AOAC Official Method 2001.05
Petrifilm™ Rapid S. aureus Count Plate Method for the Rapid Enumeration of *Staphylococcus aureus* in Selected Foods

First Action 2001

(Applicable to the enumeration of confirmed *Staphylococcus aureus* organisms in pasta filled with beef and cheese; frozen hash browns; cooked chicken patty; egg custard; frozen ground raw pork; and instant nonfat dried milk.)

Safety note: Autoclave materials after use.

See Table **2001.05** for results of the interlaboratory study supporting acceptance of the method.

A. Principle

Method uses bacterial culture plates of dry media and cold-water-soluble gel. Undiluted or diluted test portions are added to plates at 1.0 mL/plate. Pressure, when applied to plastic spreader placed on overlay film, spreads the product evenly over a 30 cm^2 growth area. Gelling agent is allowed to solidify, and plates are incubated at 35 ± 1°C for 24 ± 2 h. The plate is incubated at a high temperature (62 ± 2°C) to deactivate heat labile deoxyribonuclease; then an indicator disk which detects the heat stable enzyme TNase is placed between the top and bottom films of the plate. Plates with disks are incubated at 35 ± 1°C for 1–3 h to identify TNase-positive *staphylococci*.

B. Apparatus and Reagents

(**a**) *Petrifilm Rapid S. aureus* (*RSA*) *plates*.—Plates, available from 3M Microbiology Products (St. Paul, MN 55144, USA) contain modified Baird-Parker nutrients and a cold-water-soluble gelling agent.

(**b**) *Petrifilm TNase Reactive disk*.—Disks (3M Microbiology Products) contain DNA, Toluidine Blue-O, and a tetrazolium indicator.

(**c**) *Plastic spreader*.—With handle (3M Microbiology Products).

(**d**) *Pipets*.—Calibrated 1.0 and 10.0 mL serological pipets with 0.1 mL graduations. (Calibrated electronic pipettor 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**) *Sterile 1M NaOH*.—Dissolve 40 g NaOH in water and dilute to 1 L. Autoclave 15 min at 121°C.

(**g**) *Dilution water*.—(*1*) *Stock solution*.—Dissolve 34 g KH_2PO_4 in 500 mL water, adjust to pH 7.2 with 1M NaOH (ca 175 mL), and dilute to 1 L. (*2*) *Buffered water for dilutions*.—Dilute 1.25 mL stock solution to 1 L with boiled and cooled H_2O. Autoclave 15 min at 121°C.

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

(**i**) *Incubators*.—Maintaining 35 ± 1°C and 62 ± 2°C.

C. Preparation of Test Suspensions

Use balance with capacity of 2 kg and sensitivity of 0.1 g to aseptically weigh 50 g unthawed test portion into blender jar, **B**(**h**). Add 450 mL dilution water, **B**(**g**)(*2*), and blend at 16 000–18 000 rpm for 2 min to homogenize. If entire test sample is <50 g, weigh a portion of the test sample and add dilution water to make 1:10 dilution. As required, adjust pH of diluted test sample to 6.5–7.5 with NaOH, **B**(**f**), (ca 0.1 mL/g test portion). Do not use buffered peptone water or diluents containing citrate or thiosulfate. Prepare all decimal dilutions with 90 mL dilution water plus 10 mL from previous dilution. 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× through 30 cm arc in 7 s.

D. Analysis

Place dry Petrifilm RSA 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 for 24 ± 2 h at 35 ± 1°C. In incubator, place plates in horizontal position or in a Petrifilm plate rack, clear side up, in stacks not exceeding 20 units. After incubation, place plates in 62 ± 2°C incubator for 1 h in stacks not exceeding 10 units. Open Petrifilm plates and place Petrifilm TNase reactive disk, **B**(**b**), into each well formed by the foam dam, being careful not to trap air bubbles below disks. Disks are coated with substrate on both sides; therefore, orientation in relation to Petrifilm plate does not matter. Lower top film onto disk being careful not to trap air bubbles between film and disk. To ensure uniform contact between disk and medium, apply gentle pressure across reactive disk area. Slide a bent rod across the plate to complete contact between disk and gel by pushing the air bubbles out past edge of disk. Incubate Petrifilm plates with their disks at 35 ± 1°C. After 1 h, remove Petrifilm plates from incubator. Using a colony counter, **B**(**e**), count and record "confirmed" colonies with a pink zone around a red, blue, or colorless center. On plates where every colony has a zone, the test is considered complete and incubation should be terminated. Re-incubate all other plates for an additional 2 h at 35 ± 1°C. Count and record all typical colonies after second incubation. Select plates with 15–100 colonies. If plates are too crowded for estimated counts, or diffusion of the pink zones is so great that the color cannot be associated with individual colonies, report count as TNTC (too numerous to count).

Reference: *J. AOAC Int.* **84**, 1431(2001).

Table 2001.05. Petrifilm™ Rapid S. aureus Count Plate method for the rapid enumeration of *Staphylococcus aureus* in selected foods

		Petrifilm Rapid *S. aureus* Count Plate								Baird-Parker Agar							
Food	Level	N[a]	Mean[b]	s_r[c]	RSD_r, %[d]	r[e]	s_R[f]	RSD_R, %[g]	R[h]	N[a]	Mean[b]	s_r[c]	RSD_r, %[d]	r[e]	s_R[f]	RSD_R, %[g]	R[h]
Pasta	Low	12	2.237	0.082[i]	3.666	0.230	0.176	7.868	0.493	12	2.206	0.206	9.338	0.577	0.221	10.018	0.619
	Med	12	3.154	0.115	3.646	0.322	0.137	4.344	0.384	12	3.197	0.103	3.222	0.288	0.119	3.722	0.333
	High	12	4.200	0.139	3.310	0.389	0.139	3.310	0.389	12	4.198	0.098	2.334	0.274	0.162	3.859	0.454
Hashbrowns	Low	9	2.066	0.162	7.841	0.454	0.162	7.841	0.454	9	2.145	0.274	12.774	0.767	0.281	13.100	0.787
	Med	9	3.090	0.075	2.427	0.210	0.121	3.916	0.339	9	3.180	0.069	2.170	0.193	0.132	4.151	0.370
	High	10	4.062[j]	0.064	1.576	0.179	0.102	2.511	0.286	10	4.141	0.086	2.077	0.241	0.108	2.608	0.302
Chicken patties	Low	12	1.962	0.159[i]	8.104	0.445	0.203	10.347	0.568	12	1.928	0.291	15.093	0.815	0.304	15.768	0.851
	Med	11	3.019	0.073	2.418	0.204	0.114	3.776	0.319	11	2.956	0.042[i]	1.421	0.118	0.174	5.886	0.487
	High	11	4.000	0.074	1.850	0.207	0.142	3.550	0.398	11	3.975	0.086	2.164	0.241	0.200	5.031	0.560
Custard	Low	9	2.144	0.086[i]	4.002	0.241	0.143	6.670	0.401	9	2.164	0.192	8.891	0.539	0.221	10.203	0.618
	Med	9	3.139	0.071	2.262	0.199	0.133	4.237	0.372	9	3.114	0.074	2.376	0.207	0.149	4.785	0.417
	High	8	4.178	0.108	2.585	0.302	0.152	3.638	0.426	8	4.07	0.055[i]	1.351	0.154	0.131	3.219	0.367
Pork	Low	3	2.222	0.427	19.217	1.196	0.471	21.197	1.319	3	2.278	0.340	14.925	0.952	0.450	19.754	1.260
	Med	11	2.947	0.140	4.751	0.392	0.235	7.974	0.658	11	2.988	0.052[i]	1.740	0.146	0.179	5.991	0.501
	High	11	3.909	0.093	2.379	0.260	0.280	7.163	0.784	11	3.899	0.125	3.206	0.350	0.337	8.643	0.944
Nonfat dried milk	Low	10	1.356	0.437	32.227	1.224	0.437	32.227	1.224	10	1.521	0.244	16.042	0.683	0.244	16.042	0.683
	Med	12	2.104[j]	0.234	11.122	0.655	0.396	18.821	1.109	12	2.369	0.249	10.511	0.697	0.352	14.859	0.986
	High	12	3.170	0.216	6.814	0.605	0.308	9.716	0.862	12	3.299	0.129[i]	3.910	0.361	0.327	9.912	0.916

[a] Number of laboratories with complete data.

[b] Log *S. aureus* count/g.

[c] s_r = Repeatability standard deviation.

[d] RSD_r = repeatability relative standard deviation.

[e] r = Repeatability.

[f] s_R = Reproducibility standard deviation.

[g] RSD_R = reproducibility relative standard deviation.

[h] R = repeatability.

[i] Significantly better repeatability (p <0.05).

[j] Significantly different (p<0.05).