MicroVal and NordVal certification of Ready-To-Use Culture Media, Easy Plate EC for Enumeration of *E. coli* and Coliforms in a Broad Range of Foods, Pet Foods and Environmental Samples

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Introduction

- **Easy Plate** series (AC, EC, 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.**

- **Easy Plate EC (E-EC)** can be used for *E. coli* and coliforms.
  E. coli : blue colony
  Coliforms : purple colony

  E-EC / *E. coli* / coliforms
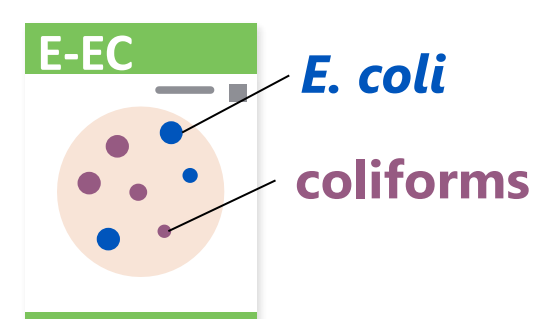
- According to ISO (16649-2:2001 and 4832:2006), tryptone bile X-glucuronic agar medium (TBX) and violet red bile lactose agar medium (VRBL) are used as conventional methods for *E. coli* and coliforms, respectively.

- In this study, the experiments were conducted to evaluate the specificity, selectivity, repeatability, accuracy and relative trueness of the E-EC for the enumeration of *E. coli* and coliforms as required by ISO 16140-2:2016.

Methods

The method validation study was done according to ISO 16140-2:2016, using ISO 16649-2:2001 and ISO 4832:2006 as the reference methods. Analysis with E-EC was performed following manufacturer's instructions.
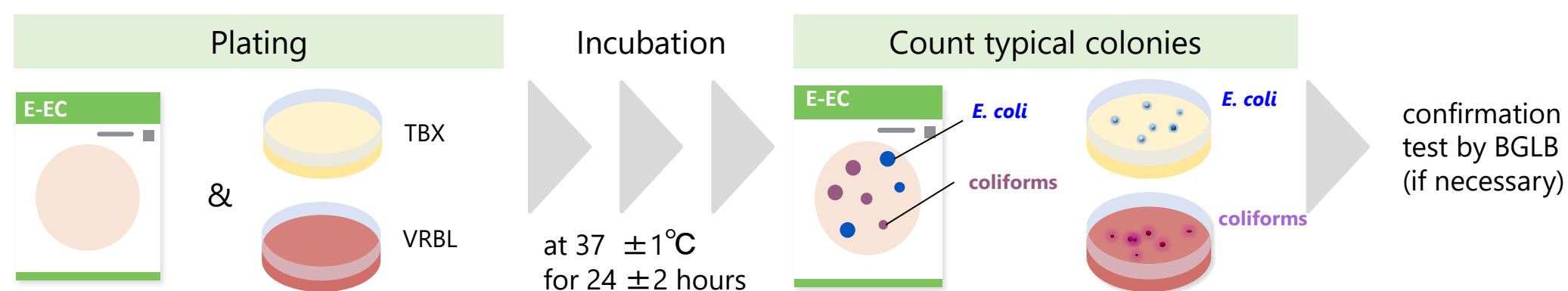
1. Inclusivity and Exclusivity

   Pure cultures of known provenance were tested for the inclusivity and the exclusivity. Each test was performed once with E-EC, TBX (for E. coli), VRBL (for coliforms) and non-selective agar.

2. Accuracy profile

   Seven categories (Milk and dairy products , Fishery products, Produce and fruits, Multi-components foods, Raw and RTC meat and poultry, Pet food and animal feed and Environmental samples) were tested for accuracy profile study.

3. Relative trueness

   Relative trueness study was conducted using a combination naturally and artificially contaminated samples across seven categories (same as the accuracy profile).

Plating | Incubation | Count typical colonies

E-EC & TBX VRBL → at 37 ±1℃ for 24 ±2 hours → E-EC / *E. coli* / coliforms → *E. coli* / coliforms → confirmation test by BGLB (if necessary)

Result 1. Inclusivity and Exclusivity

For the inclusivity study, a total of 50 pure cultures of *E. coli* and 51 of coliforms were tested. Forty-nine of the 50 *E. coli* and 50 of 51 coliform strains gave typical *E. coli* colonies on E-EC. For the exclusivity study, a total of 30 pure non-target cultures were tested separately for E-EC versus TBX and E-EC versus VRBL. E-EC and TBX showed similar selectivity for *E. coli*. Otherwise, E-EC showed better selectivity compared to VRBL for coliforms (False-positive strains are 1 and 6, respectively). **These results indicated that E-EC has similar specificity for *E. coli* as TBX and had a greater selectivity for coliforms than VRBL.**

Table 1. Summary of inclusivity and exclusivity

	E. coli	Coliforms	Non target	False positive
E-EC	49/50	50/51	57/60	Shigella sp.
ISO methods	50/50	51/51	52/60	Shigella sp. Vibrio sp.

Table 2. Result of the inclusivity study

Strains	No. of Strains	E-CC*	TBX*	VRBLA*
E. coli	50	49	50	–
Citrobacter	10	9	–	10
Enterobacter	19	19	–	19
Escherichia	5	5	–	5
Klebsiella	6	6	–	6
Cronobacter	4	4	–	4
Hafnia	1	1	–	1
Kluyvera	1	1	–	1
Serratia	2	2	–	2
Siccibacter	1	1	–	1
Franconibacter	2	2	–	2
Total	101	99/101	50/50	51/51

* Number of strains giving anticipated results

Table 3. Result of the exclusivity study

| No.	Organisms	Source or Identity	E-EC	VRBL	BGLB
1	*Aeromonas salmonicida*	NCTC 10402	✓	✓	NT
2	*Bacillus cereus*	ATCC 10876	✓	✓	NT
3	*Bacillus subtilis*	ATCC 6633	✓	✓	NT
4	*Carnobacterium divergens*	Brie	✓	✓	NT
5	*Edwardsiella tarda*	NCTC 10396	✓	✓	NT
6	*Flavobacterium species*	Bamboo shoots	✓	✓	NT
7	*Lactobacillus acidophilus*	industrial	✓	✓	NT
8	*Listeria monocytogenes*	Soft cheese	✓	✓	NT
9	*Aeromonas hydrophila*	tin of milk	✓	✓	NT
10	*Pasteurella bettyae*	NCTC 10535	✓	✓	NT
11	*Pediococcus pentasaceus*	Brine	✓	✓	NT
12	*Proteus mirabilis*	Poultry	✓	✓	NT
13	*Proteus vulgaris*	Poultry	✓	✓	NT
14	*Providencia rettgeri*	NCTC 7475	✓	✓	NT
15	*Pseudomonas aeruginosa*	NCIMB 10753	✓	✓	NT
16	*Pseudomonas fluorescens*	NCIMB 10586	✓	✓	NT
17	*Pseudomonas fluorescens*	water	✓	✓	NT
18	*Salmonella Typhimurium*	ATCC 14028	✓	✓	NT
19	*Acinetobacter calcoaceticus*	Sesame seeds	✓	✓	NT
20	*Acinetobacter lwoffii*	Tomatoes	✓	✓	NT
21	*Burkholderia gladioli*	Industrial	✓	✓	NT
22	*Shewanella putrifaciens*	NCTC 10736	✓	✓	NT
23	*Shigella boydii*	NCTC 11312	✓	✗	✓
24	*Shigella flexneri*	NCTC 9950	✓	✗	✓
25	*Shigella sonnei*	ATCC 25931	✗	✗	✓
26	*Staphylococcus aureus*	NCIMB 12702	✓	✓	NT
27	*Vibrio mimicus*	NCTC 11435	✓	✗	✓
28	*Vibrio parahaemolyticus*	NCTC 11344	✓	✗	✓
29	*Yersinia enterocolitica*	NCTC 10460	✓	✗	✓
30	*Morganella morganii*	Mince	✓	✓	NT
	Total		29/30	24/30	

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

Result 2. Accuracy profile

A total of 210 samples (comprising two items per category at three different contamination levels) were used in this study . The accuracy of the E-EC was satisfied as all 7 categories met the 0.5log AL or the re-calculated AL for both *E. coli* and coliforms.

Result 3.Relative trueness

A total of 105 samples across seven categories were tested for the relative trueness study. **The results showed that no significant difference between E-EC and the ISO methods for all seven categories**. For *E. coli*, the correlation coefficient between E-EC and TBX for all categories was 0.984 as shown in Figure 3-1. For coliforms, the correlation coefficient between E-EC and VRBL was 0.979 as shown in Figure 3-2.
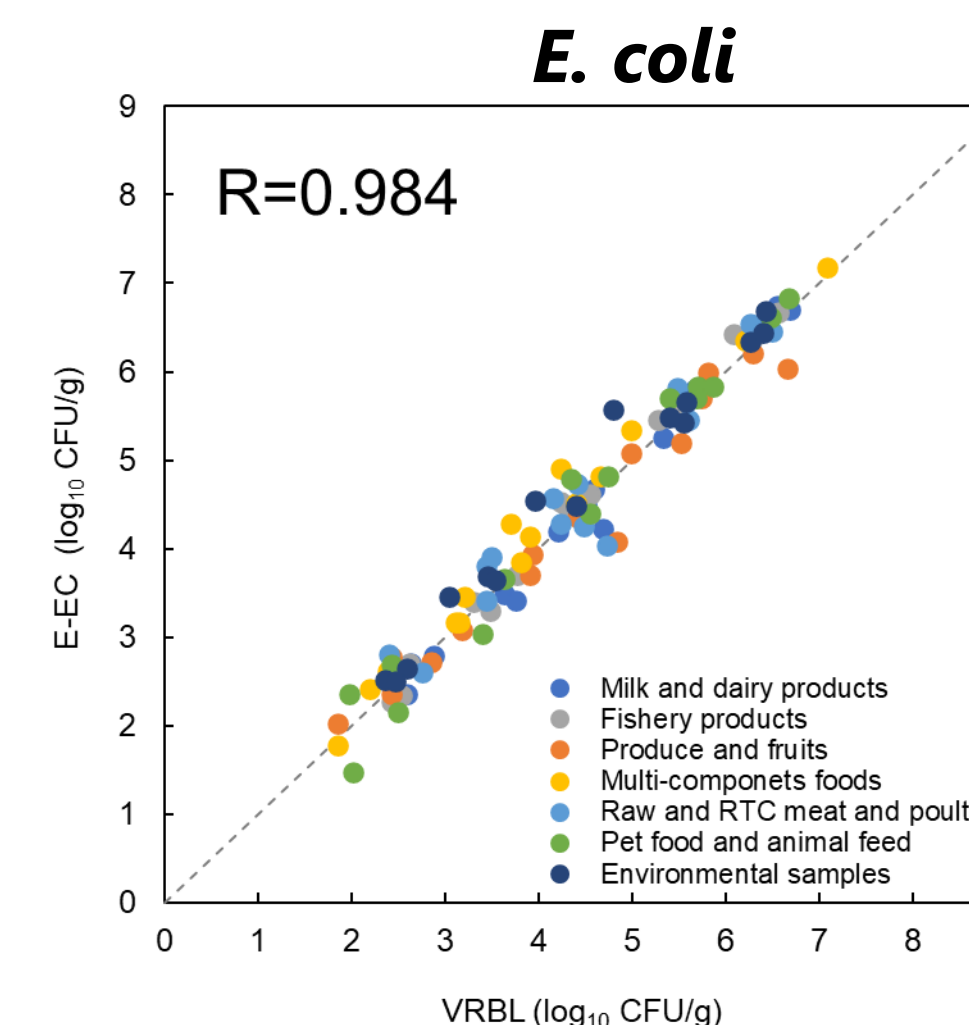
Figure 3-1. Scatter plot of the reference method versus E-EC results for *E. coli*.
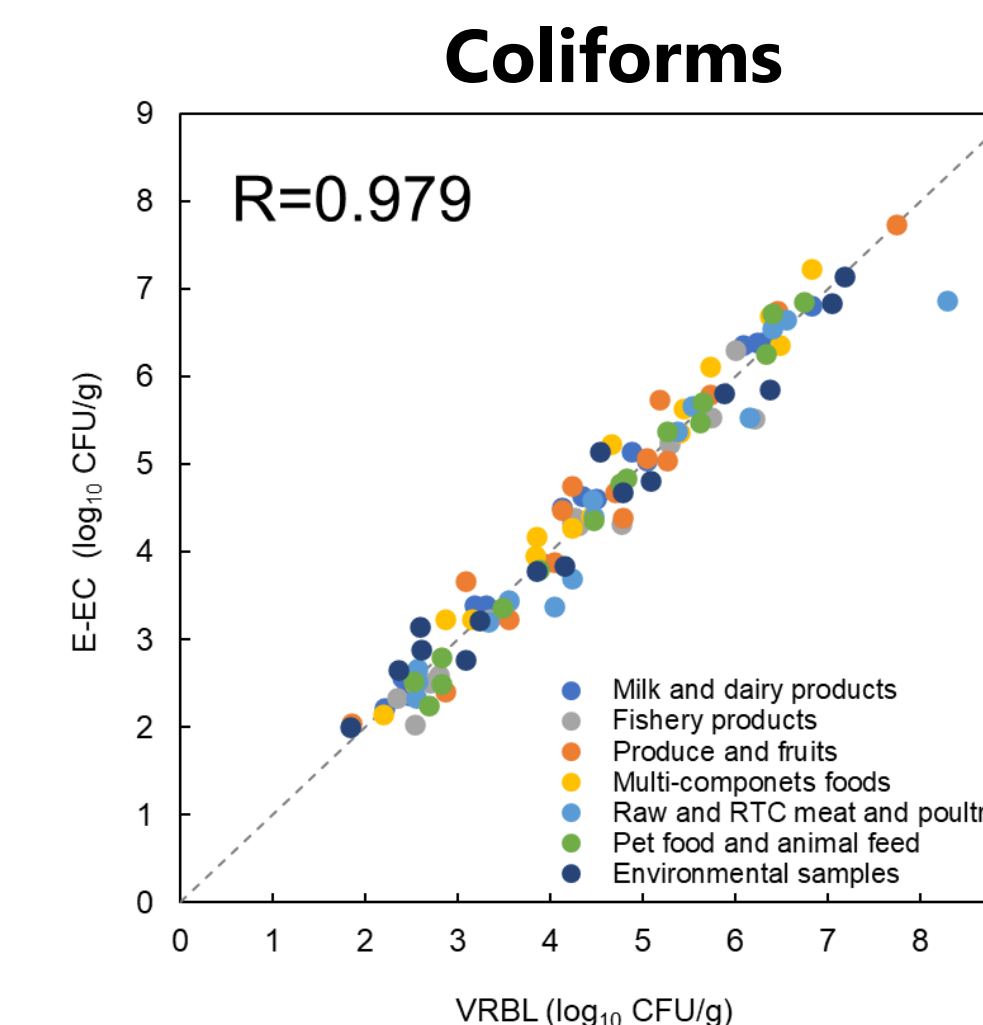
Figure 3-2. Scatter plot of the reference method versus E-EC results for **coliforms**.

Conclusion

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

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