolve 20 g NaOH in 500 mL water in 500 mL autoclavable Nalgene container. 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 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**), to aseptically weigh 50 g test portion into blender jar, **B**(**h**). Add 450 mL diluent, **B**(**g**)(*2*), 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 diluent 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 diluent plus 10 mL from the previous dilution. Pipets, **B**(**d**), must accurately deliver the required volume. 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 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 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, discard all test samples according to standard laboratory hazardous waste procedures.

Reference: J. AOAC Int. 86, 954(2003).

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Table 2003.07. Interlaboratory study results of 3M™ Petrifilm™ Staph Express Count plate method and the Baird-Parker agar method for detection of *S. aureus* in foods

		Petrifilm Staph Express Count plate								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
Lasagna	Low	12	1.84	0.24	13.29	0.68	0.34	18.62	0.96	13	1.83	0.32	17.77	0.91	0.41	22.53	1.15
	Med	13	3.21	0.27	8.39	0.75	0.27	8.39	0.75	11	3.28	0.20	5.95	0.55	0.20	5.95	0.55
	Med+	12	3.16	0.08[d]	2.59	0.23	0.20	6.40	0.57	13	3.13	0.17	5.29	0.46	0.27	8.75	0.77
Custard	Low	12	1.72	0.23	13.51	0.65	0.23	13.51	0.65	12	1.80	0.22	12.14	0.61	0.25	13.64	0.69
	Med	12	2.81	0.06[d]	2.17	0.17	0.13	4.64	0.37	12	2.80	0.12	4.42	0.35	0.20	7.17	0.56
	Med+	11	2.80	0.09	3.14	0.25	0.09	3.14	0.25	11	2.82	0.09	3.24	0.26	0.15	5.35	0.42
Mixed vegetables	Low	11	2.73	0.06[d]	2.07	0.16	0.08	3.08	0.24	12	2.74	0.12	4.42	0.34	0.15	5.55	0.43
	Med	11	3.72	0.06	1.59	0.17	0.08	2.06	0.22	10	3.76	0.07	1.83	0.19	0.11	2.87	0.30
	Med+	12	3.73	0.08	2.14	0.22	0.08	2.34	0.25	11	3.78	0.09	2.50	0.27	0.11	2.86	0.30
Hashbrowns	Low	11	2.35	0.12	5.11	0.34	0.13	5.34	0.35	11	2.39	0.12	5.19	0.35	0.17	7.08	0.47
	Med	12	3.34	0.10	2.99	0.28	0.15	4.46	0.42	12	3.34	0.12	3.62	0.34	0.21	6.29	0.59
	Med+	13	3.32	0.12	3.58	0.33	0.15	4.52	0.42	13	3.36	0.14	4.12	0.39	0.18	5.41	0.51
Batter-coated mushrooms	Low	11	2.09	0.15	7.19	0.42	0.18	8.61	0.50	9	2.23	0.15	6.61	0.41	0.15	6.61	0.41
	Med	10	3.16	0.15	4.61	0.41	0.15	4.61	0.41	11	3.11	0.16	5.00	0.43	0.23	7.43	0.65
	Med+	9	3.17	0.10[d]	3.05	0.27	0.10	3.14	0.28	11	3.07	0.30	9.68	0.83	0.30	9.68	0.83

[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 better repeatability ($p < 0.05$).