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17.3.04A

AOAC Official Method 2000.15 Rapid Enumeration of Coliforms in Foods

Dry Rehydratable Film Method, Petrifilm™ Rapid Coliform Count Plate First Action 2000 Final Action 2005

(Applicable to determination of confirmed coliforms at 14 and 24 h in foods. Not applicable to hash brown potatoes.)

See Tables 2000.15 A and B for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Method uses bacterial culture plates of dry medium and cold-water-soluble gel. Undiluted or diluted test suspensions are added to plates at a volume of 1.0 mL per plate. Pressure, when applied to plastic spreader placed on overlay film, spreads the suspension evenly over a 20 cm² growth area. Gelling agent is allowed to solidify, and plates are incubated and then counted. A serological pipet or automatic pipet can be used for suspension addition for bacterial count analysis.

B. Apparatus and Reagents

(a) *Petrifilm Rapid Coliform Count (RCC) plates*.—Plates (available from 3M Microbiology Products, St. Paul, MN 55144, USA) contain modified violet red bile (VRB) nutrients, cold-water-soluble gelling agent, tetrazolium indicator dye, and pH indicator.

(b) *Plastic spreader*.—Provided with Petrifilm plates, has a recessed side and a smooth flat side, designed to spread suspension evenly over plate growth area.

(c) *Pipets*.—1.0 and 10.0 mL serological pipets with 0.1 mL graduations. Pipets must accurately deliver required volume. Do not use <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. (Calibrated 3M™ Electronic Pipettor, or equivalent, may be used to deliver 1.0 mL.)

(d) *Colony counter*.—Quebec Dark-Field Colony Counter (VWR International; www.vwrsp.com), or equivalent.

(e) *Sterile sodium hydroxide solution*.—1M. Dissolve 40 g NaOH in 1 L water. Autoclave 15 min at 121°C.

(f) *Dilution water*.—Prepare stock solution by dissolving 34 g KH₂PO₄ in 500 mL water, adjust to pH 7.2 with 1M NaOH (ca 175 mL), and dilute to 1 L with water. Prepare buffered water for dilutions by diluting 1.25 mL stock solution to 1 L with boiled and cooled water. Autoclave 15 min at 121°C.

(g) *Blender or stomacher*.—Waring blender or equivalent, or Seward Ltd. (98 Great North Rd, London, N2 OGN UK, Tel: +44(0)20 8365 4100; www.sewardsearch.co.uk) 400 stomacher, or equivalent.

C. Preparation of Test Suspension

Prepare test suspension as in 966.23B (see 17.2.01). Specified dilutions are for maximum sensitivity. Higher dilutions may be plated as needed. Do not use diluents containing citrate or

thiosulfate. Mix all dilutions by shaking 25x through 30 cm arc in 7 s. Blend or stomach solids 2 min to homogenize.

(a) *Whole milk, 2% milk, 1% milk, skim milk, and raw milk*.—Plate 1 mL undiluted or diluted product on dry-film coliform count plate, B(a). Incubated colony count on undiluted plate is count/g.

(b) *Ice cream and mixes, chocolate milk*.—Make 1:10 dilution of product [11 g/99 mL dilution water, B(f)]. Plate 1 mL on dry-film coliform count plate, B(a). Incubate. Multiply colony count by dilution to obtain count/g.

(c) *Butter and margarine*.—Proceed as in (b) with diluent prewarmed to 40°–45°C. Do not use citrate buffer to homogenize product.

(d) *Sour cream, yogurt, and frozen yogurt*.—Proceed as in (b). After dilution adjust pH to 6.5–7.5 with 1M NaOH, B(e), (ca 0.1 mL/g product).

(e) *Cheddar cheese, cottage cheese, instant nonfat dry milk, whey powder, and related products*.—Proceed as in (b). Do not use citrate buffer to homogenize product.

(f) *Nondairy foods*.—Weigh 50 g test portion in sterile blender jar. Add 450 mL diluent and blend for 2 min in high speed blender jar at 16 000–18 000 rpm. As required, adjust pH of suspension to 6.5–7.5 with 1M NaOH, B(e), (ca 0.1 mL/g suspension). If entire test sample contains <50 g, weigh a portion of test sample and add sterile diluent to make 1:10 dilution. Prepare all decimal dilutions with 90 mL sterile diluent plus 10 mL previous dilution unless otherwise specified.

D. Analysis

Place dry Petrifilm RCC plate, B(a), on flat surface. Lift top film and inoculate 1 mL suspension onto center of bottom film. Carefully roll top film down onto inoculum. Distribute inoculum over 20 cm² growth area with downward pressure on center of plastic spreader device (flat side down). Leave plate undisturbed to permit gelling agent to solidify. Incubate plates up to 24 h at 35° ± 1°C. In incubator, place plates in horizontal position or in Petrifilm plate rack, clear side up, in stacks not exceeding 20 units. Count plates within 1 h after incubation period is completed.

Petrifilm RCC plates can be counted on a standard colony counter or other illuminated magnifier. Do not count colonies on foam dam because they are removed from selective influence of the medium. Do not count artifact bubbles that may be present. Confirmed coliforms will appear as red colonies associated with gas after 8–24 h of incubation. They are red colonies associated with one or more gas bubbles and with or without yellow acid zones. Plates with 10–150 colonies are to be selected. If no plate has at least 10 red colonies with gas, record the exact count on the least dilute inoculum. If all plates have counts >150, determine the estimated count by counting the number of colonies in one or more representative squares, determining average number per square, and then multiplying the average number by 20 (circular growth area is ca 20 cm²). If the plates are too crowded to estimate counts, report the count as too numerous to count.

Reference: [L. AOAC Int. 85, 56\(2002\)](#).

Table 2000.15A. Results of interlaboratory study of the Petrifilm RCC method at 14 and 24 h for detection of coliforms in foods

Food type	Level ^a	<i>n</i> ^b	Mean ^c	<i>s</i> _r	RSD _r , %	<i>s</i> _R	RSD _R , %
14 H							
Cheddar cheese	Low	10 (0)	1.86	0.27	14.52	0.32	17.20
	Medium	10 (0)	2.87	0.13	4.53	0.20	6.97
	High	10 (0)	3.90	0.08	2.05	0.19	4.87
Vanilla ice cream	Low	9 (0)	2.78	0.06	2.16	0.15	5.40
	Medium	10 (0)	3.67	0.11	3.00	0.19	5.18
	High	11 (0)	4.72	0.10	2.12	0.19	4.03
Flour	Uninoc.	11 (0)	3.26	0.27 ^d	8.28	0.44	13.50
	Low	11 (0)	2.92	0.29 ^e	9.93	0.34	11.64
	Medium	11 (0)	3.15	0.45	14.29	0.45	14.29
	High	11 (0)	3.49	0.21 ^d	6.02	0.35	10.03
Macaroni and cheese	Low	9 (0)	2.21	0.16 ^e	7.24	0.37	16.74
	Medium	10 (0)	3.17	0.19 ^e	5.99	0.36	11.36
	High	10 (0)	4.32	0.12 ^e	2.78	0.29	6.71
Fresh refrigerated uncooked pasta	Low	10 (0)	2.34	0.15 ^d	6.41	0.37	15.81
	Medium	10 (0)	3.33	0.14 ^e	4.20	0.36	10.81
	High	10 (0)	4.26	0.30	7.04	0.43	10.09
24 H							
Cheddar cheese	Low	11 (0)	1.89	0.26	13.76	0.31	16.40
	Medium	11 (0)	2.89	0.13	4.50	0.21	7.27
	High	11 (0)	3.93	0.09	2.29	0.19	4.83
Vanilla ice cream	Low	9 (0)	2.84	0.05	1.76	0.14	4.93
	Medium	11 (0)	3.70	0.11	2.97	0.19	5.14
	High	11 (0)	4.78	0.10	2.09	0.16	3.35
Flour	Uninoc.	11 (0)	3.33	0.26 ^d	7.81	0.41	12.31
	Low	11 (0)	2.97	0.27 ^e	9.09	0.30	10.10
	Medium	9 (2)	3.22	0.38	11.80	0.38	11.80
	High	11 (0)	3.52	0.16 ^d	4.55	0.32	9.09
Macaroni and cheese	Low	10 (0)	2.20	0.24	10.91	0.39	17.73
	Medium	10 (0)	3.24	0.17 ^d	5.25	0.30	9.26
	High	10 (0)	4.37	0.11 ^e	2.52	0.26	5.95
Hash brown potatoes	Low	11	NG ^f	NG	NG	NG	NG
	Medium	11	NG	NG	NG	NG	NG
	High	11	NG	NG	NG	NG	NG
Fresh refrigerated uncooked pasta	Low	10 (0)	2.36	0.16 ^d	6.78	0.37	15.68
	Medium	10 (0)	3.34	0.13 ^d	3.89	0.35	10.48
	High	10 (0)	4.27	0.30	7.03	0.42	9.84

^a Low = 10–100 CFU/mL, medium = 100–1000 CFU/mL, high = 1000–10 000 CFU/mL.

^b Number of laboratories with complete data; number of outliers appears in parentheses.

^c Log coliform count/g.

^d Significantly better repeatability ($p < 0.01$).

^e Significantly better repeatability ($p < 0.05$).

^f NG = Colonies with yellow acid zones were present; however, no gassing colonies were detected.

Table 2000.15B. Results of interlaboratory study of standard method for detection of coliforms in foods

Food type	Level ^a	<i>n</i> ^b	Mean ^c	<i>s</i> _r	RSD _r , %	<i>s</i> _R	RSD _R , %
Cheddar cheese	Low	11 (0)	1.90	0.29	15.26	0.30	15.79
	Medium	11 (0)	2.95	0.13	4.41	0.21	7.12
	High	11 (0)	3.99	0.12	3.01	0.20	5.01
Vanilla ice cream	Low	9 (2)	3.03	0.07	2.31	0.08	2.64
	Medium	9 (2)	3.83	0.07	1.83	0.11	2.87
	High	11 (0)	4.81	0.08	1.66	0.24	4.99
Flour	Uninoc.	11 (0)	1.97	0.67	34.01	0.95	48.22
	Low	10 (0)	1.72	0.54	31.40	0.69	40.12
	Medium	11 (0)	2.51	0.48	19.12	0.65	25.90
	High	11 (0)	2.78	0.57	20.50	0.90	32.37
Macaroni and cheese	Low	10 (0)	2.27	0.30	13.22	0.55	24.23
	Medium	10 (0)	3.20	0.41	12.81	0.65	20.31
	High	9 (1)	4.04	0.22	5.45	0.35	8.66
Hash brown potatoes	Low	10 (0)	2.06	0.41	19.90	0.69	33.50
	Medium	10 (0)	3.23	0.25	7.74	0.53	16.41
	High	10 (0)	4.00	0.96	24.00	1.09	27.25
Fresh refrigerated uncooked pasta	Low	10 (0)	2.15	0.55	25.58	0.62	28.84
	Medium	10 (0)	3.38	0.38	11.24	0.68	20.12
	High	10 (0)	4.37	0.46	10.53	0.46	10.53

^a Low = 10–100 CFU/mL, medium = 100–1000 CFU/mL, high = 1000–10 000 CFU/mL.

^b Number of laboratories with complete data; number of outliers appears in parentheses.

^c Log coliform count/g.