

NordVal International Certificate

Issued for:	Easy Plate EC for the enumeration of <i>Escherichia coli</i> and coliforms in a broad range of foods, pet food, animal feed and environmental samples
NordVal No:	061
First approval date:	01 September 2023
Valid until:	01 September 2025

Easy Plate EC

Manufactured by:

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

NordVal International has reviewed the method validation documentation. The validation was conducted by Campden BRI, UK according to ISO 16140-2.

The performance of this has been compared to the reference methods:

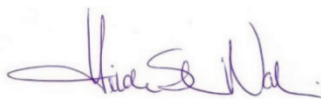
- ISO 16649-2:2001: "Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*. Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide." and
- ISO 4832:2006: "Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coliforms. Colony-count technique."

NordVal International concludes that it has been satisfactorily demonstrated that the data and interpretations comply with the EN ISO 16140-2 requirements and demonstrate comparable performance of the alternative method Easy Plate EC to the ISO reference methods for the enumeration of *Escherichia coli* and coliforms in a broad range of foods, pet food, animal feed and environmental samples.

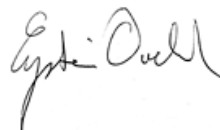
The production of Easy Plate EC is certified according to ISO 9001.

Date: 01 September 2023

Yours sincerely,



Hilde Skår Norli
Chair of NordVal International



Eystein Oveland
NMKL Executive Director



PRINCIPLE OF THE METHOD

Easy Plate EC is a prepared microbiological culture device made up of a waterproof sheet, a dry medium on the sheet and a transparent cover over the medium. The samples are pre-treated according to ISO 6687 or NMKL 91. An aliquot of 1 ml of an appropriate dilution is plated onto Easy Plate EC and incubated for $37 \pm 1^\circ\text{C}$ for $24 \pm 2\text{h}$.

E.coli colonies appear as navy blue/blue purple colonies and coliforms as pink/red purple colonies on the Easy Plate EC growth medium. In this study, the minimum incubation time of 22h was used for the Easy plate EC.

FIELD OF APPLICATION

The method has been tested on enumeration of *Escherichia coli* and coliforms in a broad range of foods, pet food, animal feed and environmental samples.

METHOD COMPARISON STUDY

Selectivity; inclusivity and exclusivity

Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains. Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

Inclusivity *E.coli*

50 pure cultures of *E. coli* were tested with the alternative method, the reference method and a non-selective agar. All 50 *E. coli* strains tested gave typical colonies on the alternate method. 49 out of the 50 *E. coli* strains tested gave typical colonies on the reference method. The level enumerated on the reference method and alternative method were similar with no negative or positive bias shown.

Exclusivity *E.coli*

28 out of the 30 non *E. coli* isolates tested gave the expected result with the reference and alternative methods. Two isolates (*Shigella boydii*, NCTC 11312) and (*Shigella sonnei*, NCTC 10352) gave colonies on both the reference and alternative methods. Further identification work with biochemical identification verified that the isolates were *Shigella* spp and *Shigella sonnei* respectively.

Inclusivity coliforms

50 pure cultures of coliforms were tested with the alternative method, the reference method and a non-selective agar. 46 out of the 50 coliform strains gave typical colonies on the alternate and reference method.

Exclusivity coliforms

31 pure cultures of non-target microorganisms were tested with the alternative method, the reference method and a non-selective agar. For the reference method, 7 strains gave presumptive positive results. For the alternative method, 3 strains gave presumptive positive results.

Conclusion:

The alternative method gave comparable performance to the reference method and is therefore selective and specific to the *E.coli* and to the coliforms.

Relative trueness study

The trueness study is a comparative study between results obtained by the reference method and the results of the alternative method.

Table 1 shows the categories, types and items tested and the ISO standards for the sample preparation used in this study.

Table 1. Categories, types and items tested and the ISO standards used for sample preparation.

Category	Types	Items	ISO
Milk and dairy products (combined category raw and heat processed Milk and dairy products)	Raw milk and dairy products	Raw milk, raw milk cheese	6887-5
	Pasteurised milk and milk based products	Processed cheese, milk based drinks, creams, ice cream, pasteurised skim milk (non-fat milk)	6887-5
	Dry milk products	Milk powders and powder for milk based desserts	6887-5
Fishery products Combined category: raw, RTE, RTRH, RTC	Raw fish (unprocessed)	Raw salmon filet, tuna, bonito	6887-3
	RTE/RTC/RTRH fish and seafoods	Smoked salmon, frozen seafoods, semi-dried fish	6887-3
	Crustaceans	Shrimp, crab	6887-3
Produce and fruits (combined category fresh and processed)	Cut ready-to-eat vegetables/leafy greens and sprouts	Bagged pre-cut lettuce shredded carrot, radish sprouts, alfalfa	6887-4
	Fresh fruit/Cut RTE fruit and vegetable products	Cut fruits, freshly squeezed juice, smoothies	6887-4
	Heat treated fruit and vegetables	Past smoothies/juice, blanched frozen vegetables	6887-4
Multi-component foods or meal components	Composite foods with substantial raw ingredients	Chilled pasta salad, egg and cress sandwich	6887-1, 6887-4
	RTRH/RTE foods (chilled, frozen)	Cooked chilled pasta, frozen fries, rice products, quiche	6887-1, 6887-4
	Mayonnaise based deli-salads	Vegetable salad, egg mayonnaise	6887-1, 6887-4
Raw and Ready to cook RTC Meat and poultry	Raw poultry and meat cuts	Raw chicken, beef, pork, turkey	6887-2
	Raw processed meat	Frozen burger patties, pork meat balls,	6887-2
	RTC processed poultry	seasoned chicken, turkey meat balls,	6887-2
Pet food and animal feed	Dry Food	Pellets, kibbles, treats	6887-4
	Wet food (raw and canned)	Pates, sausages	6887-2
	Animal feeds (poultry and fish)	Cereals and flours	6887-4

Category	Types	Items	ISO
Environmental samples (food or feed production)	Surfaces (wipes, swabs)	Equipment, floors, walls	6887-1, 18593
	Processed water	Wash water, cooling water	6887-1
	Dusts	Bakery and food manufacturing environment	6887-1, 18593

Five samples each three items of each seven categories (equals 15 samples each category) were tested by both the reference method and the alternative method in the relative trueness study.

The difference (bias) between paired samples analysed with the alternative method and the reference method, and the standard deviation thereof, are calculated. The results are provided in **Table 2** and illustrated by Bland-Altman plots. The difference is plotted against the mean values obtained by the reference method. In the plot, Upper and Lower limits are included as the bias \pm about 2 times the standard deviation of the bias.

The Bland-Altman Plot in **Figure 1** and **Figure 2** illustrates the difference obtained in the enumeration of *E.coli* and coliforms, by the alternative and the reference method, respectively.

Table 2. Summary of the calculated values per category for *E.coli* and coliforms

Category	n	<i>E.coli</i>		Coliforms	
		Bias	sD	Bias	sD
Environmental samples (food or feed production)	15	0.198	0.233	0.007	0.324
Fishery products combined category: raw, RTE RTRH, RTC	15	0.067	0.162	-0.128	0.255
Milk and dairy products (combined category raw and heat processed Milk and dairy)	15	-0.039	0.185	0.133	0.144
Multi-components foods or meal components	15	0.211	0.201	0.175	0.213
Pet food and animal feed	15	0.037	0.284	-0.035	0.182
Produce and fruits (combined category fresh and processed)	15	-0.103	0.288	0.075	0.342
Raw and ready to cook RTC meat and poultry	15	0.102	0.316	-0.136	0.286
All Categories	105	0.067	0.261	0.013	0.275

Bias is the average difference between the alternative method and the reference method, SD is the standard deviation, N is the number of samples.

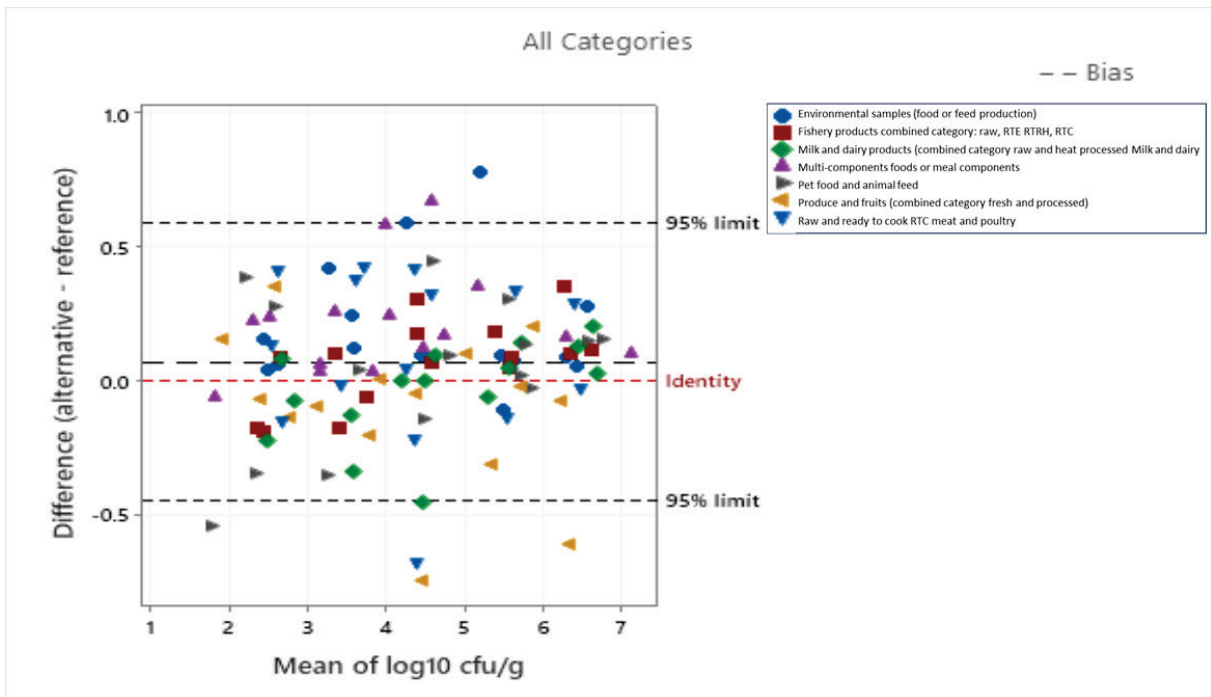


Figure 1. Bland-Altman Plot of the enumeration of E.coli in all the categories tested

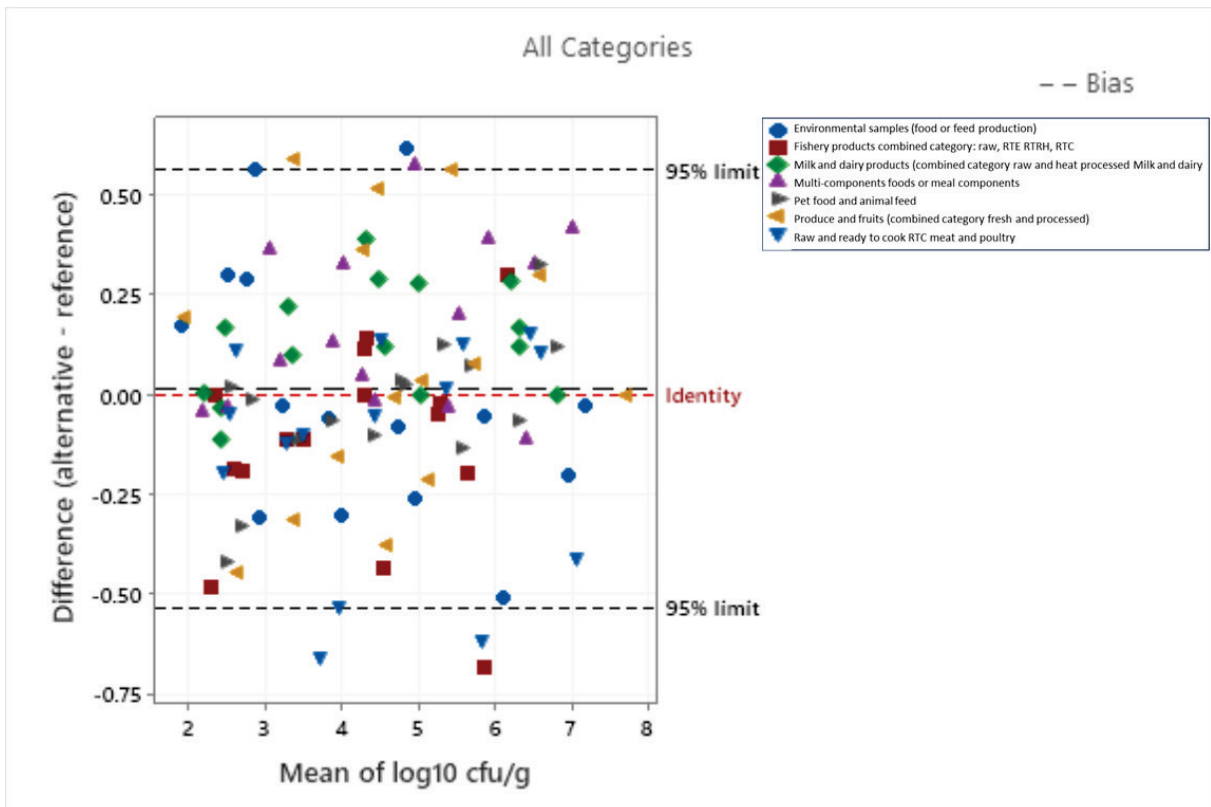


Figure 2. Bland-Altman Plot of the enumeration of coliforms in all the categories tested

In conclusion, it is expected that not more than one in 20 data values will lie outside the confidence limits.

For *E.coli*, 7 in 105 data values lie outside the confidence limits, and for coliforms, 8 in 105 data values lie outside the confidence limits. This is higher than the expectation however, there are no trends to the outlying data which represented six of the seven categories.

The relative trueness of the Alternative method is satisfied as there was a good agreement between the reference method and alternative as shown in the Bland-Altman plot.

ACCURACY PROFILE

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.

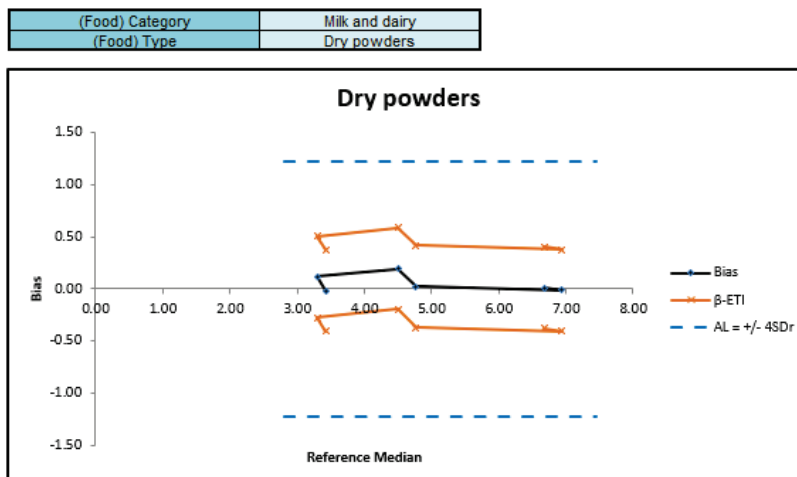
Two samples were contaminated at a low level, 2 at intermediate level, 2 at a high level. For each sample, 5 replicates (5 different test portions) were tested. A total of 30 samples were analysed per food type. The tested categories, types and items in the accuracy profile study are provided in **Table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

Table 3. Categories, types and food items

Category	Type	Items	<i>E.coli</i> (cfu/g)	Coliforms(cfu/g)
Dairy products (combined category; raw milk and heat processed)	Dry dairy products	Milk powder	1500-3000	60-200
			20000-70000	10000-610000
			1500000-250000000	8500000-23000000
		Dessert powder	1000-8000	150-350
			30000-75000	15000-25000
			2000000-90000000	14000000-9000000
Fishery products Combined category: raw, RTE, RTRH, RTC	RTC	Frozen white fish	100-900	15000-35000
			1000-3000	150000-300000
			80000-500000	7000000-16000000
		Chilled tuna steak	200-500	6000-8500
			2000-5000	5000000-1000000
			100000-800000	5000000-15000000
Produce and fruits (combined category fresh and processed)	Cut ready to eat	Lettuce	150-300	300-600
			15000-30000	20000-60000
			3000000-6000000	1500000-5000000
		Spinach	150-400	350-900
			40000-80000	20000-50000
			2000000-4500000	1000000-4000000
Meat and poultry (Combined category)	Fresh meats	Raw ground beef	30-100	300-500
			1000-6000	8000-15000
			90000-300000	100000-600000
		Chicken breast fillets	60-100	300-500
			900-3000	15000-50000
			100000-400000	900000-350000

Category	Type	Items	<i>E.coli</i> (cfu/g)	Coliforms(cfu/g)
Multi-components foods or meal components	Composite foods with raw /processed ingredients	Sandwich	150-350	75-300
			15000-40000	30000-70000
			1500000-3500000	5000000-7000000
		Pasta salad	150-300	150-400
			20000-45000	50000-75000
			2000000-4500000	6000000-9000000
Pet food and animal feed	Wet food (cooked)	Dog pate	100-350	1500-3000
			10000-40000	15000-30000
			200000-900000	800000-1500000
		Cat food with gravy	150-550	1500-3000
			10000-65000	30000-50000
			540000-1500000	1000000-2000000
Environmental samples	Process water	Wash water	200-400	400-900
			20000-400000	6000-20000
			1000000-4000000	1500000-3000000
		Cooling water	150-400	500-1000
			200000-40000	10000-60000
			1500000-4500000	1500000-8000000

All results were tabulated, calculated and interpreted according to ISO 16140-2. The accuracy profiles for the different categories for *E.coli* and coliforms are shown in **Figures 3 to Figure 16**.



Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
2 a-e	3.43	-0.016	-0.409	0.376	YES	YES
5 a-e	3.30	0.114	-0.279	0.507	NO	YES
1 a-e	4.51	0.194	-0.199	0.586	NO	YES
4 a-e	4.77	0.022	-0.371	0.414	YES	YES
3 a-e	6.94	-0.015	-0.408	0.378	YES	YES
6 a-e	6.68	0.009	-0.384	0.402	YES	YES

	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
SD Repeatability	0.306	0.272	NO	+/- 1.224

Figure 3. Accuracy profile of dairy products (combined category; raw milk and heat processed) for Easy Plate EC method for *E.coli*

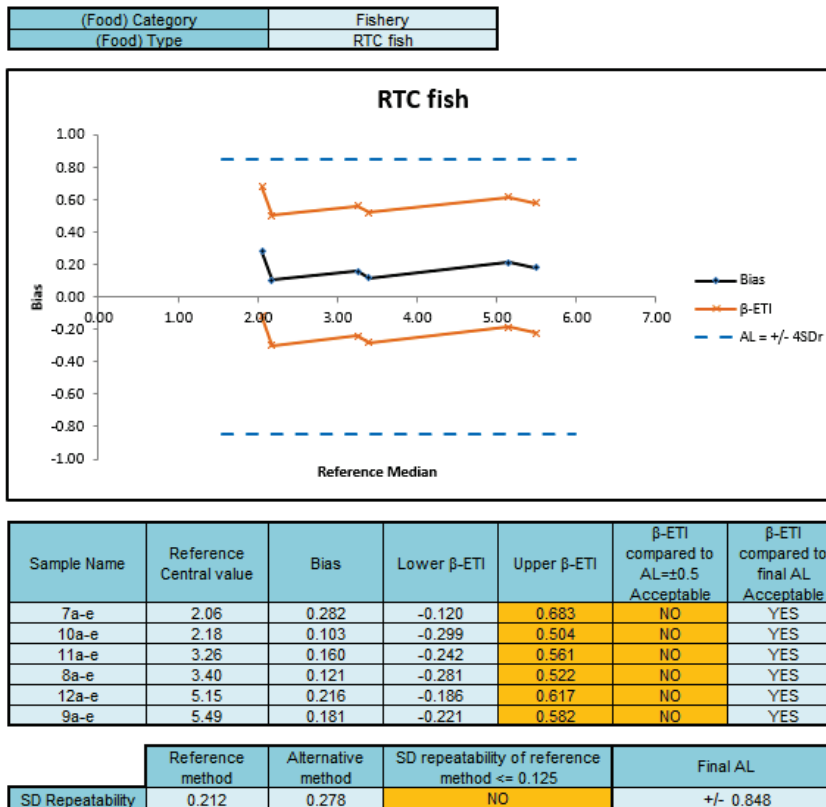


Figure 4. Accuracy profile of Fishery products (Combined category: raw, RTE, RTRH, RTC) for Easy Plate EC method for *E.coli*

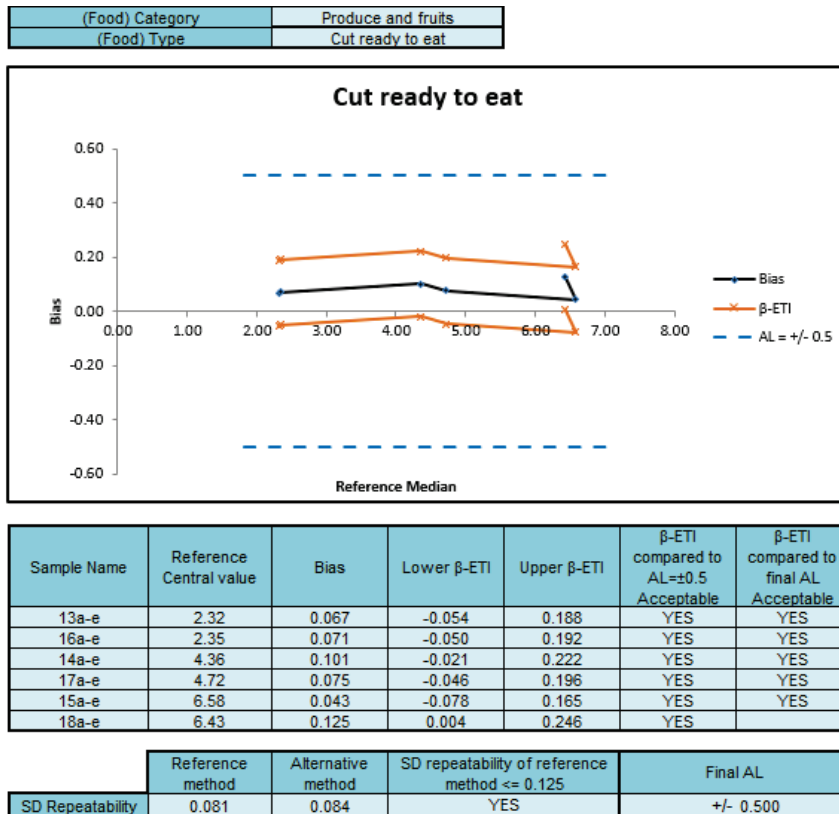


Figure 5. Accuracy profile for Produce and fruits for Easy Plate EC method for *E.coli*

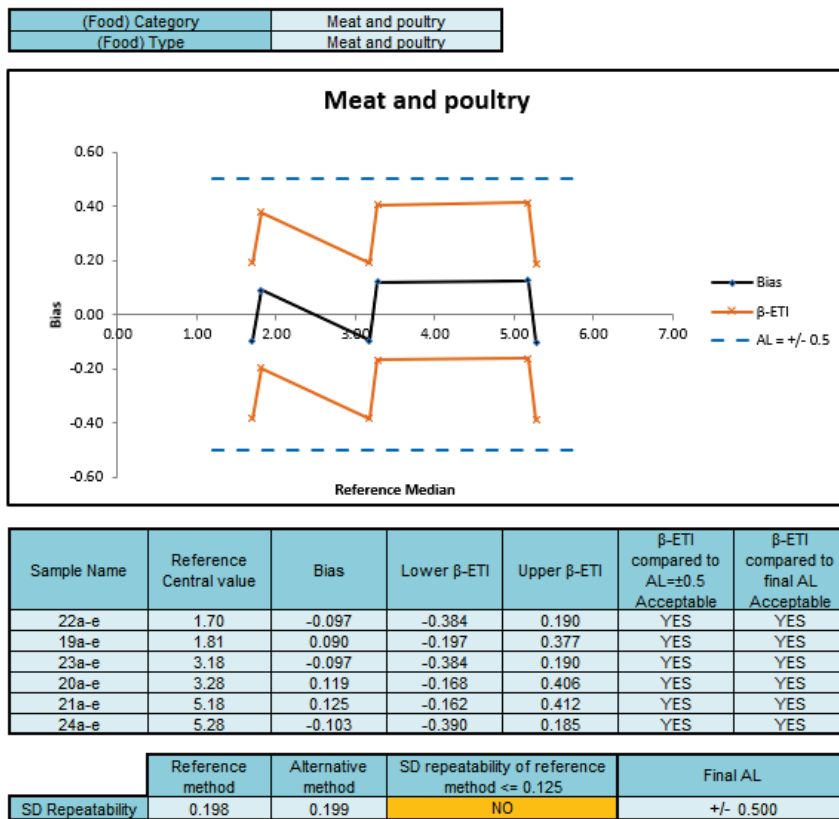


Figure 6. Accuracy profile for Meat and poultry for Easy Plate EC method for *E.coli*

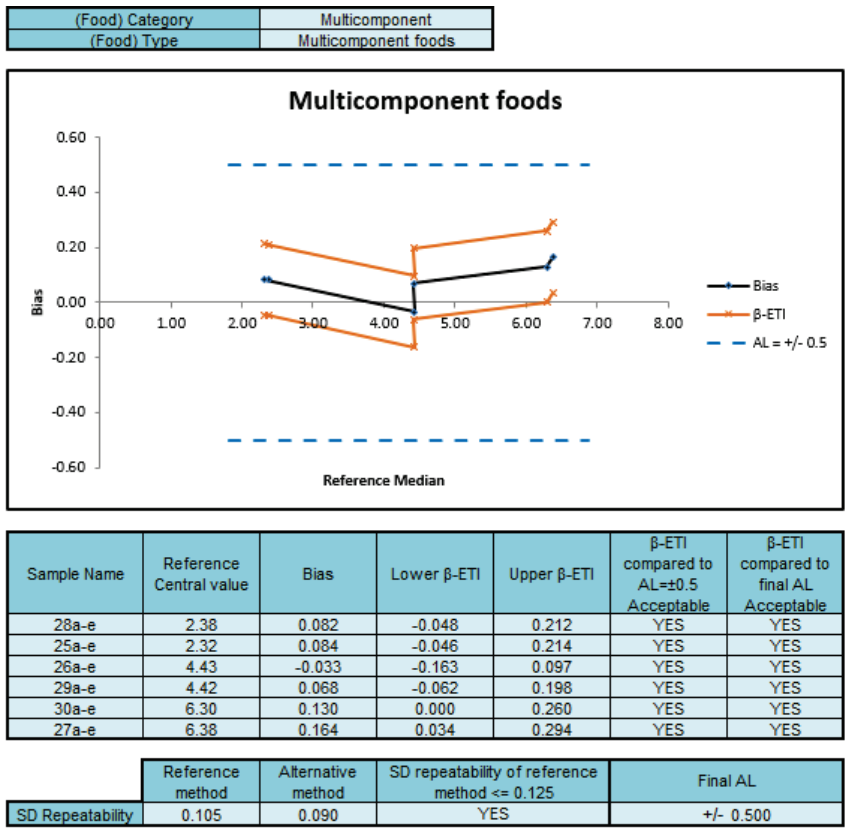


Figure 7. Accuracy profile for Multicomponent foods for Easy Plate EC method for *E.coli*

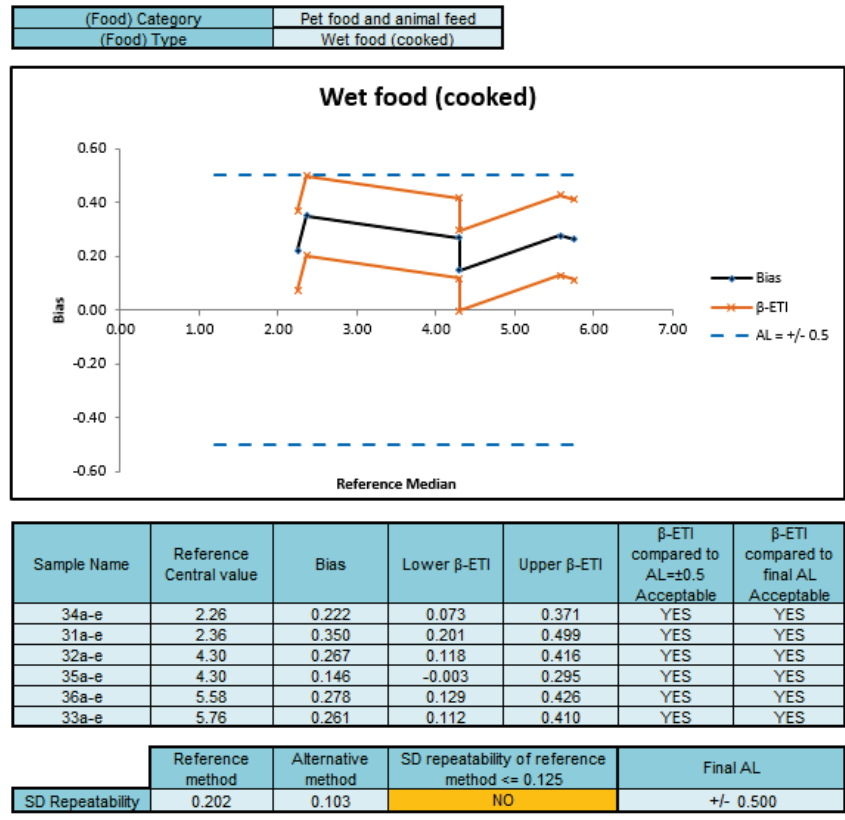


Figure 8. Accuracy profile for Pet food and animal feed for Easy Plate EC method for *E.coli*

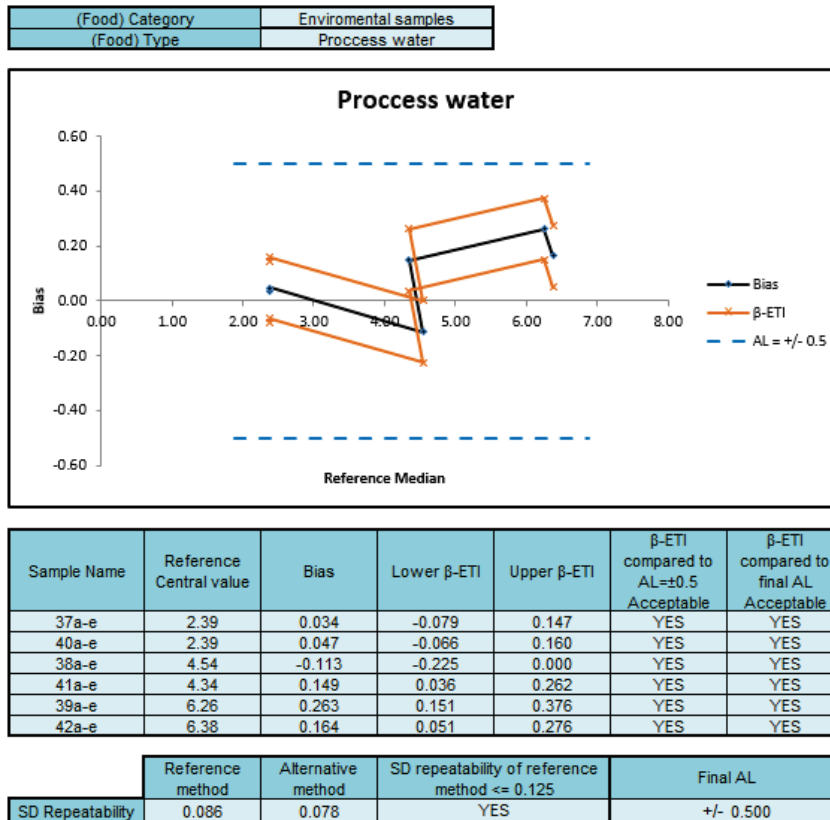


Figure 9. Accuracy profile for Environmental samples for Easy Plate EC method for *E.coli*

In conclusion, the accuracy of the alternative method (Easy Plate EC) is satisfied as all categories met the 0.5log AL or the re-calculated AL for *E.coli*.

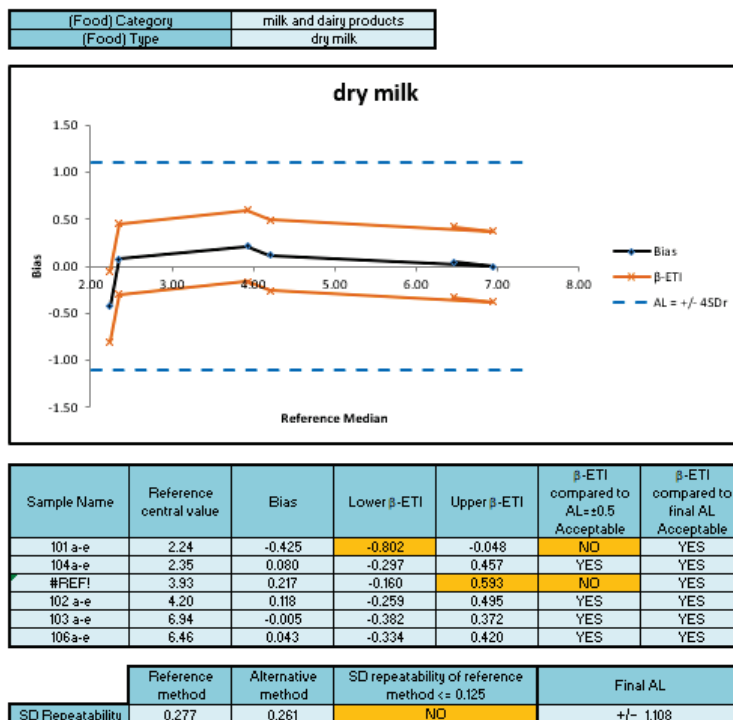


Figure 10. Accuracy profile of dairy products (combined category; raw milk and heat processed) for Easy Plate EC method for coliforms

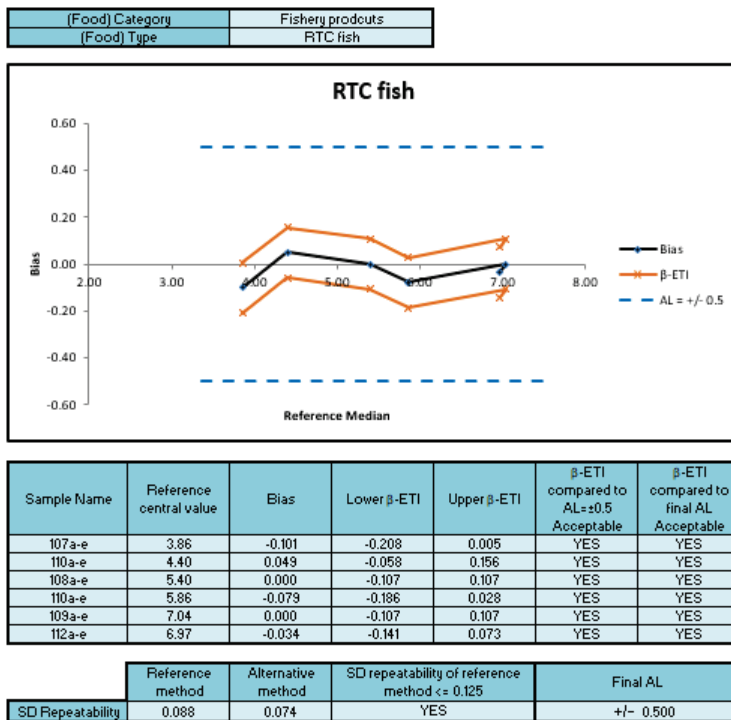


Figure 11. Accuracy profile of Fishery products (Combined category: raw, RTE, RTRH, RTC) for Easy Plate EC method for coliforms

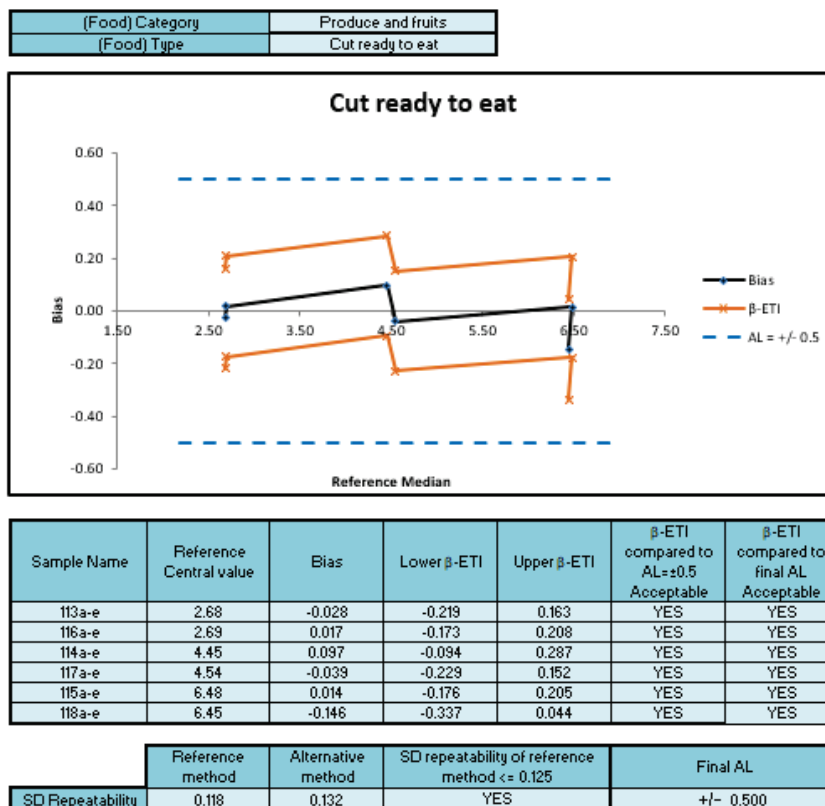


Figure 12. Accuracy profile for Produce and fruits for Easy Plate EC method for coliforms

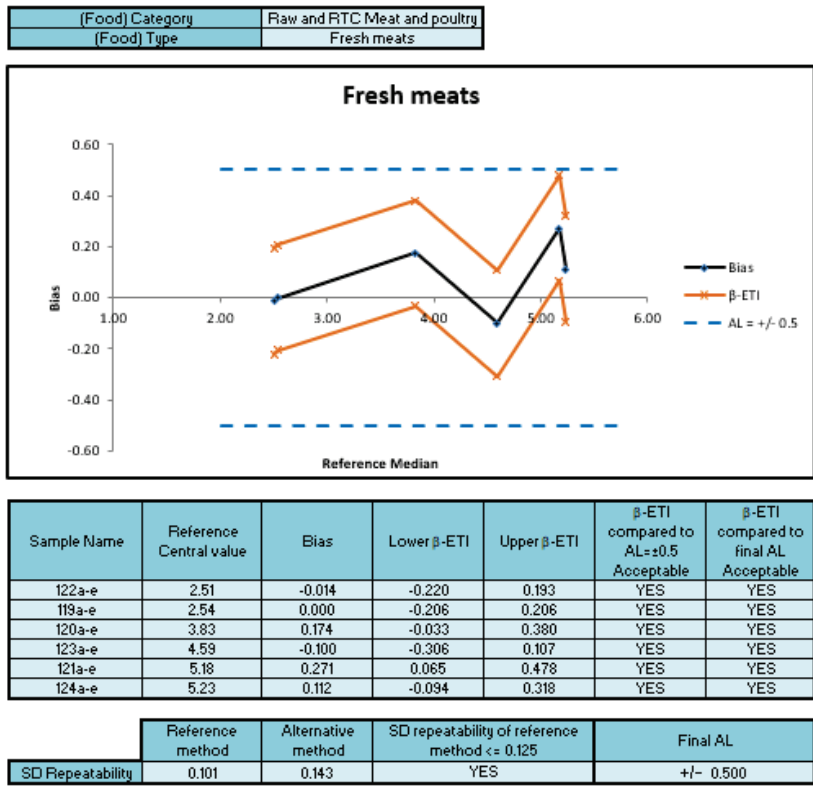


Figure 13. Accuracy profile for Meat and poultry for Easy Plate EC method for coliforms

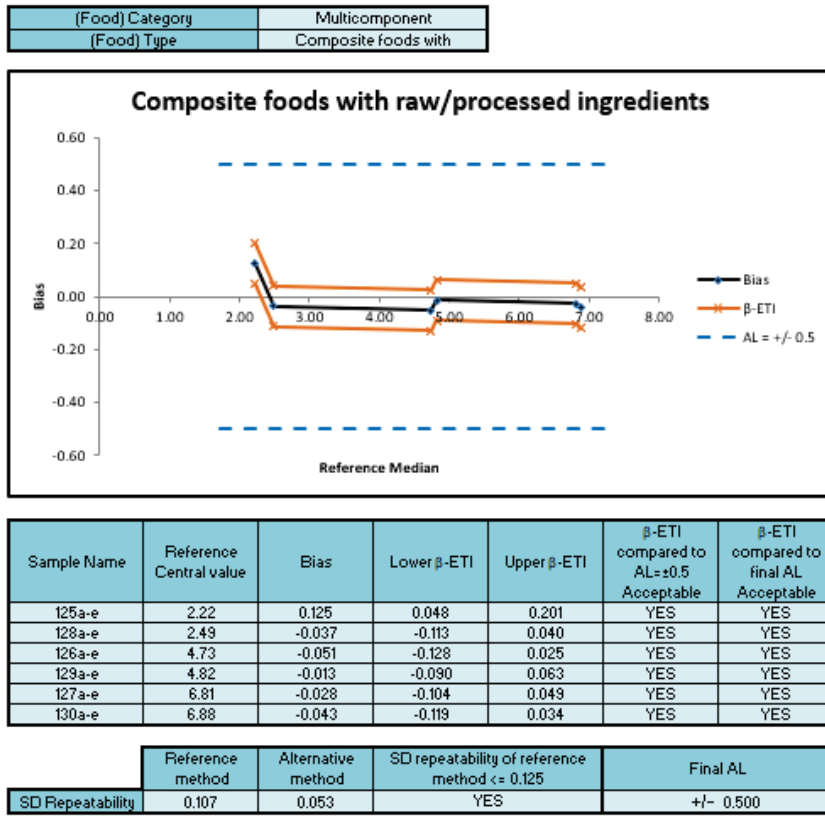
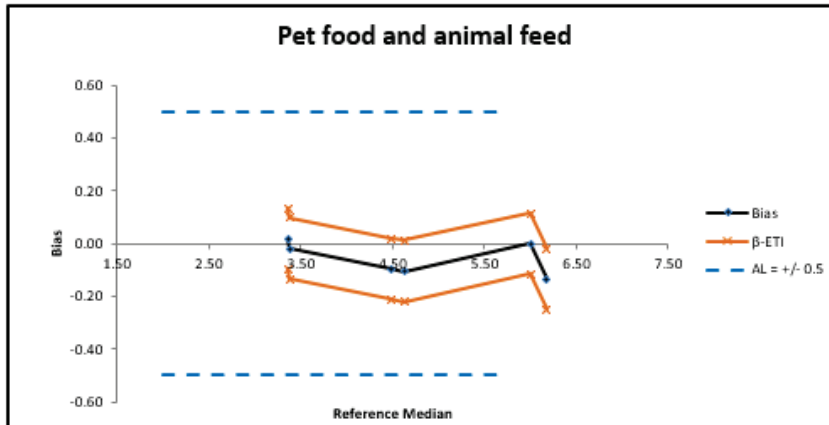


Figure 14. Accuracy profile for Multicomponent foods for Easy Plate EC method for coliforms

(Food) Category	Pet food and animal feed
(Food) Type	Pet food and animal feed

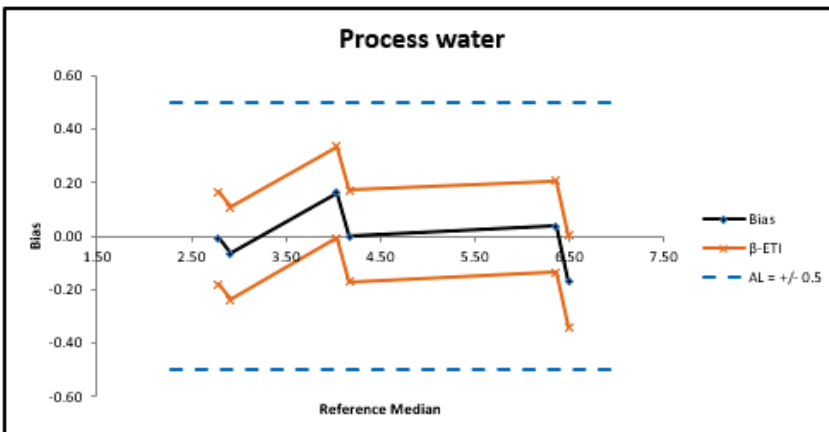


Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
131a-e	3.36	0.018	-0.097	0.134	YES	YES
134a-e	3.38	-0.018	-0.134	0.097	YES	YES
132a-e	4.48	-0.097	-0.212	0.019	YES	YES
135a-e	4.62	-0.105	-0.220	0.011	YES	YES
133a-e	6.00	0.000	-0.115	0.115	YES	YES
136a-e	6.18	-0.135	-0.250	-0.019	YES	YES

SD Repeatability	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
	0.107	0.080	YES	+/- 0.500

Figure 15. Accuracy profile for Pet food and animal feed for Easy Plate EC method for coliforms

(Food) Category	Environmental samples
(Food) Type	Process water



Sample Name	Reference Central value	Bias	Lower β -ETI	Upper β -ETI	β -ETI compared to AL=±0.5 Acceptable	β -ETI compared to final AL Acceptable
137a-e	2.79	-0.007	-0.179	0.165	YES	YES
140a-e	2.92	-0.065	-0.237	0.107	YES	YES
138a-e	4.04	0.163	-0.009	0.335	YES	YES
141a-e	4.18	0.000	-0.172	0.172	YES	YES
139a-e	6.36	0.036	-0.136	0.208	YES	YES
142a-e	6.49	-0.169	-0.341	0.003	YES	YES

SD Repeatability	Reference method	Alternative method	SD repeatability of reference method ≤ 0.125	Final AL
	0.214	0.119	NO	+/- 0.500

Figure 16. Accuracy profile for Environmental samples for Easy Plate EC method for coliforms

In conclusion, the accuracy of the alternative method (Easy Plate EC) is satisfied as all categories met the 0.5log AL or the re-calculated AL for coliforms.

This fulfils the performance criteria, and the alternative method is accepted as being equivalent to the reference method.

INTERLABORATORY STUDY

The inter-laboratory 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 10 laboratories. Smoked salmon inoculated with *Escherichia coli* CRA 108 (isolated from salmon fish cakes) and *Citrobacter diversus* CRA 7119 (an industrial isolate) were used as matrix. For each collaborator, a set of samples was prepared 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. All laboratories delivered valid results. The results are given in Table 4 and 5 and illustrated in accuracy profiles in Figure 4 and 5.

Table 4. Summary of the interlaboratory study for Easy Plate EC (*E.coli*)

Level (log cfu/g)	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
Average	2.61	4.66	5.78	2.32	4.43	5.53
Repeatability standard deviation, sr	0.109	0.108	0.101	0.236	0.319	0.166
Reproducibility standard deviation, sR	0.200	0.234	0.390	0.335	0.361	0.343
Bias	0.29	0.23	0.26			
Lower AL	-1.14	-1.14	-1.14			
Upper AL	1.14	1.14	1.14			

Table 5. Summary of the interlaboratory study for Easy Plate EC (coliforms)

Level (log cfu/g)	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
Average	2.74	4.80	5.95	2.41	4.53	5.73
Repeatability standard deviation, sr	0.090	0.304	0.078	0.333	0.214	0.153
Reproducibility standard deviation, sR	0.149	0.338	0.252	0.381	0.344	0.322
Bias	0.33	0.27	0.23			
Lower AL	-1.15	-1.15	-1.15			
Upper AL	1,15	1,15	1,15			

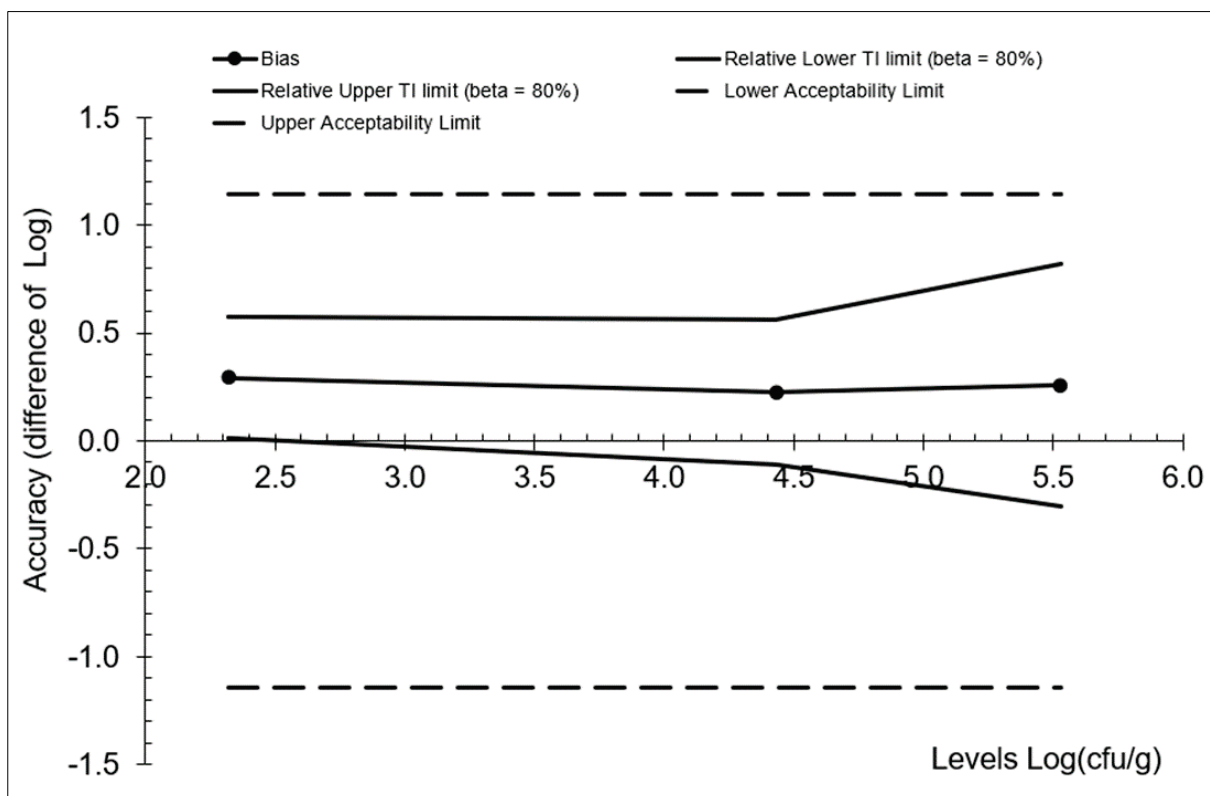


Figure 17. Accuracy profile for Easy Plate EC (*E. coli*) from the interlaboratory study

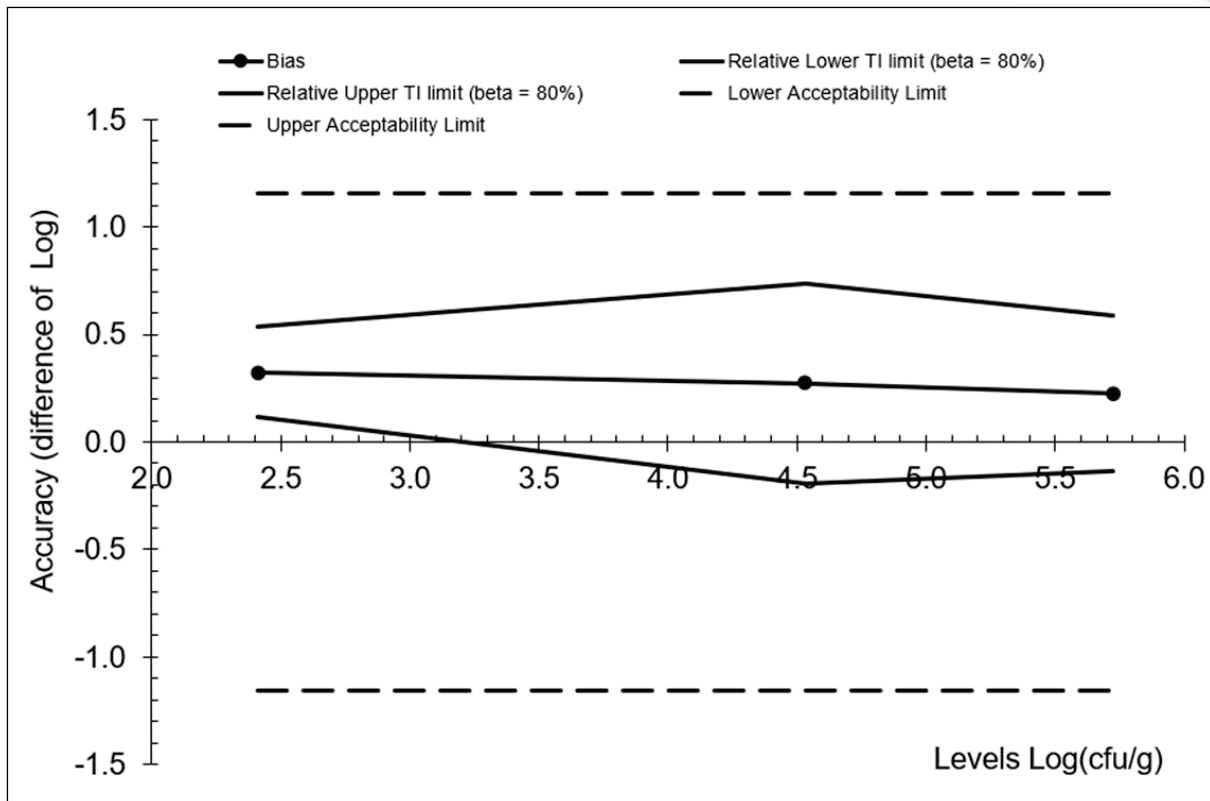


Figure 18. Accuracy profile for Easy Plate EC (coliforms) from the interlaboratory study

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

CONCLUSION

The method comparison study and the interlaboratory study performed according to ISO 16140-2, show that the alternative method Easy Plate EC for enumeration of enumeration of *Escherichia coli* and coliforms in a broad range of foods, pet food, animal feed and environmental samples provide equivalent results to the reference methods.