CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

041302

The AOAC Research Institute hereby certifies the method 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 and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. 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.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date November 19, 2024
Expiration Date December 31, 2025

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MODIFICATION MARCH 2022: Satoshi Fukuda and Shinichiro Sugiura
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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
Kikkoman Biochemifa Company
2-1-1, Nishi-shinbashi
Minato-ku, Tokyo 105-0003
Japan

METHOD NAME
Easy Plate AC
Formerly known as Medi·Ca AC

CATALOG NUMBER
61973

INDEPENDENT LABORATORY
Q Laboratories, Inc
1400 Harrison Avenue
Cincinnati, OH 45214 USA

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 annually through December 2025.

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**
6. **March 2022 Level 2**
7. **December 2023 Level 1**
8. **March 2024 Level 2**
9. **November 2024 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.**
6. **Manufacturing location change.**
7. **Editorial/clerical changes.**
8. **The outer pouch material changed from aluminum to plastic film**
9. **Editorial changes.**

Under this AOAC *Performance Tested Methods*℠ 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 Methods*℠ License Number, 041302 this method is distributed as:

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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) *Medi-Ca AC is now Easy Plate AC

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 (1) *Medi-Ca AC is now Easy Plate AC

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	
		Medium	3.98	0.07	1.64	3.96	0.07	1.86	0.02	-0.08	0.13	0.996
		High	4.88	0.07	1.42	4.96	0.02	0.44	0.08	-0.14	-0.01	

Pasteurized milk 32°C[j]	*Escherichia coli* NBRC[k] 13500	Uninoculated	<1.00			<1.00						
		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	
Pasteurized milk 35°C[j]	*E. coli* NBRC 13500	Uninoculated	<1.00			<1.00						
		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	
Natural cheese 32°C[j]	*Staphylococcus aureus* ATCC[l] 12600	Uninoculated	<1.00			<1.00						
		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	
Natural cheese 35°C[j]	*S. aureus* ATCC 12600	Uninoculated	<1.00			<1.00						
		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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