CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

021401

The AOAC Research Institute hereby certifies the test kit 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® *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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

July 21, 2021
Date

2275 Research Blvd., Suite 300, Rockville, MD 20850-3250 USA * Telephone: +1-301-924-7077 * Fax: +1-301-924-7089
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

METHOD AUTHORS

Fumihiko Saito, Mai Shimizu, Takeo Suzuki, Chie Hamada, Tatsuhiko Iwase, Norihiko Okochi, Mamoru Yamazaki, 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 CC
Formerly known as Medi·Ca CC

CATALOG NUMBERS
61981

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] US FDA, CFSAN, College Park, MD, USA
[2] Consultant, Denver, CO USA
[3] Consultant, Loganville, GA, USA
February 2020 modification reviewed internally

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 (*Bacteriological Analytical Manual*, Chapter 4, Section G) (3) for raw meat, raw poultry, raw fish and fresh fruits.

REFERENCE METHOD
***Bacteriological Analytical Manual*, Chapter 4, Section G) (3)**

ORIGINAL CERTIFICATION DATE
February 03, 2014

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 CC to Easy Plate CC.**

Under this AOAC® *Performance Tested*[SM] License Number, 021401 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® *Performance Tested*[SM] License Number, 021401 this method is distributed as:

1. **Easy Plate CC**
2. **Easy Plate CC**
3. **Easy Plate CC**
4. **Easy Plate CC**
5. **Easy Plate CC**
6. **Easy Plate CC**
7. **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.* 10^7 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)

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

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

Non-coliforms

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

Table 2. Matrix Study Results (Method Developer) (1)

Matrix	Inoculation Microorganism	Contamination Level	Medi□Ca CC Mean[a]	s_r[b]	RSD_r[c]	VRBA Mean	s_r	r	RSD_r	*p*-value	Mean Difference	95% CI[d] LCL[e]	UCL[f]	r^2[g]
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	
		Medium	5.06	0.04	0.80	5.09	0.02		0.48	0.11	0.03	-	0.07	1.00
		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	
		Medium	7.79	0.04	0.48	7.82	0.03		0.33	0.25	0.02	-	0.07	1.00
		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	
		Medium	3.62[i]	0.08	2.08	3.75	0.09		2.35	0.05	0.13	0.	0.26	0.99
		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	
		Medium	3.56	0.10	2.89	3.42	0.10		2.98	0.06	-0.13	-	0.01	1.00
		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	
		Medium	3.04	0.02	0.79	3.06	0.06		1.84	0.65	0.01	-	0.08	0.97
		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. -	-0.10	
		Medium	3.53	0.06	1.81	3.47	0.06		1.70	0.24	-0.06	-	0.06	1.00
		High	8.41[i]	0.03	0.34	8.23	0.03		0.42	0.00	-0.18	-	-0.11	
Fresh peeled banana		Uninoculated	<1.00	—	—	<1.00	—		—	—	—	—	—	
	E.coli NBRC 15034	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	0.99
		High	5.65	0.03	0.58	5.68	0.07		1.24	0.40	0.03	-	0.13	
Fresh cut pineapple		Uninoculated	<1.00	—	—	<1.00	—		—	—	—	—	—	
	ATCC 25922			i	0.68	3.34	0.03		0.93	0.01	0.06	0.	0.10	
		Medium	4.41	0.04	0.95	4.41	0.04		0.94	0.94	0.00	-	0.06	1.00
		High	5.42	0.03	0.57	5.40	0.03		0.49	0.33	-0.02	-	0.03	
Fresh cut apple		Uninoculated	<1.00	—	—	<1.00	—		—	—	—	—	—	
	E. aerogenes NBRC 13534	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	1.00
		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: $\log_{10}$(CFU/g +(1.0)f].

[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 corellation 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*℠ 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