

MicroVal and NordVal certification of Ready-To-Use Culture Media, Easy Plate CC for Enumeration of Coliforms in a Broad Range of Foods

Daiki Utsunomiya¹, Suzanne J. Jordan², Kentaro Takenaka³, Mai Shimizu³, Takeo Suzuki³ and Shinichiro Sugiura¹

(1)Kikkoman Biochemifa Company, (2)Campden BRI, (3)Kikkoman Corporation



Introduction

- **Easy Plate** series (AC, CC, EC, EB, SA and YM-R) are Ready-to-Use (RTU) medium which are manufactured and sold by Kikkoman Biochemifa Company. Compared to conventional plate media, Easy Plate provides various advantages such as **time reduction, ease of operation, space-saving and reduction of plastic.**
- In the conventional method according to ISO 4832 (2006), coliforms are enumerated on violet red bile lactose agar medium (VRBL). **Easy Plate CC (E-CC)** is an alternative media for coliform enumeration. In this study, the experiments were conducted to evaluate the specificity, selectivity, repeatability, accuracy and relative trueness of the E-CC for the enumeration of coliforms as required by ISO 16140-2.

Methods

The method validation study was done according to ISO 16140-2:2016, using ISO 4832:2006 as the reference method. Analysis with E-CC was performed following manufacturer's instructions.

1. Inclusivity and Exclusivity

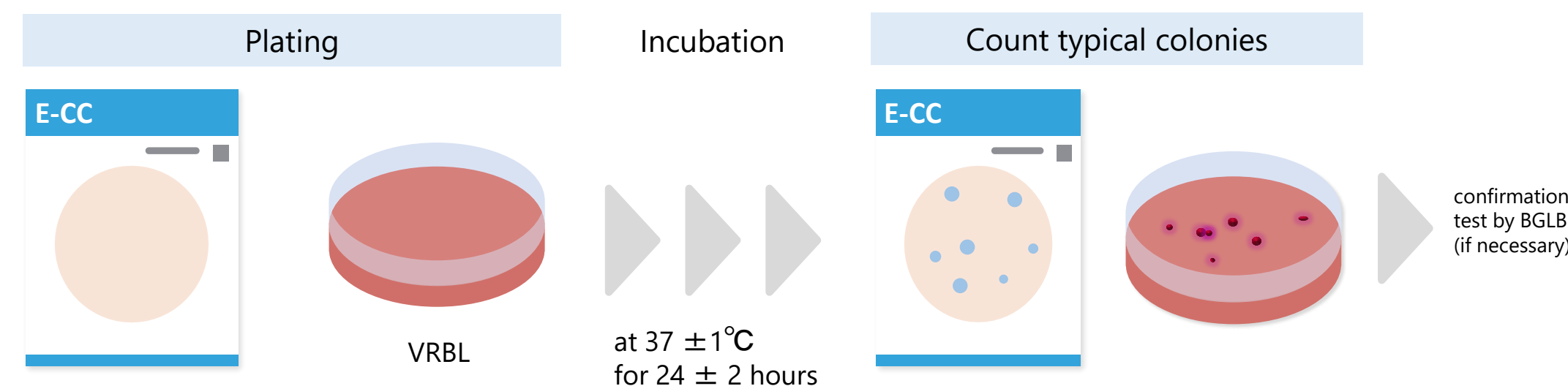
Pure cultures of known provenance were tested for the inclusivity and the exclusivity. Each test was performed once with E-CC, VRBL and a non-selective agar.

2. Accuracy profile

Five food categories (Milk and dairy products, Fishery products, Produce and fruits, Multi-components foods and Raw and ready to cook (RTC) meat and poultry) were tested for the accuracy profile study.

3. Relative trueness

Relative trueness study was conducted using a combination naturally and artificially contaminated samples. Five food categories (same as the accuracy profile) were tested by both E-CC (as an alternative method) and VRBL (as a reference method).



Result 1. Inclusivity and Exclusivity

For the inclusivity study, a total of 51 pure coliforms cultures were tested. Fifty of the 51 coliform strains gave typical colonies on E-CC. A total of 30 pure non-target cultures were tested in the exclusivity study. Seven of the exclusivity panel gave typical colonies on VRBL, and two exclusivity strains were typical on E-CC. The list of strains used in the exclusivity study and the results of E-CC, VRBL and BGLB (confirmation test) was shown in Table 3. **These results indicated that E-CC had similar specificity and a better selectivity for coliforms than VRBL.**

Table 1. Summary of inclusivity and exclusivity results

	Coliforms	Non target	False positive strain
E-CC	50/51	28/30	<i>Shigella sp.</i>
VRBL	51/51	24/30	<i>Shigella sp.</i> <i>Vibrio sp.</i>
VRBL-BGLBB	-	30/30	-

Table 2. Result of the inclusivity study

Coliforms	No. of Strains	E-CC*	VRBL*
<i>Citrobacter</i>	10	9	10
<i>Enterobacter</i>	16	16	16
<i>Leliottia</i>	1	1	1
<i>Kluyvera</i>	2	2	2
<i>Escherichia</i>	6	6	6
<i>Serratia</i>	3	3	3
<i>Klebsiella</i>	5	5	5
<i>Cronobacter</i>	4	4	4
<i>Hafnia</i>	1	1	1
<i>Siccibacter</i>	1	1	1
<i>Franconibacter</i>	2	2	2

* Number of strains giving anticipated results

Table 3. Result of the exclusivity study

No.	Organisms	Source or Identity	E-CC	VRBL	BGLB
1	<i>Acinetobacter calcoaceticus</i>	sesame seeds	✓	✓	NT
2	<i>Acinetobacter lwoffii</i>	Tomatoes	✓	✓	NT
3	<i>Aeromonas salmonicida</i>	NCTC 10402	✓	✓	NT
4	<i>Avibacterium avium</i>	NCTC 11297	✓	✓	NT
5	<i>Bacillus cereus</i>	ATCC 10876	✓	✓	NT
6	<i>Bacillus subtilis</i>	ATCC 6633	✓	✓	NT
7	<i>Burkholderia gladioli</i>	Industrial	✓	✓	NT
8	<i>Carnobacterium divergens</i>	Brie	✓	✓	NT
9	<i>Edwardsiella tarda</i>	NCTC 10396	✓	✓	NT
10	<i>Flavobacterium resinovorum</i>	soil/NCIMB 8767	✓	✓	NT
11	<i>Lactobacillus acidophilus</i>	industrial	✓	✓	NT
12	<i>Listeria monocytogenes</i>	Soft cheese	✓	✓	NT
13	<i>Morganella morgani</i>	mince	✓	✓	NT
14	<i>Pasteurella bettyae</i>	NCTC 10535	✓	✓	NT
15	<i>Pediococcus pentasaceus</i>	Brine	✓	✓	NT
16	<i>Proteus mirabilis</i>	Poultry	✓	✓	NT
17	<i>Proteus vulgaris</i>	Poultry	✓	✓	NT
18	<i>Providencia rettgeri</i>	Faeces/NCTC 7475	✓	✓	NT
19	<i>Pseudomonas aeruginosa</i>	NCIMB 10753	✓	✓	NT
20	<i>Pseudomonas fluorescens</i>	NCIMB 10586	✓	✓	NT
21	<i>Pseudomonas rhodesiae</i>	raw milk	✓	✓	NT
22	<i>Salmonella Typhimurium</i>	ATCC 14028	✓	✓	NT
23	<i>Shewanella putrifaciens</i>	NCTC 10736	✓	✓	NT
24	<i>Shigella boydii</i>	faeces/NCTC 11312	✗	✗	✓
25	<i>Shigella flexneri</i>	NCTC 9950	✓	✗	✓
26	<i>Shigella sonnei</i>	NCIMB 12702	✗	✗	✓
27	<i>Staphylococcus aureus</i>	NCTC 11435	✓	✓	NT
28	<i>Vibrio mimicus</i>	NCTC 11344	✓	✗	✓
29	<i>Vibrio parahaemolyticus</i>	NCTC 10460	✓	✗	✓
30	<i>Yersinia enterocolitica</i>	Not known	✓	✗	✓

“✓” indicates successfully suppressed
“✗” indicates false-positive. NT: Not tested

Result 2. Accuracy profile

A total of 150 samples (comprising two items per category at three different contamination levels) were used in this study. The Accuracy of the E-CC was satisfied as all five categories met the 0.5log AL or the re-calculated AL.

Result 3. Relative trueness

A total of 75 samples across five categories were tested for the relative trueness study. **The results showed that no significant difference between E-CC and the ISO method for all five categories.** The correlation coefficient between two method for all categories was 0.978 as shown in Figure 2. The Details of samples of categories and types were shown in Table 4. The correlation coefficients of each category were also shown.

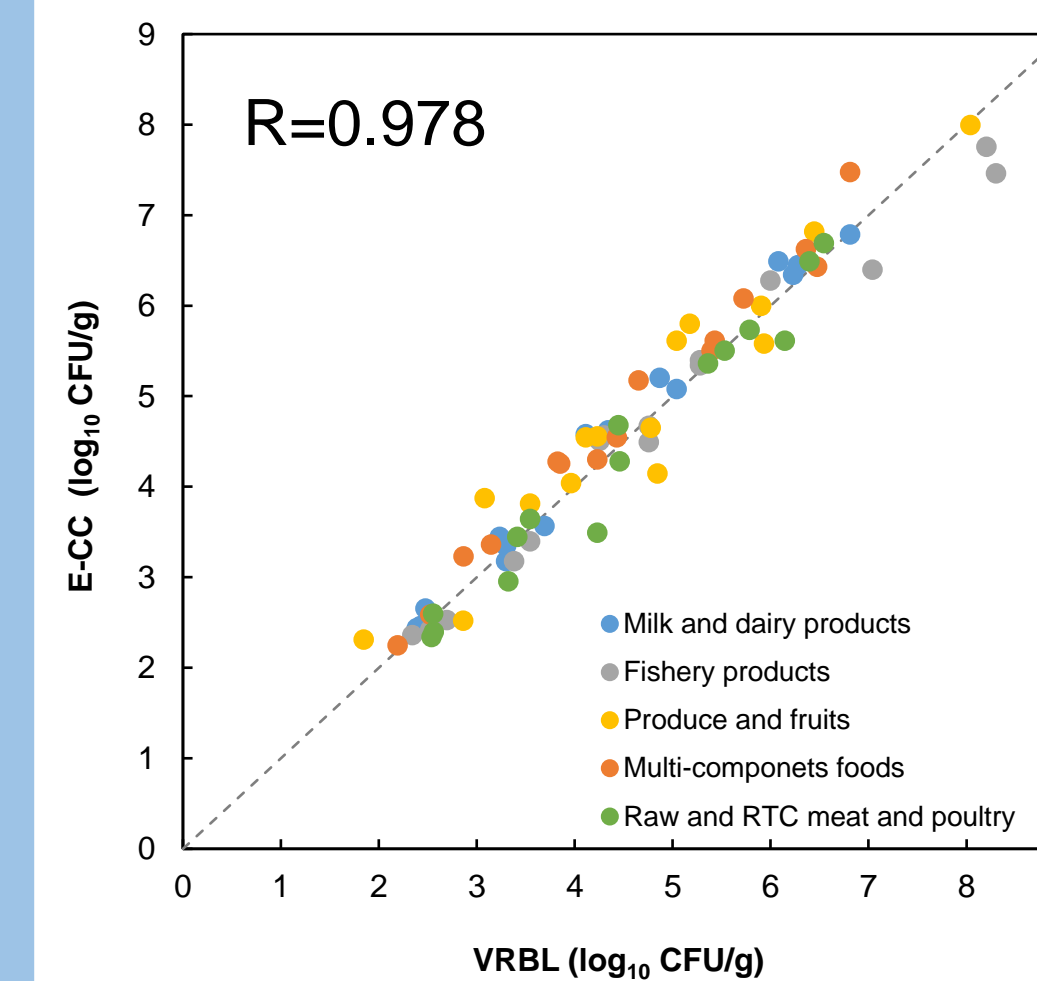


Figure 2. Scatter plot of the reference method versus E-CC results for all categories.

Table 4. List of categories, Types and correlation coefficients for each category.

Category	Types	Correlation coefficient
Milk and dairy products (combined category raw and heat processed Milk and dairy products)	Raw milk and dairy products	0.994
	Pasteurised milk and dairy products	
	Dry milk products	
Fishery products combined category: raw, RTE RTRH, RTC	Raw fish (unprocessed)	0.988
	RTE/RTC/RTRH fish and seafoods	
	Crustaceans	
Produce and fruits (combined category fresh and processed)	Cut ready-to-eat vegetables/leafy greens and sprouts	0.964
	Fresh fruit/Cut RTE fruit and vegetable products	
	Heat treated fruit and vegetables	
Multi-components foods or meal components	Composite foods with substantial raw ingredients	0.992
	RTRH/RTE foods (chilled, frozen)	
	Mayonnaise based deli-salads	
Raw and ready to cook (RTC) meat and poultry	Raw poultry and meat cuts	0.984
	RTC processed poultry	

Conclusion

- Easy Plate CC showed similar inclusivity to VRBL, and in term of exclusivity, Easy Plate CC differentiated more non-target organisms compared to VRBL.
- Easy Plate CC showed satisfactory results for accuracy profile and high correlation with VRBL.
- These results suggest that **Easy Plate CC is a comparative method to the ISO reference method** and **applicable to a broad range of foods** for enumeration of coliforms.

Contact information

Daiki Utsunomiya

✉ dutsunomiya@mail.kikkoman.co.jp

Kikkoman Biochemifa Company

<https://biochemifa.kikkoman.com/e/kit/>

LinkedIn



LinkedIn



Website

