

NordVal International Certificate

Issued for:	Easy Plate AC for the enumeration of aerobic plate count in a broad range of foods, pet food, animal feed and environmental samples
NordVal No:	059
First approval date:	01 September 2023
Renewal date:	08 September 2025
Valid until:	08 September 2027

Easy Plate AC

Manufactured by:

Kikkoman Biochemifa Company
2-1-1, Nishi-shinbashi, Minato-ku,
Tokyo 105-0003, Japan

fulfils the requirements of the NordVal Validation Protocol 1. The reference method was EN ISO 4833-1:2013 Microbiology of the food chain -- Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30°C by the pour plate technique.

NordVal International has studied the enclosures to the application and evaluated the results obtained in the validations conducted by the expert laboratory Campden BRI. The validations have been carried out according to ISO 16140-2:2016. NordVal International concludes that it has been satisfactorily demonstrated that the results document no difference in the performances between the Easy Plate AC and the reference method.

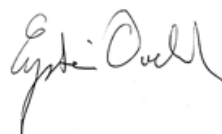
The production of the Easy Plate AC is fulfilling the requirements given in ISO 9001.

Date: 08. September 2025

Yours sincerely,

A handwritten signature in blue ink, reading "Hrólfur Sigurðsson".

Hrólfur Sigurðsson
Chair of NordVal International

A handwritten signature in black ink, reading "Eystein Oveland".

Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

Easy Plate AC is a prepared microbiological culture plate made up of a waterproof sheet, a dry medium on the sheet and a transparent cover over the medium. The Easy Plate AC method is intended to indicate the level of aerobic bacteria in food and beverage products. After incubation at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for $48\text{h} \pm 3\text{h}$, the aerobic colonies are visible as red colonies on the Easy Plate AC growth medium.

FIELD OF APPLICATION

The method is applicable for the enumeration of total aerobic bacteria in a broad range of foods, animal feed and primary production samples.

METHOD COMPARISON STUDY

Relative trueness study

The relative trueness study is a comparative study between the results obtained by the reference method and the results of the alternative method. This study was conducted using naturally or artificially contaminated samples. Different categories, types and items were tested.

A total of 7 categories were included, whereof 15 items for each category were tested by both the reference method and the alternative method. Each category was made up of 3 types, with at least 5 items representative for each type. The categories, the types and the number of samples analysed are presented in **Table 1**.

Table 1. List of categories, types, number of samples analysed and results obtained from testing within the relative trueness study

Category	Types	Items (examples)	Number of samples analysed	Number of samples with interpretable results	ISO 6887 and Diluent used
Milk and dairy products (combined category raw and heat processed Milk and dairy products)	Raw milk and dairy products	Raw milk , raw milk cheese	5	5	6887-5 sodium citrate solution
	Pasteurised milk and milk based products	Milk, ice-cream, milk based drinks	5	5	6887-5 PSD*
	Dry milk products	Milk powder, dessert powder	5	5	6887-5 PSD
	Total		15	15	
Fishery products Combined category: raw, RTE, RTRH, RTC	Raw fish (unprocessed)	Raw salmon fillet, tuna	5	5	6887-3 PSD
	RTE/RTC/RTRH fish and seafoods	Smoked salmon, frozen seafoods	5	5	6887-3 PSD
	Crustaceans	Shrimp, crab	5	5	6887-3 PSD

Category	Types	Items (examples)	Number of samples analysed	Number of samples with interpretable results	ISO 6887 and Diluent used
	Total		15	15	
Produce and fruits (combined category fresh and processed)	Cut ready-to-eat vegetables/leafy greens and sprouts	Bagged pre-cut lettuce shredded carrot	5	5	6887-4 PSD
	Fresh fruit/Cut RTE fruit and vegetable products	Cut fruits, freshly squeezed juice, smoothies	5	5	6887-4 PSD
	Heat treated fruit and vegetables	Pasteurised smoothies/juice, blanched frozen vegetables	5	5	6887-4 PSD
	Total		15	15	
Multi-component foods or meal components	Composite foods with substantial raw ingredients	Chilled pasta salad, sandwiches	5	5	6887-1 PSD
	RTRH/RTE foods (chilled, frozen)	Cooked chilled pasta, frozen fries, rice products,	5	5	6887-1 PSD
	Mayonnaise based deli-salads	Vegetable salad	5	5	6887-1 PSD
	Total		15	15	
Raw and Ready to cook RTC Meat and poultry	Raw poultry and meat cuts	Raw chicken, beef, pork, turkey	5	5	6887-2 PSD
	Raw processed meat	Frozen burger patties, pork meat balls,	5	5	6887-2 PSD
	RTC processed poultry	seasoned chicken, turkey meat balls,	5	5	6887-2 PSD
	Total		15	15	
Pet food and animal feed	Dry Food	Pellets, kibbles, treats	5	5	6887-4 PSD
	Wet food (raw and canned)	Pates, sausages	5	5	6887-2 PSD
	Animal feeds (poultry and fish)	seasoned chicken, turkey meat balls,	5	5	6887-4 PSD
	Total		15	15	
Environmental samples (food or feed production)	Surfaces (wipes, swabs)	Equipment, floors, walls	5	5	6887-1 ISO 18593:2018 PSD
	Process water	Wash water, cooling water	5	5	6887-1 PSD

Category	Types	Items (examples)	Number of samples analysed	Number of samples with interpretable results	ISO 6887 and Diluent used
	Dusts	Bakery and food manufacturing environment	5	5	6887-1 ISO 18593:2018 PSD
	Total		15	15	
Total number of samples			105	105	

*PSD = peptone salt diluent

In total, 105 samples were analysed leading to 105 exploitable results. The mean difference (bias) of the samples obtained by the the alternative method and the reference method, and the precision thereof, expressed as standard deviation, SD, was calculated and a summary is provided in **Table 2**.

Table 2. Summary of the calculated values per category

Category	N	Bias	SD
Environmental samples (food or feed production)	15	-0.282	0.336
Fishery products combined category: raw, RTE RTRH, RTC	15	0.122	0.364
Milk and dairy products (combined category raw and heat processed milk and dairy)	15	-0.167	0.272
Multi-components foods or meal components	15	0.000	0.341
Pet food and animal feed	15	0.110	0.200
Produce and fruits (combined category fresh and processed)	15	-0.066	0.131
Raw and ready to cook RTC meat and poultry	15	-0.126	0.254
All categories	105	-0.058	0.306

Bias: Average difference, SD: standard deviation of differences, N: number of samples

The results are presented in a Bland-Altman plot (**Figure 1**), including the \pm 95% confidence level for the standard deviation of all categories.

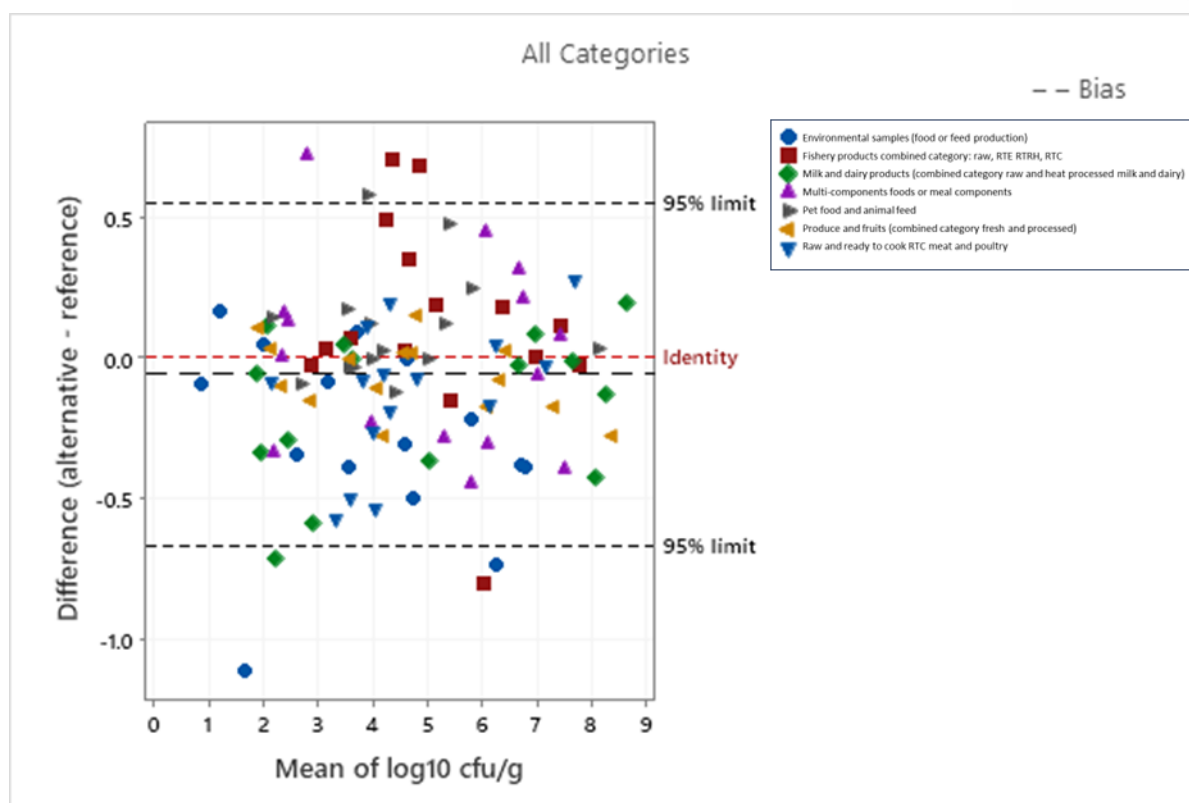


Figure 1. The Bland-Altman plot for all the samples

It is expected that not more than one in 20 data values will lie outside the confidence levels.

For this data set there are 8 in 105 data values which lie outside the levels. This is higher than the expectation, however, there are no trends to the outlying data which represented five of the seven categories.

The samples covered a diverse range of food items with different groups of naturally present organisms present and therefore these results show good agreement between the two methods for enumeration of total aerobic count.

Accuracy profile study

The accuracy profile study is a comparative study between the results obtained by the reference and the results of the alternative method. This study is conducted using artificially contaminated samples. One type per category is tested.

Two samples were contaminated at a low level, 2 at intermediate level, 2 at a high level. For each sample, 5 replicates (5 different test portions) were tested. A total of 30 samples were analysed per food type. The tested categories, types and items in the accuracy profile study are provided in **Table 3**.

All results are tabulated, calculated and interpreted according to ISO 16140-2. The statistical results are shown in Figures 2 to 8.

Table 3. Categories, types, items, strains and inoculation levels for accuracy profile study

Category	Types	Strain	Item	Level	Test portions
Dairy products (combined category; raw milk and heat processed)	Dry dairy products	<i>Bacillus cereus</i> CRA 1724 from Dried milk	Milk powder	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
			Dessert powder	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
Fishery products Combined category: raw, RTE, RTRH, RTC	RTC	<i>Pseudomonas fragi</i> CRA7222 from spoiled fish	Frozen white fish	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
			Chilled tuna steak	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
Produce and fruits (combined category fresh and processed)	Cut ready to eat	<i>E.coli</i> CRA3379 from spinach	Lettuce	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
			Grated carrot	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
Raw and RTC Meat and poultry (Combined category)	Fresh meats	<i>Citrobacter freundii</i> CRA403 from chicken	Raw stir fry beef strips	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
			Chicken breast fillets	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
Multicomponent	Composite foods with raw /processed ingredients	<i>Hafnia alvei</i> CRA7417 from pate	Sandwich	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
			Pasta salad	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
Pet food and animal feed	Wet food (cooked)	<i>Staph aureus</i> CRA 1246 from pork sausage	Dog pate	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
			Cat food with gravy	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
Environmental samples	Process water	<i>Pseudomonas fluorescens</i> CRA 7774 from wash house	Wash water	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5
			Cooling water	Low 10 ²	5
				Medium 10 ⁴	5
				High 10 ⁶	5

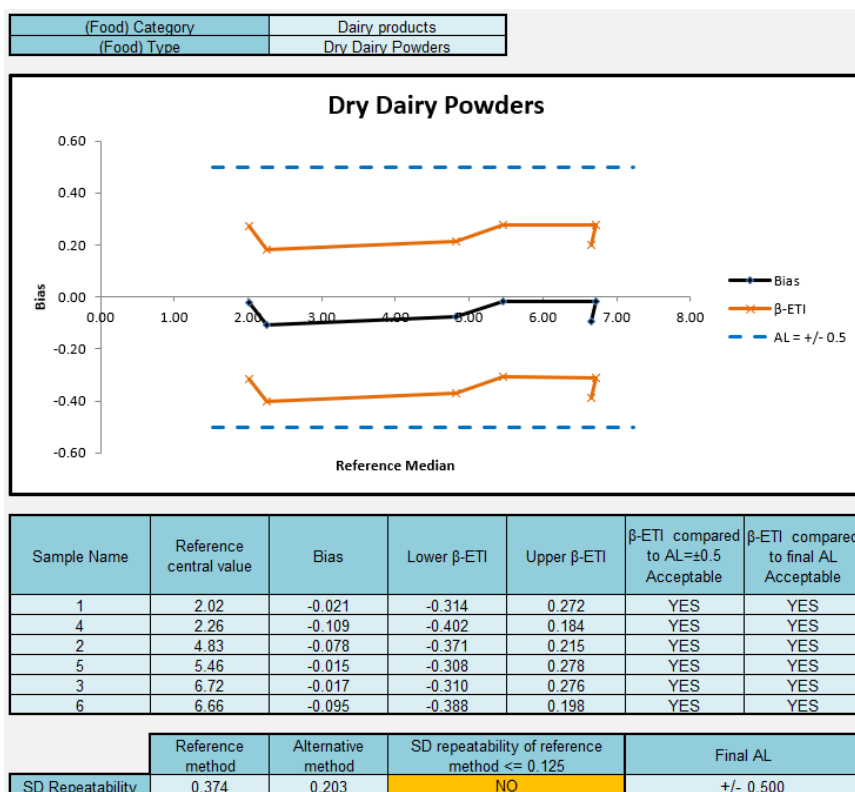


Figure 2. Accuracy profile of dairy products (dry dairy powders) for Easy Plate AC method

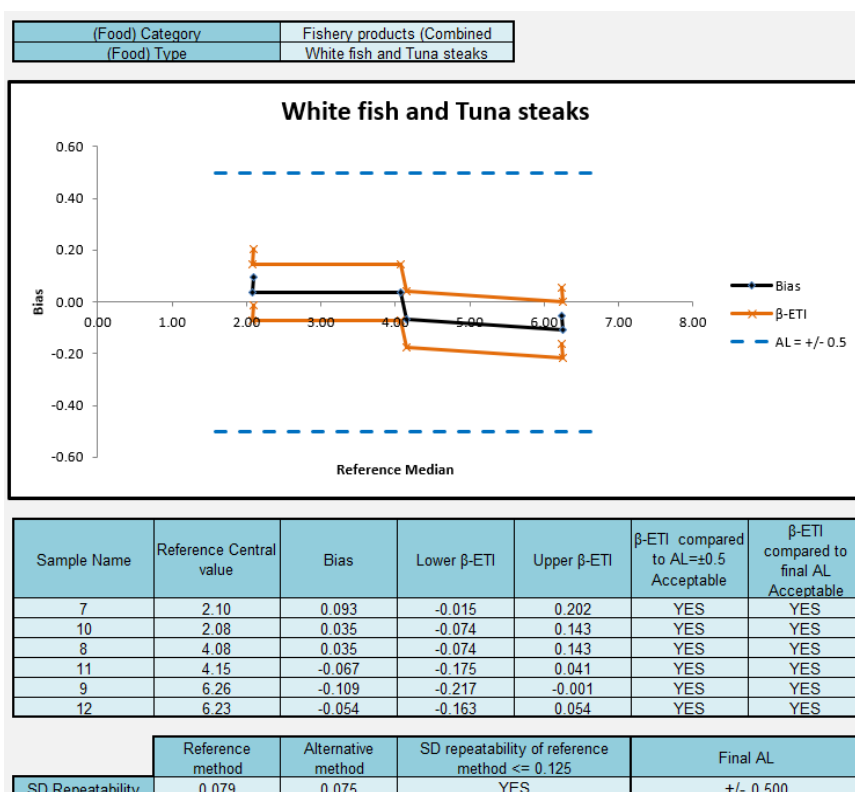


Figure 3. Accuracy profile of Fishery products for Easy Plate AC method

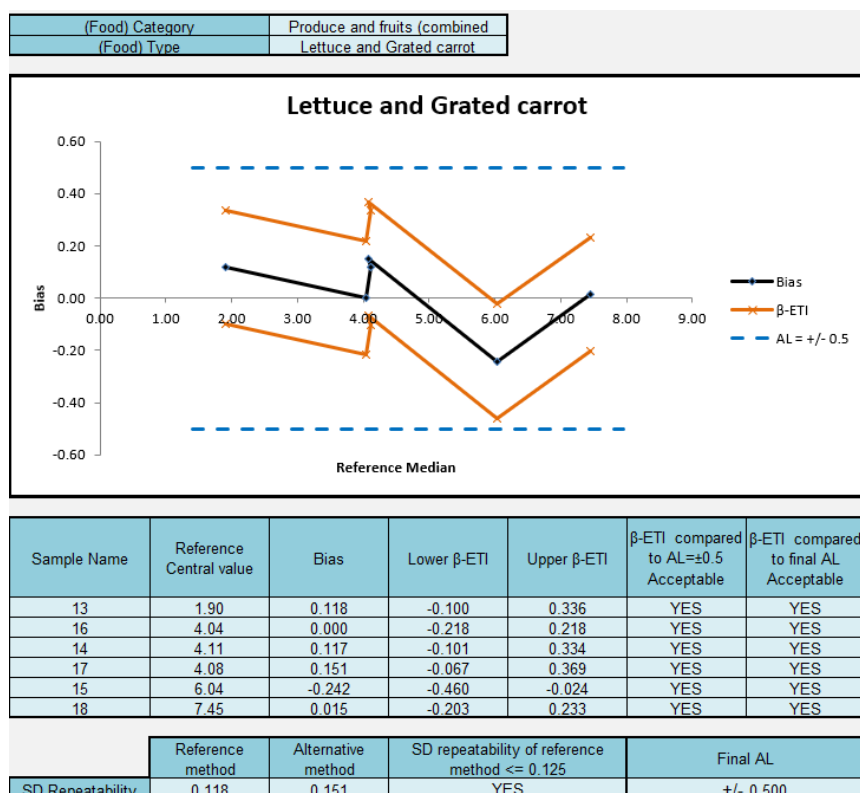


Figure 4. Accuracy profile for Produce and fruits for Easy Plate AC method

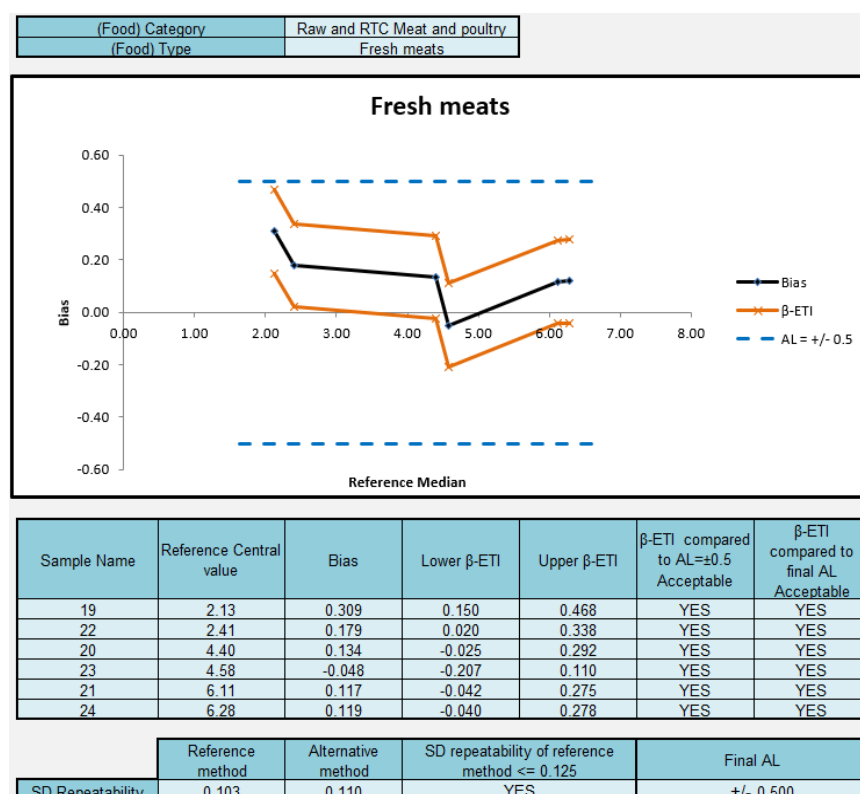


Figure 5. Accuracy profile for Meat and poultry for Easy Plate AC method

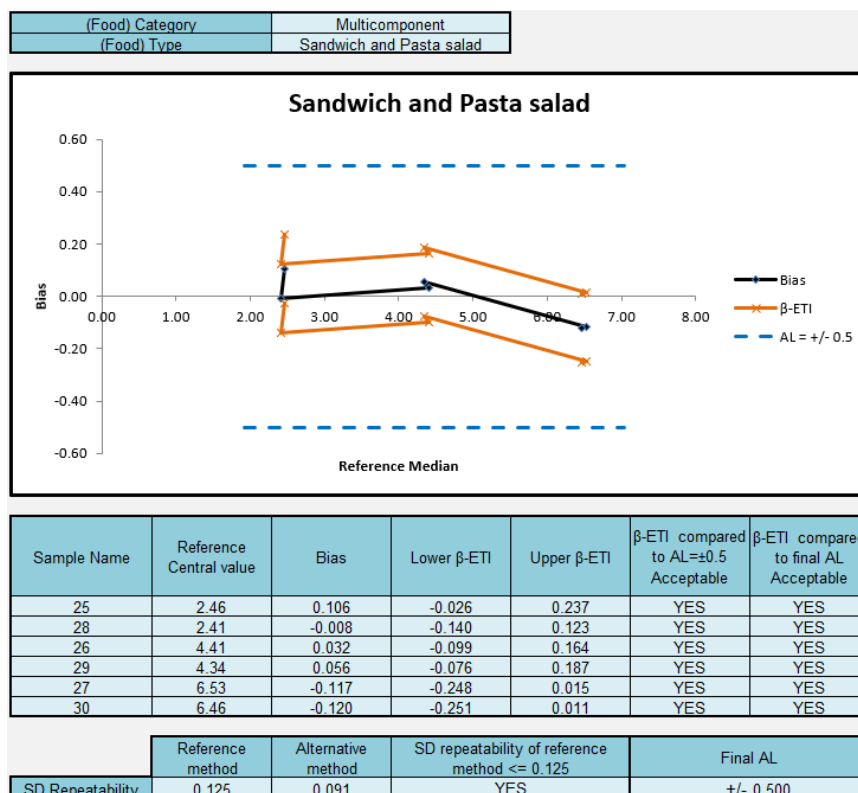


Figure 6. Accuracy profile for Multicomponent for Easy Plate AC method

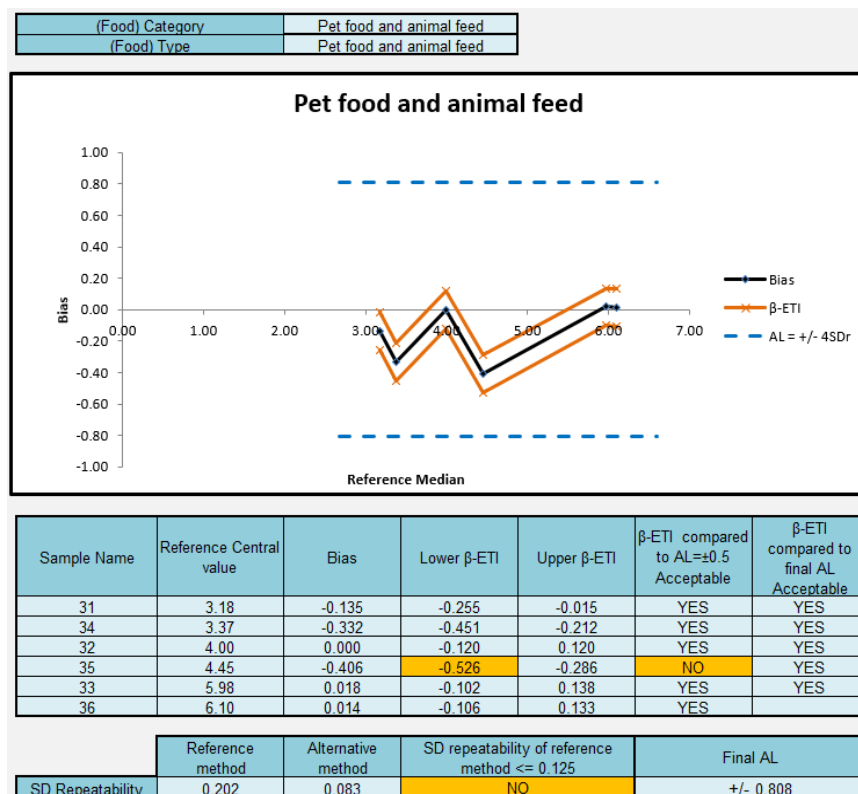


Figure 7. Accuracy profile for Pet food and animal feed for Easy Plate AC method

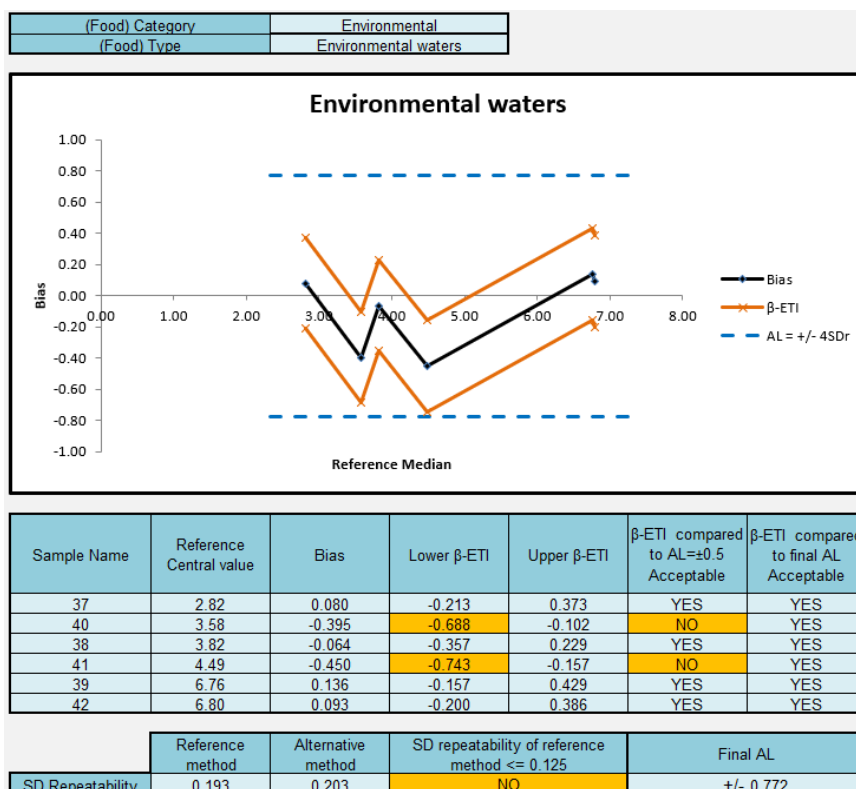


Figure 8. Accuracy profile for Environmental samples for Easy Plate AC method

Five of the seven categories met the AL of 0.5 log (dairy, fresh produce, fish and seafood, multicomponent foods, meat). Two categories (petfood and environmental samples) required the new AL to be calculated. All data met the new AL values of 0.808 and 0.772.

The accuracy of the Alternative method (Easy Plate AC) is satisfied as all categories met the 0.5 log AL or the re-calculated AL.

INTERLABORATORY STUDY

The interlaboratory study is a study performed by multiple collaborators testing identical samples at the same time, the results of which are used to estimate alternative method performance characteristics.

Samples were sent to 12 collaborators. The matrix and strain used in the study was smoked salmon inoculated with *Staphylococcus aureus* CRA 1208 (isolated from smoked fish).

Each collaborator received a set of samples containing 2 samples at a low level, two samples at a medium level, two samples at a high level and a single uninoculated blank sample. The samples were blind-coded so that the collaborators did not know the intended contamination level.

All laboratories delivered valid results. The results are given in **Table 4** and the results of the calculations given in **Table 5**. The results are illustrated in accuracy profiles in **Figure 9**.

Table 4. Summary of the results of the interlaboratory study per analyte level

Collaborator	Level	Reference method (log cfu/g)		Alternative method (log cfu/g)	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	low	2.43	2.53	2.30	2.58
2	low	4.04	2.20	3.86	2.36
3	low	3.90	2.64	3.53	2.63
4	low	3.00	3.54	2.94	3.04
5	low	2.53	2.59	2.48	2.51
6	low	3.11	3.18	3.08	3.18
7	low	3.85	2.87	3.66	2.83
8	low	3.11	4.41	2.74	3.17
9	low	3.57	2.52	3.57	2.26
10	low	4.15	4.00	3.93	3.89
11	low	2.45	2.64	2.34	2.72
12	low	2.43	2.53	2.30	2.58
1	medium	5.78	5.88	5.88	5.80
2	medium	5.67	5.94	5.86	5.99
3	medium	6.04	5.80	5.81	5.81
4	medium	5.73	5.81	5.92	5.83
5	medium	6.04	5.89	6.11	5.94
6	medium	5.89	5.88	5.86	5.92
7	medium	5.76	5.83	5.98	5.79
8	medium	5.85	5.86	5.90	5.88
9	medium	5.81	5.87	5.86	5.79
10	medium	6.00	6.04	6.08	6.00
11	medium	5.69	5.66	5.59	5.72
12	medium	5.78	5.88	5.88	5.80
1	high	6.87	6.93	6.98	6.87
2	high	6.73	6.73	6.87	6.81
3	high	7.11	7.08	7.08	7.15
4	high	6.90	6.71	6.99	6.76
5	high	7.20	7.00	7.18	7.08
6	high	6.93	6.98	6.92	6.92
7	high	6.92	6.68	6.89	6.77
8	high	6.84	6.92	7.00	6.00
9	high	9.11	6.67	9.08	6.85
10	high	7.00	6.99	7.04	6.99
11	high	6.65	6.74	6.77	6.86
12	high	6.87	6.93	6.98	6.87

Table 5. Statistical analysis of the ILS data according to the ISO spreadsheet

Levels	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
Target value	3.070	5.863	6.910	3.070	5.863	6.910
Number of participants (K)	8	8	8	8	8	8
Average for alternative method	2.992	5.884	6.949	3.070	5.863	6.910
Repeatability standard deviation (sr)	0.499	0.085	0.059	0.611	0.103	0.085
Between-labs standard deviation (sL)	0.347	0.109	0.117	0.318	0.081	0.145
Reproducibility standard deviation (sR)	0.608	0.138	0.131	0.689	0.131	0.168
Corrected number of dof	12.985	10.131	8.580	13.904	12.497	9.032
Coverage factor	1.405	1.439	1.465			
Interpolated Student t	1.350	1.371	1.389			
Tolerance interval standard deviation	0.6323	0.1449	0.1381			
Lower TI limit	2.139	5.685	6.757			
Upper TI limit	3.846	6.083	7.140			
Bias	-0.077	0.021	0.039			
Relative Lower TI limit (beta = 80%)	-0.931	-0.178	-0.153			
Relative Upper TI limit (beta = 80%)	0.777	0.220	0.231			
Lower Acceptability Limit	-1.37	-1.37	-1.37			
Upper Acceptability Limit	1.37	1.37	1.37			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.416					

TRUE
value

Select ALL blue lines to draw the accuracy profile as illustrated in the worksheet "Graph Profile"

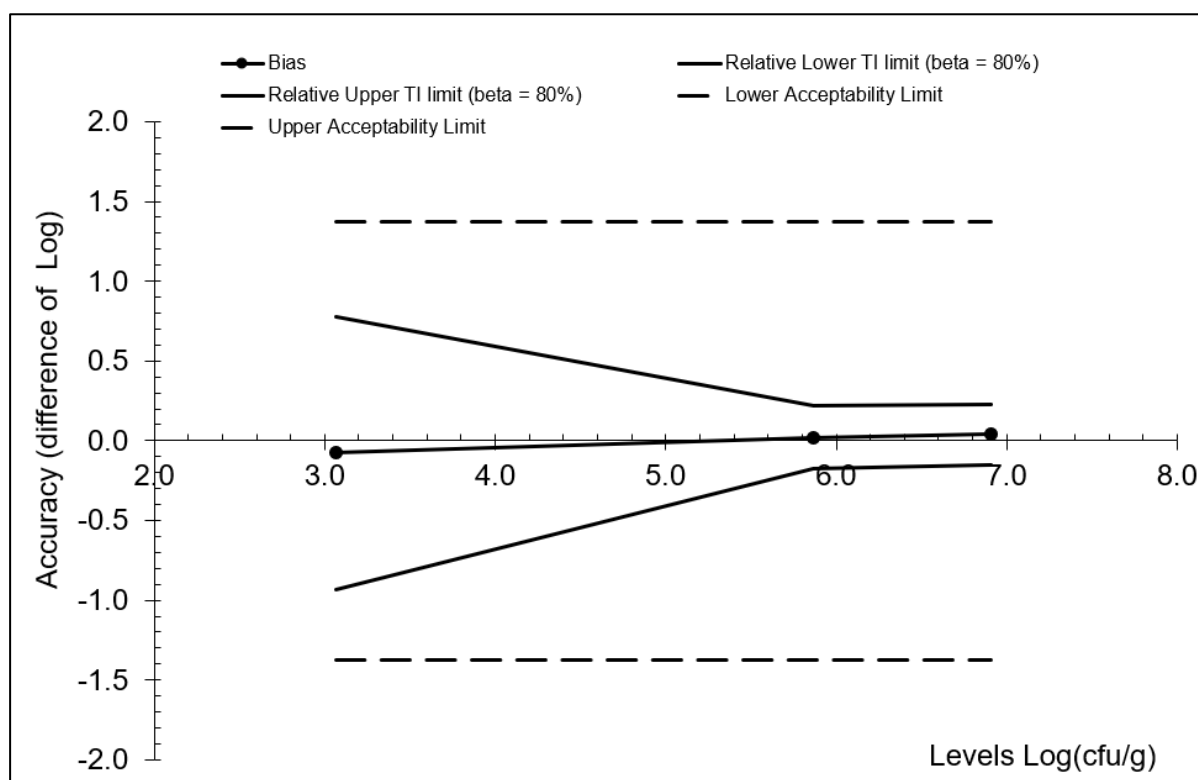


Figure 9. Accuracy profile of Easy Plate AC from the ILS

A review of the accuracy profile and statistical analysis revealed that there was a variability higher than expected in the aerobic plate values observed for the low level of contamination in the ILS samples. A root cause analysis was carried out to determine possible reasons for this. The log difference in the counts obtained in the reference method for the expert lab ranged from -0.22 to 0.12 and was -0.06 and 0.12 for the low level samples respectively. The same batches of media were used for all participants in the study and the time and temperature used for the incubation of the plates was correct.

It was noted that the reproducibility of the low-level samples was significantly higher than at the other levels being 0.611 for the reference method and 0.499 for the Easy Plate AC, compared to <0.1 recorded for both the medium and high count respectively.

Heat treated samples were used during the study due to the high level of background seen in the batch of smoked salmon used for the study. The higher variability in bacterial load could be explained by the difference in kill of the natural flora in the sample that would impact on the level of organisms able to grow at 30°C during the analysis.

In conclusion, the results in the interlaboratory study falls within the acceptability limits, and hence the alternative method show satisfactory performance.

CONCLUSION

According to the comparison and the collaborative study no statistical differences were found between the Easy Plate AC and the reference method for the enumeration of total aerobic bacteria in in a broad range of foods, pet food, animal feed and environmental samples.