



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

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CHAPTER

## 17.3.04 AOAC Official Method 991.14 Coliform and *Escherichia coli* Counts in Foods: Dry Rehydratable Film Methods Neogen® Petrifilm® *E.* *coli*/Coliform Count Plate and Neogen Petrifilm Coliform Count Plate

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First Action 1991

Final Action 1994

See Table 991.14 for results of the interlaboratory study supporting acceptance of the method.

**Table 991.14** Interlaboratory study results for total coliforms and *E. coli* on dry-film count plates

Product	Level	Mean log, CFU/g	$s_r$	$s_R$	RSD <sub>r</sub> , %	RSD <sub>R</sub> , %
<b>Total coliforms on coliform count plates</b>						
Ground turkey	Low	1.845	0.193	0.296	10.5	16.1
	Medium	2.235	0.361	0.361	16.2	16.2
	High	2.744	0.105	0.270	3.8	9.9
Fresh mushrooms	Low	4.241	0.278	0.808	6.6	19.0
	Medium	4.999	0.662	1.141	13.2	22.8
	High	5.197	0.120	0.872	2.3	16.8
Beef with gravy	Low	1.069	0.243	0.288	22.7	27.0
	Medium	2.062	0.257	0.391	12.5	18.9
	High	2.732	0.233	0.320	8.5	11.7
Cheese	Low	0.783	0.172	0.172	21.9	21.9
	Medium	2.411	0.092	0.206	3.8	8.5
	High	3.434	0.212	0.364	6.2	10.6
Wheat flour	Low	2.635	0.291	0.874	11.1	33.2
	Medium	3.460	0.746	1.291	21.6	37.3

	High	4.049	0.873	1.550	21.6	38.3
Nut meal	Low	0.896	0.336	0.443	37.5	49.5
	Medium	1.345	0.486	0.699	36.2	52.0
	High	2.370	0.267	0.831	11.3	35.1
<b>Total coliforms on <i>E. coli</i> count plates</b>						
Ground turkey	Low	1.934	0.177	0.261	9.2	13.5
	Medium	2.216	0.285	0.315	12.9	14.2
	High	2.835	0.113	0.242	4.0	8.5
Fresh mushrooms	Low	4.550	0.079	0.722	1.7	15.9
	Medium	5.278	0.600	1.001	11.4	19.0
	High	5.227	0.346	0.817	6.6	15.6
Beef with gravy	Low	1.016	0.215	0.284	21.2	27.9
	Medium	2.060	0.256	0.295	12.4	14.3
	High	2.679	0.191	0.331	7.1	12.4
Cheese	Low	0.813	0.172	0.192	21.1	23.6
	Medium	2.507	0.109	0.238	4.3	9.5
	High	3.620	0.074	0.106	2.1	2.9

Wheat flour	Low	2.639	0.243	0.883	9.2	33.4
	Medium	3.432	0.826	1.318	24.1	38.4
	High	4.064	0.929	1.557	22.9	38.8
Nut meal	Low	0.759	0.028	0.295	3.7	38.8
	Medium	1.403	0.378	0.728	26.9	51.8
	High	2.355	0.425	0.915	18.0	38.9
<b>Total <i>E. coli</i> on <i>E. coli</i> count plates</b>						
Ground turkey	Low	1.419	0.316	0.316	22.3	22.3
	Medium	1.923	0.364	0.364	18.9	18.9
	High	2.607	0.098	0.233	3.8	8.9
Fresh mushrooms	Low	2.500	0.261	0.343	10.4	13.7
	Medium	3.058	0.296	0.422	9.7	13.8
	High	3.436	0.715	0.760	20.8	22.1
Beef with gravy	Low	0.753	0.028	0.172	3.7	22.8
	Medium	0.991	0.154	0.601	15.6	60.7
	High	1.312	0.161	0.828	12.3	63.1
Cheese	Low	0.819	0.190	0.190	23.2	23.2

	Medium	2.454	0.098	0.166	4.0	6.8
	High	3.440	0.112	0.123	3.2	3.6
Wheat flour	Low	1.175	0.524	0.737	44.6	62.7
	Medium	3.095	0.967	1.362	31.3	44.0
	High	3.549	0.821	1.442	23.1	40.6
Nut meal	Low	0.716	0.067	0.236	9.4	33.0
	Medium	1.268	0.268	0.605	21.1	47.7
	High	2.314	0.265	0.857	11.4	37.0

## A Principle

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See 989.10A (see 17.3.03).

## B Apparatus and Reagent

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See 989.10B (b)–(f) (see 17.3.03).

p. C17-39E. *E. coli* count plates. — Plates are similar to coliform count plates, 989.10B (b) (see 17.3.03), with addition of 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide, an indicator of glucuronidase activity. This allows coliforms and *E. coli* to be read on the same plate. Petrifilm *E. coli* Count Plates (Neogen Corp., Lansing, MI, USA, [www.neogen.com](http://www.neogen.com)) meet these specifications.

## C Preparation of Test Suspension

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See 966.23B (see 17.2.01) and 986.33C (see 17.3.02).

## D Analysis

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- (a) *Coliform count*. — Place dry-film *E. coli* count plate, B, or coliform count plate, 989.10B (b) (see 17.3.03), 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 test suspension over prescribed growth area with downward pressure on center of plastic spreader device (flat side down). Leave plate undisturbed 1 min to permit gel to solidify. Incubate plates  $24 \pm 2$  h at  $35 \pm 1^\circ\text{C}$ .

In incubator, place plates in horizontal position, clear side up, in stacks not exceeding 20 units. Count plates promptly after incubation period. After incubation is complete, plates may be stored frozen ( $\leq -15^\circ\text{C}$ ) up to 7 days. This should be avoided as routine practice. Use standard colony counter for counting purposes. Magnifier-illuminator may also be used to facilitate counting. Coliforms appear as red colonies that have one or more gas bubbles associated (within one colony diameter) with them. Count all colonies in countable range (15–150 colonies). Red colonies without gas bubbles are not counted as coliform organisms.

- (b) *E. coli count*. — Use *E. coli* count plate and proceed as in (a). Incubate an additional  $24 \pm 2$  h ( $48 \pm 4$  h total). *E. coli* colonies appear as blue colonies associated with gas bubbles; other coliforms appear as red colonies with gas.

## Reference:

JAOAC 74, 635(1991)

Revised: March 2002; November 2023 (brand update and minor editorial corrections)