Official Methods of Analysis of AOAC INTERNATIONAL (22nd Edition)

Dr. George W Latimer, Jr. (ed.)

https://doi.org/10.1093/9780197610145.001.0001

Published: 2023 **Online ISBN:** 9780197610145 **Print ISBN:** 9780197610138

CHAPTER

17.3.10 AOAC Official Method 2003.01 Enumeration of *Enterobacteriaceae* in Selected Foods: Neogen® Petrifilm® Enterobacteriaceae Count Plate Method

https://doi.org/10.1093/9780197610145.003.2199

Published: January 2023

Collection: Official Methods of Analysis of AOAC INTERNATIONAL

First Action 2003

Final Action 2006

(Applicable to enumeration of *Enterobacteriaceae* organisms in cheddar cheese, milk, flour, frozen prepared meals, frozen broccoli, and nut pieces.)

See Tables 2003.01A and B for results of the interlaboratory study supporting acceptance of the method.

Table 2003.01A Repeatability performance of Neogen Petrifilm Enterobacteriaceae Count Plate, violet red bile glucose (VRBG), and most probable number (MPN) methods for detection of *Enterobacteriaceae* in foods

Food	Level	Petrifilm Enterobacteriaceae Count Plate					VRBG					MPN				
		n_a	Mean[b]	s_r	RSD_r, %	r	n_a	Mean[b]	s_r	RSD_r, %	r	n_a	Mean[b]	s_r	RSD_r, %	r
Cheese	Uninoculated	0					0					9	2.09	0.35	16.75	0.98
	Low	12	3.02[c]	0.18[d]	5.96	0.51	12	3.03	0.06[e]	1.94	0.17	11	3.12	0.31	9.79	0.86
	Medium	12	4.07[c]	0.14[d]	3.33	0.38	12	4.06	0.11	2.69	0.31	12	4.23	0.38	8.89	1.05
	High	10	4.91[c]	0.05[d,f]	0.92	0.13	12	4.93	0.13	2.59	0.36	9	4.82	0.26	5.31	0.72
Milk	Uninoculated	0					0					7	1.67	0.31	18.60	0.87
	Low	11	2.49[c,g]	0.06[d,f]	2.57	0.18	12	2.27	0.50	22.19	1.42	12	1.93	0.12	6.00	0.32
	Medium	12	3.34[c]	0.10[d,f]	3.00	0.28	12	3.27	0.21	6.46	0.60	12	2.65	0.42	15.79	1.17
	High	11	4.34[c]	0.10[d,f]	2.37	0.29	11	4.19	0.20	4.69	0.55	11	3.24	0.63	19.34	1.75
Flour	Uninoculated	12	2.70	0.25[d]	9.33	0.71	12	2.98	0.28	9.23	0.78	12	2.44	0.47	19.20	1.31
	Low	12	2.73[c]	0.32	11.78	0.91	12	2.95	0.30	10.28	0.86	12	2.24	0.40	18.02	1.13

	Medium	11	2.83[c]	0.38	13.31	1.06	12	3.06	0.28	9.15	0.79	12	2.20	0.51	23.36	1.44
	High	12	2.89[g]	0.26[d]	8.90	0.72	12	3.16	0.28	8.84	0.79	12	2.87	0.52	18.13	1.46
Prepared meals	Uninoculated	0					0					9	1.47	0.34	23.04	0.95
	Low	11	2.61[c]	0.13[d]	4.81	0.35	11	2.52	0.14	5.69	0.40	9	2.42	0.42	17.43	1.18
	Medium	11	3.51[g]	0.08[d, f]	2.21	0.22	12	3.30	0.14	4.35	0.40	12	3.34	0.38	11.42	1.07
	High	11	4.47[g]	0.08[d, f]	1.74	0.22	12	3.98	0.21	5.24	0.59	8	4.30	0.52	12.21	1.47
Frozen broccoli	Uninoculated	0					0					11	1.32	0.34	26.06	0.96
	Low	10	1.60	0.32	19.91	0.90	5	1.36	0.32	23.62	0.91	11	1.58	0.36	23.02	1.02
	Medium	12	2.66[c, g]	0.14[d, f]	5.15	0.39	11	2.02	0.29	14.28	0.81	11	2.11	0.45	21.18	1.25
	High	11	3.44[c, g]	0.29	8.49	0.82	10	2.84	0.55	19.38	1.55	10	2.71	0.48	17.73	1.34
Nuts	Uninoculated	10	2.74	0.21	7.53	0.58	10	2.89	0.24	8.45	0.69	9	2.67	0.34	12.76	0.95
	Low	10	3.50	0.29[d]	8.40	0.83	9	3.64	0.44	12.06	1.24	9	3.48	0.53	15.31	1.49
	Medium	7	4.09	0.50	12.10	1.40	7	4.13	0.47	11.38	1.32	5	3.82	0.68	17.89	1.91
	High	7	4.20	0.29[d]	6.92	0.82	7	4.14	0.49	11.86	1.39	7	3.83	0.85	22.13	2.37

a Number of laboratories with positive results.

b Log_{10} *Enterobacteriaceae* count/g.

c Significantly different from MPN method *(p* < 0.05).

d Significantly better repeatability than for the MPN method.

e Significantly better repeatability than for the Petrifilm plate method.

f Significantly better repeatability than for the VRBG method.

g Significantly different from VRBG method (p < 0.05).

Table 2003.01B Reproducibility performance of Neogen Petrifilm Enterobacteriaceae Count Plate, violet red bile glucose (VRBG), and most probable number (MPN) methods for detection of *Enterobacteriaceae* in foods

Food	Level	Petrifilm Enterobacteriaceae Count Plate					VRBG					MPN				
		n [a]	Mean[b]	S_R	RSD_R, %	R	n [a]	Mean[b]	S_R	RSD_r, %	R	n [a]	Mean[b]	S_R	RSD_R, %	R
Cheese	Control	0					0					9	2.09	0.98	46.73	2.73
	Low	12	3.02	0.20	6.69	0.57	12	3.03	0.10	3.43	0.29	11	3.12	0.34	10.80	0.94
	Medium	12	4.07	0.14	3.44	0.39	12	4.06	0.12	2.93	0.34	12	4.23	0.38	8.98	1.06
	High	10	4.91	0.24	4.89	0.68	12	4.93	0.29	5.84	0.81	9	4.82	0.27	5.60	0.76
Milk	Control	0					0					7	1.67	0.58	34.61	1.62
	Low	11	2.49	0.44	17.67	1.24	12	2.27	0.50	22.19	1.42	11	1.93	0.53	27.60	1.49
	Medium	12	3.34	0.18	5.27	0.50	12	3.27	0.39	11.87	1.09	11	2.65	0.73	27.73	2.05
	High	10	4.34	0.18	4.17	0.51	10	4.19	0.32	7.57	0.89	9	3.24	0.93	28.77	2.61
Flour	Control	12	2.70	0.33	12.15	0.92	12	2.98	0.37	12.34	1.04	12	2.44	0.47	19.20	1.31
	Low	12	2.73	0.37	13.46	1.04	12	2.95	0.44	14.99	1.25	12	2.24	0.45	20.21	1.27
	Medium	11	2.83	0.41	14.35	1.15	12	3.06	0.42	13.70	1.18	12	2.20	0.63	28.72	1.77
	High	12	2.89	0.29	10.10	0.82	12	3.16	0.37	11.74	1.04	12	2.87	0.65	22.52	1.81

Prepared meals	Control	0					0					9	1.47	0.34	23.04	0.95
	Low	11	2.61	0.17	6.43	0.47	11	2.52	0.28	11.23	0.80	9	2.42	0.42	17.43	1.18
	Medium	11	3.51	0.10	2.72	0.27	12	3.30	0.28	8.39	0.78	12	3.34	0.64	19.30	1.80
	High	11	4.47	0.21	4.71	0.59	12	3.98	0.37	9.22	1.03	8	4.30	0.52	12.21	1.47
Frozen broccoli	Control	0					0					11	1.32	0.51	38.43	1.42
	Low	10	1.60	0.43	26.99	1.22	5	1.36	0.35	26.03	1.00	11	1.58	0.48	30.27	1.34
	Medium	12	2.66	0.40	14.98	1.12	11	2.02	0.61	30.30	1.72	11	2.11	0.78	36.72	2.17
	High	11	3.44	0.55	16.14	1.56	10	2.84	0.68	23.87	1.91	10	2.71	0.87	32.07	2.43
Nuts	Control	10	2.74	0.48	17.47	1.35	10	2.89	0.42	14.70	1.20	9	2.67	0.34	12.76	0.95
	Low	10	3.50	0.68	19.33	1.91	9	3.64	0.71	19.57	2.01	9	3.48	0.65	18.58	1.81
	Medium	7	4.09	0.66	16.20	1.87	7	4.13	0.68	16.50	1.92	5	3.82	0.69	18.02	1.93

a Number of laboratories with positive results.

b Log_{10} *Enterobacteriaceae* count/g.

A Principle

Method uses bacterial culture plates of dry medium with pH indicator and cold-water-soluble gel. Undiluted or diluted test portions are added to plates at 1.0 mL per plate. Pressure applied to plastic spreader placed on overlay film spreads the test portion evenly over 20 cm^2 growth area. Gelling agent is allowed to solidify, and plates are incubated for 24 ± 2 h at 37 ± 1°C and then counted.

B Apparatus and Reagents

(a) *Petrifilm Enterobacteriaceae (EB) Count Plates.*—Plates, available from Neogen Corp. (Lansing, MI, USA, www.neogen.com), contain violet red bile glucose (VRBG) nutrients, pH indicator, cold-water-soluble gelling agent, and tetrazolium indicator dye.

(b) *Plastic spreader.*—Provided with Petrifilm plates; has smooth flat side designed to spread test portion evenly over plate growth area.

(c) *Pipets.*—1.0 and 10.0 mL serological pipets with 0.1 mL graduations. (Calibrated electronic pipettor and tips, or equivalent, may be used to deliver 1.0 mL.) Pipets must accurately deliver 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.

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

(e) *Butterfield's Phosphate Buffered Dilution water.*—Prepare stock solution as follows: Dissolve 34 g KH_2PO_4 in 500 mL water, adjust to pH 7.2 with 1 M 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.

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

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

(h) *Incubator.*—Maintaining 37 ± 1°C.

(i) *Balance.*—2000 ± 0.1 g capacity.

C Sample Preparation

Aseptically weigh 11.0 g unthawed test portion into blender jar, **B**(**g**). Add 99 mL dilution water, **B**(**e**), 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 1:10 dilution. Adjust pH of diluted test portion to 6.5–7.5 with 1 M 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 previous dilution. Mix all dilutions by shaking 25 times through 30 cm arc in 7 s.

D Analysis

Place dry Petrifilm EB plate, **B(a)**, on flat surface. Lift top film and inoculate 1 mL test suspension onto center of film base. Carefully roll top film down onto inoculum. Distribute test portion over 20 cm^2 growth area with downward pressure on center of plastic spreader device, **B(b)**, flat side down. Leave plate undisturbed for minimum of 1 min to permit gel to solidify. Incubate plates for 24 ± 2 h at 37 ± 1°C. In incubator, place plates in horizontal position, clear side up, in stacks not exceeding 20 plates. Count plates promptly (within 2 h) after incubation period.

Count Petrifilm EB plates on standard colony counter, **B(d)**, or other illuminated magnifier. Count all red colonies producing gas regardless of acid production. Also count all non-gassing colonies with yellow acid zones. Select plates with 10–150 colonies. If no plate has at least 10 qualifying colonies, record the exact raw count on the least dilute test suspension. If all the plates have counts >150, make estimates on plates by counting the number of colonies in one or more representative squares, determining the average number per square, and multiplying the average number by 20. If plates are too crowded for estimated counts, report the count as too numerous to count (TNTC). Do not count colonies on the foam dam because they are removed from the selective influence of the medium. Do not count artifact bubbles that may be present.

Reference:

J. AOAC Int. 86, 802(2003)Crossref

Revised: November 2023 (brand update and minor editorial corrections)