



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
021401

The AOAC Research Institute hereby certifies the method known as:

Easy Plate CC

manufactured by

Kikkoman Biochemifa Company
2-1-1, Nishi-shinbashi
Minato-ku, Tokyo 105-0003
Japan

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date 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

Issue Date	November 28, 2022
Expiration Date	December 31, 2023

AUTHORS ORIGINAL VALIDATION: Fumihiko Saito, Mai Shimizu, Takeo Suzuki, Chie Hamada, Tatsuhiko Iwase, Norihiko Okochi, Mamoru Yamazaki, Hitoshi Kyotani MODIFICATION MARCH 2022: Satoshi Fukuda and Shinichiro Sugiura		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
METHOD NAME Easy Plate CC Formerly known as Medi-Ca CC		CATALOG NUMBER 61974	
INDEPENDENT LABORATORY Q Laboratories, Inc 1400 Harrison Avenue Cincinnati, OH 45214 USA		AOAC EXPERTS AND PEER REVIEWERS Yi Chen ¹ , Yvonne Salfinger ² , Wayne Ziemer ³ ¹ Food and Drug Administration, Center for Food Safety and Applied Nutrition, Maryland, USA ² Consultant, Colorado, USA ³ Consultant, Georgia, USA Modifications February 2020 and March 2022 reviewed internally by AOAC Research Institute.	
APPLICABILITY OF METHOD Target organism – Coliform bacteria Matrixes – (50 g) - raw ground pork, raw lamb, raw ground chicken, raw tuna fillet, raw salmon fillet, raw shrimp, fresh peeled banana, fresh cut pineapple, and fresh cut apples Performance claims – The method is a reasonable alternative to the Violet Red Bile Agar method (<i>Bacteriological Analytical Manual</i> , Chapter 4, Section G) (3) for raw meat, raw poultry, raw fish and fresh fruits.		REFERENCE METHOD <i>Bacteriological Analytical Manual</i> , Chapter 4, Section G) (3)	
ORIGINAL CERTIFICATION DATE February 03, 2014		CERTIFICATION RENEWAL RECORD Renewed annually through December 2023.	
METHOD MODIFICATION RECORD <ol style="list-style-type: none"> February 2020 Level 2 November 2020 Level 1 June 2021 Level 1 March 2022 Level 2 		SUMMARY OF MODIFICATION <ol style="list-style-type: none"> Manufacturing location change from Tokyo, Japan to Kanagawa, Japan. Editorial and formatting changes to insert. Rebranded kit to reflect Kikkoman and method name change from Medi-Ca CC to Easy Plate CC. Manufacturing location change. 	
Under this AOAC <i>Performance Tested Methods</i> SM License Number, 021401 this method is distributed by: <ol style="list-style-type: none"> AS ONE Corporation KENIS LIMITED Nippon Bacterial Test CO., LTD. FUJIFILM Wako Pure Chemical Corporation Microgene Co. LTD Weber Scientific ELMEX Limited 		Under this AOAC <i>Performance Tested Methods</i> SM License Number, 021401 this method is distributed as: <ol style="list-style-type: none"> Easy Plate CC Easy Plate CC Easy Plate CC Easy Plate CC Easy Plate CC Easy Plate CC Easy Plate CC 	

PRINCIPLE OF THE METHOD (1)

Easy Plate CC (formerly Medi-Ca CC) is a ready-made dry medium for coliform count made up of four components: a waterproof sheet, a dry medium containing a gelling agent and the chromogenic enzyme substrate, 5-Bromo-4-chloro-3-indolyl β-D galactopyranoside (X-gal), a hydrophobic resin ring surrounding the medium, and a transparent cover over the medium. Sample suspension is dispensed on the center of the medium while the cover is lifted. After that, the cover is dropped gently to spread the suspension on the medium evenly. 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 blue colonies because of the enzymatic reaction involving the substrate: the β-galactosidase produced by bacteria catalyzes the hydrolysis of the X-gal to yield an insoluble blue product.

DISCUSSION OF THE VALIDATION STUDY (1)

The Easy Plate CC method was compared to the VRBA method (BAM, Chapter 4, Section G) for the nine raw foods from the four food categories. The 95% CI for the mean difference between the two methods at each contamination level for seven matrixes from all four categories fell within the range of -0.50 to 0.50, and no statistical difference was observed at all three contamination levels for four matrixes from three categories (Table 2 and 5). In addition, the repeatability of the Easy Plate CC method was overall similar to that of the VRBA method. These results demonstrated that the Easy Plate CC method is a reasonable alternative to the VRBA method for raw meat, raw poultry, raw fish and fresh fruits.

In contrast to the internal lab study, the Easy Plate CC method produced the remarkably lower coliform count than the VRBA method at the high level for raw salmon fillet in the independent lab study (Table 5). The explanation for this is that non-coliform bacteria in large numbers competed with total coliform and made it difficult for coliforms to be detected: approximately 80% of the total Coliform colonies could not be recognized due to their tiny sizes or slight intensities. The Easy Plate CC method is subject to that kind of growth inhibition because a gel volume of a Easy Plate CC medium is roughly ten times smaller than an agar volume of VRBA. In fact, a total viable count of the sample stored in a storage condition in the independent lab study (at 2-5°C for 5 days) was *ca.* 10⁷ CFU/g, being one hundred times higher than that of the one stored in a storage condition in the internal lab study (at 10 ± 1°C for 24 ± 1 h). In addition, the third party pointed out that the low level had distinct dark blue colonies and the high level had small light blue colonies. Probably, psychrophiles such as *Pseudomonas* selectively grew to inhibit the growth of coliforms in the independent lab study. In that case, an additional incubation for a few hours for making the colony size larger or the colony color intensity darker is recommended to obtain the colony count results equivalent to the VRBA method.

Table 1. Inclusivity/Exclusivity Panel Results (1)

Strain Name	Source ^a	Coliforms		Results ^b
		Origin	Medi-Ca CC	
<i>Citrobacter koseri</i>	NBRC 105690	Unknown	+	+
<i>Citrobacter amalonaticus</i>	NBRC 13547	Unknown	+	+
<i>Citrobacter freundii</i>	ATCC 8090	Unknown	+	+
<i>Citrobacter freundii</i>	NBRC	unknown	+	+
<i>Citrobacter koseri</i>	Natural isolate #20	White radish sprouts	+	+
<i>Cronobacter sakazakii</i>	NBRC 102416T	Child's throat	+	+
<i>Cronobacter sakazakii</i>	NBRC 105698	Child's throat	+	+
<i>Cronobacter sakazakii</i>	Natural isolate #6	Green soybeans	+	+
<i>Enterobacter aerogenes</i>	NBRC 13534T	Sputum	+	+
<i>Enterobacter aminigenus</i>	NBRC 105700T	soil	+	+
<i>Enterobacter cloacae</i>	NBRC 13535T	Spinal fluid	+	+
<i>Enterobacter cloacae</i>	NBRC 13536	Unknown	+	+
<i>Enterobacter cloacae</i>	NBRC 12935	Unknown	+	+
<i>Enterobacter cloacae</i>	NBRC 12937	Diseased silk-worm	+	+
<i>Enterobacter cloacae</i>	ATCC 222	Unknown	+	+
<i>Enterobacter cloacae</i>	Natural isolate #21	White radish sprouts	+	+
<i>Enterobacter gergoviae</i>	NBRC 105706T	Urine	+	+
<i>Escherichia blattae</i>	NBRC 105725T	Hindgut of cockroach	-	+
<i>Escherichia coli</i>	NBRC 15034	Clinical specimen	+	+
<i>Escherichia coli</i>	NBRC 102203T	urine	+	+
<i>Escherichia coli</i>	NBRC 13500	Unknown	+	+
<i>Escherichia coli</i>	NBRC 15034	Clinical specimen	+	+
<i>Escherichia coli</i>	ATCC 25922	Unknown	+	+
<i>Escherichia coli</i>	NBRC 13966	unknown	+	+
<i>Escherichia coli</i>	NBRC 13898	Unknown	+	+
<i>Escherichia coli</i>	NBRC 3301	unknown	+	+
<i>Escherichia coli</i>	NBRC 3302	Unknown	+	+
<i>Escherichia coli</i>	NBRC 13540	unknown	+	+
<i>Escherichia coli</i>	NBRC 3366	Unknown	+	-
<i>Escherichia coli</i>	NBRC 3543	unknown	+	+
<i>Escherichia coli</i>	NBRC 3544	Unknown	+	+
<i>Escherichia coli</i>	NBRC 14129	unknown	+	+
<i>Escherichia coli</i>	NBRC 15484	Unknown	+	+
<i>Escherichia coli</i>	NBRC 12062	unknown	+	+
<i>Escherichia coli</i>	NBRC 12433	Unknown	+	+
<i>Escherichia coli</i>	NBRC 12734	unknown	+	+
<i>Escherichia coli</i>	NBRC 3972	Feces	+	+
<i>Escherichia coli</i>	NBRC 3991	unknown	+	+
<i>Escherichia coli</i>	NBRC 13891	Unknown	+	-
<i>Escherichia coli</i>	NBRC 13892	unknown	+	-
<i>Escherichia coli</i>	NBRC 3545	Unknown	+	+
<i>Escherichia coli</i>	NBRC 3546	unknown	+	+
<i>Escherichia coli</i>	NBRC 3806	Unknown	+	+
<i>Escherichia coli</i>	NBRC 3993	Unknown	+	-
<i>Escherichia fergusonii</i>	NBRC 102419	Feces of human (one-year-old boy)	+	+
<i>Escherichia hermannii</i>	NBRC 105704T	Toe of 17-year-old female	+	+
<i>Escherichia vulneris</i>	NBRC 102420	Human wound	+	+

<i>Klebsiella oxytota</i>	NBRC 105695	Pharyngeal tonsil	+	+
<i>Klebsiella pneumoniae</i>	NBRC 14940T	Unknown	+	+
<i>Klebsiella pneumoniae</i>	ATCC 13883	Unknown	+	+
<i>Klebsiella pneumoniae</i>	Natural isolate #31	Raw yellowtail	+	+
<i>Kluyvera cryocrescens</i>	Natural isolate #2	food	+	+
<i>Kluyvera intermedia</i>	NBRC 102594T	Surface water	+	+
<i>Leclercia adecarboxylata</i>	NBRC 102595	Drinking water	+	+
<i>Pantoea agglomerans</i>	Natural isolate #3	Cake	+	+
<i>Rahnella aquatilis</i>	Natural isolate #10	Raw ground pork	+	+
<i>Raoultella planticola</i>	NBRC 14939	Radish root	+	+
<i>Raoultella terrigena</i>	Natural isolate #33	Raw salmon	+	+
<i>Raoultella terrigena</i>	NBRC 14941T	Drinking water	+	+

Non-coliforms

Strain Name	Source	Origin	Medi-Ca CC	Results	BGLB
<i>Achromobacter denitrificans</i>	NBRC 15125T	soil	-	-	-
<i>Achromobacter xylosoxidans</i>	NBRC 15126	Ear discharge	-	-	-
<i>Aeromonas hydrophila</i>	NBRC 12658	unknown	-	-	-
<i>Alcaligenes faecalis</i>	NBRC 13111T	Unknown	-	-	-
<i>Bacillus amyloliquefaciens</i>	Natural isolate #8	Powdered paprika	-	-	-
<i>Bacillus cereus</i>	NBRC 15305T	Unknown	-	-	-
<i>Bacillus cereus</i>	NBRC 3836	Unknown	-	-	-
<i>Bacillus cereus</i>	NBRC 13494	Unknown	-	-	-
<i>Bacillus licheniformis</i>	Natural isolate #4	Cheese cake	-	-	-
<i>Bacillus subtilis</i>	Natural isolate #14	Chinese barbecued pork	-	-	-
<i>Bacillus subtilis</i>	NBRC 3134	Unknown	-	-	-
<i>Corynebacterium variabile</i>	NBRC 15286	Food	-	-	-
<i>Edwardsiella tarda</i>	NBRC 105688T	Human feces	-	-	-
<i>Kocuria kristinae</i>	Natural isolate #5	Cheese cake	-	-	-
<i>Lactobacillus delbrueckii</i>	NBRC 3202	Sour grain mash	-	-	-
<i>Lactococcus lactis</i>	Natural isolate #40	Yoghurt	-	-	-
<i>Lactobacillus casei</i>	Natural isolate #42	Lactic acid drink	-	-	-
<i>Micrococcus luteus</i>	NBRC 3333T	Unknown	-	-	-
<i>Micrococcus luteus</i>	NBRC 13867	Air	-	-	-
<i>Micrococcus lylae</i>	NBRC 15355T	Human skin	-	-	-
<i>Proteus hauseri</i>	NBRC 3851	Unknown	-	-	-
<i>Proteus hauseri</i>	NBRC 105696	Unknown	-	-	-
<i>Proteus mirabilis</i>	NBRC 105697T	Unknown	-	-	-
<i>Providencia alcalifaciens</i>	NBRC 105687T	Feces	-	-	-
<i>Pseudomonas mendocina</i>	NBRC 14162	Soil enrichment with ethanol as carbon source	-	-	-
<i>Pseudomonas aeruginosa</i>	NBRC 3453	Unknown	-	-	-
<i>Pseudomonas aeruginosa</i>	NBRC 12689	Unknown	-	-	-
<i>Pseudomonas aeruginosa</i>	ATCC 9027	Unknown	-	-	-
<i>Pseudomonas aeruginosa</i>	NBRC 3446	Urine	-	-	-
<i>Pseudomonas aeruginosa</i>	NBRC 3449	Urine	-	-	-
<i>Pseudomonas fluorescens</i>	Natural isolate #16	Raw lamb	-	-	-
<i>Pseudomonas fluorescens</i>	Natural isolate #22	White radish sprouts	-	-	-
<i>Pseudomonas pseudodalis</i>	NBRC 14167	Sinus drainage	-	-	-
<i>Pseudomonas stutzeri</i>	NBRC 14165	Human spinal fluid	-	-	-
<i>Serratia liquefaciens</i>	Natural isolate #12	Raw ground chicken	-	-	-
<i>Serratia marcescens</i>	NBRC 102204	Pond water	+	-	-
<i>Staphylococcus aureus</i>	ATCC 33862	Unknown	-	-	-
<i>Staphylococcus aureus</i>	NBRC 14462	Clinical isolate	-	-	-
<i>Staphylococcus aureus</i>	NBRC 100910T	Human pleural fluid	-	-	-
<i>Staphylococcus aureus</i>	NBRC 12732	Human lesion	-	-	-
<i>Streptococcus equines</i>	NBRC 12553T	Unknown	-	-	-
<i>Streptococcus thermophilus</i>	Natural isolate #41	Yoghurt	-	-	-

^a The natural isolate strains were isolated and numbered in our laboratory, and then identified by molecular and biochemical analyses.

^b + = detected; - = not detected

Table 2. Matrix Study Results (Method Developer) (1) *Medi-Ca CC is now Easy Plate CC

Matrix	Inoculation Microorganism	Contamination Level	Medi-Ca CC		RSD _r ^c	VRBA		RSD _r	<i>p</i> -value	Mean Difference	95% LCL ^e	CI ^d UCL ^f	<i>r</i> ^{2g}
			Mean ^a	<i>s</i> ^b		Mean _r	<i>s</i> _r						
Raw ground pork	N/A ^h	Low	4.19	0.03	0.71	4.18	0.06	1.36	0.69	-0.01	-	0.06	1.00
		Medium	5.06	0.04	0.80	5.09	0.02	0.48	0.11	0.03	-	0.07	
		High	8.42	0.06	0.69	8.47	0.11	1.30	0.53	0.23	-	0.25	
Raw lamb	N/A	Low	2.31 ⁱ	0.09	3.83	2.48	0.05	1.95	0.01	0.18	0.	0.27	1.00
		Medium	7.79	0.04	0.48	7.82	0.03	0.33	0.25	0.02	-	0.07	
		High	8.59 ⁱ	0.04	0.46	8.69	0.07	0.86	0.04	0.09	0.	0.18	
Raw ground chicken	N/A	Low	2.11	0.07	3.47	2.22	0.09	4.20	0.08	0.11	-	0.23	0.99
		Medium	3.62 ⁱ	0.08	2.08	3.75	0.09	2.35	0.05	0.13	0.	0.26	
		High	5.02	0.02	0.43	4.93	0.12	2.43	0.14	-0.09	-	0.04	
Raw tuna fillet	N/A	Low	2.53	0.06	2.23	2.54	0.05	1.85	0.76	0.01	-	0.13	1.00
		Medium	3.56	0.10	2.89	3.42	0.10	2.98	0.06	-0.13	-	0.01	
		High	6.41	0.03	0.52	6.27	0.13	2.12	0.09	-0.13	-	0.03	
Raw salmon fillet	N/A	Low	2.16	0.19	8.99	2.02	0.08	4.06	0.25	-0.15	-	0.15	0.97
		Medium	3.04	0.02	0.79	3.06	0.06	1.84	0.65	0.01	-	0.08	
		High	3.97	0.03	0.71	3.96	0.06	1.45	0.61	-0.01	-	0.00	
Raw shrimp	N/A	Low	2.42 ⁱ	0.08	3.41	2.16	0.17	7.66	0.01	-0.26	-	-0.10	1.00
		Medium	3.53	0.06	1.81	3.47	0.06	1.70	0.24	-0.06	-	0.06	
		High	8.41 ⁱ	0.03	0.34	8.23	0.03	0.42	0.00	-0.18	-	-0.11	
Fresh peeled banana	<i>E.coli</i> NBRC 15034	Uninoculated	<1.00	—	—	<1.00	—	—	—	—	—	—	0.99
		Low	3.51	0.11	3.28	3.49	0.06	1.74	0.73	-0.02	-	0.12	
		Medium	4.75	0.09	1.86	4.76	0.09	1.84	0.88	0.01	-	0.19	
		High	5.65	0.03	0.58	5.68	0.07	1.24	0.40	0.03	-	0.13	
Fresh cut pineapple	ATCC 25922	Uninoculated	<1.00	—	—	<1.00	—	—	—	—	—	—	1.00
		Medium	4.41	0.04	0.95	4.41	0.04	0.94	0.94	0.00	-	0.06	
		High	5.42	0.03	0.57	5.40	0.03	0.49	0.33	-0.02	-	0.03	
Fresh cut apple	<i>E. aerogenes</i> NBRC 13534	Uninoculated	<1.00	—	—	<1.00	—	—	—	—	—	—	1.00
		Low	3.60	0.06	1.54	3.58	0.05	1.46	0.34	-0.02	-	0.03	
		Medium	4.67	0.08	1.71	4.64	0.11	2.38	0.70	-0.03	-	0.19	
		High	5.74	0.04	0.63	5.65	0.08	1.46	0.11	-0.09	-	0.03	

^a Mean of 5 replicates after the logarithmic transformation: $\log_{10}[\text{CFU/g} + (1.0)]$.^b RSD_r = standard deviation.^c RSD_r = relative standard deviation.^d CI = confidence interval.^e LCL = lower confidence limit.^f UCL = upper confidence limit.^g *r*² = square of the correlation coefficient.^h N/A — Not applicable. Samples are naturally contaminated.ⁱ Significantly different (*p*<0.05).

REFERENCES CITED

1. Saito, Fumihiko, Shimizu, Mai, Suzuki, Takeo, Hamada, Chie, Iwase, Tatsuhiko, Okochi, Norihiko, Yamazaki, Mamoru, and Kyotani, Hitoshi., Evaluation of the Dai Nippon Printing Co., LTD. Easy Plate CC (formerly Medi-Ca CC) for the Detection of Coliform bacteria, AOAC *Performance Tested Methods*SM certification number 021401.
2. AOAC Research Institute Validation Outline for Dai Nippon Printing Co., LTD. Medi-Ca CC , Approved – February 2014.
3. US Food and Drug Administration Bacteriological Analytical Manual (BAM), Enumeration of *Escherichia coli* and the Coliform Bacteria, Chapter 4, Section G, 2013 (<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064948.htm>)
4. US Food and Drug Administration Bacteriological Analytical Manual (BAM), Media Index for BAM, 2013 (<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm055778.htm>)
5. AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (2012) Official Methods of Analysis of AOAC INTERNATIONAL, 19th Ed., Appendix J, AOAC INTERNATIONAL, Gaithersburg, MD.
6. Fukuda, S. and Sugiura, S., Modification Validation of Easy Plate Series (AC, CC, EC, and SA) for the Manufacturing Location Change, AOAC *Performance Tested Methods*SM certification number 021401. Approved March 2022.