Performance Evaluation of Ready-to-Use Culture Media, Easy Plate SA for Enumeration of *Staphylococcus Aureus* in a Broad Range of Foods

Shinichiro Sugiura[1], Suzanne J. Jordan[2], Kentaro Takenaka[3], Mai Shimizu[3] and Takeo Suzuki[3]
(1)Kikkoman Biochemifa Company, (2)Campden BRI, (3)Kikkoman Corporation

1. Introduction

・For the **enumeration of *Staphylococcus aureus*, Baird-Parker agar (BPA)** is traditionally used according to the ISO 6888-1:2021: Enumeration of coagulase-positive staphylococci.

・A type of Ready-to-use (RTU) media, Easy Plate SA (E-SA) (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 the traditional method.

2. Summary

E-SA showed comparativeness to ISO 6888-1:2021 for a broad range of foods.

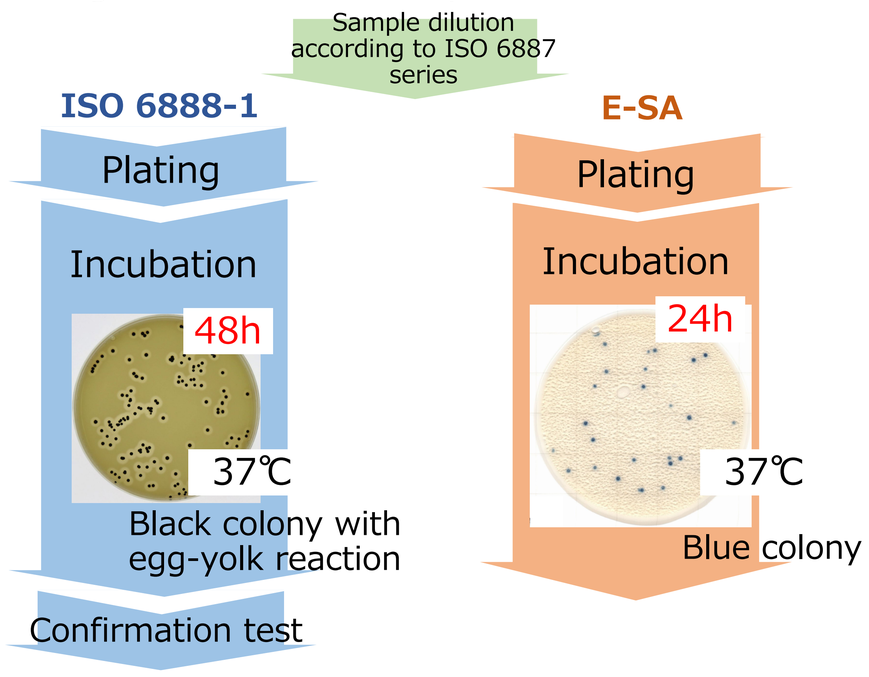
E-SA is found to be

1. **Reliable method to enumerate *S. aureus***
   ✓Comparative to the traditional ISO 6888-1 method
2. **Applicable to a broad range of foods**
   ✓With reasonable repeatability and accuracy
3. **Provide results in 24h**
   ✓Saves 24h compared with the traditional BPA method
4. **Excels at differentiating *S. aureus*.**
   ✓Clear colonies with greater selectivity to reduce misinterpretation

3. Methods & Results

The Method validation study was done **according to ISO 16140-2:2016,** with ISO 6888-1 (2021) as the reference method. Analysis using E-SA was performed following manufacturer's instructions.

Figure 1. Method comparison work flow



3.1. Relative trueness study

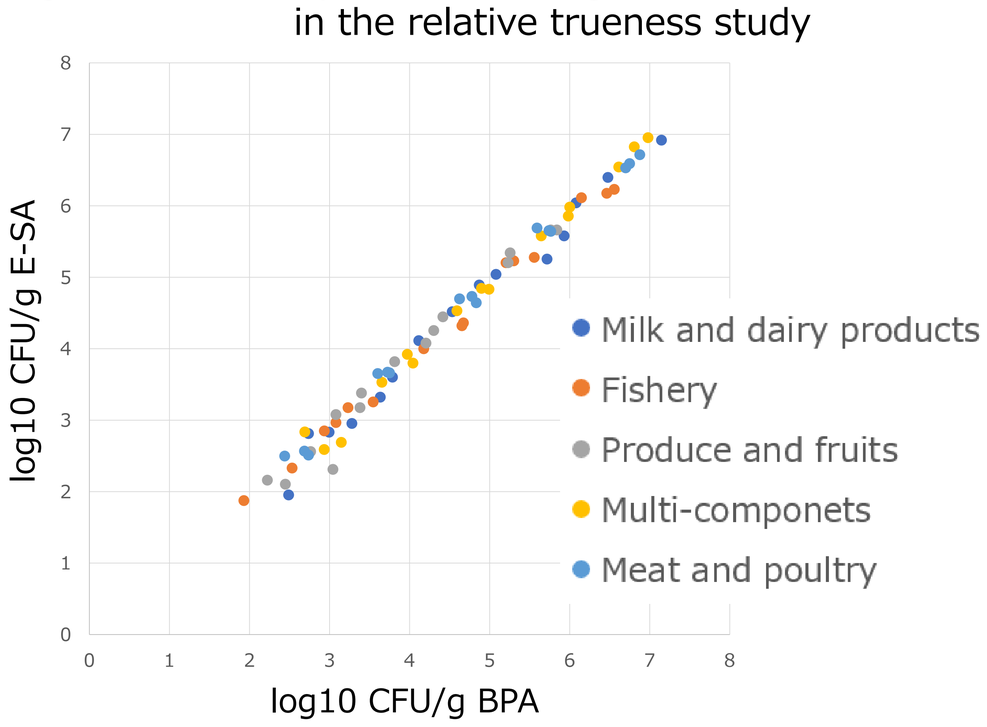
・A total of **75 foods across 5 categories** was tested. Each category contained 3 types and 15 items per category. Details of categories and types used are shown in Table 1.

→ **No significant difference between E-SA and the ISO method** was noted as revealed in the scatter plot displayed in Figure 2 below.

Table 1. Categories and types tested in Relative trueness study

	Types
Dairy (raw and processed) **and egg products**	Raw milk and dairy products
	Pasterurised milk and dairy products
	Processed egg products
Fishery (raw, ready-to-eat, ready-to-reheat, ready-to-cook)	Raw fish (unprocessed)
	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 (ready-to-eat, ready-to-reheat)	Cooked meat and poultry products
	Fermented or dried products
	Raw cured products

Figure 2. Scatter plot for all categories in the relative trueness study



3.2. Accuracy profile study

・A total of 150 artificially contaminated samples **across 5 categories with 2 items per category was tested in this section. Each item used was** contaminated at 3 levels, with 5 replicates analyzed per level

→ **All 5 categories passed** the 0.5log acceptability limits or the recalculated limits.

3.3. Inclusivity and Exclusivity

・Inclusivity was examined **with 50 *S. aureus* strains** from various sources.

→ E-SA was found to be **comparative to the ISO method**.

・Exclusivity was examined with 31 **non-target isolates**.

→ E-SA **outperformed the ISO method**.

Table 2. Summary results of inclusivity and exclusivity

Inclusivity (Target)		**Exclusivity (Non-target)**	
ISO 6888-1 (BPA)	**E-SA**	**ISO 6888-1 (BPA)***	**E-SA**
100% (50/50)	100% (50/50)	71% (22/31)	97% (30/31)

* Presumptive results recorded for exclusivity isolates

Table 3. Microorganisms tested for exclusivity

No.	Organism	Source	Identity if available	BPA ISO 6888-1	E-SA
1	***Bacillus cereus***	Dairy product	Industrial strain	✓	✓
2	***Bacillus cereus***	Unknown	ATCC 10876, NCTC 7464	✓	✓
3	***Bacillus subtilis***	Unknown	ATCC 6633, NCTC 10400	✓	✓
4	***Brochothrix thermospacta***	Pork sausage	NCTC 10822	✓	✓
5	***Flavobacterium species***	Unknown	Industrial strain	✓	✓
6	***Enterobacter agglomerans***	Raw mince	Industrial Strain	✓	✓
7	***Enterococcus faecalis***	Urine	NCIMB 13280, ATCC 29212	✓	✓
8	***Enterococcus faecalis***	Unknown	NCTC 775	✓	✓
9	***Escherichia coli***	Raw ground beef	Industrial Strain	✓	✓
10	***Lactobacillus brevis***	Unknown	NCTC13386	✓	✓
11	***Lactobacillus gasseri***	Human source	NCIMB 13081	✓	✓
12	***Leuconostoc mesenteroides***	Ham	Industrial strain	✓	✓
13	***Listeria monocytogenes***	Soft cheese	Industrial strain	✓	✓
14	***Micrococcus luteus***	Unknown	NCTC 2665, ATCC 13507	✓	✓
15	***Pediococcus pentosaceus***	Brine	Industrial strain	✓	✓
16	***Pseudomonas rhodesiae***	Unknown	Industrial strain	✓	✓
17	***Salmonella Enteritidis***	Fish cakes	Industrial strain	✓	✓
18	***Salmonella typhimurium***	Chicken	Industrial strain	✓	✓
19	***Staphylococcus caprae***	Goat	Industrial strain	×	✓
20	***Staphylococcus carnosus***	Fermented sausage	Industrial strain	×	✓
21	***Staphylococcus cohnii***	Unknown	Industrial strain	×	×
22	***Staphylococcus epidemidis***	Human skin	Industrial strain	×	✓
23	***Staphylococcus hominis***	Dried milk powder	Industrial strain	✓	✓
24	***Staphylococcus hyicus***	Pig skin	Industrial strain	×	✓
25	***Staphylococcus intermedius***	Pigeons	NCTC 11048, ATCC 29663	×	✓
26	***Staphylococcus piscifermentans***	Unknown	Industrial strain	×	✓
27	***Staphylococcus sciuri***	Unknown	Industrial strain	×	✓
28	***Staphylococcus simulans***	Human skin	Industrial strain	✓	✓
29	***Staphylococcus warneri***	German salami	Industrial strain	×	✓
30	***Staphylococcus xylosus***	Mettwurst sausage	Industrial strain	✓	✓
31	***Streptococcus lactis***	Milk powder	Industrial strain	✓	✓

"×" indicates false-positive while "✓" indicates successfully suppressed.
NB: 8/9 presumptive positive colonies on BPA were confirmed as coagulase negative.