



# NordVal International Certificate

Issued for:	<b>Easy Plate CC for the enumeration of coliforms in a broad range of foods</b>
NordVal No:	060
First approval date:	1 September 2023
Valid until:	1 September 2025

## Easy Plate CC

### Manufactured by:

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

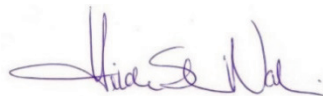
fulfils the requirements of the NordVal Validation Protocol 1. The reference method was EN ISO 4832:2006 Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coliforms - Colony Count Method for coliforms.

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 results document no difference in the performances between the Easy Plate CC and the reference method.

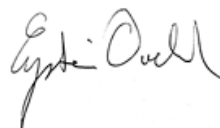
The production of the Easy Plate CC is fulfilling the requirements given in ISO 9001.

Date: 01 September 2023

Yours sincerely,

A handwritten signature in purple ink, appearing to read 'Hilde Skår Norli'.

Hilde Skår Norli  
Chair of NordVal International

A handwritten signature in black ink, appearing to read 'Eystein Oveland'.

Eystein Oveland  
NMKL Executive Director

## PRINCIPLE OF THE METHOD

Easy Plate CC is a microbiological culture device made up of a waterproof sheet, a readymade dry medium on the sheet and a transparent cover over the medium. The Easy Plate CC method is intended to indicate the level of coliform bacteria in food and beverage products. After incubation at 37°C for 24h ±2h, coliform colonies grow as blue colonies on the growth medium of the Easy plate CC plate.

## FIELD OF APPLICATION

The method is applicable for the enumeration of total coliforms in a broad range of foods.

## 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 5 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	No of samples	ISO 6887
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	6887-5
	Pasteurised milk and milk based products	Processed cheese, milk based drinks, creams, ice cream, pasteurised skim milk (non-fat milk)	5	6887-5
	Dry milk products	Milk powders and powder for milk based desserts	5	6887-5
Fishery products Combined category: raw, RTE, RTRH, RTC	Raw fish (unprocessed)	Raw salmon filet, tuna, bonito	5	6887-3
	RTE/RTC/RTRH fish and seafoods	Smoked salmon, frozen seafoods, semi-dried fish	5	6887-3
	Crustaceans	Shrimp, crab	5	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	5	6887-4
	Fresh fruit/Cut RTE fruit and vegetable products	Cut fruits, freshly squeezed juice, smoothies	5	6887-4

Category	Types	Items	No of samples	ISO 6887
	Heat treated fruit and vegetables	Past smoothies/juice, blanched frozen vegetables	5	6887-4
Multi-component foods or meal components	Chilled pasta salad, egg and cress sandwich	Chilled pasta salad, egg and cress sandwich	5	6887-1, 6887-4
	Cooked chilled pasta, frozen fries, rice products, quiche	Cooked chilled pasta, frozen fries, rice products, quiche	5	6887-1, 6887-4
	Vegetable salad, egg mayonnaise	Vegetable salad, egg mayonnaise	5	6887-1, 6887-4
Raw and Ready to cook RTC Meat and poultry	Raw poultry and meat cuts	Raw chicken, beef, pork, turkey	5	6887-2
	Raw processed meat	Frozen burger patties, pork meat balls,	5	6887-2
	RTC processed poultry	seasoned chicken, turkey meat balls,	5	6887-2

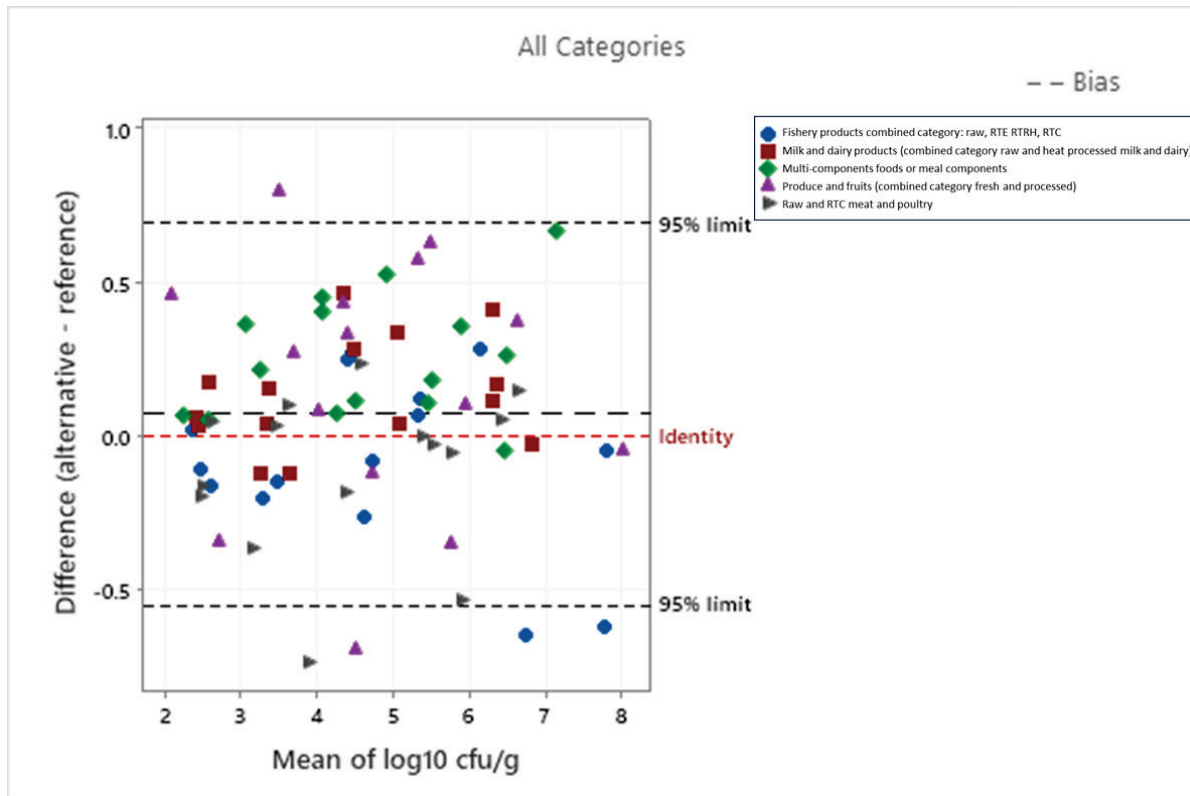
In total, 75 samples were analysed leading to 75 exploitable results. The mean difference (bias) of the samples obtained by the the alternative method and the reference method, and the precision thereof, expressed as standard deviation, SD, was calculated and a summary is provided in **Table 2**.

**Table 2. Summary of the calculated values per category**

Category	N	Bias	SD
Fishery products combined category: raw, RTE RTRH, RTC	15	-0.097	0.286
Milk and dairy products (combined category raw and heat processed milk and dairy)	15	0.133	0.178
Multi-components foods or meal components	15	0.253	0.202
Produce and fruits (combined category fresh and processed)	15	0.163	0.416
Raw and RTC meat and poultry	15	-0.110	0.264
<b>All Categories</b>	<b>75</b>	<b>0.071</b>	<b>0.309</b>

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 5 in 75 data values which lie outside the levels. This is higher than the expectation however, there are no trends to the outlying data.

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

### 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**.

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

Category	Types	Strain	Item	Target Level* cfu/g	Test portions
Milk and dairy products (combined)	Pasteurised dairy products	<i>Leclercia adecarboxylata</i> (previously known as	Pasteurised cream	100-250	5
				10000-20000	5
				1000000-3000000	5

Category	Types	Strain	Item	Target Level* cfu/g	Test portions
category raw and heat processed Milk and dairy products)		<i>Escherichia adecarboxylata</i> ) CRA 5501 Isolated from skimmed milk powder	Cream cheese	80-250	5
				10000-20000	5
				800000-2000000	5
Produce and fruits (combined category fresh and processed)	Fresh produce	<i>Enterobacter taylorae</i> CRA 7530 isolated from seeds	Ready to cook Vegetable preparation	200-300	5
				20000-30000	5
				2000000-5500000	5
			Vegetable juice	200-500	5
				30000-50000	5
				3000000-5500000	5
Raw poultry and meats (Combined category raw/ RTC meats and poultry)	Fresh meat	<i>Escherichia fergusonii</i> CRA 7522 Isolated from sausages	Pork mince	100-200	5
				9500-36000	5
				600000-2500000	5
			Raw bacon	150-250	5
				1000-13000	5
				820000-1500000	5
Fishery products Combined category: raw, RTE, RTRH, RTC	Cooked fish products e.g. prawns	<i>Leliottia amingena</i> , previously known as ( <i>Enterobacter amingenus</i> ) NCIMB 2118 Isolated from seawater	Fresh prawns	200-400	5
				10000-60000	5
				1500000-4500000	5
			Fish pate	250-400	5
				25000-40000	5
				1900000-4000000	5
Multi-component foods or meal components	Composite foods with raw ingredients	<i>Escherichia hermanii</i> CRA 7477 Isolated from sesame seeds	Sandwiches	400-700	5
				10000-60000	5
				7000000-1800000	5
			Cooked chilled rice	200-400	5
				15000-55000	5
				300000-700000	5

All results are tabulated, calculated and interpreted according to ISO 16140-2. The statistical results are shown in **Figures 2 to 6**.

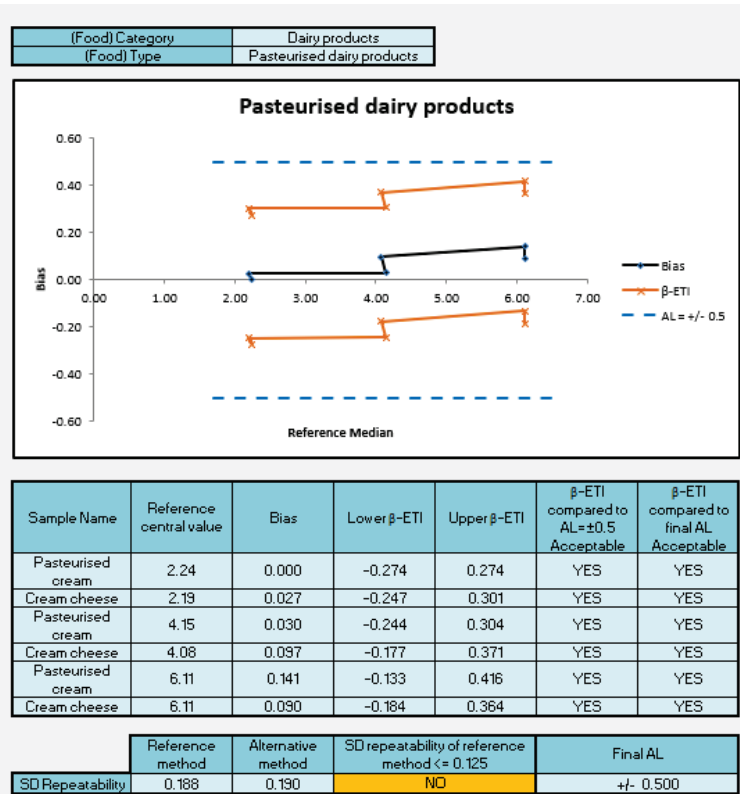


Figure 2. Accuracy profile of dairy products (combined category; raw milk and heat processed) for Easy Plate CC method

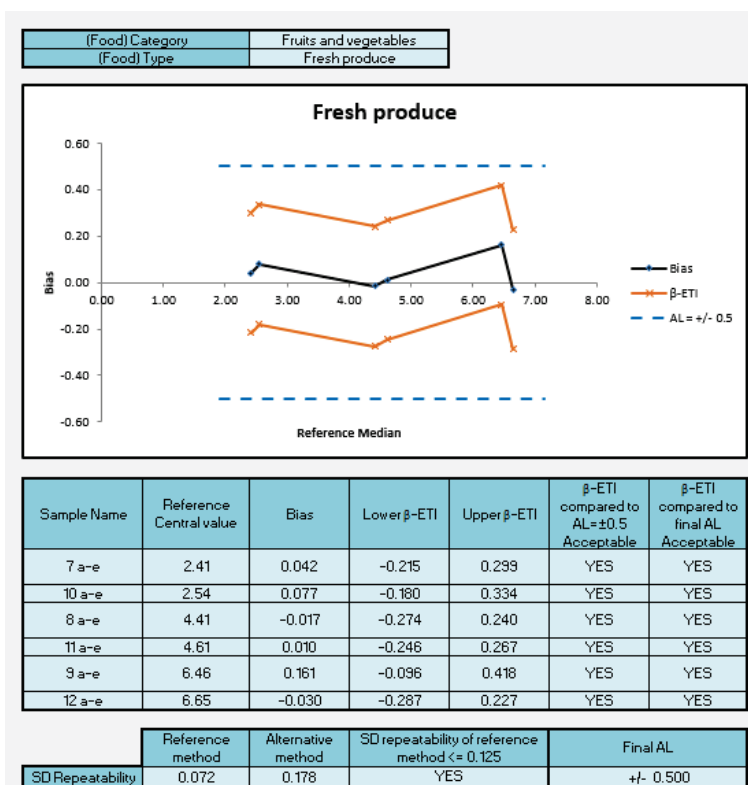


Figure 3. Accuracy profile for Fruit and vegetables for Easy Plate CC method

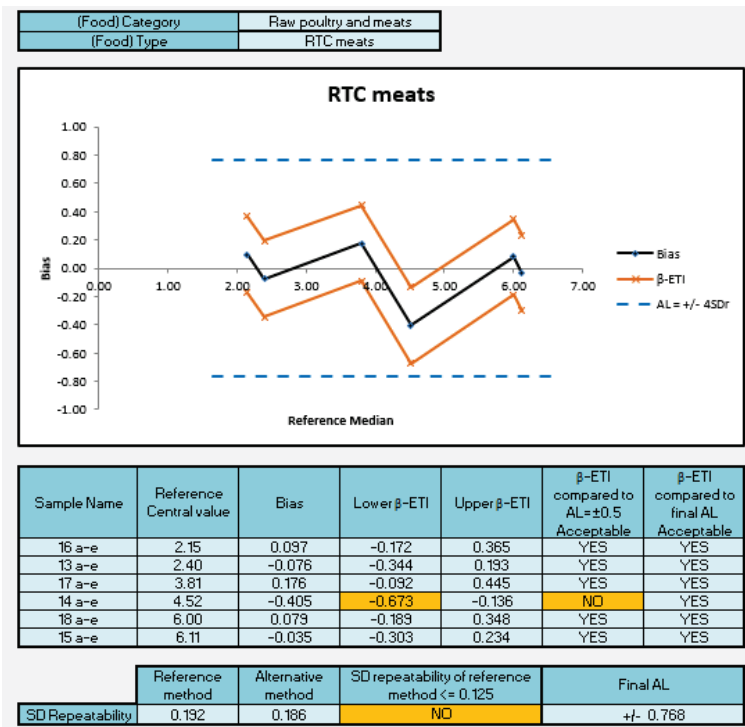


Figure 4. Accuracy profile for Meat and poultry for Easy Plate CC method

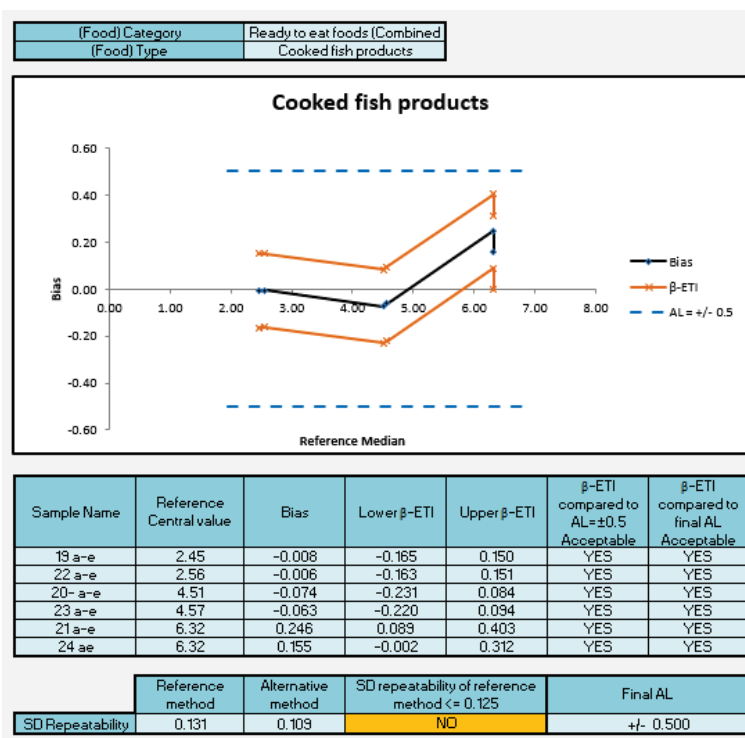
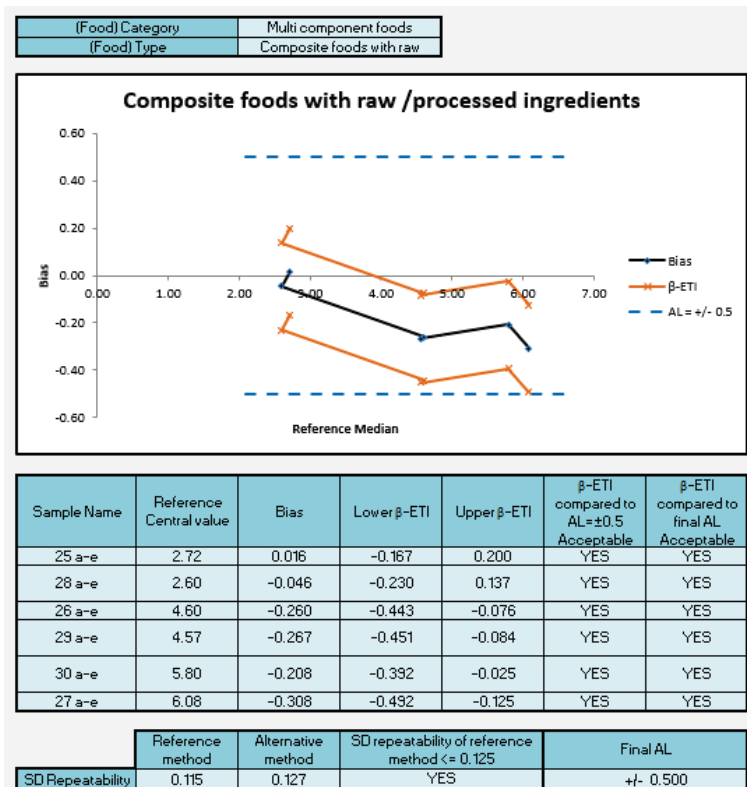


Figure 5. Accuracy profile of Fishery products (Combined category: raw, RTE, RTRH, RTC) for Easy Plate CC method



**Figure 6. Accuracy profile for Multicomponent foods for Easy Plate CC method**

Four of the five categories met the acceptability level, AL, of 0.5 log. One category (raw meat and poultry) required the new AL to be calculated. All data met the new AL value of 0.768.

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

### Selectivity of the method (Inclusivity/exclusivity)

**Inclusivity** is the ability of an alternative method to detect the target analyte from a wide range of strains. 50 out of the 54 isolates were correctly identified following the alternative method. Three out of the four isolates giving unexpected results in the inclusivity panel were confirmed to be negative for BGLBB, indicating that they should be considered to be atypical coliforms. Taking this into account, 49 out of the 50 typical coliforms were correctly identified following the alternative method.

**Exclusivity** is the lack of interference from a relevant range of non-target strains of the alternative method. In this study, 28 out of the 31 isolates in the exclusivity panel gave the expected results with the alternative method.

The alternative method gave comparable performance to the reference method and is therefore selective and specific to the coliform group.





## 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 10 collaborators. The matrix and strain used in the study was smoked salmon inoculated with *Escherichia coli* CRA 108 (isolated from salmon fish cakes) and *Citrobacter diversus* CRA 7119 (an industrial isolate).

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 7**.

**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	2.5	2.0	2.7	2.4
2	low	2.6	2.0	2.7	2.6
4	low	2.6	2.4	2.8	2.9
5	low	2.4	2.4	2.9	2.6
6	low	2.7	2.6	2.9	2.7
7	low	3.4	2.4	2.8	2.7
9	low	2.3	2.0	2.7	2.5
10	low	2.2	1.5	2.6	2.4
11	low	2.4	2.6	2.8	2.9
12	low	2.5	2.0	2.7	2.4
1	medium	4.5	4.4	4.7	4.6
2	medium	4.3	4.4	4.4	4.7
4	medium	4.5	4.4	4.8	4.9
5	medium	4.5	4.7	4.8	5.0
6	medium	4.7	4.7	4.9	4.9
7	medium	4.4	4.5	4.8	4.9
9	medium	4.3	4.1	4.6	4.5
10	medium	4.0	4.3	4.6	4.6
11	medium	4.6	4.8	4.9	4.8
12	medium	4.5	4.4	4.7	4.6
1	high	5.6	5.6	5.7	5.6
2	high	5.8	5.6	5.6	5.7
4	high	5.9	5.9	6.1	6.1
5	high	6.0	6.5	6.5	6.5
6	high	6.4	6.3	6.2	6.2
7	high	5.7	5.6	5.9	5.8
9	high	5.3	5.3	5.7	5.8
10	high	5.4	5.3	5.7	5.7
11	high	5.5	5.8	5.9	5.0
12	high	5.6	5.6	5.7	5.6
1	blank	<1		<1	
2	blank	<1		<1	
3	blank	<1		<1	
4	blank	<1		<1	
5	blank	<1		<1	
6	blank	<1		<1	
7	blank	<1		<1	
9	blank	<1		<1	
10	blank	<1		<1	
11	blank	<1		<1	
12	blank	<1		<1	

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

Accuracy profile			
Study Name	Free text		
Date	Free text		
Coordinator	Free text		
Tolerance probability (beta)	80%	80%	80%
Acceptability limit in log (lambda)	1.07	1.07	1.07

Application of clause 6.2.3  
 Step 8: If any of the values for the  $\beta$ -ETI fall outside the acceptability limits, calculate the pooled average reproducibility standard deviation of the reference method.  
 Step 9: Calculate new acceptability limits as a function of this standard deviation.

Levels	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
Target value	2.408	4.481	5.745			
Number of participants (K)	10	10	10	10	10	10
Average for alternative method	2.727	4.756	5.850	2.408	4.481	5.745
Repeatability standard deviation (sr)	0.110	0.092	0.310	0.328	0.117	0.145
Between-labs standard deviation (sL)	0.119	0.138	0.236	0.183	0.189	0.322
Reproducibility standard deviation (sR)	0.162	0.166	0.389	0.376	0.222	0.354
Corrected number of dof	14.103	12.211	16.152	17.522	11.852	10.655
Coverage factor	1.395	1.411	1.381			
Interpolated Student t	1.345	1.355	1.336			
Tolerance interval standard deviation	0.1681	0.1727	0.4025			
Lower TI limit	2.501	4.521	5.312			
Upper TI limit	2.953	4.990	6.388			
Bias	0.319	0.275	0.105			
Relative Lower TI limit (beta = 80%)	0.093	0.040	-0.432			
Relative Upper TI limit (beta = 80%)	0.545	0.509	0.643			
Lower Acceptability Limit	-1.07	-1.07	-1.07			
Upper Acceptability Limit	1.07	1.07	1.07			

**New acceptability limits may be based on reference method pooled variance**

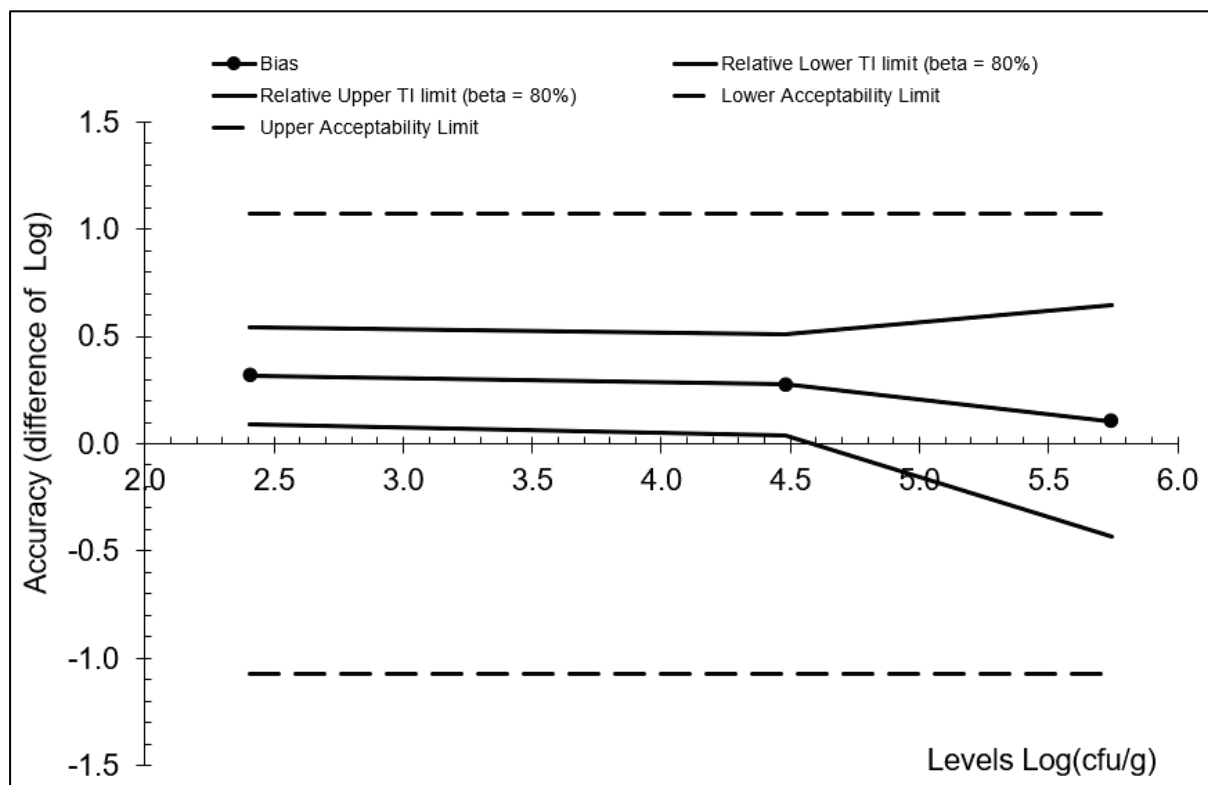
Pooled repro standard dev of reference	0.324
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TRUE

FALSE

TRUE

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



**Figure 7. Accuracy profile of Easy Plate CC from the ILS**

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 CC and the reference method for the enumeration of coliforms in a broad range of foods.