COPYRIGHT NOTICE & PERMISSION TO USE

AOAC Official Method 998.08
Confirmed *Escherichia coli* Counts in Poultry, Meats, and Seafood

Dry Rehydratable Film Method
Petrifilm™ *E. coli*/Coliform Count Plate
First Action 1998
Final Action 2002

(Applicable to determination of confirmed *Escherichia coli* in poultry, meats, and seafood in 24 h.)

See Table **998.08** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Undiluted or diluted 1.0 mL test portions are added to plates of dry medium and cold-water-soluble gel. Pressure, when applied to plastic spreader placed on overlay film, spreads suspension evenly over ca 20 cm^2 growth area. Gelling agent is allowed to solidify and plates are incubated and counted. The Petrifilm *E. coli*/Coliform (EC) Count Plate is a ready-made culture medium system that contains modified violet red bile (VRB), nutrients, a cold-water-soluble gelling agent, a tetrazolium indicator that facilitates colony enumeration, and the glucuronidase indicator, 5-bromo-4-chloro-3-indolyl-β-D-glucuronide. Glucuronidase, which is produced by most *E. coli*, reacts with the indicator dye to form a blue precipitate around the colony. Thus, glucuronidase-positive *E. coli* appear as blue colonies with gas. Non-*E. coli* coliforms that are glucuronidase-negative appear as red colonies with gas.

B. Apparatus and Reagents

(**a**) *Petrifilm EC Plates*.—Plates contain VRB nutrients conforming to American Public Health Association standards as given in *Compendium of Methods for the Microbiological Examination of Foods* (1992) 3rd Ed., American Public Health Association, Washington, DC 20001-3710, USA, cold-water-soluble gelling agent, 2,3,5,-triphenyltetrazolium chlorine indicator (for coliforms), and 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (for *E. coli*) as an indicator of glucuronidase activity. This allows presence of coliforms and *E. coli* to be read on the same plate. (Available from 3M Microbiology Products, 3M Center Bldg, 275-5W-05, St Paul, MN 55144-1000, USA.)

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

(**c**) *Pipets*.—Calibrated for bacteriological use, or plate loop continuous pipetting syringe to deliver 1.0 mL (with 0.1 mL graduation). Automatic pipets may be used. 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.

(**d**) *Colony counter.*—Standard apparatus, Quebec Model preferred (Fisher Laboratory Products, 200 Park Ln, Pittsburgh, PA 15275, USA, No. 07-908-7), or one providing equivalent magnification and visibility.

(**e**) *Dilution water.*—*See* 940.36A(**a**) (*see* 17.1.02).

C. Test Suspension

Prepare food suspensions as in 966.23B (*see* 17.2.01). Weigh 50 g test portion in sterile blender jar. Add 450 mL diluent and blend 2 min in a high-speed blender jar at 10 000 to 12 000 rpm. If entire test sample contains <50 g, weigh a portion of the test sample and add sterile diluent to make a 1 + 10 dilution. Prepare all decimal dilutions with 90 mL sterile diluent plus 10 mL previous dilution unless otherwise specified. Shake all dilutions vigorously 25× in 30 cm arc for 7 s.

D. Analysis

Place dry Petrifilm EC Plate on flat surface. Lift top film and inoculate 1 mL test suspension onto center of film base. Carefully place top film down onto inoculum. Distribute suspension over 20 cm^2 growth area with downward pressure on center of plastic

Table 998.08. Interlaboratory study results of Petrifilm™ EC Plate and MPN for detection of *E. coli* in foods

	Petrifilm *E. coli*/Coliform Count Plate								Most probable number							
Level	n^a	Mean^b	s_r	RSD_r, %	r	s_R	RSD_R, %	R	n^a	Mean	s_r	RSD_r, %	r	s_R	RSD_R, %	R
							Fish									
Low	11	2.32	0.23	9.91	0.65	0.42	18.10	1.18	11	2.42	0.27	11.16	0.76	0.58	23.97	1.64
Medium	11	2.95	0.14^c	4.75	0.39	0.32	10.88	0.90	11	3.11	0.37	11.90	1.04	0.51	16.45	1.44
High	11	3.77	0.12^c	3.18	0.31	0.27	7.16	0.76	8	3.71	0.27	7.28	0.82	0.32	8.44	0.90
							Turkey									
Low	11	2.06	0.24	11.65	0.67	0.38	18.45	1.06	11	2.32	0.37	15.95	1.04	0.38	16.38	1.07
Medium	10	2.55	0.10^c	3.92	0.28	0.22	8.63	0.62	11	2.73	0.39	14.29	1.10	0.45	16.48	1.27
High	10	3.15	0.09^c	2.86	0.25	0.19	6.03	0.54	11	3.36	0.34	10.12	0.96	0.35	10.42	0.99
							Beef									
Low	11	2.08	0.14^c	6.73	0.39	0.26	12.50	0.73	11	2.17	0.46	21.20	1.30	0.49	22.58	1.38
Medium	11	2.74	0.15^c	5.47	0.42	0.25	9.12	0.71	11	2.76	0.61	22.10	1.72	0.61	22.10	1.72
High	11	3.45	0.32	9.28	0.90	0.40	11.59	1.13	10	3.61	0.32	8.86	0.90	0.41	11.36	1.16

[a] No. of laboratories with complete data.

[b] Log *E. coli* count/g.

[c] Significantly greater repeatability ($p < 0.01$).

spreader device (flat side down). Leave plate undisturbed for a minimum of 1 min to permit gel to solidify. Incubate plates 24 ± 1 h at 35°C. In incubator, place plates in horizontal position, clear side up, in stacks not exceeding 20 units. Count plates promptly after incubation period. If impossible to count at once after required incubation, store plates at below –15°C no longer than 1 week. This should be avoided as routine practice.

Petrifilm EC Plates can be counted on a standard colony counter or other magnified light source. 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. Most *E. coli* colonies appear as blue colonies associated with gas bubbles within one colony diameter. All other coliforms appear as red colonies that have one or more gas bubbles associated (within one colony diameter) with them. Plates with 10–150 colonies are to be selected. If no plate has at least 10 blue colonies with gas, the exact count on the least dilute suspension is recorded. If all plates have counts >150, the estimated count is determined by counting the number of colonies in one or more representative squares, determining the average number per square, and then multiplying the average number by 20 (circular growth area is ca 20 cm^2). If the plates are too crowded to estimate counts, the count is reported as too numerous to count.

Reference: J. AOAC Int. 82, 73(1999).