Performance Evaluation of Easy Plate EB (for Enumeration of *Enterobacteriaceae*)

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Introduction

- *Enterobacteriaceae* encompass both lactose-fermenting coliforms and major non-lactose-fermenting enteric pathogens, including *Salmonella*, *Shigella*, and *Yersinia*. Their clear taxonomic classification makes them a widely recognized hygiene indicator, particularly in regions like the EU. In Japan, they have been designated as a hygiene indicator under the Food Sanitation Act since 2011, specifically for compositional standards of raw meat intended for consumption.
- The ISO 21528-2:2017 method recommends the use of Violet Red Bile Glucose Agar (VRBG). However, preparing this medium is time-consuming and involves labor-intensive steps such as serial dilution and overlaying, making the process cumbersome.
- To address these challenges, we developed the **Easy Plate EB (E-EB)**, a simplified medium for *Enterobacteriaceae*, and a new algorithm for the **Easy Plate Colony Counter System (CCS)** specifically designed for **E-EB**. Here, we report the results of performance evaluations for both.

Easy Plate EB (E-EB)

Conclusion

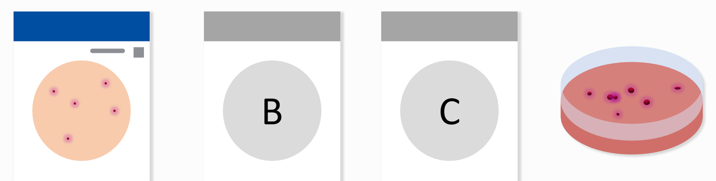
Easy Plate EB demonstrated **a high correlation** with VRBG and other existing simplified media. However, in certain food samples, it showed the potential to detect Enterobacteriaceae more effectively compared to VRBG.

When **combined with the CCS**, Easy Plate EB ensures testing accuracy while **reducing microbiological testing time, standardizing processes, and lowering testing costs.** This combination is expected to significantly enhance the efficiency of microbiological testing.

1. Food Evaluation

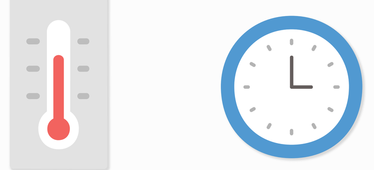
A total of 62 food samples, as listed in the table below, were used as test specimens. A 9-fold volume of phosphate-buffered saline (PBS) was added to each sample to prepare the stock solution, which was further serially diluted with PBS as needed to create the test solutions. The test solutions were inoculated onto the respective media, and correlation evaluations were conducted under the incubation conditions specified below.

【Media for *Enterobacteriaceae* detection】



E-EB RTU-B RTU-C VRBG

【Incubation condition】

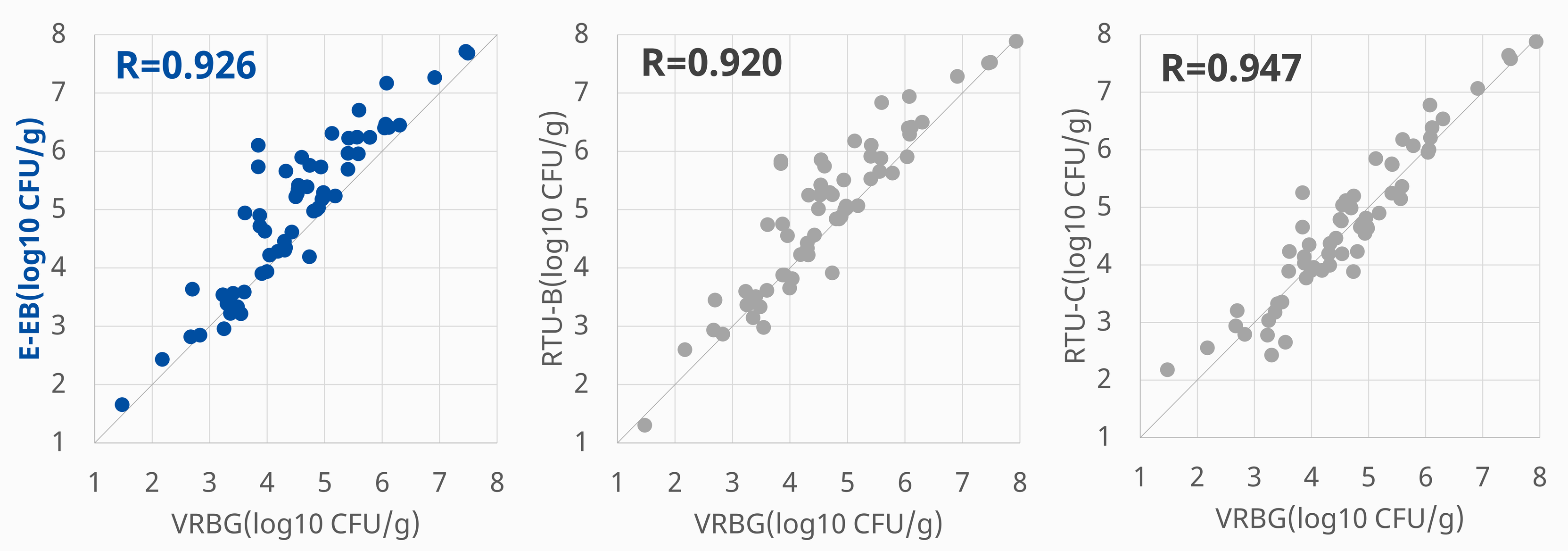


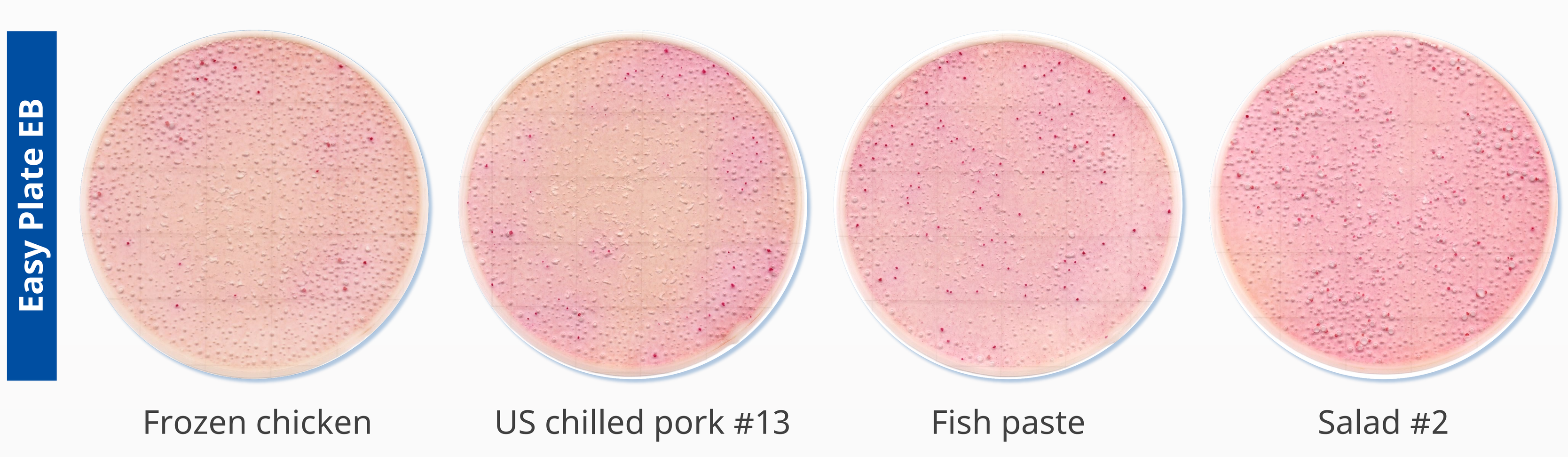
35±1°C 24±1hrs

【Food Matrices】

Category	Examples	Kikkoman	Tokyo Kenbikyo-in	Sum
Chocolate, bakery products and confectionary	Mille crepes	1	0	1
Dairy (raw and processed) and egg products	Milk shakes, natural cheese	1	3	4
Dried vegetables	Dried enoki mushroom	0	2	2
Fresh produces	Chopped green onions, Salad	5	11	16
Multi-component foods or meal components	Tortilla, Sushi roll *"Futomaki"*	6	3	9
Raw/RTC seafoods	Fish paste, Frozen shrimp	2	1	3
Raw/RTC meat・chicken	US chilled pork, Ground chicken	5	17	22
RTE/RTRH fishery products	Bonito flake powder, Tuna	0	5	5
		20	42	**62**

1.1. Correlation Evaluation



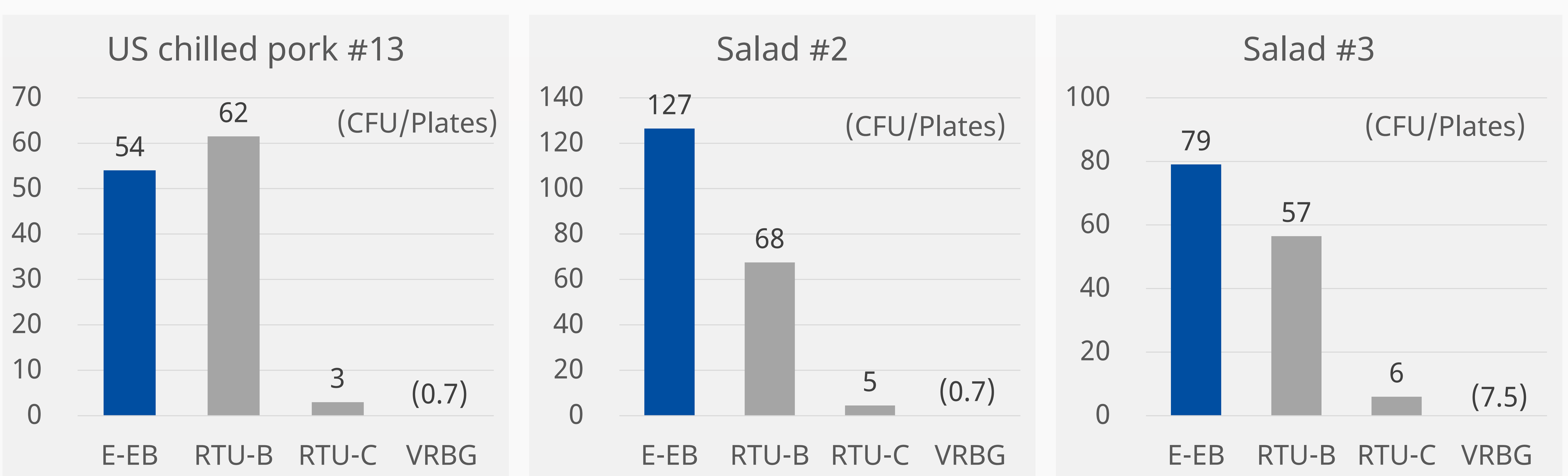


Frozen chicken US chilled pork #13 Fish paste Salad #2

A high correlation was confirmed between VRBG and each simplified medium. For VRBG, 10 samples were identified where the bacterial count on E-EB exceeded VRBG by more than 10 times. The following food items were confirmed:
US chilled pork #4, #13, Salad #1~4, Green onions, Tortilla, Korean lettuce *"Sanchu"*, Korean-style salad *"Choregi salad"*

1.2. Microbial Identification

The three food samples that showed the greatest discrepancy in bacterial counts between E-EB and VRBG were selected for further analysis. Colonies were isolated and purified from either the E-EB or RTU-B medium, and microbial identification was performed using the Bruker MALDI Biotyper Sirius system.
The bacterial count on VRBG was calculated as the count at the lower dilution divided by 10.



▼ A total of 14 CFU were isolated from higher dilutions.

Identification Results (US chilled pork #13)	
Yersinia ruckeri	8
Serratia liquefaciens	3
Not detected	3

▼ 16 CFU were isolated

Identification Results (Salad #2)	
Lelliottia amnigena	13
Rahnella aquatilis	1
Rahnella bruchi	1
Rahnella inusitata	1

▼ 10 CFU were isolated

Identification Results (Salad #3)	
Rahnella aquatilis	9
Serratia plymuthica	1

Out of the 40 colonies isolated, 37 were identified as members of the Enterobacteriaceae family (3 colonies could not be identified using the smear method). These results indicate that, for certain foods (such as some raw vegetables and raw meats), the detection sensitivity of E-EB and RTU-B was observed to be higher than that of VRBG.

2. Automated Counting Evaluation

The newly developed **CCS algorithm for E-EB** was evaluated using food samples tested at the Tokyo Metropolitan Institute of Public Health. A total of **222 images** from 31 samples were analyzed, including 126 images suitable for visual counting and 96 images classified as NC (Not Countable) due to TNTC (Too Numerous to Count) or similar reasons. A precision comparison between manual visual counts and the CCS algorithm was conducted.

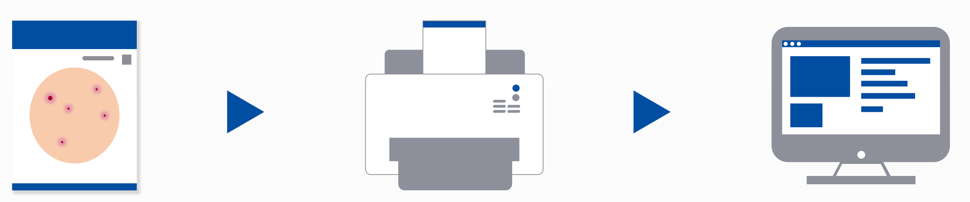
【Evaluation Details】

Correlation Assessment : The correlation between manual visual counts and CCS algorithm counts was evaluated using 126 images where visual counting was possible.

Negative Determination Accuracy : The accuracy of negative results was assessed by checking whether the 13 plates visually determined to have 0 CFU were also identified as 0 CFU by the CCS algorithm.

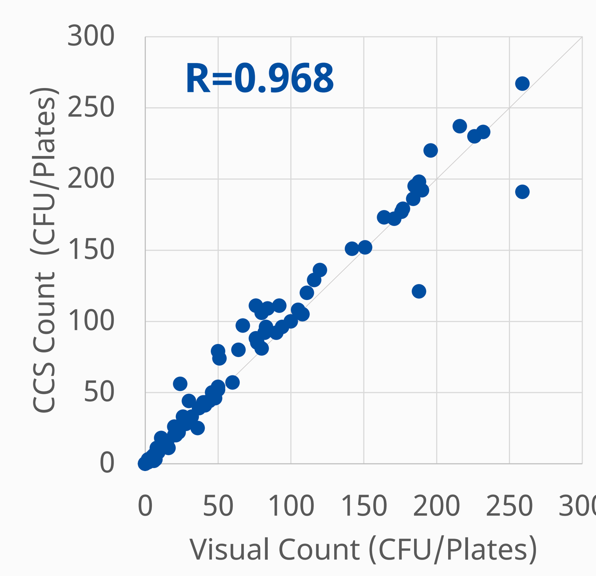
NC Determination Precision : The consistency between visual judgment and the CCS algorithm in determining Countable/Not Countable (NC) status was evaluated.

Flow for CCS



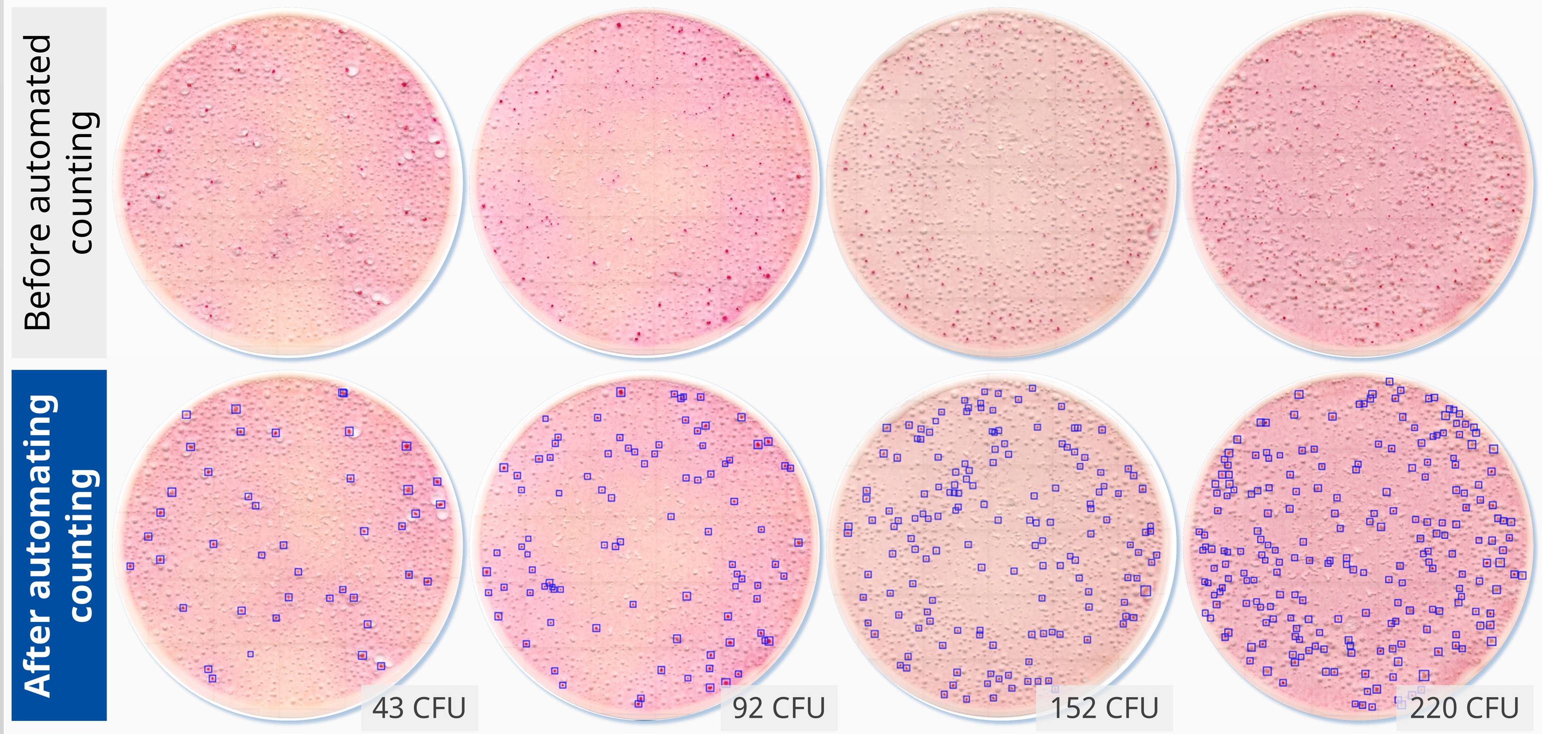
Cultured E-EB ▶ **Media Image Acquisition** ▶ **Automated Counting**

2.1. Correlation Assessment



The correlation coefficient was **R=0.968**, and the Root Mean Squared Logarithmic Error **(RMSLE) was 8.11%**, indicating high counting accuracy and a strong correlation with manual visual counts. These results confirm the reliability and precision of the CCS algorithm in comparison to traditional manual counting methods.

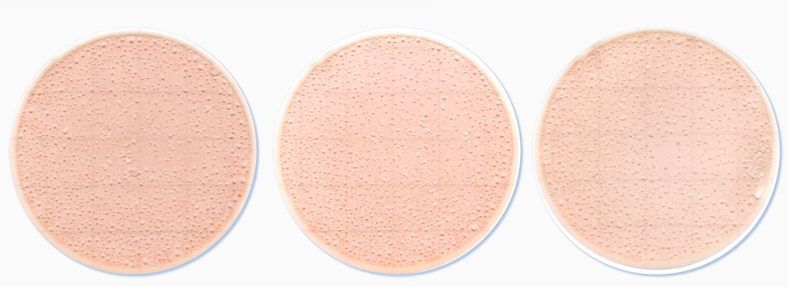
$$RMSLE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\log(y_i+1)-\log(\hat{y}_i+1))^2}$$



Milk shake #3 US chilled pork #15 Chopped green onions #2 Skinless chicken

2.2. Negative Determination Assessment

Plates determined as negative
- US chilled pork 1 plate
- Milk shake #1~3 9 plates
- Chopped green onions #3~4 3 plates



▲ An example image of an E-EB plate with 0 CFU

All 13 plates visually determined as negative (0 CFU) were also accurately classified as negative by the CCS.

2.3. NC Determination Precision Assessment

NC Determination:
For images visually judged as Not Countable (NC), such as those determined to be TNTC (Too Numerous To Count), the CCS algorithm includes a feature to issue an alert in cases where the automated counting accuracy might be compromised.

Visual	CCS	Number of plates	
Countable	Countable	125	Match 94.6%
NC/TNTC	NC	85	
Countable	NC	1	Mismatch 5.4%
NC/TNTC	Countable	11	

The NC determination between visual inspection and the CCS algorithm matched for **94.6%** of the samples, demonstrating high accuracy in issuing alerts. Further improvements in the training dataset are expected to enhance NC determination precision even more.