CERTIFICATE OF COMPLIANCE

LRQA

hereby declares that the certification assessment has demonstrated that

Easy Plate AC

Supplied by:	Manufactured by:	Manufactured by:
Kikkoman Biochemifa Company 2-1-1 Nishi-shinbashi Minato-ku, Tokyo 105-0003 Japan	Kikkoman Biochemifa Company Edogawa Plant 376-2 Kamihanawa Noda-shi, Chiba-ken Japan	Kikkoman Biochemifa Company Edogawa Plant Imagami area 2470 Imagami Noda-shi, Chiba-ken Japan

has been validated and revealed to be at least equivalent to the reference method as demonstrated by the validation study report. The summary of the validation report is available on the MicroVal website: www.microval.org

Reference method:
ISO 4833-1:2013 Microbiology of the food chain -- Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30°C by the pour plate technique

Scope: A broad range of foods, pet food and animal feed
environmental samples

The validation and certification has been performed in accordance with EN ISO 16140-2:2016 and the MicroVal Rules and Certification Scheme version 9.1.

Certificate no.: 2021LR102

First approval date: 23 March 2023
Expiry date: 22 March 2027

ISSUED BY: LRQA Nederland B.V.
Rotterdam, The Netherlands

CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

041302

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

Easy Plate AC

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

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Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

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CURRENT COMPANY
Kikkoman Biochemifa Company
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KIT NAME(S)
Easy Plate AC
Formerly known as Medi·Ca AC

CATALOG NUMBERS
61980

INDEPENDENT LABORATORY
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AOAC EXPERTS AND PEER REVIEWERS
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[1] US Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA
[2] Consultant, Denver, CO USA
[3] Consultant, Loganville, GA, USA
Modification February 2020 reviewed internally.

APPLICABILITY OF METHOD
Target organism – Aerobic bacteria

Matrixes – (50) - cooked roast beef, Chinese barbecued pork, bacon, cooked ham, frankfurter (made from beef and pork), boiled and cooked pork sausage
MODIFICATION DECEMBER 2017: (50 g): raw beef (steak meat), raw ground beef (80 % lean), raw ground pork, raw chicken, raw ground chicken, bagged lettuce, blueberries, raw shrimp (without head), raw salmon filet, raw tuna filet, dry pet food
pasteurized milk (3.6 % fat, 11 mL), natural cheese (Camembert, 11 g)

Performance claims - The method is a reasonable alternative to the Microbiological Methods (AOAC Official Method 966.23) (3) for heat-processed meat matrixes.

REFERENCE METHODS
***Official Methods of Analysis* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method 966.23 (3)**
U.S. Food and Drug Administration (2013) *FDA Bacteriological Analytical Manual,* Chapter 3 (9)

ORIGINAL CERTIFICATION DATE
April 10, 2013

CERTIFICATION RENEWAL RECORD
Renewed annual through December 2021

METHOD MODIFICATION RECORD
1. **January 2016 Level 2**
2. **December 2017 Level 3**
3. **February 2020 Level 2**
4. **November 2020 Level 1**
5. **June 2021 Level 1**

SUMMARY OF MODIFICATION
1. **Shelf life extension approved**
2. **Matrix extension**
3. **Manufacturing location change from Tokyo, Japan to Kanagawa, Japan.**
4. **Editorial and formatting changes to insert.**
5. **Rebranded kit to reflect Kikkoman and method name change from Medi·Ca AC to Easy Plate AC.**

Under this AOAC® *Performance Tested*SM License Number, 041302 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, 041302 this method is distributed as:
1. **Easy Plate AC**
2. **Easy Plate AC**
3. **Easy Plate AC**
4. **Easy Plate AC**
5. **Easy Plate AC**
6. **Easy Plate AC**
7. **Easy Plate AC**

PRINCIPLE OF THE METHOD (1)

Easy Plate AC (formerly known as Medi·Ca AC) is a ready-made dry medium for aerobic count made up of four components: a waterproof sheet, a dry medium containing a gelling agent, a hydrophobic resin ring surrounding the medium, and a transparent cover over the medium. The cover is lifted, sample suspension is placed on the center of the medium, and the cover is dropped onto the sample. The sample soaks into the medium and turns into a gel in 3 minutes. The gelled medium contains the redox indicator 2,3,5-triphenyl tetrazolium chloride (TTC) derived from a coating on the cover. The incubation of the sheet at 35 ± 1°C for 48 ± 2 hours develops red colonies because of the redox reaction involving the indicator.

DISCUSSION OF THE VALIDATION STUDY (1)

The Easy Plate AC method was compared to the AOAC **966.23** method for seven different heat-processed meat matrixes in the two Matrix studies. The 95% CI for the mean difference between the two methods at each contamination level for each matrix fell within the range of -0.50 to 0.50, and no statistical difference was observed at all three contamination levels for five matrixes (Table 1 and 4). In addition, the repeatability of the Easy Plate AC method was overall similar to that of the AOAC **966.23** method. These results demonstrated that the Easy Plate AC method is a reasonable alternative to the AOAC **966.23** method for heat-processed meat matrixes.

The mean $\log_{10}$ counts of the Medi·Ca AC method for boiled pork sausage contaminated with the heat-stressed strain were significantly lower than those of the AOAC **966.23** method. Interestingly, the same matrix contaminated with the same strain without any heat treatment provided no significant difference (data not shown). These results suggest that the Medi·Ca AC medium cannot grow heat-stressed microorganisms as vigorously as the PCA, depending on the microorganisms.

The Medi·Ca AC method is similar to the Aerobic Plate Count in Foods (AOAC Official Method **990.12**), also known as the Petrifilm™ Aerobic Count Plate method (4). Morita et al. has pointed out that liquefaction of the gel by bacteria which caused diffusion of colonies was observed on the Petrifilm™ Aerobic Count plates (5). The same phenomenon, which sometimes interfered with counting, was also observed for Chinese barbecued pork, cooked ham, and frankfurter in this study (data not shown). On the other hand, Medi·Ca AC medium did not appear to be subject to the liquefaction by bacteria for all the matrixes, which made counting easier.

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

Matrix	Inoculation Micoorganism	Contamination Level	Medi·Ca AC			AOAC **966.23**			Mean Difference	95% CI[d]		r^{2g}
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[e]	UCL[f]	
Cooked roast beef	N/A[h]	Low	3.51	0.03	0.89	3.44	0.10	2.97	-0.07	-0.23	0.10	0.99
		Medium	6.20	0.14	2.31	6.28	0.08	1.26	0.08	-0.04	0.19	
		High	8.59	0.15	1.69	8.63	0.14	1.67	0.04	-0.31	0.39	
Chinese barbecued pork	N/A	Low	4.61	0.07	1.58	4.56	0.03	0.61	-0.05	-0.13	0.04	1.00
		Medium	7.93	0.05	0.64	8.00	0.04	0.52	0.07	0.00	0.14	
		High	8.56	0.06	0.65	8.64	0.06	0.64	0.08	0.00	0.16	
Bacon	N/A	Low	4.34	0.03	0.66	4.34	0.05	1.13	0.01	-0.05	0.07	0.99
		Medium	6.35	0.02	0.32	6.30	0.04	0.60	-0.04	-0.11	0.03	
		High	7.43	0.08	1.03	7.57	0.06	0.74	0.14	-0.01	0.28	
Cooked ham	N/A	Low	2.61	0.04	1.57	2.60	0.04	1.40	-0.01	-0.10	0.08	0.99
		Medium	7.09[i]	0.04	0.54	7.48	0.04	0.54	0.39	0.34	0.43	
		High	9.26[i]	0.03	0.35	9.12	0.06	0.70	-0.14	-0.25	-0.03	
Frankfurter	N/A	Low	4.88	0.05	0.93	4.91	0.04	0.78	0.04	-0.04	0.11	0.99
		Medium	5.74	0.04	0.70	5.71	0.05	0.82	-0.03	-0.07	0.01	
		High	6.12	0.04	0.59	6.13	0.03	0.55	0.02	-0.04	0.08	
Boiled pork sausage	*E. cloacae*[j] ATCC 222	Uninoculated	<1.00	—	—	<1.00	—	—	—	—	—	0.99
		Low	2.60[i]	0.06	2.18	2.97	0.04	1.47	0.37	0.27	0.47	
		Medium	3.58[i]	0.04	1.11	3.81	0.05	1.40	0.24	0.12	0.35	
		High	4.55[i]	0.09	1.87	4.74	0.05	0.96	0.19	0.05	0.33	

[a] Mean of 5 replicates after the logarithmic transformation: $Log_{10}[CFU/g + (0.1)f]$.
[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] r^2 = square of the corellation coefficient.
[h] N/A—Not applicable. Samples are naturally contaminated.
[i] Significantly different ($p<0.05$).
[j] A heat-stressed culture with 71% injury was used.

DISCUSSION OF A MODIFICATION STUDY APPROVED DECEMBER 2017 (8)

Easy Plate AC method was compared to the AOAC **966.23** and BAM Chapter 3 for 13 food matrixes in five different categories: meat products, vegetable and fruits, seafood, dairy products and pet food including heat processed food. According to the validation results in method developer study and independent laboratory study, the mean differences between the Easy Plate AC and reference methods were less than 0.16 $\log_{10}$, and much smaller in most cases. The 95% CIs for the mean differences between the two methods fell within the range of -0.5 to 0.5 (Table 1). These results demonstrated that the Medi·Ca AC method produced statistically similar results when compared to the reference method.

Furthermore, for the dairy products (pasteurized milk and natural cheese), incubation temperature in the reference method is 32°C. In this study, Medi·Ca AC was incubated at 32 and 35 ± 1°C in the Method Developer Study for the dairy products and only the pasteurized milk in the Independent Study. As a result, there were no differences in these two incubation temperature (Table 1). Therefore, both dairy and non-dairy foods can be incubated in 35 ± 1°C by using Medi·Ca AC, and it is not necessary to use a different incubator for each food type.

Table 1. Matrix Study: Medi·Ca AC vs. AOAC 966.23 and BAM Chapter 3

Matrix	Inoculation Microorganism	Contamination Level	Medi·Ca AC			AOAC 966.23			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 beef	N/A[h]	Low	4.93	0.03	0.68	4.91	0.08	1.53	0.02	-0.12	0.08	
		Medium	6.37	0.03	0.44	6.35	0.03	0.49	0.02	-0.04	0.01	0.998
		High	7.15	0.02	0.30	7.12	0.04	0.57	0.03	-0.06	0.01	
Raw beef[i]	N/A	Low	1.88	0.19	10.1	1.92	0.17	8.83	0.04	-0.24	0.15	
		Medium	2.95	0.15	5.10	3.02	0.11	3.72	0.08	-0.18	0.03	0.979
		High	3.76	0.12	3.21	3.75	0.14	3.65	0.01	-0.14	0.15	
Raw ground beef	N/A	Low	6.90	0.03	0.50	6.85	0.05	0.75	0.05	-0.11	0.02	
		Medium	7.87	0.05	0.67	7.81	0.04	0.50	0.06	-0.15	0.04	0.996
		High	8.81	0.04	0.44	8.79	0.04	0.48	0.02	-0.06	0.02	
Raw ground pork	N/A	Low	5.47	0.05	0.94	5.63	0.11	1.91	0.16	0.02	0.29	
		Medium	8.93	0.06	0.67	8.95	0.06	0.65	0.03	-0.10	0.15	0.998
		High	9.77	0.01	0.14	9.80	0.04	0.41	0.03	-0.02	0.08	
Raw chicken	N/A	Low	4.33	0.04	0.97	4.41	0.08	1.73	0.08	-0.05	0.20	
		Medium	8.73	0.04	0.42	8.63	0.09	1.00	0.11	-0.22	0.01	0.998
		High	9.63	0.07	0.70	9.60	0.04	0.45	0.03	-0.13	0.07	
Raw ground chicken	N/A	Low	7.34	0.04	0.49	7.33	0.03	0.47	0.00	-0.09	0.08	
		Medium	8.35	0.02	0.28	8.34	0.02	0.21	0.01	-0.06	0.04	0.996
		High	9.37	0.03	0.28	9.29	0.03	0.27	0.08	-0.13	-0.03	
Lettuce	N/A	Low	4.63	0.08	1.70	4.46	0.05	1.17	0.16	-0.22	-0.11	
		Medium	6.02	0.03	0.44	6.02	0.06	0.97	0.00	-0.07	0.07	0.997
		High	7.74	0.08	1.07	7.74	0.04	0.58	0.01	-0.11	0.12	
Blueberries	N/A	Low	2.61	0.05	1.90	2.76	0.06	2.24	0.15	0.03	0.26	
		Medium	3.26	0.02	0.70	3.29	0.06	1.90	0.02	-0.05	0.10	0.987
		High	4.47	0.07	1.60	4.39	0.05	1.18	0.08	-0.21	0.05	
Raw shrimp	N/A	Low	7.30	0.02	0.29	7.29	0.04	0.50	0.01	-0.05	0.03	
		Medium	8.15	0.03	0.36	8.19	0.04	0.48	0.04	-0.04	0.11	0.995
		High	9.19	0.05	0.59	9.25	0.03	0.34	0.06	-0.03	0.15	
Raw salmon	N/A	Low	7.57	0.09	1.15	7.66	0.05	0.70	0.09	-0.03	0.20	
		Medium	8.65	0.06	0.65	8.68	0.07	0.77	0.03	-0.07	0.12	0.990
		High	9.63	0.07	0.76	9.62	0.05	0.50	0.01	-0.15	0.13	
Raw tuna	N/A	Low	6.96	0.03	0.40	6.90	0.03	0.44	0.06	-0.09	-0.03	
		Medium	7.89	0.04	0.53	7.90	0.03	0.41	0.01	-0.03	0.06	0.997
		High	8.84	0.05	0.55	8.87	0.03	0.33	0.03	-0.05	0.10	
Dry pet food[i]	N/A	Low	1.76	0.15	8.38	1.76	0.16	9.18	0.00	-0.12	0.13	0.996
		Medium	3.98	0.07	1.64	3.96	0.07	1.86	0.02	-0.08	0.13	

		High	4.88	0.07	1.42	4.96	0.02	0.44	0.08	-0.14	-0.01	
		Uninoculated	<1.00			<1.00						
Pasteurized milk 32°C[j]	*Escherichia coli* NBRC[k] 13500	Low	2.52	0.08	3.33	2.57	0.04	1.70	0.04	-0.01	0.10	
		Medium	3.62	0.07	2.00	3.50	0.08	2.17	0.12	-0.21	-0.03	0.977
		High	4.57	0.12	2.58	4.52	0.09	2.07	0.05	-0.30	0.19	
		Uninoculated	<1.00			<1.00						
Pasteurized milk 35°C[j]	*E. coli* NBRC 13500	Low	2.55	0.07	2.81	2.57	0.04	1.70	0.02	-0.04	0.08	
		Medium	3.58	0.07	2.09	3.50	0.08	2.17	0.08	-0.26	0.10	0.988
		High	4.58	0.05	1.10	4.52	0.09	2.07	0.06	-0.14	0.02	
Pasteurized milk[i] 32°C[j]	N/A	Low	1.36	0.22	15.9	1.39	0.09	6.67	0.03	-0.33	0.27	
		Medium	3.91	0.03	0.71	3.92	0.05	1.30	0.01	-0.09	0.06	0.991
		High	4.92	0.02	0.47	4.85	0.08	1.70	0.07	-0.03	0.16	
Pasteurized milk[i] 35°C[j]	N/A	Low	1.41	0.13	9.41	1.39	0.09	6.67	0.02	-0.04	0.09	
		Medium	3.90	0.06	1.61	3.92	0.05	1.30	0.02	-0.10	0.06	0.999
		High	4.90	0.05	0.97	4.85	0.08	1.70	0.05	-0.02	0.11	
		Uninoculated	<1.00			<1.00						
Natural cheese 32°C[j]	*Staphylococcus aureus* ATCC[l] 12600	Low	2.86	0.04	1.39	2.78	0.07	2.70	0.08	-0.21	0.04	
		Medium	4.01	0.07	1.84	3.91	0.03	0.75	0.10	-0.21	0.01	0.985
		High	4.70	0.08	1.66	4.67	0.10	2.11	0.03	-0.17	0.11	
		Uninoculated	<1.00			<1.00						
Natural cheese 35°C[j]	*S. aureus* ATCC 12600	Low	2.87	0.04	1.41	2.78	0.07	2.70	0.09	-0.15	-0.02	
		Medium	4.01	0.05	1.16	3.91	0.03	0.75	0.10	-0.18	-0.02	0.993
		High	4.74	0.02	0.48	4.67	0.10	2.11	0.07	-0.18	0.04	

[a] Mean of 5 replicates after the logarithmic transformation: Log_{10}[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] r^2 = square of the correlation coefficient.
[h] N/A = Not applicable. Samples are naturally contaminated.
[i] Matrix study conducted by the independent laboratory.
[j] Incubation temperature for Medi·Ca AC
[k] Biological Resource Center, National Institute of Technology and Evaluation, Chiba, Japan
[l] American Type Culture Collection, Manassas, VA

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CERTIFICATE OF COMPLIANCE

LRQA

hereby declares that the certification assessment has demonstrated that

Easy Plate CC

Supplied by:	Manufactured by:	Manufactured by:
Kikkoman Biochemifa Company 2-1-1 Nishi-shinbashi Minato-ku, Tokyo 105-0003 Japan	Kikkoman Biochemifa Company Edogawa Plant 376-2 Kamihanawa Noda-shi, Chiba-ken Japan	Kikkoman Biochemifa Company Edogawa Plant Imagami area 2470 Imagami Noda-shi, Chiba-ken Japan

has been validated and revealed to be at least equivalent to the reference method as demonstrated by the validation study report. The summary of the validation report is available on the MicroVal website: www.microval.org

Reference method:
ISO4832:2006 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony Count Method for coliforms.

Scope: a broad range of food

The validation and certification has been performed in accordance with EN ISO 16140-2:2016 and the MicroVal Rules and Certification Scheme version 9.1.

Certificate no.: 2021LR104

First approval date: 25 May 2023
Expiry date: 24 May 2027

ISSUED BY: LRQA Nederland B.V.
Rotterdam, The Netherlands

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

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KIT NAME(S)
Easy Plate CC
Formerly known as Medi·Ca CC

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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

CERTIFICATE OF COMPLIANCE

LRQA

hereby declares that the certification assessment has demonstrated that

Easy Plate EC

Supplied by:	Manufactured by:	Manufactured by:
Kikkoman Biochemifa Company 2-1-1 Nishi-shinbashi Minato-ku, Tokyo 105-0003 Japan	Kikkoman Biochemifa Company Edogawa Plant 376-2 Kamihanawa Noda-shi, Chiba-ken Japan	Kikkoman Biochemifa Company Edogawa Plant Imagami area 2470 Imagami Noda-shi, Chiba-ken Japan

has been validated and revealed to be at least equivalent to the reference method as demonstrated by the validation study report. The summary of the validation report is available on the MicroVal website: www.microval.org

Reference methods:

- ISO 16649-2:2001 – Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration ß glucuronidase-positive *escherichia coli* — Part 2: Colony-count technique at 44° C using 5-bromo-4-chloro-3-indolyl ß-D-glucuronide
- ISO 4832:2006 Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coliforms - Colony Count Method for coliforms.

Scope: Broad range of food plus pet food and environmental samples

The validation and certification has been performed in accordance with EN ISO 16140-2:2016 and the MicroVal Rules and Certification Scheme version 9.1.

Certificate no.: 2021LR103

First approval date: 25 May 2023
Expiry date: 24 May 2027

ISSUED BY: LRQA Nederland B.V.
Rotterdam, The Netherlands

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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

July 21, 2021
Date

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SUBMITTING COMPANY
Dai Nippon Printing Co., Ltd.
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Shinjuku-ku, Tokyo, 162-8001
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CURRENT COMPANY
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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

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February 2020 modification reviewed internally.

APPLICABILITY OF METHOD
Target organism – *Escherichia coli* 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 *Official Method*℠ 966.24, Coliform Group and *Escherichia coli* 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® *Performance Tested*℠ License Number, 031601 this method is distributed by:
1. **AS ONE Corporation**
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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-ß-D-glucuronic acid (X-Gluc) and 6-bromo-5-chloro3-indolyl-ß-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 ß-glucuronidase produced by bacteria catalyzes the hydrolysis of the X-Gluc to yield an insoluble blue product and the ß-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 ß-glucuronidase and ß-galactosidase and non-*E. coli* coliform only produce ß-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 ß-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 ß-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	*Buttiauxella noackiae*	#D0077[b]	Chicken	+ (Pink)
2	*Citrobacter amalonaticus*	NBRC[c] 13547	Unknown	+ (Red-purple)
3	*Citrobacter freundii*	NBRC 12681	Unknown	+ (Red-purple)
4	*Citrobacter freundii*	ATCC[d] 8090	Unknown	+ (Red-purple)
5	*Citrobacter koseri*	NBRC 105690	Unknown	+ (Red-purple)
6	*Cronobacter sakazakii*	#D0003	Soybean	+ (Red-purple)
7	*Enterobacter aerogenes*	NBRC 13534	Sputum	+ (Red-purple)
8	*Enterobacter amnigenus*	#D0037	Cabbage	+ (Red-purple)
9	*Enterobacter asburiae*	#D0029	Radish sprout	+ (Red-purple)
10	*Enterobacter cloacae*	#D0030	Radish sprout	+ (Red-purple)
11	*Enterobacter cloacae*	ATCC222	Unknown	+ (Red-purple)
12	*Enterobacter cloacae*	#D0033	Bean sprout	+ (Pink)
13	*Escherichia blattae (Shimwellia blattae)*	NBRC 105725	Hindgut of cockroach	-
14	*Escherichia coli*	NBRC 102203	Urine	+ (Navy-blue)
15	*Escherichia coli*	NBRC 12062	Unknown	+ (Navy-blue)
16	*Escherichia coli*	NBRC 12433	Unknown	+ (Navy-blue)
17	*Escherichia coli*	NBRC 12734	Unknown	+ (Navy-blue)
18	*Escherichia coli*	NBRC 13500	Unknown	+ (Navy-blue)
19	*Escherichia coli*	NBRC 15034	Clinical specimen	+ (Navy-blue)
20	*Escherichia coli*	NBRC 3972	Feces	+ (Blue-purple)
21	*Escherichia coli*	ATCC 25922	Unknown	+ (Navy-blue)
22	*Escherichia coli*	NBRC 3301	Unknown	+ (Blue-purple)
23	*Escherichia coli*	#D0100	Ground beef and pork	+ (Navy-blue)
24	*Escherichia coli*	#D0099	Ground chicken	+ (Navy-blue)
25	*Escherichia coli*	#D0101	Chicken	+ (Navy-blue)
26	*Escherichia coli*	#D0102	Chicken	+ (Navy-blue)
27	*Escherichia coli*	NBRC 13540	Unknown	+ (Navy-blue)
28	*Escherichia coli*	NBRC 3543	Unknown	+ (Blue-purple)
29	*Escherichia coli*	NBRC 3806	Unknown	+ (Navy-blue)
30	*Escherichia coli*	NBRC 3991	Unknown	+ (Navy-blue)
31	*Escherichia coli*	NBRC 13898	Unknown	+ (Navy-blue)
32	*Escherichia coli*	#D0104	Coconut water	+ (Navy-blue)
33	*Escherichia coli*	NBRC 14195	Unknown	+ (Navy-blue)
34	*Escherichia coli*	NBRC 3302	Unknown	+ (Blue-purple)
35	*Escherichia coli*	NBRC 3544	Unknown	+ (Navy-blue)
36	*Escherichia coli*	NBRC 14129	Unknown	+ (Blue-purple)
37	*Escherichia coli* O157	ATCC 43895	Raw hamburger meat	+ (Red-purple)
38	*Escherichia coli* O26	RIMD[e] 05091876	Patient	+ (Blue-purple)
39	*Escherichia fergusonii*	NBRC 102419	Feces of human	+ (Red-purple)
40	*Escherichia hermanii*	NBRC 105704	Toe of 17-year-old female	+ (Red-purple)
41	*Escherichia vulneris*	NBRC 102420	Human wound	+ (Red-purple)
42	*Klebsiella oxytoca*	#D0032	Yellowtail	+ (Pink)
43	*Klebsiella oxytoca*	NBRC 105695	Pharyngeal tonsil	+ (Red-purple)
44	*Klebsiella pneumoniae*	ATCC 13883	Unknown	+ (Red-purple)
45	*Kluyvera cryocrescens*	NBRC 102467	Food	+ (Red-purple)
46	*Leclercia adecarboxylata*	NBRC 102595	Drinking water	+ (Red-purple)
47	*Pantoea agglomerans*	#D0004	Cake	+ (Pink)
48	*Rahnella aquatilis*	#D0038	Pork	+ (Red-purple)
49	*Rahnella aquatilis*	#D0053	Salmon	+ (Red-purple)
50	*Raoultella terrigena*	#D0022	Salmon	+ (Red-purple)
51	*Raoultella planticola*	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	*Achromobacter xylosoxidans*	NBRC[b] 15126	Ear discharge	-
2	*Aeromonas hydrophila*	NBRC 12658	Unknown	+ (Pink)
3	*Bacillus amylolychefaciens*	#D0015[c]	Paprika powder	-
4	*Bacillus cereus*	NBRC 3836	Unknown	-
5	*Bacillus circulans*	NBRC 13626	Soil	-
6	*Bacillus coagulans*	NBRC 12583	Evaporated milk	-
7	*Bacillus licheniformis*	#D0010	Cheese cake	-
8	*Bacillus megaterium*	NBRC 15308	Unknown	-
9	*Bacillus subtilis*	#D0021	Chinese barbecued pork	-
10	*Bacillus thuringiensis*	NBRC 3951	Unknown	-
11	*Corynebacterium variabile*	NBRC 15286	Food	-
12	*Edwardsiella tarda*	NBRC 105688	Human feces	-
13	*Enterococcus faecalis*	ATCC[d] 29212	Urine	-
14	*Enterococcus faecium*	NBRC 100486	Unknown	-
15	*Kocuria rhizophila*	#D0008	Raw pork	-
16	*Lactobacillus casei*	#D0025	Lactic acid drink	-
17	*Lactobacillus delbrueckii*	NBRC 3202	Sour grain mash	-
18	*Lactococcus lactis*	#D0026	Yogurt	-
19	*Leuconostoc mesenteroides*	#D0057	Korean pickle	-
20	*Micrococcus luteus*	NBRC 3333	Unknown	-
21	*Proteus hauseri*	NBRC 3851	Unknown	-
22	*Proteus mirabilis*	NBRC 105697	Unknown	-
23	*Pseudomonas aeruginosa*	NBRC 3899	Well water	-
24	*Pseudomonas aeruginosa*	ATCC 9027	Unknown	-
25	*Pseudomonas mendocina*	NBRC 14162	soil enrichment with ethanol as carbon source	-
26	*Pseudomonas sp.*	#D0054	Salmon	-
27	*Salmonella enterica*	NBRC 105726	human feces (food poisoning in man)	-
28	*Serratia liquefaciens*	#D0027	Chicken	-
29	*Serratia marcescens*	NBRC 102204	Pond water	+ (Pink)
30	*Serratia rubidaea*	NBRC 12973	Seawater	+ (Red-purple)
31	*Staphylococcu epidermidis*	NBRC 100911	Nose	-
32	*Staphylococcus aureus*	#D0072	Ground beef and pork	-
33	*Staphylococcus aureus*	ATCC 25923	Clinical isolate	-
34	*Staphylococcus carnosus*	#D0086	Roast beef	-
35	*Staphylococcus gallinarum*	#D0061	Japanese tea leaf	-
36	*Staphylococcus intermedius*	ATCC 29663	Pigeon nares	-
37	*Staphylococcus saprophyticus*	#D0009	Pork	-
38	*Staphylococcus simulans*	NBRC 109714	Human skin	-
39	*Staphylococcus sp.*	#D0058	Ground beef and pork	-
40	*Staphylococcus xylosus*	NBRC 109770	Human skin	-
41	*Yersinia frederiksenii*	#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]		
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[e]	UCL[f]	r^2[g]
Raw pork	*E. coli* 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	
		Medium	3.90	0.12	2.98	3.67	0.19	5.12	0.24	-0.01	0.48	0.92
		High	4.39	0.09	2.01	4.61	0.13	2.74	-0.22	-0.45	0.02	
Raw frozen pork	*E. coli* #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	
		Medium	3.06	0.07	2.30	2.77	0.18	6.59	0.29	0.09	0.49	0.94
		High	4.12	0.02	0.58	4.16	0.19	4.62	-0.04	-0.30	0.22	
Raw beef	*E. coli* ATCC 25922 *K.oxytoca* 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	
		Medium	3.16	0.10	3.10	3.12	0.25	7.86	0.04	-0.25	0.34	0.95
		High	4.86	0.03	0.53	4.70	0.21	4.48	0.16	-0.09	0.41	
Raw beef[h]	*E. coli* ATCC 25922 *K. oxytoca* 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	
		Medium	3.50	0.09	2.47	3.22	0.17	5.35	0.28	0.01	0.55	0.95
		High	4.23	0.13	2.95	4.04	0.00	0.00	0.18	0.03	0.34	
Raw lamb	*E. coli* #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	
		Medium	4.01	0.04	0.96	4.01	0.09	2.31	0.00	-0.10	0.10	0.99
		High	5.85	0.06	0.99	6.18	0.15	2.36	-0.32	-0.46	-0.19	
Raw salmon	*E. coli* 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	
		Medium	3.03	0.04	1.30	3.24	0.14	4.17	-0.21	-0.36	-0.05	0.93
		High	4.23	0.11	2.52	4.29	0.10	2.42	-0.05	-0.31	0.20	
Frankfurter sausage	*E. coli* NBRC 12433 *E. cloacae* 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	
		Medium	4.05	0.05	1.20	4.01	0.09	2.31	0.04	-0.13	0.21	0.97
		High	4.89	0.07	1.42	4.73	0.13	2.78	0.16	-0.03	0.35	
Cooked ham	*E. coli* NBRC 13500 *E. cloacae* 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	
		Medium	2.69	0.05	1.71	2.80	0.15	5.48	-0.10	-0.34	0.13	0.95
		High	3.36	0.03	0.92	3.33	0.09	2.65	0.03	-0.10	0.16	
Cooked ham[h]	*E. coli* NBRC 13500 *E. cloacae* 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	
		Medium	3.12	0.18	5.74	2.89	0.21	7.18	0.23	-0.06	0.52	0.93
		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: Log_{10}[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] r^2 = 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	*E. coli*									
			Medi·Ca EC			EC			Mean Difference	95% CI[d]		
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[e]	UCL[f]	r^{2g}
Raw pork	*E. coli* 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	
		Medium	2.47	0.16	6.44	2.33	0.08	3.50	0.14	-0.09	0.37	0.93
		High	3.80	0.04	1.12	3.81	0.25	6.57	-0.01	-0.29	0.27	
Raw frozen pork	*E. coli* #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	
		Medium	2.84	0.04	1.44	3.03	0.09	3.08	-0.19	-0.31	0.22	0.97
		High	3.95	0.05	1.21	4.12	0.21	5.10	-0.17	-0.44	0.10	
Raw beef	*E. coli* ATCC 25922 *K. oxytoca* 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	
		Medium	2.35	0.08	3.21	2.32	0.08	3.41	0.02	-0.09	0.14	0.91
		High	3.15	0.08	2.48	3.18	0.21	6.47	-0.03	-0.31	0.25	
Raw beef[h]	*E. coli* ATCC 25922 *K. oxytoca* 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	
		Medium	3.22	0.06	1.89	3.14	0.16	5.00	0.07	-0.06	0.21	0.93
		High	3.81	0.14	3.72	3.61	0.31	8.46	0.20	-0.14	0.54	
Raw lamb	*E. coli* #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	
		Medium	3.75	0.08	2.01	3.77	0.18	4.84	-0.02	-0.23	0.19	0.95
		High	4.79	0.06	1.18	4.84	0.18	3.78	-0.05	-0.24	0.15	
Raw salmon	*E. coli* 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	
		Medium	2.42	0.01	0.49	2.46	0.16	6.60	-0.04	-0.25	0.17	0.96
		High	3.67	0.07	2.01	3.70	0.24	6.38	-0.02	-0.25	0.20	
Frankfurter sausage	*E. coli* NBRC 12433 *E. cloacae* 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	
		Medium	3.92	0.04	0.90	3.84	0.18	4.76	0.08	-0.16	0.32	0.98
		High	5.69	0.04	0.62	5.81	0.21	3.57	-0.12	-0.41	0.17	
Cooked ham	*E. coli* NBRC 13500 *E. cloacae* 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	
		Medium	2.52	0.09	3.75	2.49	0.22	8.80	0.03	-0.17	0.23	0.93
		High	3.23	0.09	2.80	3.33	0.09	2.65	-0.10	-0.25	0.05	
Cooked ham[h]	*E. coli* NBRC 13500 *E. cloacae* 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	
		Medium	2.80	0.16	5.58	2.76	0.27	9.82	0.04	-0.17	0.25	0.93
		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: Log_{10}[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] r^2 = square of the correlation coefficient.
[h] Matrix study conducted by the independent laboratory.

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CERTIFICATE OF COMPLIANCE

LRQA

hereby declares that the certification assessment has demonstrated that

Easy Plate SA

Supplied by:	Manufactured by:	Manufactured by:
Kikkoman Biochemifa Company 2-1-1 Nishi-shinbashi Minato-ku, Tokyo 105-0003 Japan	Kikkoman Biochemifa Company Edogawa Plant 376-2 Kamihanawa Noda-shi, Chiba-ken Japan	Kikkoman Biochemifa Company Edogawa Plant Imagami area 2470 Imagami Noda-shi, Chiba-ken Japan

has been validated and revealed to be at least equivalent to the reference method as demonstrated by the validation study report. The summary of the validation report is available on the MicroVal website: www.microval.org

Reference method:
ISO 6888-1: 2021 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase - positive staphylococci (*Staphylococcus aureus* and other species) - Part 1 - Technique using Baird-Parker
agar medium.

Scope: a broad range of food

The validation and certification has been performed in accordance with EN ISO 16140-2:2016 and the MicroVal Rules and Certification Scheme version 9.1.

Certificate no.: 2021LR105

First approval date: 23 June 2023
Expiry date: 22 June 2027

ISSUED BY: LRQA Nederland B.V.
Rotterdam, The Netherlands

CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

111703

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

Easy Plate SA

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

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KIT NAME(S)
Easy Plate SA
Formerly known as Medi·Ca SA

CATALOG NUMBERS
61983

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[2] Consultant, Denver, CO, USA
[3] Consultant, Loganville, GA, USA
Modification February 2020 reviewed internally.

APPLICABILITY OF METHOD
Target organisms – *Staphylococcus aureus*

Matrixes – raw beef, raw ground beef, raw lamb, cooked ham, raw salmon, frozen prawn, fresh chilled pasta, pasteurized milk, natural cheese, cream puff and potato salad

Performance claims - Performance is comparable to that of the AOAC *Official Methods*℠ 975.55, *Staphylococcus aureus* in Foods

REFERENCE METHOD
AOAC *Official Method*℠ 975.55, *Staphylococcus aureus* in Foods (2)

ORIGINAL CERTIFICATION DATE
November 15, 2017

CERTIFICATION RENEWAL RECORD
New approval 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 SA to Easy Plate SA.**

Under this AOAC® *Performance Tested*℠ License Number, 111703 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*℠ License Number, 111703 this method is distributed as:
1. **Easy Plate SA**
2. **Easy Plate SA**
3. **Easy Plate SA**
4. **Easy Plate SA**
5. **Easy Plate SA**
6. **Easy Plate SA**
7. **Easy Plate SA**

PRINCIPLE OF THE METHOD (1)
Easy Plate SA (formerly Medi·Ca SA) is a ready-made dry medium for *Staphylococcus aureus* count made up of four components: a waterproof sheet, a dry medium containing a gelling agent and the chromogenic enzyme substrates, 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° or 37 ± 1°C for 24 ± 1 h develops blue colonies for *S. aureus* because of the enzymatic reaction involving the substrate.

DISCUSSION OF THE VALIDATION STUDY (1)

In the exclusivity study, *S. gallinarum* (NBRC 109767), *B. cereus* (NBRC 13494, #D0068) and *B. licheniformis* (NBRC 12200) formed pink colony. The reason for this was that the type of enzyme produced from these strains was different from *S. aureus.* Those strains were easily distinguished from *S. aureus* by colony color. However, *S. schleiferi* (ATCC 43808) formed blue colony, which was the same as *S. aureus*. In this case, coagulase test is necessary to identify *S. aureus* or not. *S. schleiferi* showed negative coagulase reaction.

Some of the *S. aureus* related bacteria such as *Staphylococci* and *Bacillus* species grow in Baird-Parker medium. When testing the raw food matrixes, these colonies sometimes make enumeration of *S. aureus* difficult. In case of Medi·Ca SA method, the growth of these bacteria is inhibited, and *S. aureus* colony is stained clearly. Medi·Ca SA method does not need to confirm halo of colony.

In the matrix study, all matrixes were incubated at two temperatures, 35 or 37 ± 1°C. It was observed that the colony intensity was stronger at 37 °C than 35 °C for some of the matrixes. However, the number of colonies were almost the same at these two temperatures, and there were no significant differences.

Overall, it was generally observed that the Medi·Ca SA method produced statistically similar results when compared to the reference method. This rapid method makes it possible to simultaneously detect and enumerate *S. aureus* in only 24 hours, while the reference method requires 48 hours.

Table 1. Inclusivity Study (1)

	Strain Name	Source	Origin	Medi·Ca SA[a]
1	*Staphylococcus aureus*	ATCC 6538 [b]	Human lesion	**Blue**
2	*Staphylococcus aureus*	ATCC 8095	Cream pie	**Blue**
3	*Staphylococcus aureus*	ATCC 9144	Unknown	**Blue**
4	*Staphylococcus aureus*	ATCC 13565	Ham	**Blue**
5	*Staphylococcus aureus*	ATCC 25904	Unknown	**Blue**
6	*Staphylococcus aureus*	ATCC 25923	Clinical isolate	**Blue**
7	*Staphylococcus aureus*	ATCC 27664	Chicken tetrazzini	**Blue**
8	*Staphylococcus aureus*	ATCC 33862	Unknown	**Blue**
9	*Staphylococcus aureus*	NBRC 12732 [c]	Unknown	**Blue**
10	*Staphylococcus aureus*	NBRC 13276	Human lesion	**Blue**
11	*Staphylococcus aureus*	NBRC 15035	Wound	**Blue**
12	*Staphylococcus aureus*	NBRC 100910	Human pleural fluid	**Blue**
13	*Staphylococcus aureus*	NCTC 10788 [d]	Human lesion	**Blue**
14	*Staphylococcus aureus*	#D0072 [e]	Ground beef and pork	**Blue**
15	*Staphylococcus aureus*	#D0075	Chicken	**Blue**
16	*Staphylococcus aureus*	#D0076	Chicken	**Blue**
17	*Staphylococcus aureus*	#D0088	Ground pork	**Blue**
18	*Staphylococcus aureus*	#D0106	Human skin	**Blue**
19	*Staphylococcus aureus*	#D0107	Human skin	**Blue**
20	*Staphylococcus aureus*	#D0108	Human skin	**Blue**
21	*Staphylococcus aureus*	#D0109	Human skin	**Blue**
22	*Staphylococcus aureus*	#D0112	Food poisoning	**Blue**
23	*Staphylococcus aureus*	#D0113	Food poisoning	**Blue**
24	*Staphylococcus aureus*	#D0116	Food poisoning	**Blue**
25	*Staphylococcus aureus*	#D0117	Food poisoning	**Blue**
26	*Staphylococcus aureus*	#D0118	Food poisoning	**Blue**
27	*Staphylococcus aureus*	#D0120	Food poisoning	**Blue**
28	*Staphylococcus aureus*	#D0121	Food poisoning	**Blue**
29	*Staphylococcus aureus*	#D0124	Food poisoning	**Blue**
30	*Staphylococcus aureus*	#D0125	Food poisoning	**Blue**
31	*Staphylococcus aureus*	#D0130	Food poisoning	**Blue**
32	*Staphylococcus aureus*	#D0131	Food poisoning	**Blue**
33	*Staphylococcus aureus*	#D0133	Food poisoning	**Blue**
34	*Staphylococcus aureus*	#D0134	Food poisoning	**Blue**
35	*Staphylococcus aureus*	#D0135	Food poisoning	**Blue**
36	*Staphylococcus aureus*	#D0138	Food poisoning	**Blue**
37	*Staphylococcus aureus*	#D0151	Milk	**Blue**
38	*Staphylococcus aureus*	#D0152	Milk	**Blue**
39	*Staphylococcus aureus*	#D0153	Milk	**Blue**
40	*Staphylococcus aureus*	#D0154	Milk	**Blue**
41	*Staphylococcus aureus*	#D0156	Milk	**Blue**
42	*Staphylococcus aureus*	#D0182	Ground pork	**Blue**
43	*Staphylococcus aureus*	#D0183	Ground pork	**Blue**
44	*Staphylococcus aureus*	#D0185	Chicken	**Blue**
45	*Staphylococcus aureus*	#D0206	Unknown	**Blue**
46	*Staphylococcus aureus*	#D0207	Unknown	**Blue**
47	*Staphylococcus aureus*	#D0208	Pork	**Blue**
48	*Staphylococcus aureus*	#D0209	Pork	**Blue**
49	*Staphylococcus aureus*	#D0210	Pork	**Blue**
50	*Staphylococcus aureus*	#D0211	Pork	**Blue**
51	*Staphylococcus aureus*	#D0216	Food	**Blue**
52	*Staphylococcus aureus*	#D0217	Food	**Blue**

[a] Colony color.

[b] American Type Culture Collection, Manassas, VA.

[c] Biological Resource Center, National Institute of Technology and Evaluation, Chiba, Japan.

[d] National Collection of Type Cultures, a Culture Collection of Public Health England, Salisbury, UK

[e] Numbers starting with #D indicates strains that were isolated by Dai Nippon Printing Co., Ltd.

Table 2. Exclusivity Study (1)

	Strain Name	Source	Origin	Medi・Ca SA[a]
1	*Staphylococcus auricularis*	ATCC 33753 [b]	External auditory meatus	-
2	*Staphylococcus capitis*	ATCC 27840	Human skin	-
3	*Staphylococcus caprae*	ATCC 35538	Goat milk	-
4	*Staphylococcus carnosus*	NBRC 109622 [c]	Dry sausage	-
5	*Staphylococcus carnosus*	#D0086 [d]	Roast beef	-
6	*Staphylococcus cohnii*	NBRC 109713	Human skin	-
7	*Staphylococcus epidermidis*	NBRC 12993	Unknown	-
8	*Staphylococcus epidermidis*	NBRC 100911	Nose	-
9	*Staphylococcus gallinarum*	NBRC 109767	Chicken nares	**Pink**
10	*Staphylococcus* sp.	#D0058	Ground beef and pork	-
11	*Staphylococcus haemolyticus*	NBRC 109768	Human skin	-
12	*Staphylococcus hominis*	ATCC 700586	Blood	-
13	*Staphylococcus hyicus*	ATCC 11249	Pig with exudative epidermitis	-
14	*Staphylococcus intermedius*	ATCC 29663	Pigeon nares	-
15	*Staphylococcus lentus*	ATCC 29070	Goat udder	-
16	*Staphylococcus saprophyticus*	NBRC 102446	Urine	-
17	*Staphylococcus schleiferi*	ATCC 43808	Jugular catheter	**Blue**
18	*Staphylococcus sciuri*	ATCC 29062	Eastern gray squirrel skin	-
19	*Staphylococcus simulans*	NBRC 109714	Human skin	-
20	*Staphylococcus warneri*	NBRC 109769	Human skin	-
21	*Staphylococcus xylosus*	NBRC 109770	Human skin	-
22	*Bacillus circulans*	NBRC 13626	Soil	-
23	*Bacillus cereus*	NBRC 3836	Unknown	-
24	*Bacillus cereus*	NBRC 15305	Unknown	-
25	*Bacillus cereus*	NBRC 13494	Unknown	**Pink**
26	*Bacillus cereus*	#D0068	Food powder	**Pink**
27	*Bacillus licheniformis*	NBRC 12200	Unknown	**Pink**
28	*Bacillus subtilis*	NBRC 3134	Unknown	-
29	*Bacillus thuringiensis*	NBRC 3951	Unknown	-
30	*Bacillus pumilus*	NBRC 12092	Unknown	-
31	*Enterococcus faecalis*	NBRC 100481	Unknown	-
32	*Enterococcus faecalis*	ATCC 29212	Urine	-
33	*Enterococcus faecium*	NBRC 100486	Unknown	-
34	*Leuconostoc mesenteroides*	NBRC 3426	Unknown	-
35	*Macrococcus caseolyticus*	ATCC 13548	Dairy products	-
36	*Macrococcus caseolyticus*	#D0073	Ground beef	-
37	*Micrococcus luteus*	NBRC 3333	Unknown	-
38	*Aeromonas hydrophila*	NBRC 12658	Unknown	-
39	*Citrobacter freundii*	ATCC 8090	Unknown	-
40	*Enterobacter aerogenes*	NBRC 13534	Sputum	-
41	*Enterobacter cloacae*	NBRC 13535	Spinal fluid	-
42	*Escherichia coli*	NBRC 3972	Feces	-
43	*Escherichia coli*	NBRC 102203	Urine	-
44	*Escherichia coli*	ATCC 25922	Clinical isolate	-
45	*Klebsiella oxytoca*	NBRC 105695	Pharyngeal tonsil	-
46	*Klebsiella pneumoniae*	ATCC 13883	Unknown	-
47	*Kluyvera cryocrescens*	NBRC 102467	Kitchen food	-
48	*Proteus mirabilis*	NBRC 105697	Unknown	-
49	*Pseudomonas aeruginosa*	NBRC 3899	Well water	-
50	*Pseudomonas aeruginosa*	ATCC 9027	Outer ear infection	-
51	*Salmonella enterica*	NBRC 105726	Human feces	-
52	*Serratia marcescens*	NBRC 102204	Pond water	-
53	*Aspergillus niger*	NBRC 33023	Tannin gallic acid fermentation	-
54	*Candida albicans*	NBRC 1594	Clinical bronchomycosis	-
55	*Saccharomyces cerevisiae*	NBRC 10217	Brewer's top yeast	-

[a] Colony color, - = not detected.

[b] American Type Culture Collection, Manassas, VA.

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

[d] Numbers starting with #D indicates strains that were isolated by Dai Nippon Printing Co., Ltd.

Table 3. Matrix Study: Easy Plate SA vs. AOAC 975.55 – 35°C (1)

Matrix	Inoculation Micoorganism	Contamination Level	35°C									
			Medi·Ca SA			Baird-Parker			Mean Difference	95% CI[d]		
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[e]	UCL[f]	r^{2g}
Cooked ham	*Staphylococcus aureus* D0109	Uninoculated	<1.00			<1.00						
		Low	2.56	0.05	1.90	2.70	0.07	2.42	0.15	0.06	0.23	
		Medium	3.53	0.05	1.35	3.65	0.07	1.95	0.13	0.04	0.22	0.99
		High	4.48	0.06	1.24	4.70	0.04	0.90	0.22	0.13	0.32	
Cream puff	*Staphylococcus aureus* ATCC 8095	Uninoculated	<1.00			<1.00						
		Low	2.05	0.07	3.29	2.11	0.13	6.21	0.06	-0.12	0.24	
		Medium	2.95	0.04	1.30	3.07	0.07	2.13	0.12	0.01	0.23	0.99
		High	3.96	0.05	1.33	4.10	0.02	0.59	0.14	0.08	0.21	
Fresh chilled pasta	*Staphylococcus aureus* NBRC 100910	Uninoculated	<1.00			<1.00						
		Low	3.16	0.04	1.36	3.16	0.02	0.75	0.00	-0.04	0.04	
		Medium	3.88	0.06	1.41	4.01	0.05	1.36	0.13	0.02	0.23	0.99
		High	4.86	0.07	1.33	4.96	0.01	0.17	0.11	0.03	0.18	
Frozen prawn	*Staphylococcus aureus* NBRC 13276	Uninoculated	<1.00			<1.00						
		Low	2.93	0.04	1.45	3.10	0.06	1.93	0.17	0.10	0.25	
		Medium	4.14	0.09	2.25	4.21	0.05	1.17	0.07	-0.06	0.20	0.99
		High	4.95	0.03	0.50	5.05	0.08	1.58	0.10	-0.03	0.23	
Natural cheese	*Staphylococcus aureus* ATCC 25923	Uninoculated	<1.00			<1.00						
		Low	2.45	0.07	3.01	2.61	0.14	5.37	0.16	-0.03	0.34	
		Medium	3.36	0.08	2.42	3.46	0.05	1.41	0.10	0.01	0.20	0.99
		High	5.07	0.04	0.79	5.27	0.04	0.69	0.19	0.15	0.23	
Natural cheese [h]	*Staphylococcus aureus* ATCC 25923	Uninoculated	<1.00			<1.00						
		Low	1.60	0.17	10.63	1.74	0.07	3.86	0.25	-0.50	0.00	
		Medium	2.53	0.03	1.14	2.48	0.06	2.41	0.06	-0.15	0.03	0.98
		High	3.56	0.04	1.12	3.50	0.03	0.91	0.05	-0.12	0.03	
Pasteurized milk	*Staphylococcus aureus* D0152	Uninoculated	<1.00			<1.00						
		Low	2.87	0.03	1.01	3.08	0.05	1.74	0.21	0.12	0.29	
		Medium	3.82	0.04	0.96	4.08	0.07	1.61	0.26	0.18	0.34	1.00
		High	5.14	0.02	0.45	5.38	0.03	0.58	0.24	0.19	0.29	
Potato salad	*Staphylococcus aureus* D0138	Uninoculated	<1.00			<1.00						
		Low	2.65	0.04	1.65	2.81	0.04	1.52	0.17	0.09	0.24	
		Medium	4.27	0.02	0.53	4.38	0.04	0.93	0.11	0.05	0.18	1.00
		High	5.07	0.04	0.79	5.27	0.04	0.69	0.19	0.15	0.23	
Raw beef	*Staphylococcus aureus* NBRC 15035	Uninoculated	<1.00			<1.00						
		Low	2.03	0.09	4.21	1.99	0.20	10.1	0.04	-0.30	0.21	
		Medium	3.05	0.06	2.07	3.04	0.03	0.95	0.01	-0.10	0.08	0.98
		High	3.87	0.04	1.03	3.91	0.04	0.89	0.09	0.00	0.17	
Raw beef [h]	*Staphylococcus aureus* ATCC 12600	Uninoculated	<1.00			<1.00						
		Low	2.76	0.04	1.50	2.68	0.07	2.57	0.08	-0.13	-0.04	
		Medium	3.66	0.09	2.32	3.57	0.06	1.80	0.09	-0.20	0.02	0.99
		High	4.56	0.05	0.99	4.52	0.08	1.71	0.05	-0.12	0.03	
Raw ground beef [h]	*Staphylococcus aureus* ATCC 29213	Uninoculated	<1.00			<1.00						
		Low	2.15	0.12	5.79	2.05	0.12	5.70	0.11	-0.01	0.23	
		Medium	3.30	0.04	1.20	3.23	0.14	4.42	0.07	-0.12	0.27	0.99
		High	4.23	0.08	1.93	4.20	0.08	1.89	0.04	-0.81	0.21	
Raw lamb	*Staphylococcus aureus*	Uninoculated	<1.00			<1.00						
		Low	2.85	0.04	1.49	2.89	0.05	1.69	0.04	-0.06	0.15	1.00

	ATCC 12600	Medium	3.79	0.04	0.93	3.86	0.04	1.08	0.07	0.04	0.10	
		High	4.69	0.02	0.47	4.82	0.05	1.12	0.14	0.07	0.21	
Raw salmon	*Staphylococcus aureus* D0076	Uninoculated	<1.00			<1.00						
		Low	2.34	0.05	1.98	2.36	0.11	4.72	0.02	-0.08	0.12	
		Medium	3.48	0.06	1.76	3.43	0.05	1.43	0.05	-0.10	0.01	0.99
		High	4.73	0.04	0.90	4.80	0.07	1.48	0.08	0.00	0.15	

[a] Mean of 5 replicates after the logarithmic transformation: Log_{10}[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] r^2 = square of the correlation coefficient.
[h] Matrix study conducted by the independent laboratory.

Table 4. Matrix Study: Easy Plate SA vs. AOAC 975.55 – 37°C (1)

Matrix	Inoculation Micoorganism	Contamination Level	37°C									
			Medi·Ca SA			Baird-Parker			Mean Difference	95% CI[d]		
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[e]	UCL[f]	r^2[g]
Cooked ham	*Staphylococcus aureus* D0109	Uninoculated	<1.00			<1.00						
		Low	2.49	0.08	3.25	2.74	0.08	2.96	0.25	0.09	0.40	
		Medium	3.57	0.07	1.90	3.66	0.03	0.81	0.09	-0.02	0.19	0.98
		High	4.48	0.03	0.57	4.69	0.07	1.40	0.21	0.12	0.30	
Cream puff	*Staphylococcus aureus* ATCC 8095	Uninoculated	<1.00			<1.00						
		Low	2.02	0.07	3.58	2.15	0.13	6.12	0.13	-0.05	0.31	
		Medium	2.97	0.05	1.62	3.10	0.04	1.14	0.14	0.04	0.23	0.99
		High	3.97	0.05	1.21	4.12	0.03	0.78	0.15	0.10	0.20	
Fresh chilled pasta	*Staphylococcus aureus* NBRC 100910	Uninoculated	<1.00			<1.00						
		Low	3.18	0.04	1.21	3.12	0.04	1.31	0.06	-0.12	0.01	
		Medium	3.93	0.04	1.08	4.05	0.06	1.54	0.12	0.01	0.24	0.99
		High	4.76	0.10	2.01	4.93	0.03	0.67	0.17	0.06	0.29	
Frozen prawn	*Staphylococcus aureus* NBRC 13276	Uninoculated	<1.00			<1.00						
		Low	3.06	0.05	1.65	3.07	0.05	1.55	0.00	-0.08	0.09	
		Medium	4.19	0.03	0.70	4.22	0.05	1.18	0.02	-0.03	0.07	0.99
		High	4.96	0.08	1.53	5.06	0.05	0.97	0.10	-0.04	0.23	
Natural cheese	*Staphylococcus aureus* ATCC 25923	Uninoculated	<1.00			<1.00						
		Low	2.47	0.07	3.02	2.57	0.09	3.56	0.10	-0.02	0.22	
		Medium	3.32	0.12	3.47	3.36	0.10	2.97	0.04	-0.20	0.28	0.98
		High	4.60	0.04	0.77	4.81	0.06	1.14	0.21	0.16	0.27	
Natural cheese [h]	*Staphylococcus aureus* ATCC 25923	Uninoculated	<1.00			<1.00						
		Low	1.61	0.12	7.55	1.74	0.07	3.86	0.23	-0.43	-0.02	
		Medium	2.59	0.03	1.05	2.49	0.06	2.40	0.02	-0.05	0.12	0.98
		High	3.59	0.04	1.02	3.51	0.03	0.87	0.00	-0.08	0.08	
Pasteurized milk	*Staphylococcus aureus* D0152	Uninoculated	<1.00			<1.00						
		Low	2.84	0.07	2.60	3.06	0.04	1.45	0.22	0.11	0.33	
		Medium	3.84	0.06	1.44	4.06	0.03	0.64	0.23	0.17	0.29	0.99
		High	5.06	0.06	1.23	5.39	0.04	0.65	0.33	0.22	0.44	
Potato salad	*Staphylococcus aureus* D0138	Uninoculated	<1.00			<1.00						
		Low	2.66	0.07	2.50	2.86	0.06	2.02	0.20	0.13	0.27	0.99
		Medium	4.29	0.01	0.33	4.33	0.05	1.21	0.04	-0.02	0.09	

		High	5.09	0.04	0.80	5.30	0.04	0.83	0.21	0.13	0.30	
Raw beef	*Staphylococcus aureus* NBRC 15035	Uninoculated	<1.00			<1.00						
		Low	1.91	0.16	8.12	2.03	0.07	3.55	0.13	-0.07	0.32	
		Medium	3.06	0.04	1.30	3.05	0.02	0.66	0.01	-0.08	0.06	0.98
		High	3.85	0.06	1.62	3.87	0.03	0.78	0.02	-0.08	0.12	
Raw beef [h]	*Staphylococcus aureus* ATCC 12600	Uninoculated	<1.00			<1.00						
		Low	2.73	0.04	1.48	2.68	0.07	2.57	0.05	-0.17	0.07	
		Medium	3.68	0.09	2.39	3.57	0.06	1.80	0.11	-0.20	-0.03	0.99
		High	4.63	0.03	0.59	4.52	0.08	1.71	0.12	-0.22	-0.01	
Raw ground beef [h]	*Staphylococcus aureus* ATCC 29213	Uninoculated	<1.00			<1.00						
		Low	2.20	0.15	6.73	2.09	0.11	5.07	0.16	0.00	0.32	
		Medium	3.29	0.05	1.51	3.26	0.13	4.07	0.06	-0.11	0.23	0.99
		High	4.26	0.04	0.97	4.21	0.08	1.82	0.06	-0.08	0.21	
Raw lamb	*Staphylococcus aureus* ATCC 12600	Uninoculated	<1.00			<1.00						
		Low	2.87	0.02	0.72	2.91	0.03	1.10	0.04	0.01	0.07	
		Medium	3.81	0.05	1.34	3.86	0.06	1.54	0.04	-0.08	0.16	0.99
		High	4.68	0.09	1.91	4.73	0.04	0.90	0.05	-0.11	0.21	
Raw salmon	*Staphylococcus aureus* D0076	Uninoculated	<1.00			<1.00						
		Low	2.35	0.05	2.07	2.36	0.06	2.48	0.01	-0.07	0.09	
		Medium	3.42	0.06	1.68	3.38	0.06	1.72	0.05	-0.12	0.03	1.00
		High	4.73	0.05	1.03	4.80	0.01	0.19	0.07	0.01	0.13	

[a] Mean of 5 replicates after the logarithmic transformation: $Log_{10}[CFU/g + (0.1)f]$.
[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] r^2 = square of the correlation coefficient.
[h] Matrix study conducted by the independent laboratory.

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