



CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

031601

The AOAC Research Institute hereby certifies the test kit known as:

Easy Plate EC

manufactured by

Kikkoman Biochemifa Company

2-1-1, Nishi-shinbashi

Minato-ku, Tokyo 105-0003

Japan

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (July 13, 2021 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

July 21, 2021

Date

METHOD AUTHORS Mai Shimizu, Fumihiko Saito, Kentaro Takenaka, Narumi Kimura, Takeo Suzuki, Tatsuhiko Iwase, and Hitoshi Kyotani	SUBMITTING COMPANY Dai Nippon Printing Co., Ltd. 1-1-1, Ichigaya Kagacho Shinjuku-ku, Tokyo, 162-8001 Japan	CURRENT COMPANY Kikkoman Biochemifa Company 2-1-1, Nishi-shinbashi Minato-ku, Tokyo 105-0003 Japan
KIT NAME(S) Easy Plate EC Formerly known as Medi-Ca EC	CATALOG NUMBERS 61982	
INDEPENDENT LABORATORY Q Laboratories, Inc. 1400 Harrison Ave. Cincinnati, OH USA	AOAC EXPERTS AND PEER REVIEWERS Yi Chen ¹ , Wayne Ziemer ² , Yvonne Salfinger ³ ¹ USDA FDA CFSAN, College Park, MD, USA ² Consultant, Loganville, GA, USA ³ Consultant, Denver, CO, USA February 2020 modification reviewed internally.	
APPLICABILITY OF METHOD Target organism – <i>Escherichia coli</i> and coliform Matrixes – (50 g samples) - raw beef, raw pork, raw frozen pork, raw lamb, raw salmon, Frankfurter sausage, cooked ham Performance claims - Performance comparable to that of the reference method.	REFERENCE METHOD AOAC <i>Official Method</i> SM 966.24, Coliform Group and <i>Escherichia coli</i> in Tree Nut Meats (3)	
ORIGINAL CERTIFICATION DATE March 27, 2016	CERTIFICATION RENEWAL RECORD Renewed annually through December 2021	
METHOD MODIFICATION RECORD 1. February 2020 Level 2 2. November 2020 Level 1 3. June 2021 Level 1	SUMMARY OF MODIFICATION 1. Manufacturing location change from Tokyo, Japan, to Kanagawa, Japan. 2. Editorial and formatting changes to insert. 3. Rebranded kit to reflect Kikkoman and method name change from Medi-Ca EC to Easy Plate EC.	
Under this AOAC® <i>Performance Tested</i> SM License Number, 031601 this method is distributed by: 1. AS ONE Corporation 2. KENIS LIMITED 3. Nippon Bacterial Test CO., LTD. 4. FUJIFILM Wako Pure Chemical Corporation 5. Microgiene Co. LTD 6. Weber Scientific 7. ELMEX Limited	Under this AOAC® <i>Performance Tested</i> SM License Number, 031601 this method is distributed as: 1. Easy Plate EC 2. Easy Plate EC 3. Easy Plate EC 4. Easy Plate EC 5. Easy Plate EC 6. Easy Plate EC 7. Easy Plate EC	

PRINCIPLE OF THE METHOD (1)

Easy Plate EC (formerly Medi-Ca EC) is a ready-made dry medium for *E. coli* and coliform count made up of four components: a waterproof sheet, a dry medium containing a gelling agent and the chromogenic enzyme substrates, 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-Gluc) and 6-bromo-5-chloro-3-indolyl-β-D-galactopyranoside (Magenta-Gal), a hydrophobic resin ring surrounding the medium, and a transparent cover over the medium. A sample suspension is dispensed on the center of the medium while the cover is lifted. After that, the cover is gently dropped to evenly spread the suspension on the medium. The suspension rapidly soaks into the medium, which turns into a gel in 3 minutes. The incubation of the sheet at 35 ± 1°C for 24 ± 1 h develops navy-blue/blue-purple and pink/red-purple colonies because of the enzymatic reaction involving the substrate: the β-glucuronidase produced by bacteria catalyzes the hydrolysis of the X-Gluc to yield an insoluble blue product and the β-galactosidase produced by bacteria catalyzes the hydrolysis of the Magenta-Gal to yield an insoluble red-purple product. Navy-blue/blue-purple colonies indicate *E. coli* and pink/red-purple colonies indicate non-*E. coli* coliform. Ninety-eight percent of *E. coli* produce both β-glucuronidase and β-galactosidase and non-*E. coli* coliform only produce β-galactosidase (4).

DISCUSSION OF THE VALIDATION STUDY (1)

In inclusivity study, *Escherichia blattae* (NBRC105725) was not detected. The reason for this was that production of β -galactosidase of *Escherichia blattae* do not occur. In the exclusivity study, Medi-Ca EC detected some *Serratia* and *Aeromonas* as non-*E. coli* coliform. Generally, most of these species produce β -galactosidase but some of them do not have the ability of lactose fermentation. Therefore, those species are classified as non-coliform. This shows Easy Plate EC has the ability to detect coliform related bacteria such as *Serratia*.

In the results of the matrix study conducted by the independent laboratory, some of the 95% CIs for the mean differences fell outside of -0.5 to 0.5, but all mean differences were $<0.5 \log_{10}$. In each instance, a difference of means with a positive numerical value indicated higher recovery of the target analyte for the alternative method. In addition, most of s_r and RSD_r values of the Medi-Ca EC method were lower than those of the reference method. Statistical differences may have been the result of comparing a direct plate count to the MPN estimate, which is limited in the numerical values that can be generated. The test principle of the MPN method is inherently more variable than a direct plate count method.

Overall, it was generally observed that the Easy Plate EC method produced statistically similar results when compared to the reference method. This rapid method makes it possible to simultaneously detect and enumerate *E. coli* and coliform in only 24 hours, while the reference method requires 7 to 10 days.

Table 1. Inclusivity Study (1)

Strain Name	Source	Origin	Medi-Ca EC ^a
1 <i>Buttiauxella noackiae</i>	#D0077 ^b	Chicken	+ (Pink)
2 <i>Citrobacter amalonaticus</i>	NBRC ^c 13547	Unknown	+ (Red-purple)
3 <i>Citrobacter freundii</i>	NBRC 12681	Unknown	+ (Red-purple)
4 <i>Citrobacter freundii</i>	ATCC ^d 8090	Unknown	+ (Red-purple)
5 <i>Citrobacter koseri</i>	NBRC 105690	Unknown	+ (Red-purple)
6 <i>Cronobacter sakazakii</i>	#D0003	Soybean	+ (Red-purple)
7 <i>Enterobacter aerogenes</i>	NBRC 13534	Sputum	+ (Red-purple)
8 <i>Enterobacter amnigenus</i>	#D0037	Cabbage	+ (Red-purple)
9 <i>Enterobacter asburiae</i>	#D0029	Radish sprout	+ (Red-purple)
10 <i>Enterobacter cloacae</i>	#D0030	Radish sprout	+ (Red-purple)
11 <i>Enterobacter cloacae</i>	ATCC222	Unknown	+ (Red-purple)
12 <i>Enterobacter cloacae</i>	#D0033	Bean sprout	+ (Pink)
13 <i>Escherichia blattae</i> (<i>Shimwellia blattae</i>)	NBRC 105725	Hindgut of cockroach	-
14 <i>Escherichia coli</i>	NBRC 102203	Urine	+ (Navy-blue)
15 <i>Escherichia coli</i>	NBRC 12062	Unknown	+ (Navy-blue)
16 <i>Escherichia coli</i>	NBRC 12433	Unknown	+ (Navy-blue)
17 <i>Escherichia coli</i>	NBRC 12734	Unknown	+ (Navy-blue)
18 <i>Escherichia coli</i>	NBRC 13500	Unknown	+ (Navy-blue)
19 <i>Escherichia coli</i>	NBRC 15034	Clinical specimen	+ (Navy-blue)
20 <i>Escherichia coli</i>	NBRC 3972	Feces	+ (Blue-purple)
21 <i>Escherichia coli</i>	ATCC 25922	Unknown	+ (Navy-blue)
22 <i>Escherichia coli</i>	NBRC 3301	Unknown	+ (Blue-purple)
23 <i>Escherichia coli</i>	#D0100	Ground beef and pork	+ (Navy-blue)
24 <i>Escherichia coli</i>	#D0099	Ground chicken	+ (Navy-blue)
25 <i>Escherichia coli</i>	#D0101	Chicken	+ (Navy-blue)
26 <i>Escherichia coli</i>	#D0102	Chicken	+ (Navy-blue)
27 <i>Escherichia coli</i>	NBRC 13540	Unknown	+ (Navy-blue)
28 <i>Escherichia coli</i>	NBRC 3543	Unknown	+ (Blue-purple)
29 <i>Escherichia coli</i>	NBRC 3806	Unknown	+ (Navy-blue)
30 <i>Escherichia coli</i>	NBRC 3991	Unknown	+ (Navy-blue)
31 <i>Escherichia coli</i>	NBRC 13898	Unknown	+ (Navy-blue)
32 <i>Escherichia coli</i>	#D0104	Coconut water	+ (Navy-blue)
33 <i>Escherichia coli</i>	NBRC 14195	Unknown	+ (Navy-blue)
34 <i>Escherichia coli</i>	NBRC 3302	Unknown	+ (Blue-purple)
35 <i>Escherichia coli</i>	NBRC 3544	Unknown	+ (Navy-blue)
36 <i>Escherichia coli</i>	NBRC 14129	Unknown	+ (Blue-purple)
37 <i>Escherichia coli</i> O157	ATCC 43895	Raw hamburger meat	+ (Red-purple)
38 <i>Escherichia coli</i> O26	RIMD ^e 05091876	Patient	+ (Blue-purple)
39 <i>Escherichia fergusonii</i>	NBRC 102419	Feces of human	+ (Red-purple)
40 <i>Escherichia hermanii</i>	NBRC 105704	Toe of 17-year-old female	+ (Red-purple)
41 <i>Escherichia vulneris</i>	NBRC 102420	Human wound	+ (Red-purple)
42 <i>Klebsiella oxytoca</i>	#D0032	Yellowtail	+ (Pink)
43 <i>Klebsiella oxytoca</i>	NBRC 105695	Pharyngeal tonsil	+ (Red-purple)
44 <i>Klebsiella pneumoniae</i>	ATCC 13883	Unknown	+ (Red-purple)
45 <i>Kluyvera cryocrescens</i>	NBRC 102467	Food	+ (Red-purple)
46 <i>Leclercia adecarboxylata</i>	NBRC 102595	Drinking water	+ (Red-purple)
47 <i>Pantoea agglomerans</i>	#D0004	Cake	+ (Pink)
48 <i>Rahnella aquatilis</i>	#D0038	Pork	+ (Red-purple)
49 <i>Rahnella aquatilis</i>	#D0053	Salmon	+ (Red-purple)
50 <i>Raoultella terrigena</i>	#D0022	Salmon	+ (Red-purple)
51 <i>Raoultella planticola</i>	NBRC 14939	Radish root	+ (Red-purple)

^a + = detected, - = not detected, () = color.

^b Numbers starting with #D indicates strains that were isolated by Dai Nippon Printing Co., Ltd.

^c Biological Resource Center, National Institute of Technology and Evaluation.

^d American Type Culture Collection, Manassas, VA.

^e Research Institute of Microbial Diseases, Osaka University.

Table 2. Exclusivity Study (1)

	Strain Name	Source	Origin	Medi•Ca EC ^a
1	<i>Achromobacter xylosoxidans</i>	NBRC ^b 15126	Ear discharge	-
2	<i>Aeromonas hydrophila</i>	NBRC 12658	Unknown	+ (Pink)
3	<i>Bacillus amyloxycheifaciens</i>	#D0015 ^c	Paprika powder	-
4	<i>Bacillus cereus</i>	NBRC 3836	Unknown	-
5	<i>Bacillus circulans</i>	NBRC 13626	Soil	-
6	<i>Bacillus coagulans</i>	NBRC 12583	Evaporated milk	-
7	<i>Bacillus licheniformis</i>	#D0010	Cheese cake	-
8	<i>Bacillus megaterium</i>	NBRC 15308	Unknown	-
9	<i>Bacillus subtilis</i>	#D0021	Chinese barbecued pork	-
10	<i>Bacillus thuringiensis</i>	NBRC 3951	Unknown	-
11	<i>Corynebacterium variabile</i>	NBRC 15286	Food	-
12	<i>Edwardsiella tarda</i>	NBRC 105688	Human feces	-
13	<i>Enterococcus faecalis</i>	ATCC ^d 29212	Urine	-
14	<i>Enterococcus faecium</i>	NBRC 100486	Unknown	-
15	<i>Kocuria rhizophila</i>	#D0008	Raw pork	-
16	<i>Lactobacillus casei</i>	#D0025	Lactic acid drink	-
17	<i>Lactobacillus delbrueckii</i>	NBRC 3202	Sour grain mash	-
18	<i>Lactococcus lactis</i>	#D0026	Yogurt	-
19	<i>Leuconostoc mesenteroides</i>	#D0057	Korean pickle	-
20	<i>Micrococcus luteus</i>	NBRC 3333	Unknown	-
21	<i>Proteus hauseri</i>	NBRC 3851	Unknown	-
22	<i>Proteus mirabilis</i>	NBRC 105697	Unknown	-
23	<i>Pseudomonas aeruginosa</i>	NBRC 3899	Well water	-
24	<i>Pseudomonas aeruginosa</i>	ATCC 9027	Unknown	-
25	<i>Pseudomonas mendocina</i>	NBRC 14162	soil enrichment with ethanol as carbon source	-
26	<i>Pseudomonas sp.</i>	#D0054	Salmon	-
27	<i>Salmonella enterica</i>	NBRC 105726	human feces (food poisoning in man)	-
28	<i>Serratia liquefaciens</i>	#D0027	Chicken	-
29	<i>Serratia marcescens</i>	NBRC 102204	Pond water	+ (Pink)
30	<i>Serratia rubidua</i>	NBRC 12973	Seawater	+ (Red-purple)
31	<i>Staphylococcus epidermidis</i>	NBRC 100911	Nose	-
32	<i>Staphylococcus aureus</i>	#D0072	Ground beef and pork	-
33	<i>Staphylococcus aureus</i>	ATCC 25923	Clinical isolate	-
34	<i>Staphylococcus carnosus</i>	#D0086	Roast beef	-
35	<i>Staphylococcus gallinarum</i>	#D0061	Japanese tea leaf	-
36	<i>Staphylococcus intermedius</i>	ATCC 29663	Pigeon nares	-
37	<i>Staphylococcus saprophyticus</i>	#D0009	Pork	-
38	<i>Staphylococcus simulans</i>	NBRC 109714	Human skin	-
39	<i>Staphylococcus sp.</i>	#D0058	Ground beef and pork	-
40	<i>Staphylococcus xylosus</i>	NBRC 109770	Human skin	-
41	<i>Yersinia frederiksenii</i>	#D0052	Salmon	-

^a + = detected, - = not detected, () = color.^b Biological Resource Center, National Institute of Technology and Evaluation.^c Numbers starting with #D indicates strains that were isolated by Dai Nippon Printing Co., Ltd.^d American Type Culture Collection, Manassas, VI.

Table 3. Matrix Study: Easy Plate EC vs. AOAC 966.24 – Total Coliforms (1)

Matrix	Inoculation Micoorganism	Contamination Level	Total Coliform									
			Medi-Ca EC			BGLB			Mean Difference	95% CI ^d		r ^{2g}
			Mean ^a	s _r ^b	RSD _r ^c	Mean	s _r	RSD _r		LCL ^e	UCL ^f	
Raw pork	<i>E. coli</i> ATCC 9637	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	2.40	0.08	3.42	2.49	0.15	6.20	-0.10	-0.24	0.05	0.92
		Medium	3.90	0.12	2.98	3.67	0.19	5.12	0.24	-0.01	0.48	
		High	4.39	0.09	2.01	4.61	0.13	2.74	-0.22	-0.45	0.02	
Raw frozen pork	<i>E. coli</i> #D0099	Uninoculated	2.14	0.09	4.27	1.93	0.20	10.16	0.21	0.04	0.38	-
		Low	2.36	0.05	2.20	2.30	0.19	8.26	0.06	-0.14	0.26	0.94
		Medium	3.06	0.07	2.30	2.77	0.18	6.59	0.29	0.09	0.49	
		High	4.12	0.02	0.58	4.16	0.19	4.62	-0.04	-0.30	0.22	
Raw beef	<i>E. coli</i> ATCC 25922 <i>K. oxytoca</i> NBRC 105695	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	2.52	0.05	1.88	2.53	0.26	10.39	-0.02	-0.34	0.31	0.95
		Medium	3.16	0.10	3.10	3.12	0.25	7.86	0.04	-0.25	0.34	
		High	4.86	0.03	0.53	4.70	0.21	4.48	0.16	-0.09	0.41	
Raw beef ^h	<i>E. coli</i> ATCC 25922 <i>K. oxytoca</i> NBRC 105695	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	2.44	0.10	4.07	2.30	0.19	8.39	0.13	-0.01	0.34	0.95
		Medium	3.50	0.09	2.47	3.22	0.17	5.35	0.28	0.01	0.55	
		High	4.23	0.13	2.95	4.04	0.00	0.00	0.18	0.03	0.34	
Raw lamb	<i>E. coli</i> #D0101	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	2.94	0.05	1.81	3.05	0.11	3.73	-0.11	-0.31	0.09	0.99
		Medium	4.01	0.04	0.96	4.01	0.09	2.31	0.00	-0.10	0.10	
		High	5.85	0.06	0.99	6.18	0.15	2.36	-0.32	-0.46	-0.19	
Raw salmon	<i>E. coli</i> NBRC 3806	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	1.67	0.08	4.69	1.64	0.22	13.12	0.03	-0.27	0.32	0.93
		Medium	3.03	0.04	1.30	3.24	0.14	4.17	-0.21	-0.36	-0.05	
		High	4.23	0.11	2.52	4.29	0.10	2.42	-0.05	-0.31	0.20	
Frankfurter sausage	<i>E. coli</i> NBRC 12433 <i>E. cloacae</i> ATCC 222	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	3.12	0.04	1.36	2.99	0.11	3.70	0.13	-0.02	0.28	0.97
		Medium	4.05	0.05	1.20	4.01	0.09	2.31	0.04	-0.13	0.21	
		High	4.89	0.07	1.42	4.73	0.13	2.78	0.16	-0.03	0.35	
Cooked ham	<i>E. coli</i> NBRC 13500 <i>E. cloacae</i> NBRC 13536	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	1.67	0.26	15.61	1.88	0.14	7.66	-0.22	-0.47	0.03	0.95
		Medium	2.69	0.05	1.71	2.80	0.15	5.48	-0.10	-0.34	0.13	
		High	3.36	0.03	0.92	3.33	0.09	2.65	0.03	-0.10	0.16	
Cooked ham ^h	<i>E. coli</i> NBRC 13500 <i>E. cloacae</i> NBRC 13536	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	1.92	0.11	5.68	1.73	0.37	21.52	0.19	-0.27	0.66	0.93
		Medium	3.12	0.18	5.74	2.89	0.21	7.18	0.23	-0.06	0.52	
		High	4.15	0.07	1.68	3.93	0.17	4.25	0.22	0.05	0.38	

^a Mean of 5 replicates after the logarithmic transformation: Log₁₀[CFU/g + (0.1)]^f.^b s_r = standard deviation.^c RSD_r = relative standard deviation.^d CI = confidence interval.^e LCL = lower confidence limit.^f UCL = upper confidence limit.^g r² = square of the correlation coefficient.^h Matrix study conducted by the independent laboratory.

Table 4. Matrix Study: Easy Plate EC vs. AOAC 966.24 – *E. coli* (1)

Matrix	Inoculation Micoorganism	Contamination Level	<i>E. coli</i>									
			Medi-Ca EC			EC			Mean Difference	95% CI ^d		r ^{2g}
			Mean ^a	s _r ^b	RSD _r ^c	Mean	s _r	RSD _r		LCL ^e	UCL ^f	
Raw pork	<i>E. coli</i> ATCC 9637	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	1.53	0.33	21.79	1.53	0.15	9.64	0.00	-0.46	0.46	0.93
		Medium	2.47	0.16	6.44	2.33	0.08	3.50	0.14	-0.09	0.37	
		High	3.80	0.04	1.12	3.81	0.25	6.57	-0.01	-0.29	0.27	
Raw frozen pork	<i>E. coli</i> #D0099	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	1.80	0.11	5.90	1.99	0.22	11.1	-0.18	-0.44	0.07	0.97
		Medium	2.84	0.04	1.44	3.03	0.09	3.08	-0.19	-0.31	0.22	
		High	3.95	0.05	1.21	4.12	0.21	5.10	-0.17	-0.44	0.10	
Raw beef	<i>E. coli</i> ATCC 25922 <i>K. oxytoca</i> NBRC 105695	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	1.91	0.09	4.73	1.77	0.18	10.32	0.14	-0.15	0.43	0.91
		Medium	2.35	0.08	3.21	2.32	0.08	3.41	0.02	-0.09	0.14	
		High	3.15	0.08	2.48	3.18	0.21	6.47	-0.03	-0.31	0.25	
Raw beef ^h	<i>E. coli</i> ATCC 25922 <i>K. oxytoca</i> NBRC 105695	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	2.12	0.14	6.41	2.20	0.15	6.91	-0.08	-0.22	0.06	0.93
		Medium	3.22	0.06	1.89	3.14	0.16	5.00	0.07	-0.06	0.21	
		High	3.81	0.14	3.72	3.61	0.31	8.46	0.20	-0.14	0.54	
Raw lamb	<i>E. coli</i> #D0101	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	2.83	0.04	1.58	3.01	0.20	6.74	-0.19	-0.46	0.09	0.95
		Medium	3.75	0.08	2.01	3.77	0.18	4.84	-0.02	-0.23	0.19	
		High	4.79	0.06	1.18	4.84	0.18	3.78	-0.05	-0.24	0.15	
Raw salmon	<i>E. coli</i> NBRC 3806	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	1.68	0.11	6.59	1.69	0.15	9.14	-0.01	-0.24	0.21	0.96
		Medium	2.42	0.01	0.49	2.46	0.16	6.60	-0.04	-0.25	0.17	
		High	3.67	0.07	2.01	3.70	0.24	6.38	-0.02	-0.25	0.20	
Frankfurter sausage	<i>E. coli</i> NBRC 12433 <i>E. cloacae</i> ATCC 222	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	2.72	0.06	2.28	2.52	0.17	6.56	0.20	-0.07	0.47	0.98
		Medium	3.92	0.04	0.90	3.84	0.18	4.76	0.08	-0.16	0.32	
		High	5.69	0.04	0.62	5.81	0.21	3.57	-0.12	-0.41	0.17	
Cooked ham	<i>E. coli</i> NBRC 13500 <i>E. cloacae</i> NBRC 13536	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	1.58	0.20	12.95	1.70	0.15	8.75	-0.13	-0.34	0.08	0.93
		Medium	2.52	0.09	3.75	2.49	0.22	8.80	0.03	-0.17	0.23	
		High	3.23	0.09	2.80	3.33	0.09	2.65	-0.10	-0.25	0.05	
Cooked ham ^h	<i>E. coli</i> NBRC 13500 <i>E. cloacae</i> NBRC 13536	Uninoculated	<1.00	-	-	<1.00	-	-	-	-	-	-
		Low	1.52	0.21	13.77	1.46	0.22	15.25	0.06	-0.31	0.43	0.93
		Medium	2.80	0.16	5.58	2.76	0.27	9.82	0.04	-0.17	0.25	
		High	3.83	0.09	2.29	3.51	0.25	7.24	0.32	-0.04	0.68	

^a Mean of 5 replicates after the logarithmic transformation: Log₁₀[CFU/g + (0.1)f].^b s_r = standard deviation.^c RSD_r = relative standard deviation.^d CI = confidence interval.^e LCL = lower confidence limit.^f UCL = upper confidence limit.^g r² = square of the correlation coefficient.^h Matrix study conducted by the independent laboratory.

REFERENCES CITED

- Shimizu, M., Saito, F., Takenaka, K., Kimura, N., Suzuki, T., Iwase, T., and Kyotani, H., Evaluation of Medi-Ca EC, AOAC® Performance TestedSM certification number 031601.
- AOAC Research Institute Validation Outline for Medi-Ca EC, Approved – March 2016.
- AOAC Official MethodSM 966.24, Coliform Group and *Escherichia coli* in Tree Nut Meats, (<http://www.eoma.aoac.org>)
- T. Teramoto, *Japanese Journal of Food Microbiology*, 9(4), 211-216, 1993
- Centers for Disease Control and Prevention (CDC). *E. coli* (*Escherichia coli*) Homepage. Page last updated: December 21, 2015. (<http://www.cdc.gov/ecoli/general/>)
- U.S. Food and Drug Administration (2013) *FDA Bacteriological Analytical Manual*, Chapter 4, (<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064948.htm>)
- Official Methods of Analysis* (2012), 19th Ed., Appendix J, AOAC INTERNATIONAL, Rockville, MD, http://www.eoma.aoac.org/app_j.pdf
- Least Cost Formulations, Ltd., Paired Method Analysis for Micro Testing (2010), Version 1.0, <http://lcfstd.com/AOAC/paired-method-analysis-for-micro.xlsx>
- AOAC Research Institute Performance Tested MethodsSM Program validation outline protocol: *Independent Laboratory Study for the Dai Nippon Printing Compant, Ltd. for the Medi-Ca EC Medium for Enumeration of Escherichia coli and Coliform Bacteria* (Version 1, September 2015)
- J. J. FARMER III et al., *Journal of Clinical Microbiology*, Jan. 1985, p. 46-76