

Performance Evaluation of Ready-to-Use Culture Media, Easy Plate AC for Enumeration of Aerobic Plate Count in a Broad Range of Foods, Environmental Samples and Pet Foods

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1. Introduction

•For the enumeration of an aerobic count, plate count agar (PCA) is traditionally used according to ISO 4833-1 (2013)

•A type of Ready-to-use (RTU) media, Easy Plate AC (E-AC) (Kikkoman Biochemifa Company) can be alternatively used to provide many benefits including **reduced time to result, simplicity of use** and is **plastic-saving** compared to PCA.



2. Summary

E-AC was shown to be comparative to the ISO reference method 4833-1 :2013 for a broad range of foods, environmental samples and pet foods.

E-AC is found to be

- 1. Reliable method to enumerate aerobic plate count**
 ✓ Comparative to the ISO 4833-1:2013 method
- 2. Applicable to a broad range of foods, environmental samples and pet foods**
 ✓ With reasonable repeatability and accuracy
- 3. Provide results in 48h, a saving of 24h compared with traditional PCA**
- 4. Compatible with foods containing *Bacillus* spp. that are liable to spreading**

3. Methods & Results

The Method validation study was done according to **ISO 16140-2:2016**, using ISO 4833-1:2013 as the reference method. Analysis with E-AC was performed following manufacturer's instructions. The incubation condition used for the study are displayed in Table 1.

Table 1. Incubation conditions used for the study

Media	Temperature	Time
PCA	30°C	72h
E-AC	30°C	48h

3.1. Relative trueness study

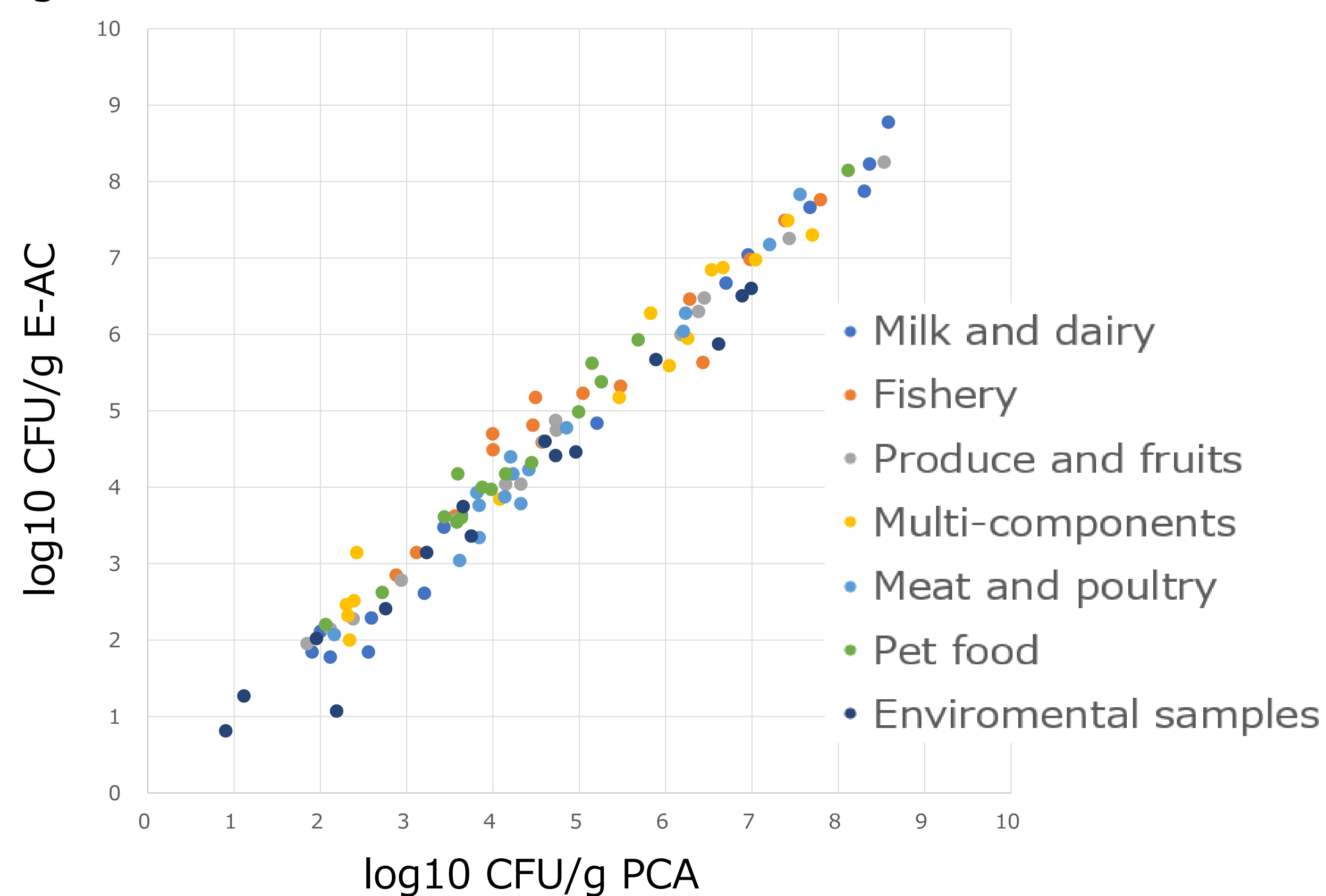
•A total of **105 samples across 7 categories** was tested. Each category contained 3 types and 15 items per category. Details of categories and types used are shown in Table 2.

→ **No significant difference between E-AC and the ISO method** was noted as revealed in the scatter plot displayed in Figure 1 below.

Table 2. Categories and types tested in the relative trueness study

Milk and dairy (raw and heat processed)	Raw milk and dairy products
	Pasteurized milk and milk based products
	Dry milk products
Fishery (raw, ready-to-eat, ready-to-reheat and ready-to-cook)	Raw fish
	RTE/RTC/RTRH fish and seafoods
	Crustaceans
Produce and fruits (fresh and processed)	Cut ready-to-eat vegetables/leafy greens and sprouts
	Fresh fruit/Cut RTE fruit and vegetable products
	Heat treated fruit and vegetables
Multi-component foods or meal components	Composite foods with substantial raw ingredients
	RTRH/RTE foods (chilled, frozen)
	Mayonnaise based deli-salads
Meat and poultry (raw and ready to cook)	Raw poultry and meat cuts
	Raw processed meat
	RTC processed poultry
Pet food and animal feed	Dry Food
	Wet food (raw and canned)
	Animal feeds (poultry and fish)
Environmental samples (food or feed production)	Surfaces (wipes, swabs)
	Process water
	Dusts

Figure 1. Correlation between E-AC and the ISO method



3.2. Accuracy profile study

•A total of 210 artificially contaminated samples **across 7 categories with 2 items per category** were tested in this section. Each item used was contaminated at 3 levels, with 5 replicates analyzed per level.

→ **All 7 categories passed** the 0.5log acceptability limits or the recalculated limits.

Additional work to determine the impact of E-AC on the growth of *Bacillus* spp. was also performed

3.3. Evaluating the spread of colonies

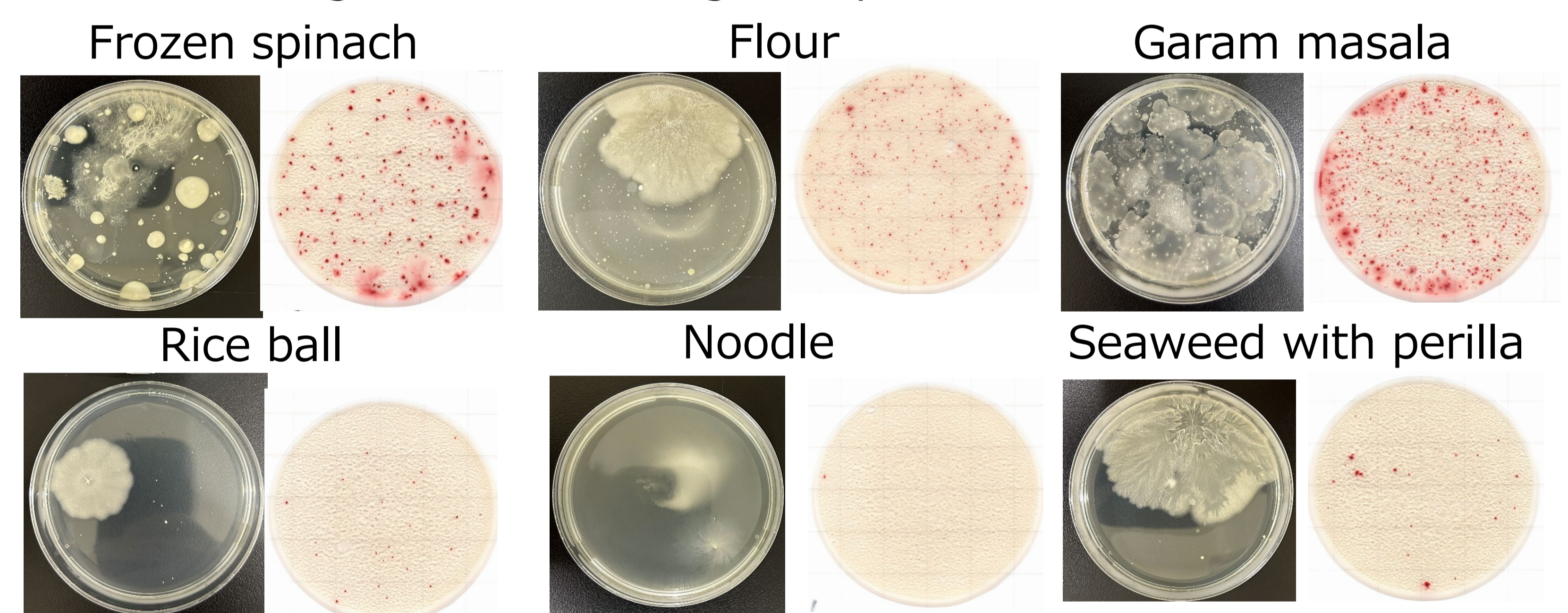
3.3.1. Analysis with foods containing *Bacillus* spp.

•Six foods were plated to E-AC and PCA

→ **Colonies spread over the surface of PCA** (Almost unable to count colonies)

→ **The spread was prevented on E-AC.**

Figure 2. Evaluating the spread of colonies



3.3.2. Evaluating the spread of *Bacillus* spp. pure cultures

•Six spreading colonies of 3.3.1 were **identified with 16S rRNA sequencing.**

→ All of them were **identified as *Bacillus* spp.**

•Isolates were plated to PCA, E-AC and another type of RTU media (RTU1) after pure culture.

→ **The spread was prevented on E-AC** for all species while not on PCA and RTU1 for some species.

Table 3. Evaluating the spread of *Bacillus* spp. with pure culture

Organism	Top hits	Origin	PCA	E-AC	RTU1
<i>Bacillus</i> sp.	<i>B. cabrialesii</i> , <i>B. inaquosorum</i>	Garam masala	×	✓	×
<i>B. velezensis</i>	<i>B. Velezensis</i>	Flour	×	✓	×
<i>B. cereus</i> group	<i>B. pseudomycooides</i>	Frozen spinach	×	✓	×
<i>Bacillus</i> sp.	<i>B. amyloliquefaciens</i> , <i>B. velezensis</i>	Seaweed with perilla	×	✓	×
<i>Bacillus</i> sp.	<i>B. subtilis</i> , <i>B. stercoris</i>	Rice ball	×	✓	✓
<i>B. altitudinis</i>	<i>B. aerius</i> , <i>B. altitudinis</i> , <i>B. aerophilus</i> , <i>B. stratosphericus</i>	Noodle	Spread modestly	✓	✓

“✓” indicates “easy to count” while “×” indicates “difficult or unable to count”