



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

021401

The AOAC Research Institute hereby certifies the method known as:

Easy Plate CC

manufactured by

Kikkoman Biochemifa Company

2-1-1, Nishi-shinbashi

Minato-ku, Tokyo 105-0003

Japan

This method has been evaluated in the AOAC[®] Performance Tested MethodsSM 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 TestedSM 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 (March 7, 2022 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

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

Scott Coates, Senior Director
Signature for AOAC Research Institute

March 7, 2022

Date

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| METHOD NAME Easy Plate CC Formerly known as Medi-Ca CC | CATALOG NUMBERS 61974 |
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| 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 Modifications February 2020 and March 2022 reviewed internally by AOAC Research Institute. |
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| APPLICABILITY OF METHOD Target organism – Coliform bacteria | REFERENCE METHOD <i>Bacteriological Analytical Manual</i> , Chapter 4, Section G) (3) |
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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.

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| ORIGINAL CERTIFICATION DATE February 03, 2014 | CERTIFICATION RENEWAL RECORD Renewed annually through December 2022. |
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| METHOD MODIFICATION RECORD | SUMMARY OF MODIFICATION |
| 1. February 2020 Level 2 | 1. Manufacturing location change from Tokyo, Japan to Kanagawa, Japan. |
| 2. November 2020 Level 1 | 2. Editorial and formatting changes to insert. |
| 3. June 2021 Level 1 | 3. Rebranded kit to reflect Kikkoman and method name change from Medi-Ca CC to Easy Plate CC. |
| 4. March 2022 Level 2 | 4. Manufacturing location change. |

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| Under this AOAC® Performance TestedSM License Number, 021401 this method is distributed by: | Under this AOAC® Performance TestedSM License Number, 021401 this method is distributed as: |
| 1. AS ONE Corporation | 1. Easy Plate CC |
| 2. KENIS LIMITED | 2. Easy Plate CC |
| 3. Nippon Bacterial Test CO., LTD. | 3. Easy Plate CC |
| 4. FUJIFILM Wako Pure Chemical Corporation | 4. Easy Plate CC |
| 5. Microgiene Co. LTD | 5. Easy Plate CC |
| 6. Weber Scientific | 6. Easy Plate CC |
| 7. ELMEX Limited | 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. 10⁷ CFU/g, being one hundred times higher than that of the one stored in a storage condition in the internal lab study (at 10 ± 1°C for 24 ± 1 h). In addition, the third party pointed out that the low level had distinct dark blue colonies and the high level had small light blue colonies. Probably, psychrophiles such as *Pseudomonas* selectively grew to inhibit the growth of coliforms in the independent lab study. In that case, an additional incubation for a few hours for making the colony size larger or the colony color intensity darker is recommended to obtain the colony count results equivalent to the VRBA method.

Table 1. Inclusivity/Exclusivity Panel Results (1)

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

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

Non-coliforms

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

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

^b + = detected; - = not detected

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

| Matrix | Inoculation Microorganism | Contamination Level | Medi@Ca CC | | RSD _r ^c | VRBA | | | p-value | Mean Difference | 95% CI ^d | | r ^{2g} |
|---------------------|-----------------------------------|---------------------|-------------------|----------------|-------------------------------|-------------------|----------------|------------------|---------|-----------------|---------------------|------------------|-----------------|
| | | | Mean ^a | s ^b | | Mean _r | s _r | RSD _r | | | LCL ^e | UCL ^f | |
| Raw ground pork | N/A ^h | Low | 4.19 | 0.03 | 0.71 | 4.18 | 0.06 | 1.36 | 0.69 | -0.01 | - | 0.06 | 1.00 |
| | | Medium | 5.06 | 0.04 | 0.80 | 5.09 | 0.02 | 0.48 | 0.11 | 0.03 | - | 0.07 | |
| | | High | 8.42 | 0.06 | 0.69 | 8.47 | 0.11 | 1.30 | 0.53 | 0.23 | - | 0.25 | |
| Raw lamb | N/A | Low | 2.31 ⁱ | 0.09 | 3.83 | 2.48 | 0.05 | 1.95 | 0.01 | 0.18 | 0. | 0.27 | 1.00 |
| | | Medium | 7.79 | 0.04 | 0.48 | 7.82 | 0.03 | 0.33 | 0.25 | 0.02 | - | 0.07 | |
| | | High | 8.59 ⁱ | 0.04 | 0.46 | 8.69 | 0.07 | 0.86 | 0.04 | 0.09 | 0. | 0.18 | |
| Raw ground chicken | N/A | Low | 2.11 | 0.07 | 3.47 | 2.22 | 0.09 | 4.20 | 0.08 | 0.11 | - | 0.23 | 0.99 |
| | | Medium | 3.62 ⁱ | 0.08 | 2.08 | 3.75 | 0.09 | 2.35 | 0.05 | 0.13 | 0. | 0.26 | |
| | | High | 5.02 | 0.02 | 0.43 | 4.93 | 0.12 | 2.43 | 0.14 | -0.09 | - | 0.04 | |
| Raw tuna fillet | N/A | Low | 2.53 | 0.06 | 2.23 | 2.54 | 0.05 | 1.85 | 0.76 | 0.01 | - | 0.13 | 1.00 |
| | | Medium | 3.56 | 0.10 | 2.89 | 3.42 | 0.10 | 2.98 | 0.06 | -0.13 | - | 0.01 | |
| | | High | 6.41 | 0.03 | 0.52 | 6.27 | 0.13 | 2.12 | 0.09 | -0.13 | - | 0.03 | |
| Raw salmon fillet | N/A | Low | 2.16 | 0.19 | 8.99 | 2.02 | 0.08 | 4.06 | 0.25 | -0.15 | - | 0.15 | 0.97 |
| | | Medium | 3.04 | 0.02 | 0.79 | 3.06 | 0.06 | 1.84 | 0.65 | 0.01 | - | 0.08 | |
| | | High | 3.97 | 0.03 | 0.71 | 3.96 | 0.06 | 1.45 | 0.61 | -0.01 | - | 0.00 | |
| Raw shrimp | N/A | Low | 2.42 ⁱ | 0.08 | 3.41 | 2.16 | 0.17 | 7.66 | 0.01 | -0.26 | - | -0.10 | 1.00 |
| | | Medium | 3.53 | 0.06 | 1.81 | 3.47 | 0.06 | 1.70 | 0.24 | -0.06 | - | 0.06 | |
| | | High | 8.41 ⁱ | 0.03 | 0.34 | 8.23 | 0.03 | 0.42 | 0.00 | -0.18 | - | -0.11 | |
| Fresh peeled banana | <i>E. coli</i> NBRC 15034 | Uninoculated | <1.00 | — | — | <1.00 | — | — | — | — | — | — | 0.99 |
| | | Low | 3.51 | 0.11 | 3.28 | 3.49 | 0.06 | 1.74 | 0.73 | -0.02 | - | 0.12 | |
| | | Medium | 4.75 | 0.09 | 1.86 | 4.76 | 0.09 | 1.84 | 0.88 | 0.01 | - | 0.19 | |
| | | High | 5.65 | 0.03 | 0.58 | 5.68 | 0.07 | 1.24 | 0.40 | 0.03 | - | 0.13 | |
| Fresh cut pineapple | ATCC 25922 | Uninoculated | <1.00 | — | — | <1.00 | — | — | — | — | — | — | 1.00 |
| | | Medium | 4.41 | 0.04 | 0.68 | 3.34 | 0.03 | 0.93 | 0.01 | 0.06 | 0. | 0.10 | |
| | | High | 5.42 | 0.03 | 0.57 | 5.40 | 0.03 | 0.49 | 0.33 | -0.02 | - | 0.03 | |
| Fresh cut apple | <i>E. aerogenes</i> NBRC 13534 | Uninoculated | <1.00 | — | — | <1.00 | — | — | — | — | — | — | 1.00 |
| | | Low | 3.60 | 0.06 | 1.54 | 3.58 | 0.05 | 1.46 | 0.34 | -0.02 | - | 0.03 | |
| | | Medium | 4.67 | 0.08 | 1.71 | 4.64 | 0.11 | 2.38 | 0.70 | -0.03 | - | 0.19 | |
| | | High | 5.74 | 0.04 | 0.63 | 5.65 | 0.08 | 1.46 | 0.11 | -0.09 | - | 0.03 | |

^a Mean of 5 replicates after the logarithmic transformation: $\log_{10}[\text{CFU/g} + (1.0)]$.

^b RSD_r = standard deviation.

^c RSD_r = relative standard deviation.

^d CI = confidence interval.

^e LCL = lower confidence limit.

^f UCL = upper confidence limit.

^g r² = square of the correlation coefficient.

^h N/A — Not applicable. Samples are naturally contaminated.

ⁱ Significantly different ($p < 0.05$).

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