Evaluation of Easy Plate™ EB for Enterobacteriaceae enumeration using ISO 16140-2, and AOAC validation standards

Daiki Utsunomiya(1), Mariko Kobayashi(1), Suzanne J. Jordan(2), Kentaro Takenaka(3), and Shinichiro Sugiura(1)

(1)Kikkoman Biochemifa Company, Noda-city, Chiba-prefecture, Japan, (2)Campden BRI, Chipping Campden, United Kingdom, (3)Kikkoman Corporation, Noda-city, Chiba-prefecture, Japan

INTRODUCTION

Background

- Enterobacteriaceae are commonly used as hygiene indicators in food and food processing environments.
- Conventional agar-based methods using Violet Red Bile Glucose (VRBG) require media preparation, over-lay procedures, incubation space, and manual colony counting.
- The Easy Plate series offers several advantages, including simplified preparation, reduced preparation time, space efficiency, lower plastic consumption, and compatibility with automated colony counting.
- **Easy Plate EB (E-EB)** can detect Enterobacteriaceae within 24 hours and the colonies on the plate can be automatically counted using CCS (colony counting system for Easy Plate).

Objective

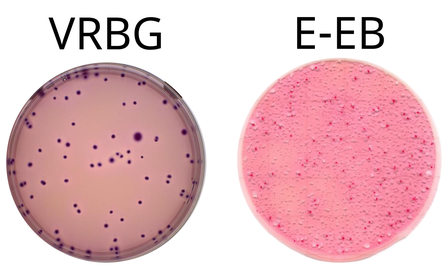
In this study, the performance of E-EB and CCS for Enterobacteriaceae enumeration was evaluated.

MATERIALS and METHODS

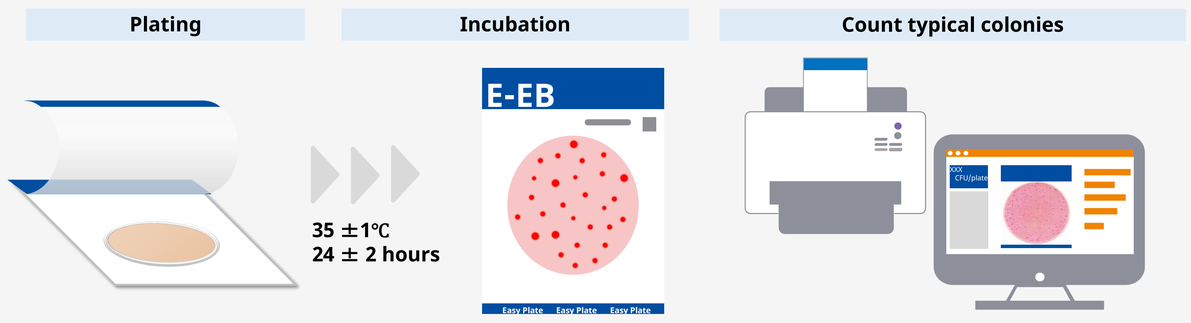
The performance of E-EB was evaluated according to ISO 16140-2 and AOAC study designs. All samples were analyzed following the manufacturer's instructions for E-EB and ISO 21528-2:2017 for the reference method.

Evaluation according to ISO and AOAC standards
- Inclusivity (50 target strains)
- Exclusivity (30 non-target strains)
- Accuracy (not presented in this poster)
- Relative Trueness (using 105 samples (Table 1.))

Additional evaluation (Not required by ISO 16140-2)
- CCS vs Manual counting. (CCS version 1.3.1)



Easy Plate method workflow



※Only manual count is in the validation method.

Table 1. Food and environmental samples used in the relative trueness study

Category	Type	natural contamination※
Heat processed milk and dairy products	Pasteurised milk-based products, Pasteurised dairy products, Dry milk products	0%
Multicomponent foods	Composite foods with substantial raw ingredients, Mayonnaise based deli salads with raw ingredients, Ready to reheat foods (refrigerated)	13.3%
Fresh produce	Cut ready to eat fruit, Cut ready to eat vegetables, Leafy greens	73.3%
Fishery products	Raw fish (unprocessed), Ready to cook fish (processed), Cooked fishery products (RTE)	13.3%
Raw meat and poultry	Fresh meats (unprocessed), Ready to cook products (processed raw), Fresh poultry (unprocessed)	53.3%
Pet food and animal feed	Dry Food, Wet food (raw and canned), Animal feeds (poultry and fish)	6.7%
Environmental samples	Process water, Swabs, Dust wipes	20.0%

※Rate of natural contamination. Samples without natural contamination were artificially inoculated according to the MicroVal protocol.

RESULTS and DISCUSSIONS

Inclusivity and Exclusivity

E-EB demonstrated inclusivity and exclusivity comparable to those of the conventional VRBG method.

Table 2. Summary of inclusivity and exclusivity results

	Enterobacteriaceae	Atypical result	Non target	Atypical result
VRBG	50/51	*Erwinia carotovora*※	29/30	*Vibrio parahaemolyticus*
E-EB	50/51	*Leminorella richardii*	29/30	*Vibrio parahaemolyticus*

※*Pectobacterium carotovorum*

Table 3. Results of inclusivity study.

Target strains	No. of strains	E-EB	VRBG
Buttiauxella spp.	1	1	1
Citrobacter spp.	7	7	7
Erwinia spp.	2	2	1
Cronobacter spp.	2	2	2
Enterobacter spp.	9	9	9
Siccibacter spp.	1	1	1
Escherichia spp.	4	4	4
Hafnia spp.	1	1	1
Klebsiella spp.	5	5	5
Leclercia spp.	1	1	1
Leminorella spp.	1	0	1
Morganella spp.	1	1	1
Pantoea spp.	1	1	1
Proteus spp.	2	2	2
Providencia spp.	2	2	2
Raoultella spp.	1	1	1
Salmonella spp.	5	5	5
Serratia spp.	3	3	3
Shigella spp.	1	1	1
Shimwellia spp.	1	1	1
Franconobacter spp.	1	1	1

Number of giving anticipated results

Table 4. Results of exclusivity study.

Non-Target strains	Source or Identity	E-EB	VRBG
Acinetobacter calcoaceticus	sesame seeds	✓	✓
Acinetobacter lwoffii	Tomatoes	✓	✓
Aeromonas salmonicida	NCTC10402	✓	✓
Avibacterium avium	Chicken	✓	✓
Bacillus coagulans	Sterilised milk	✓	✓
Bacillus subtilis	Custard	✓	✓
Brochothrix thermosphacta	Fresh pork sausage	✓	✓
Burkholderia gladioli	Industrial	✓	✓
Burkholderia stabilis	soft drinks	✓	✓
Candida magnoliae	Strawberries	✓	✓
Flavobacterium indologenes	ATCC 29212	✓	✓
Flavobacterium resinovorum	Bamboo shoots	✓	✓
Lactobacillus brevis	NCIMB 8767	✓	✓
Lactobacillus casei	Fermenting olives	✓	✓
Listeria innocua	Industrial isolate	✓	✓
Listeria monocytogenes	Mammal Brain	✓	✓
Novosphingobium capsulatum	Soft cheese	✓	✓
Pasteurella multocida	Distilled water	✓	✓
Pediococcus pentasaceus	Cattle	✓	✓
Pseudomonas aeruginosa	Brine	✓	✓
Pseudomonas fluorescens	Blood	✓	✓
Shewanella putrefaciens	Soil	✓	✓
Enterococcus faecalis	Industrial isolate	✓	✓
Staphylococcus aureus	NCIMB 12702	✓	✓
Stenotrophomonas maltophila	NCIMB 9428	✓	✓
Streptococcus agalactiae	Milk	✓	✓
Streptococcus pyogenes	Clinical	✓	✓
Vibrio parahaemolyticus	Human faeces	X	X
Xanthomonas maltophilia	Bamboo shoots	✓	✓
Zygosaccharomyces bailii	Fruit Jam	✓	✓

"✓" indicates successfully suppressed
"X" indicates false-positive

Relative trueness

A total of 105 samples across seven categories were evaluated in the relative trueness study. No significant differences were observed between E-EB and the reference ISO method in any category. The coefficient of determination (R^2) between the two methods for all samples was 0.964 (Figure 1). The sample categories and corresponding coefficients of determination are summarized in Table 4.

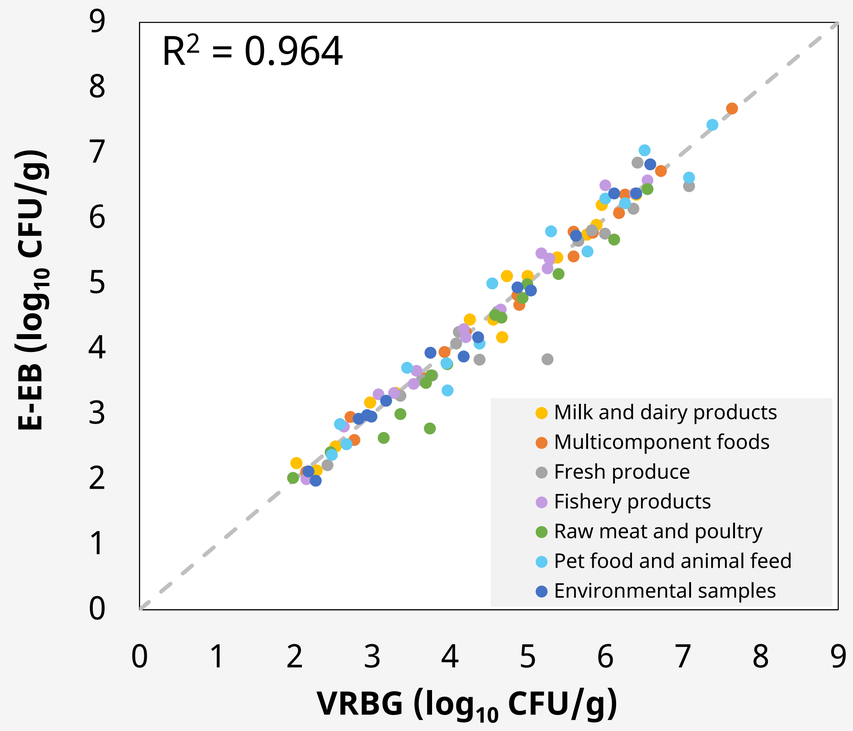


Table 4. Sample categories and correlation coefficients for each category.

category	Correlation coefficient
Heat processed milk and dairy products	0.990
Multicomponent foods	0.997
Fresh produce	0.953
Fishery products	0.994
Raw meat and poultry	0.981
Pet food and animal feed	0.978
Environmental samples	0.995

Figure 1. Scatter plot of the reference method versus E-EB results for all categories.

Performance of automated colony counting (additional study)

Automated enumeration of E-EB using CCS showed excellent agreement with manual counting (R^2>0.99). This result indicates that CCS enables faster colony enumeration while maintaining accuracy comparable to the manual method.

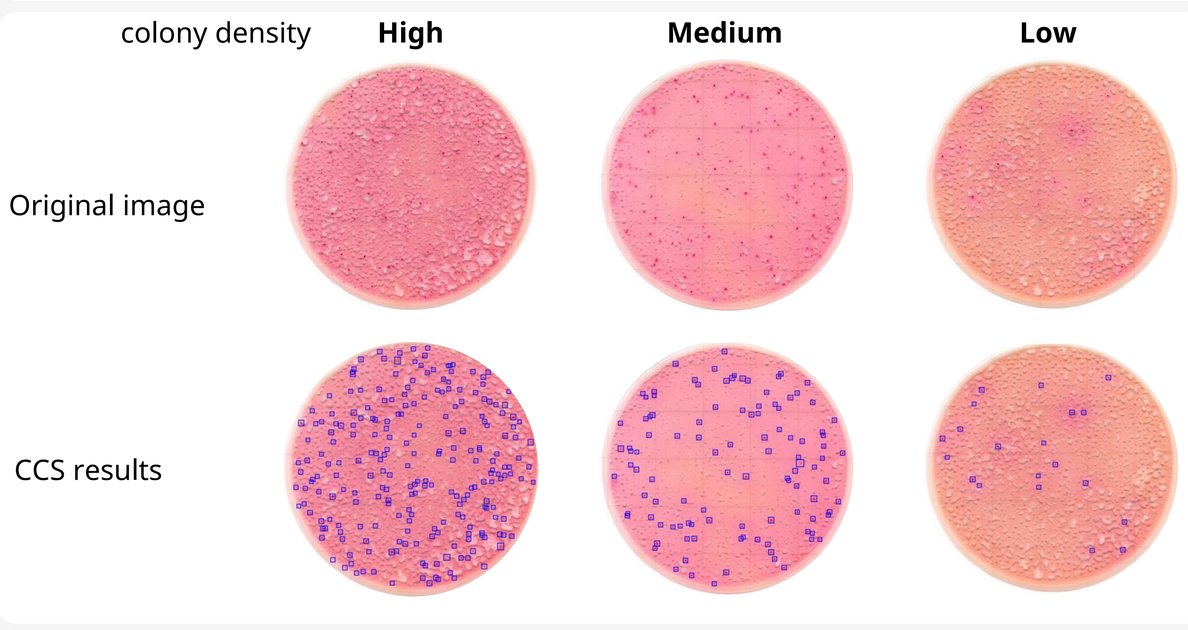


Figure 2. CCS performance at different colony levels

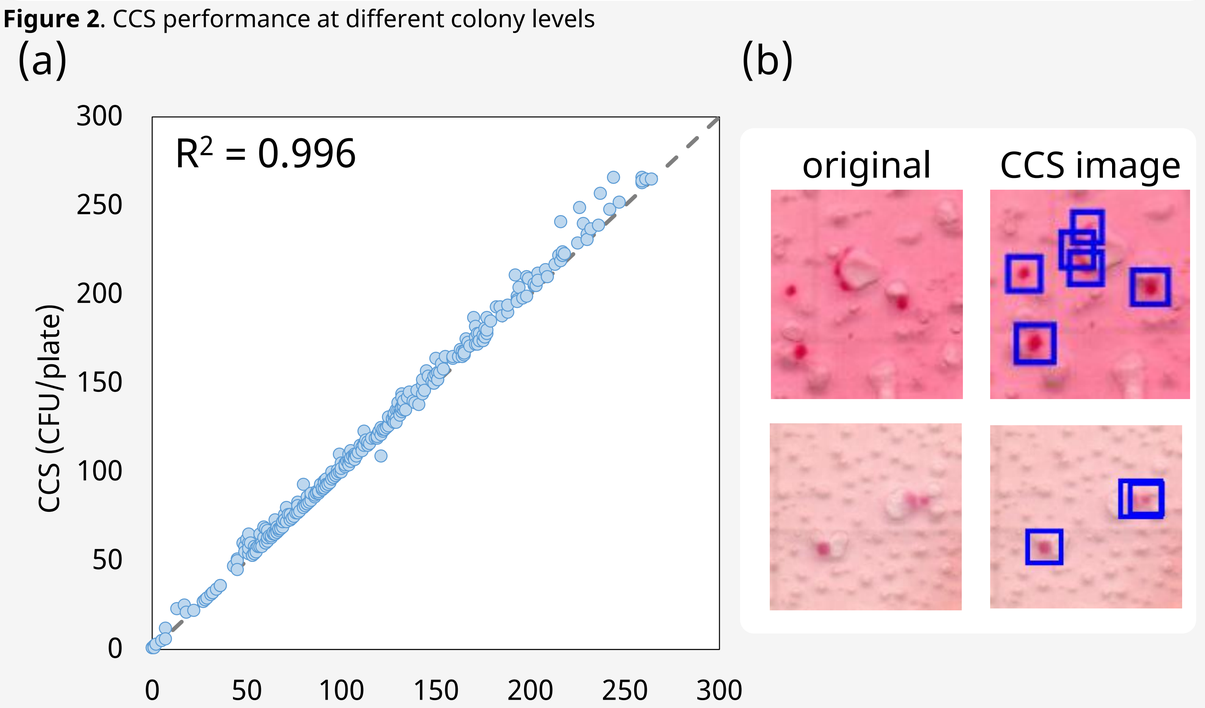


Figure 3. (a) Correlation of Enterobacteriaceae counts obtained by manual counting and CCS. (b) Representative misdetection case. A slight positive bias was observed, primarily due to occasional overcounting caused by incorrect segmentation of individual colonies.

CONCLUSIONS

- E-EB demonstrated **selectivity comparable** to that of the conventional VRBG method for Enterobacteriaceae detection.
- Easy Plate EB showed **good agreement** with ISO 21528-2:2017 reference method across a wide range of food and environmental samples.
- CCS for Easy Plate enabled **automated colony enumeration** with accuracy comparable to manual method, supporting improved laboratory productivity.

CONTACT INFORMATION

Daiki Utsunomiya
dutsunomiya@mail.Kikkoman.co.jp
Kikkoman Biochemifa Company
http://biochemifa.kikkoman.com/e/kit/

Website
LinkedIn