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NordVal International Certificate

Issued for:	**Easy Plate YM-R for enumeration of yeasts and moulds in food, feed and environmental samples**
NordVal No:	**063**
First approval date:	14 October 2024
Valid until:	14 October 2026

Easy Plate YM-R

Manufactured and supplied by:
Kikkoman Biochemifa Company
2-1-1, Nishi-shinbashi, Minato-ku,
Tokyo 105-0003,
Japan

fulfills the requirements of the NordVal validation protocol / ISO 16140-2. The reference method was ISO 21527:2008 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds.

NordVal International has reviewed the method and the validation study conducted by Campden BRI, United Kingdom. The results of the validation document show that the alternative method performs equivalently to the reference method for the enumeration of the sum of yeasts and moulds in a broad range of food, in pet food, animal feed, and environmental samples. NordVal International has concluded that it has been satisfactorily demonstrated that the method performance characteristic requirements have been fulfilled.

The production of the Easy Plate YM-R fulfills the requirements outlined by ISO 9001.

Date: 14. October 2024

Yours sincerely,

Hrólfur Sigurðsson

Hrólfur Sigurðsson
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

Easy Plate YM-R 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. 1 ml volumes of appropriately diluted samples are plated onto the YM-R plates, allowed to soak in and then incubated at 25 ± 1°C for 48 ± 2h and for 72h-76h. Yeasts tend to form small colonies with defined edges, and moulds tend to form colonies with diffuse edges. In this study, the combined yeasts and moulds was enumerated in alignment with the reference method.

FIELD OF APPLICATION

The method is applicable for the enumeration of yeasts and moulds for a broad range of food, pet food, animal feed and environmental samples.

The validation study was performed with both incubation times, 46h and 72h, for all samples. Based on the data from the validation study the incubation time of 46h was satisfactory for three categories. The results were satisfactory for all categories after 72h of incubation, and thus NordVal International recommends incubating for 72h. **Table 1** summarises the appropriate incubation time for each category.

Table 1. Incubation times for categories

Category	Meets ISO 16140-2 criteria at 46h incubation	Meets ISO 16140-2 criteria at 72h incubation
Heat processed milk and dairy products	✗	✓
Bakery products	✗	✓
Fresh produce and fruits	✓	✓
Ready to eat/ready to reheat meat and poultry products	✗	✓
Multicomponent foods or meal components	✓	✓
Dried cereals, fruits, nuts, seeds and vegetables	✗	✓
Chocolate and confectionery	✗	✓
Pet food and animal feed	✗	✓
Environmental samples	✓	✓

METHOD COMPARISON STUDY

Relative trueness study

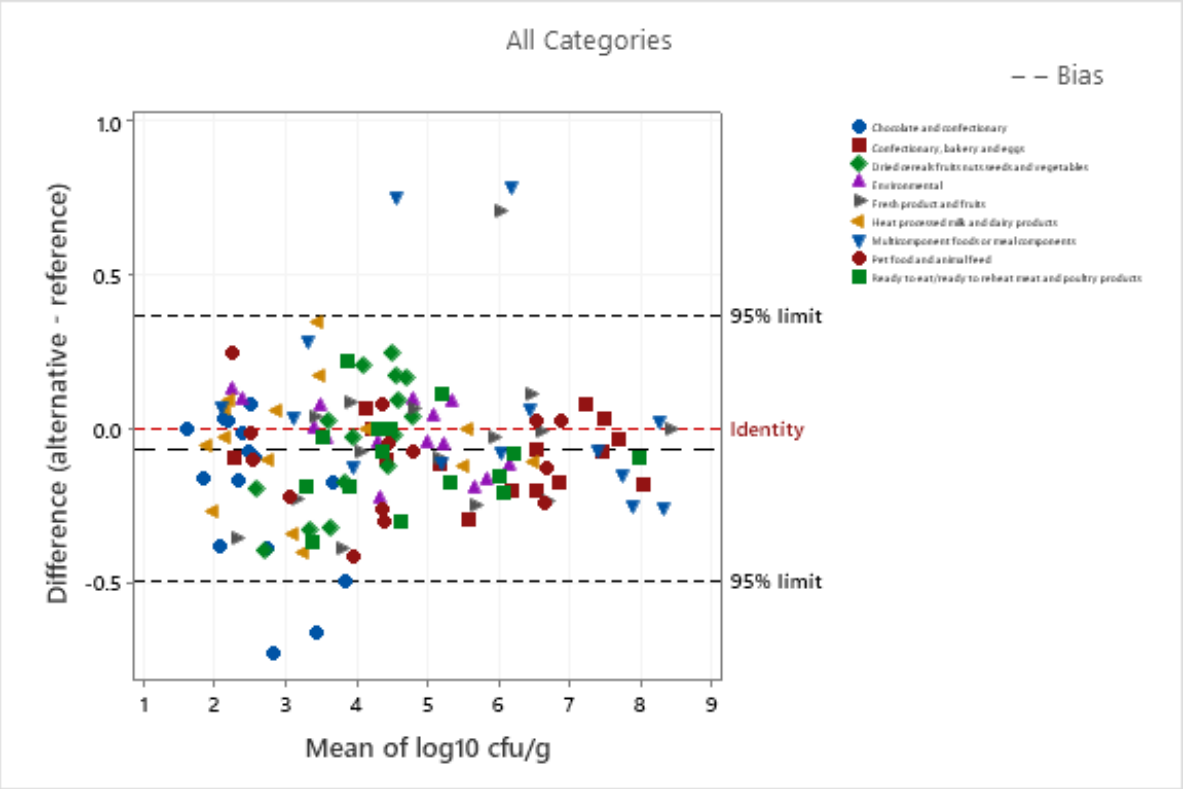
The relative trueness is illustrated using a Bland-Altman plot, i.e. the difference (bias) between paired samples analysed with the reference method and the alternative method respectively, plotted against the mean values obtained by the reference method. In the plot, Upper and Lower limits are included as the bias ± 2 times the standard deviation of the bias. Each category was made up of 3 types, with at least 5 items representative for each type.

Table 2 shows the different categories, types and items tested. The preparation of the samples were conducted according to ISO 6887-4. The Bland-Altman Plot in **Figure 1** illustrates the difference obtained in the enumeration of yeasts and moulds in foods by the alternative (after 72h) and the reference method, respectively. **Figure 2** shows the Bland-Altman Plot for the three categories; fresh produce and fruits, multi components foods or meal components and environmental samples incubated for 46h.

Table 2. Categories, types, and preparation of the samples

Category	Types/Items
Heat processed milk and dairy products	Pasteurised dairy products e.g. milk-based dessert, drinks, creams
	Cheese e.g. hard and semi hard cheese
	Fermented and acidified pasteurised milk and yogurt e.g. Yogurts with fruit, fermented milk drinks
Fresh produce and fruits	Fresh fruit salad and fruit purees
	Chilled fruit juices
	Fermented vegetables e.g. sauerkraut, olives
Multi component foods or meal components	Composite foods with raw ingredients e.g. tuna and cucumber sandwich, pasta salads.
	Mayonnaise based chilled salads e.g. prawn layered salad
	Ambient stable acidified foods e.g. ketchup, fish sauce
Bakery products	Bakery products with custard
	Baked chill products without additives e.g. chilled quiches
	Par baked bread products Aw >0.95
RTE/RTRH meat and poultry products	Cooked meat and poultry e.g. cooked hams, pate, cooked poultry
	Fermented or dried products e.g. Salami, chicken sausage
	Raw cured products e.g. dry cured hams, smoked turkey
Dried cereals fruits nuts seeds and vegetables	Dried cereals
	Nuts and seeds, nut butters
	Low and IF fruits aw <0.85
Chocolate, and confectionary	Dry and sugared low moisture aw<0.85 e.g. praline
	Dry and sugared low moisture aw<0.65 e.g. syrups
	Dry powders e.g. cake mixes
Pet food and animal feed	Dry Food
	Wet food (raw and canned)
	Animal feeds (poultry and fish)
Environmental samples (food or feed production)	Surfaces (sponges, swabs) Equipment, floors, walls
	Wash water, cooling water
	Dusts - Bakery and food manufacturing environment

Figure 1. Bland-Altman plot for all categories, 72 h

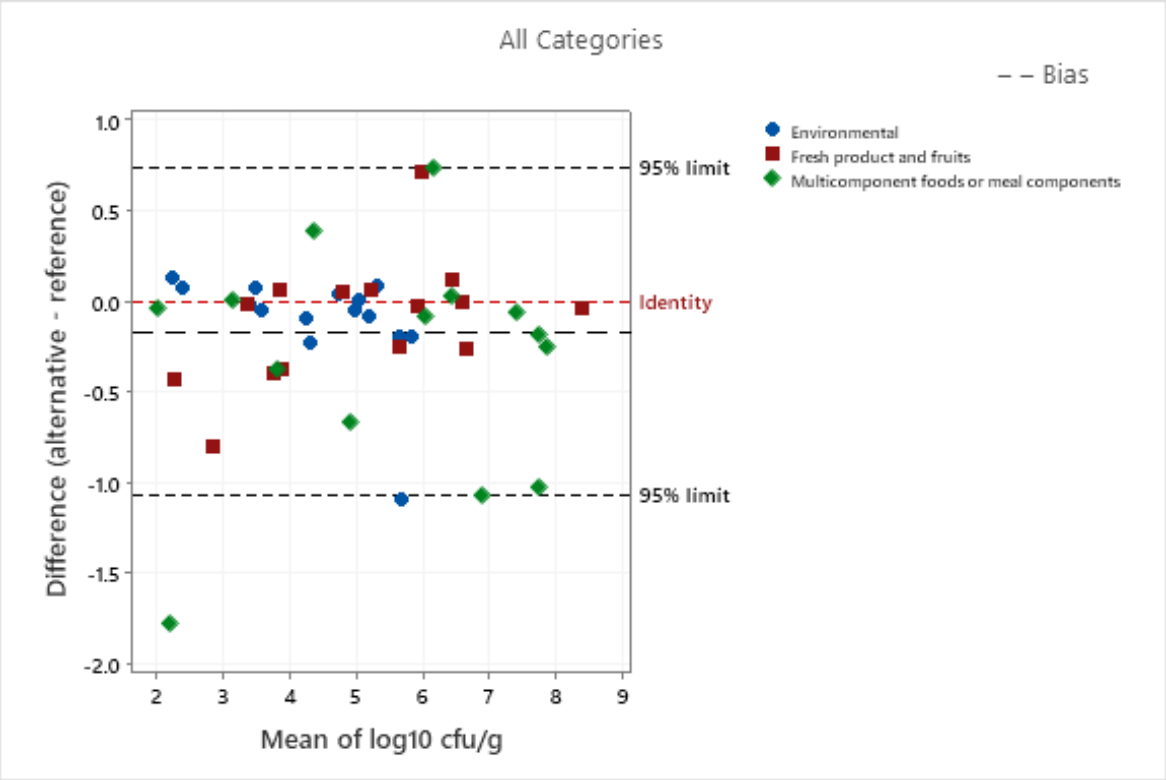


It is expected that no more than 1 in 20 data values will lie outside the 95% confidence levels (upper limit and lower limits). In this study there were 5 data points from a total of 135 data points which were outside of the accepted limits.

The five food items with differences outside of the limits belonged to 3 out of the 8 categories included in the study, suggesting that they are random outliers. Three out of the five food items showed positive bias and the remaining three food items gave negative bias. In addition, there was no impact on the type of sample contamination observed, with equal numbers of food items being naturally contaminated and artificially contaminated.

The results are in accordance with the expectations.

Figure 2. Bland-Altman plot for selected categories multicomponent foods and fresh produce and fruits, and environmental samples, 46 h



It is expected that no more than 1 in 20 data values will lie outside the 95% confidence levels (upper limit and lower limits). In this study, two of the 30 data points was outside of the accepted limits. The results meet the expectations.

Accuracy Profile

The accuracy profile study is a comparative study between the results obtained by the reference method and the results of the alternative method. Nine categories were tested with a single batch of two different food items using 6 samples per item. Two samples were contaminated at a low level, 2 at intermediate level, and 2 at a high level. For each sample, 5 replicates (5 different test portions) were tested. A total of 30 samples were analysed per category. The tested categories, types, items, and inoculated strains are provided in the **Table 3**.

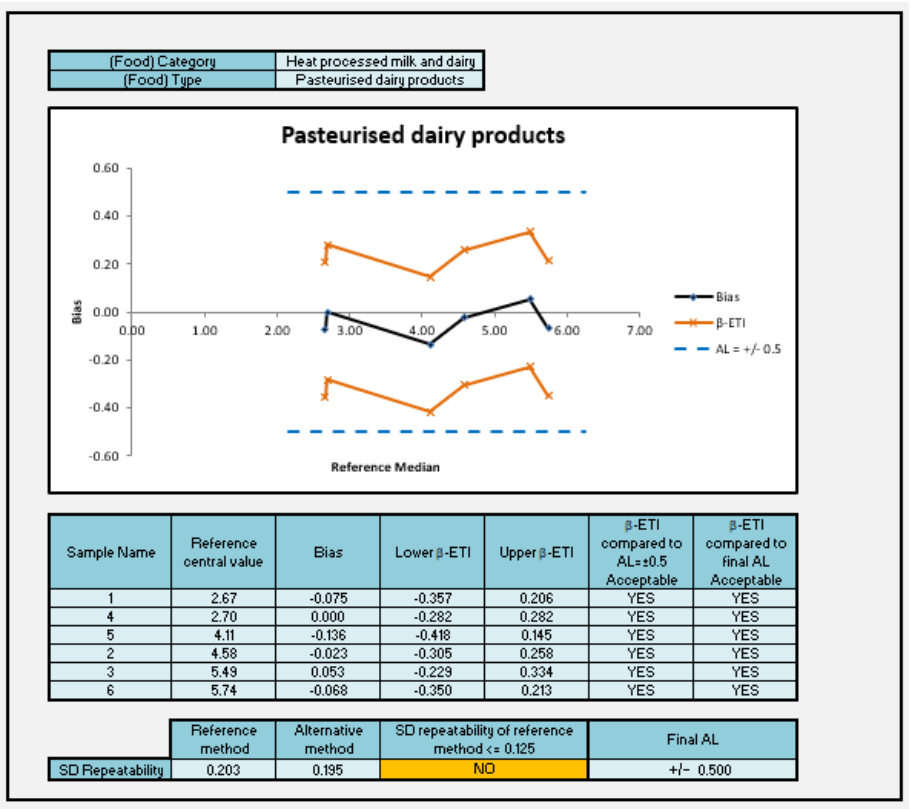
Table 3. Categories, types, items and inoculated strains tested

Category	Types	Inoculated Strain	Item
Heat processed milk and dairy products	Pasteurised dairy products	*Saccharomyces cerevisiae* CRA 15968 isolated from frozen fruit juice	Fermented yogurt drink
		Kluyveromyces marxianus CRA 6749 Dairy isolate	Cream cheese
Fruits and vegetables	Fresh produce	*Debaryomyces hansenii CRA 15969* Factory isolate	Vegetable juice
		Pichia galeiformis CRA16015 isolated from spoiled tomato juice	Beetroot salad
Multi component foods or meal components	Composite foods with raw ingredients e.g. sandwiches, pasta salads.	*Geotrichum conidium* CRA 14398, Factory isolate	Raw vegetable salad with dressing
		Saccharomyces exiguous CRA16017 isolated from spoiled mayonnaise	Frozen ready to reheat pizza
Confectionery bakery and eggs	Chilled RTE foods	*Aspergillus niger* CRA 16667 isolated from grapes	Quiche
		Kloeckera apiculata CRA6412 bakery isolate	Custard tart
RTE/RTRH foods	Ready to eat meat and poultry	*Penicillium chrysogeum* DSM 848 source unknown	Cooked breaded chicken patties
		Metschenikowia pulcherrina CRA16167 food spoilage isolate	Deli turkey
Dried cereals fruits nut seeds and vegetables	Nuts and seeds	*Eurotium amstelodami CRA* 8155 isolated from a mouldy kernel	Dried apricots
		Zygosaccharomy-ces rouxii CRA16128 isolated from spoiled fruit	Almond butter
Chocolate, bakery and confectionary	Dry and sugared low moisture aw<0.85	*Zygosaccharomyces bailli* CRA16125 isolated from nougat	Milk chocolate
		Paecilomyces variotii CRA16670 isolated from fruit concentrate	Cream puff
Environmental surfaces	Stainless steel (4” x 4”)	*Candia sojae* CRA16138 isolated from the soft drinks environment	Stainless steel sampled with sponges
	Sealed concrete (1” x 1”)	*Cryptococcus laurenti* CRA16139 isolated from a soft drinks factory	Sealed concrete sampled with swabs
Pet food and animal feed	Dry dog food	*Trichosporon coremiiforme* CRA15962 *isolated from powder*	Kibbles
	Animal origin	*Yarrowia lipolytica* CRA16146 isolated from a factory	Meat and bone meal

All categories incubated for 72h

The statistical results and the accuracy profiles for all categories incubated for 72h are provided in the **Figures 3 to 11**.

Figure 3. Accuracy profile for heat processed milk and dairy products category, 72h

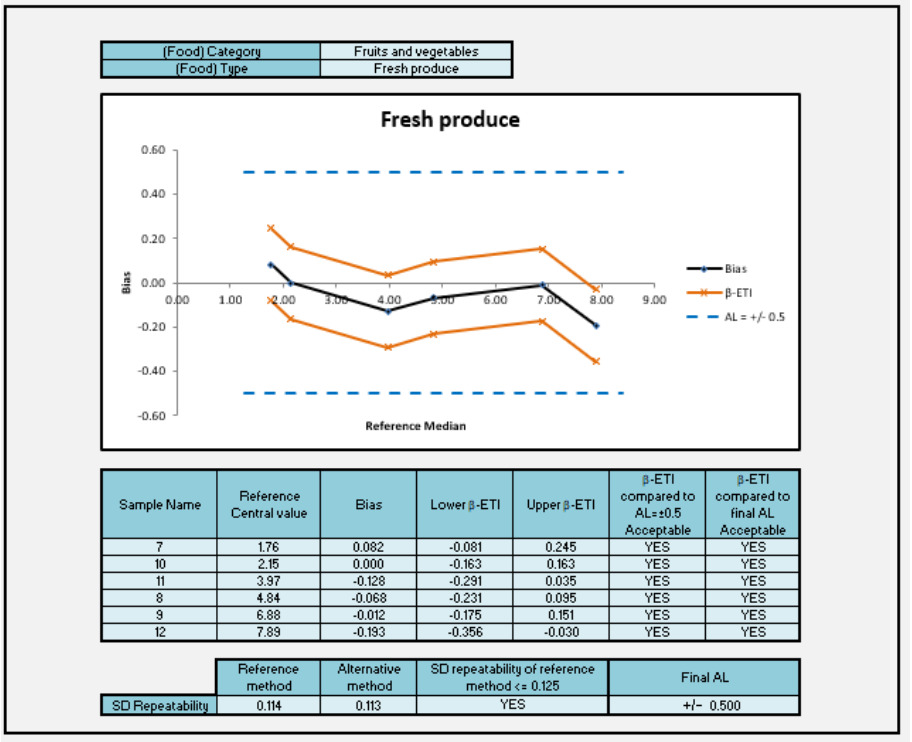


(Food) Category	Heat processed milk and dairy
(Food) Type	Pasteurised dairy products

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
1	2.67	-0.075	-0.357	0.206	YES	YES
4	2.70	0.000	-0.282	0.282	YES	YES
5	4.11	-0.136	-0.418	0.145	YES	YES
2	4.58	-0.023	-0.305	0.258	YES	YES
3	5.49	0.053	-0.229	0.334	YES	YES
6	5.74	-0.068	-0.350	0.213	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.203	0.195	NO	+/- 0.500

Figure 4. Accuracy profile for the fresh produce and fruits category, 72h



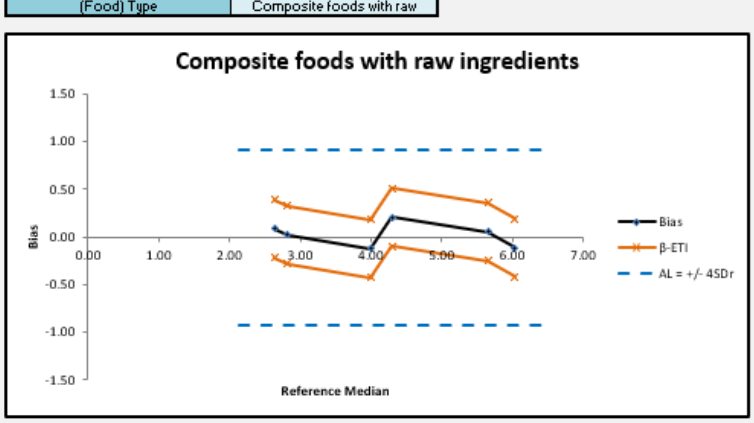
(Food) Category	Fruits and vegetables
(Food) Type	Fresh produce

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
7	1.76	0.082	-0.081	0.245	YES	YES
10	2.15	0.000	-0.163	0.163	YES	YES
11	3.97	-0.128	-0.291	0.035	YES	YES
8	4.84	-0.068	-0.231	0.095	YES	YES
9	6.88	-0.012	-0.175	0.151	YES	YES
12	7.89	-0.193	-0.356	-0.030	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.114	0.113	YES	+/- 0.500

Figure 5. Accuracy profile for the multicomponent foods category, 72h

(Food) Category	Multi component foods or meal
(Food) Type	Composite foods with raw

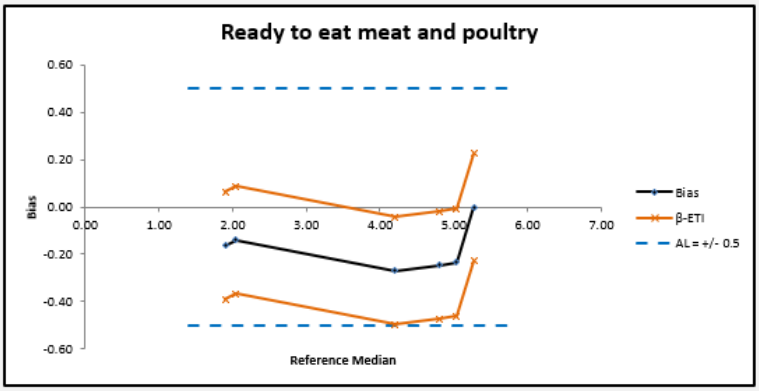


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
16	2.64	0.082	-0.221	0.386	YES	YES
13	2.82	0.019	-0.284	0.322	YES	YES
17	4.00	-0.125	-0.428	0.178	YES	YES
14	4.30	0.204	-0.099	0.507	NO	YES
18	5.66	0.053	-0.250	0.356	YES	YES
15	6.04	-0.117	-0.420	0.186	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.230	0.210	NO	+/- 0.920

Figure 6. Accuracy profile for the ready to eat/ready to reheat meat and poultry, 72h

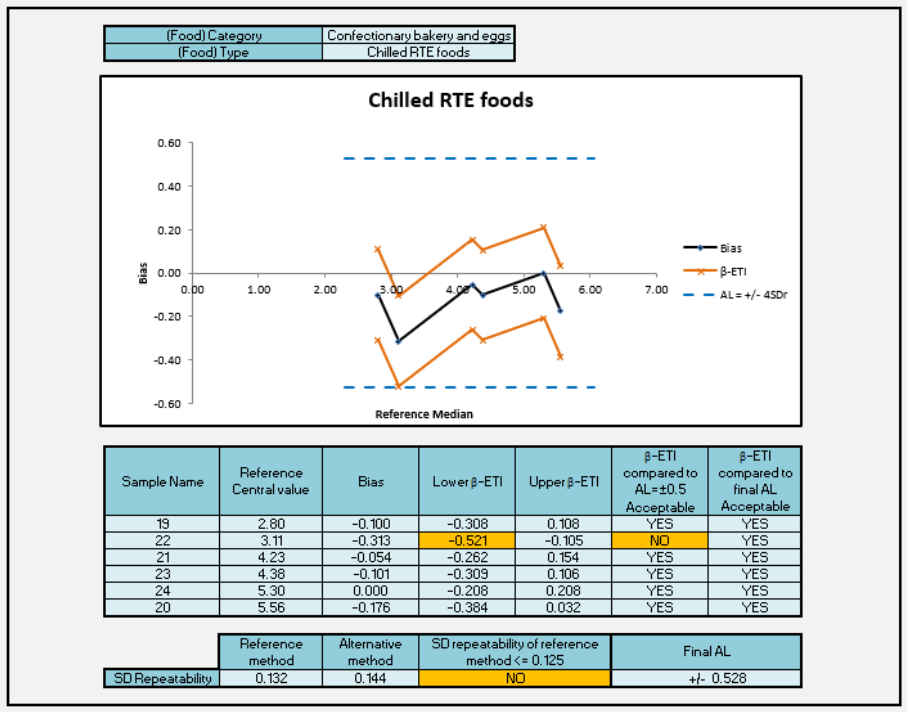
(Food) Category	RTE and RTRH foods
(Food) Type	Ready to eat meat and poultry



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
28	1.90	-0.163	-0.389	0.064	YES	YES
25	2.04	-0.138	-0.365	0.088	YES	YES
26	4.20	-0.270	-0.496	-0.043	YES	YES
29	4.81	-0.245	-0.471	-0.018	YES	YES
30	5.04	-0.235	-0.462	-0.009	YES	YES
27	5.28	0.000	-0.227	0.227	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.092	0.157	YES	+/- 0.500

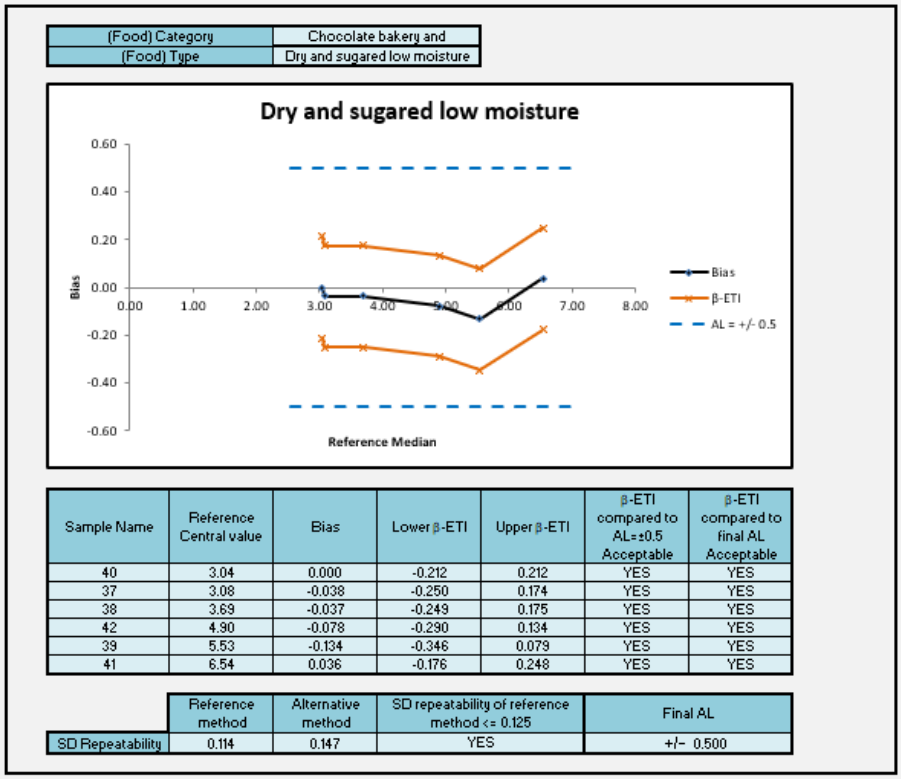
Figure 7. Accuracy profile for the the confectionery, bakery and eggs category, 72h



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
19	2.80	-0.100	-0.308	0.108	YES	YES
22	3.11	-0.313	-0.521	-0.105	NO	YES
21	4.23	-0.054	-0.262	0.154	YES	YES
23	4.38	-0.101	-0.309	0.106	YES	YES
24	5.30	0.000	-0.208	0.208	YES	YES
20	5.56	-0.176	-0.384	0.032	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.132	0.144	NO	+/- 0.528

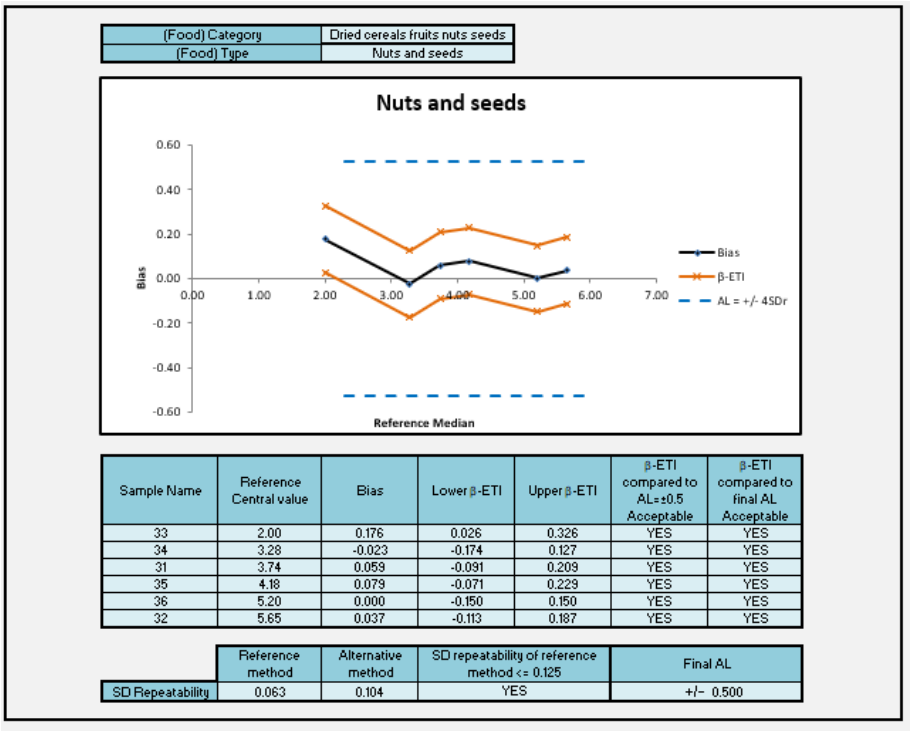
Figure 8. Accuracy profile for the chocolate and confectionery category, 72h



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
40	3.04	0.000	-0.212	0.212	YES	YES
37	3.08	-0.038	-0.250	0.174	YES	YES
38	3.69	-0.037	-0.249	0.175	YES	YES
42	4.90	-0.078	-0.290	0.134	YES	YES
39	5.53	-0.134	-0.346	0.079	YES	YES
41	6.54	0.036	-0.176	0.248	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.114	0.147	YES	+/- 0.500

Figure 9. Accuracy profile for the dried cereals, fruits and vegetables category, 72h

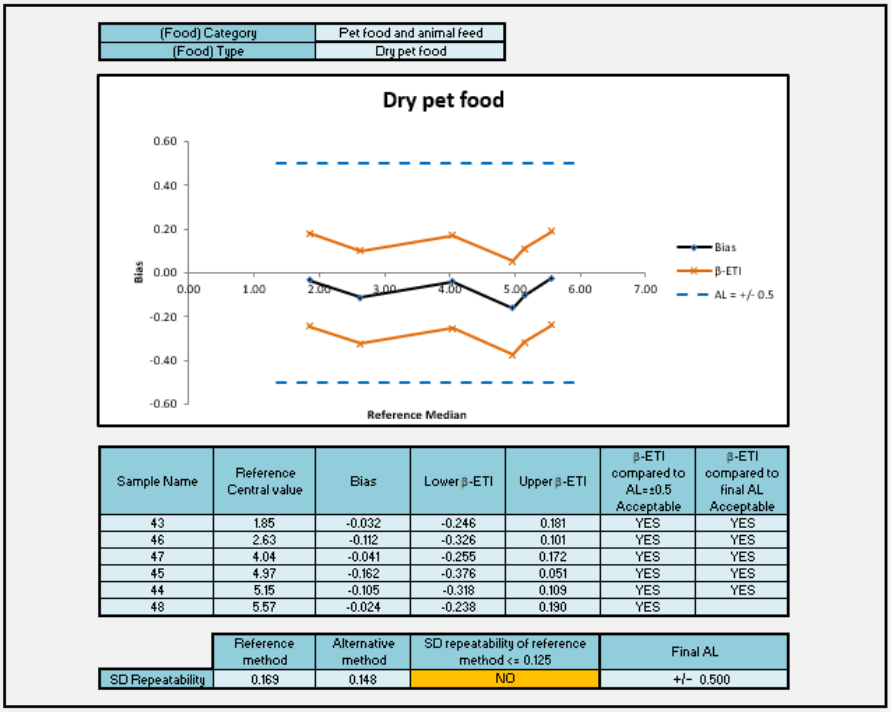


(Food) Category	Dried cereals fruits nuts seeds
(Food) Type	Nuts and seeds

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
33	2.00	0.176	0.026	0.326	YES	YES
34	3.28	-0.023	-0.174	0.127	YES	YES
31	3.74	0.059	-0.091	0.209	YES	YES
35	4.18	0.079	-0.071	0.229	YES	YES
36	5.20	0.000	-0.150	0.150	YES	YES
32	5.65	0.037	-0.113	0.187	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.063	0.104	YES	+/- 0.500

Figure 10. Accuracy profile for the pet food and animal feed category, 72h

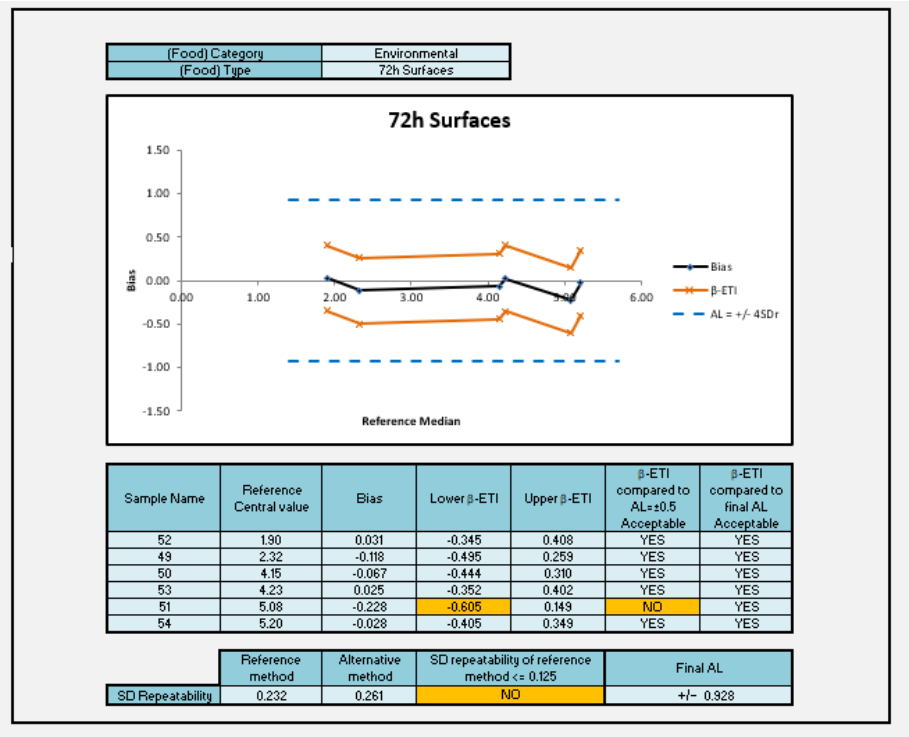


(Food) Category	Pet food and animal feed
(Food) Type	Dry pet food

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
43	1.85	-0.032	-0.246	0.181	YES	YES
46	2.63	-0.112	-0.326	0.101	YES	YES
47	4.04	-0.041	-0.255	0.172	YES	YES
45	4.97	-0.162	-0.376	0.051	YES	YES
44	5.15	-0.105	-0.318	0.109	YES	YES
48	5.57	-0.024	-0.238	0.190	YES	

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.169	0.148	NO	+/- 0.500

Figure 11. Accuracy profile for the environmental category, 72h



(Food) Category	Environmental
(Food) Type	72h Surfaces

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
52	1.90	0.031	-0.345	0.408	YES	YES
49	2.32	-0.118	-0.495	0.259	YES	YES
50	4.15	-0.067	-0.444	0.310	YES	YES
53	4.23	0.025	-0.352	0.402	YES	YES
51	5.08	-0.228	-0.605	0.149	NO	YES
54	5.20	-0.028	-0.405	0.349	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.232	0.261	NO	+/- 0.928

Results for all categories after 72h incubation

In this study the following categories met the Acceptance Limit, AL, of 0.5 log: pasteurised dairy products, fresh produce, pet food, dried cereals, RTE/RTRH meat and poultry, and chocolate and confectionary.

If any of the upper or lower limits exceeded the 0.5log Acceptance Limits (AL) and the standard deviation of the reference method is >0.125 log cfu/g, a new AL can be calculated as 4xSD. In this study, the following categories required the new AL to be calculated: Multicomponent foods, environmental samples and Confectionary bakery and eggs. All of these categories met the new AL value of 0.920, 0.928 and 0.528 respectively.

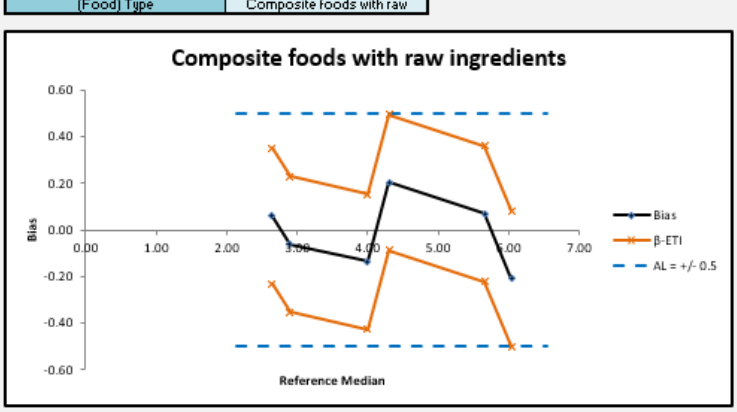
The accuracy of the alternative method met the AL of 0.50 log cfu/g or the re-calculated AL.

Selected categories incubated for 46h

The statistical results and the accuracy profiles for multicomponent food and fresh produce categories and environmental samples, incubated for 46h are provided in the **Figure 12**, **Figure 13** and **Figure14**.

Figure 12. Accuracy profile for the multicomponent food, 46h

(Food) Category	Multi component foods or meal
(Food) Type	Composite foods with raw

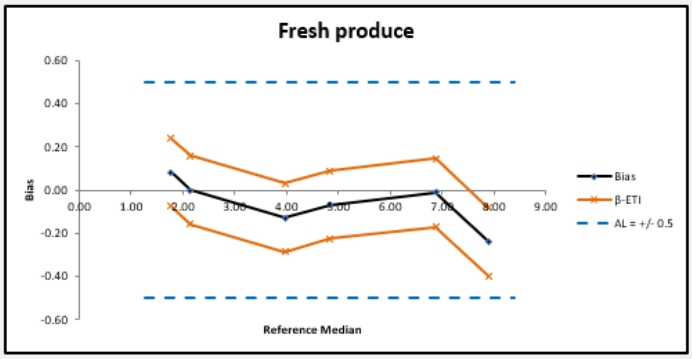


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
16	2.64	0.060	-0.230	0.350	YES	YES
13	2.89	-0.062	-0.352	0.229	YES	YES
17	4.00	-0.137	-0.427	0.153	YES	YES
14	4.30	0.204	-0.086	0.494	YES	YES
18	5.66	0.070	-0.221	0.360	YES	YES
15	6.04	-0.209	-0.499	0.081	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.231	0.201	NO	+/- 0.500

Figure 13. Accuracy profile for the fresh produce and fruits, 46h

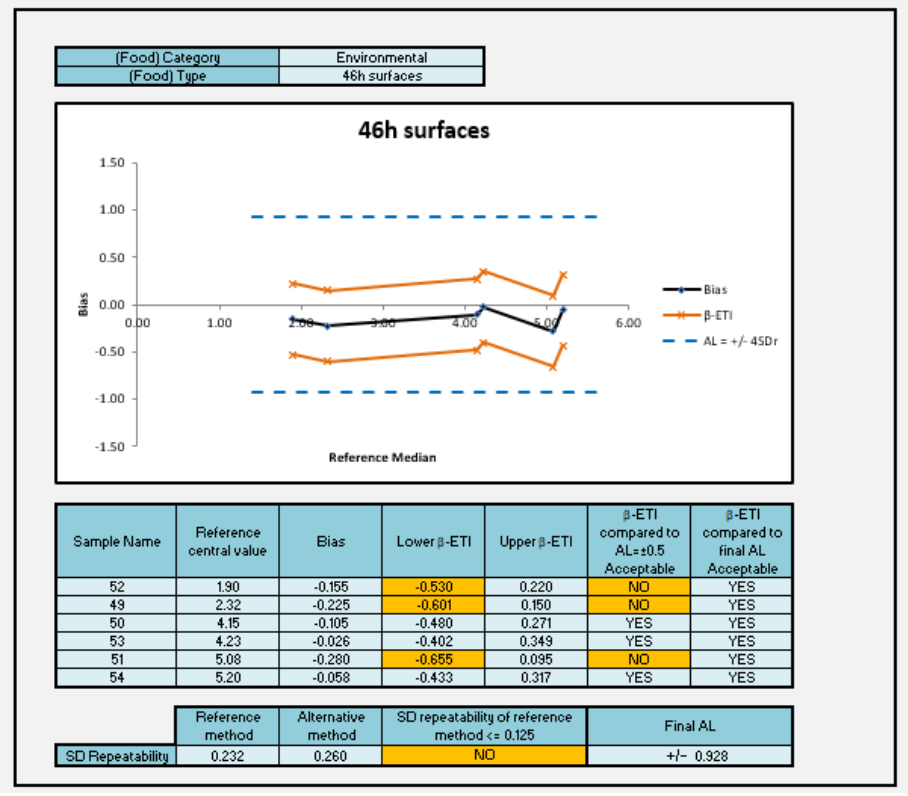
(Food) Category	Fruits and vegetables
(Food) Type	Fresh produce



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
7	1.76	0.082	-0.076	0.239	YES	YES
10	2.15	0.000	-0.157	0.157	YES	YES
11	3.97	-0.128	-0.285	0.029	YES	YES
8	4.84	-0.068	-0.225	0.089	YES	YES
9	6.88	-0.012	-0.169	0.146	YES	YES
12	7.89	-0.239	-0.396	-0.082	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.114	0.109	YES	+/- 0.500

Figure 14. Accuracy profile for environmental samples, 46h



(Food) Category	Environmental
(Food) Type	46h surfaces

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
52	1.90	-0.155	-0.530	0.220	NO	YES
49	2.32	-0.225	-0.601	0.150	NO	YES
50	4.15	-0.105	-0.480	0.271	YES	YES
53	4.23	-0.026	-0.402	0.349	YES	YES
51	5.08	-0.280	-0.655	0.095	NO	YES
54	5.20	-0.058	-0.433	0.317	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.232	0.260	NO	+/- 0.928

Results of selected categories after 46h incubation

In this study the multicomponent foods and fresh produce categories met the AL of 0.5 log. For the environmental category, the AL required to be recalculated, and the results met the new AL value of 0.928 log.

Inclusivity / 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.

46h incubation – selected categories

- **Inclusivity**: A total of 50 strains were tested for inclusivity and 49 of these strains showed a positive result at 46h. One strain, *Eurotium amstelomdami,* did not produce a count following 46h incubation. *Eurotium amstelomdami* is a xerophilic mould and therefore unlikely to be a source of contamination with the three categories (multicomponent foods fresh produce and environmental samples) that are selected for validation at 46h.
- **Exclusivity**: A total of 30 strains were tested for exclusivity and 30 of these strains showed a negative result on the alternative method.

72h incubation – all categories

- **Inclusivity**: A total of 50 strains were tested for inclusivity and 50 of these strains showed a positive result at 72h.
- **Exclusivity**: A total of 30 strains were tested for exclusivity and 30 of these strains showed a negative result on the alternative method after 72h.

INTERLABORATORY STUDY

Valid results from 10 collaborators were received for the analysis of yeasts and moulds in pasta salad samples. The samples had been co-inoculated with *Candida sojae* CRA 16136 isolated from a soft drinks factory and *Penicillium chrysogenum* DSM 848 isolated from cheese. The laboratories analysed duplicate samples inoculated at four levels (10^2, 10^3, 10^5, 10^6 cfu/g) using both the reference and the alternative method.

The results from the interlaboratory study (ILS) after 46h incubation are given in **Table 3** and the accuracy profile of the results are shown in **Figure 15**. The results after 72h incubation are given in **Table 4** and illustrated in **Figure 16**.

Table 3. Statistical analysis of the ILS data – 46h incubation

	Alternative method			Reference method		
Levels (log)	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Average	2.37	4.16	5.91	2.36	4.17	5.93
Repeatability standard deviation (sr)	0.31	0.40	0.13	0.17	0.31	0.19
Reproducibility standard deviation (sR)	0.51	0.48	0.50	0.41	0.40	0.48
Bias	0.01	-0.01	-0.02			
Lower limit	1.65	3.50	5,19			
Upper limit	3.08	4.81	6,63			
Lower Acceptability limit	-1.43					
Upper Acceptability limit	1.43					

Figure 15. Accuracy profile for ILS 46h

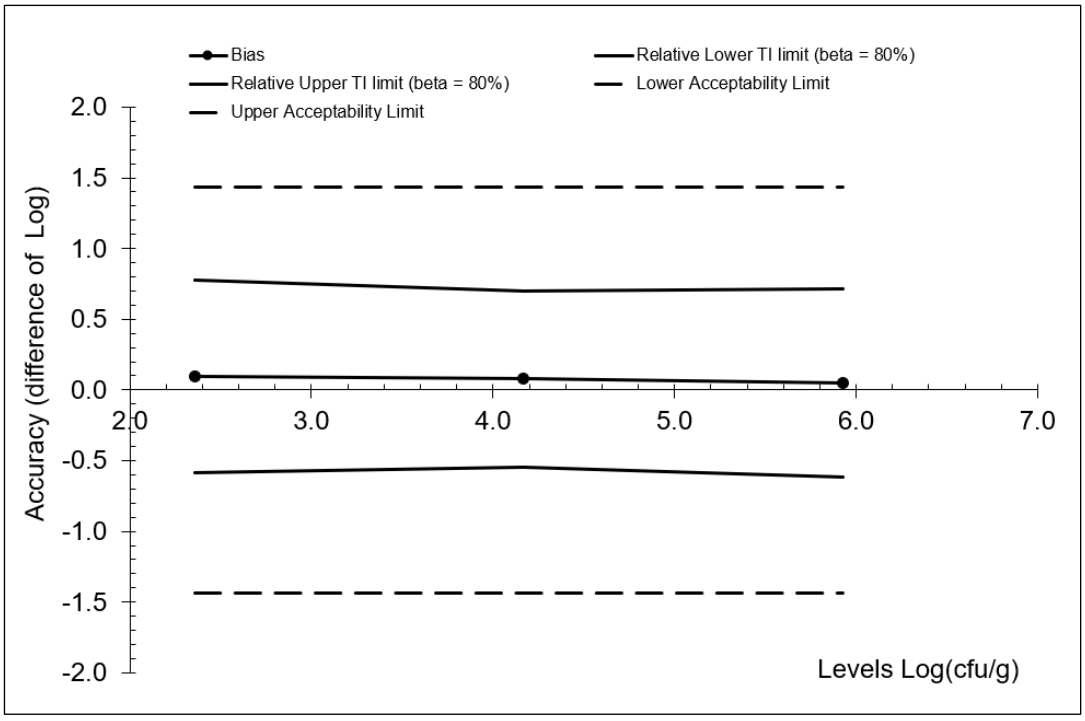
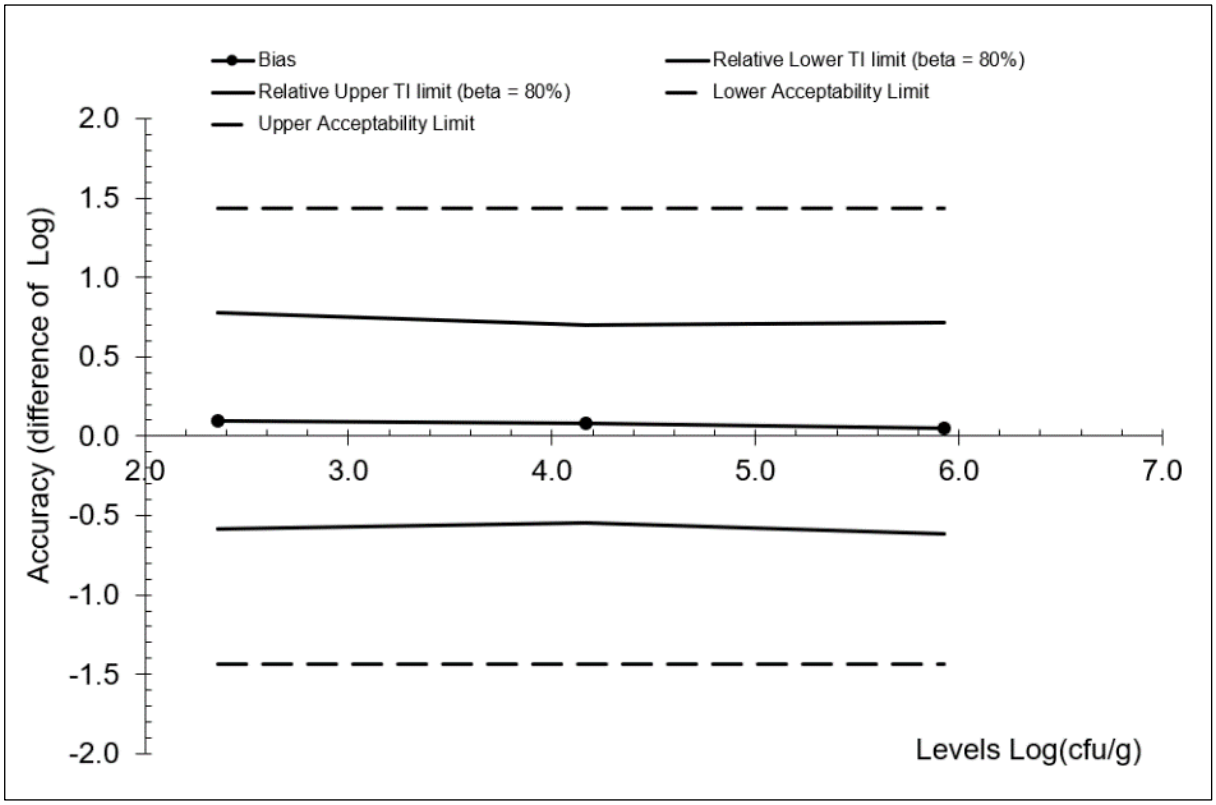


Table 4. Statistical analysis of the ILS data – 72h incubation

	Alternative method			Reference method		
Levels (log)	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Average	2.45	4.25	5.98	2.36	4.17	5.93
Repeatability standard deviation (sr)	0.38	0.38	0.12	0.17	0.31	0.19
Reproducibility standard deviation (sR)	0.49	0.45	0.46	0.41	0.40	0.48
Bias	0.01	0.08	0.05			
Lower limit	1.77	3.62	5,31			
Upper limit	3.13	4.87	6,6			
Lower Acceptability limit	-1.43					
Upper Acceptability limit	1.43					

Figure 16. Accuracy profile for ILS 72h



The alternative method provides equivalent result to the reference method. There is no significant bias, and the results are within the calculated lower and upper acceptability limits.

CONCLUSION

After 46 hours incubation, the alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows equivalent results to ISO 21527:2008 for multicomponent foods, fresh produce and environmental samples categories.

After 72 hours incubation, the alternative method Easy Plate YM-R for enumeration of yeasts and moulds shows equivalent results to ISO 21527:2008 for a broad range of food, pet food, animal feed and environmental samples. NordVal International recommends using 72h of incubation.