



**NMKL**



**NMKL - NordVal International**  
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# NordVal International Certificate

Issued for:	Compact Dry ETC Method for the Enumeration of Enterococci in Food and Water intended for Human Consumption
NordVal No:	047
First approval date:	01 September 2014
Renewal date:	15 February 2023
Valid until:	15 February 2025

## Compact Dry ETC

Manufactured and supplied by:  
**Nissui Pharmaceutical Co.Ltd,**  
3-23-9 Ueno,  
Taito-ku, Tokyo, 110-8736  
Japan

For the enumeration of Enterococci in water, the reference method was ISO 7899-2:2000 Water quality - Detection and enumeration of intestinal *Enterococci* – Part 2: Membrane filtration method.

For the enumeration Enterococci in foods, the reference method was NMKL 68, 5<sup>th</sup> Edition, 2011: *Enterococcus*. Determination in foods and feeds.

The validation studies have been conducted by Campden, UK according to ISO 16140-2:2016 and the requirements of ISO 17994 Water quality – Criteria for establishing equivalence between microbiological methods. NordVal International concludes that results document no statistical difference in the performances between Compact Dry ETC and the reference methods for the samples tested.

The production of Compact Dry ETC is certified according to ISO 9001

Yours sincerely,

Hilde Skår Norli  
Chair of NordVal International

Date: 13 February 2023

Eystein Oveland  
NMKL Executive Director



## PRINCIPLE OF THE METHOD

Compact Dry are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent, rehydrated by inoculating 1 ml of the pre-treated food sample into the centre of the self diffusible medium. The pre-treatment of food samples is carried out according to NMKL 91 or ISO 6887. For water samples, 100 ml or 250 ml are filtered and the membrane is incubated on the Compact Dry ETC. The Compact Dry ETC method contains chromogenic medium and selective agents for the detection and enumeration of Enterococci, which according to the manufacturer's instructions appear as blue colonies after 24h incubation at  $36 \pm 1^\circ\text{C}$ .

## FIELD OF APPLICATION

Determination of *Enterococcus* in a broad range of foods and in water products intended for human consumption.

## HISTORY

In 2014, the Compact Dry ETC method was certified for water products intended for human consumption. The comparison study and interlaboratory study were conducted by CCFRA Technology Limited, Chipping Campden, UK according to ISO 16140 and ISO 17994.

In 2015-2017, extensive studies were carried out on Compact Dry ETC method on food. The studies were carried out by CCFRA Technology Limited, Chipping Campden, according to the new ISO 16140-2:2016.

In 2017, the results obtained for the Compact Dry ETC method on water have been recalculated according to the ISO 16140-2:2016.

## RESULTS OF THE COMPARISONS STUDIES

### Selectivity; inclusivity and exclusivity

Selectivity studies were carried out in 2014 and in 2016.

#### **Study conducted in 2014:**

Inclusivity: Of the 31 strains of *Enterococcus*, 28 showed typical blue colonies on the Compact dry ETC. The 3 strains that did not have typical colonies were a strain of *Ent. faecalis* (CRA3460) which was re-identified as a *Lactococcus* strain, *Ent. cecorum* and *Ent. aquamarines*. There were 7 strains that did not show typical colonies following the reference method. These included the 3 that were not detected by Compact Dry ETC plus 4 other strains: *Ent. durans* (CRA8211 & NCTC 8130 (and *Ent. faecalis* CRA5401 and CRA5402). It would appear that the alternative method was better at detecting the target organisms than the reference method.

#### Exclusivity:

Of the 20 non-target strains tested, one of the strains grew on both the alternative and the reference method, this being *Streptococcus lactis* CRA 527 showing a typical morphology on all media. One other strain grew on the ETC only; this was *Streptococcus cremoris* CRA 556.

#### **Study conducted in 2016:**

Inclusivity: Of the 50 strains tested, 36 strains were detected and 14 were not detected using the alternative method. For the reference method 33 of the strains were detected and 17 were not detected.

The strains not detected for either method were: *E. cecorum*, 16849; *E. aquamarinus*, 16813; *E. dispar*, 16850, *E. columbae*, 16851; *E. pseudoavium*, 16852; *E. sulfureus*, 16853; *E. seriolicida*, 16854; *E. flavescens*, 16855; *E. sacharolyticus*, 16863; *E. dispar*, 16864; *E. xiangfangensis*, 16865; *E. solitarus*, 16867.



Strains not detected by the alternative method but detected by the reference method were: *E. durans*, 16810; *E. porcinus*16857.

Strains not detected by the reference method but detected by the alternative method were: *E. durans*, 16464; *E. haemoperoxidus*, 16858; *E. thailandicus*, 16859; *E. malodoratus*, 16860; *E. gallinarum*, 16861.

It would appear that both methods were good at detecting the more usual Enterococcus species, i.e.

*E. faecalis* and *E. faecium*, but less good at detecting other species. In the inclusivity study there were 50 strains of Enterococci covering 23 different species. The Compact Dry ETC method was more specific as it detected 11 of the 23 different species whereas the reference method only detected 8 of the different species.

#### Exclusivity:

Of the 30 strains tested, 28 were not detected and 2 were detected using both the reference and alternative methods. The 2 detected cultures were *Lactobacillus gasseri* CRA 6804 and *Streptococcus lactis* CRA 527.

#### **Relative trueness**

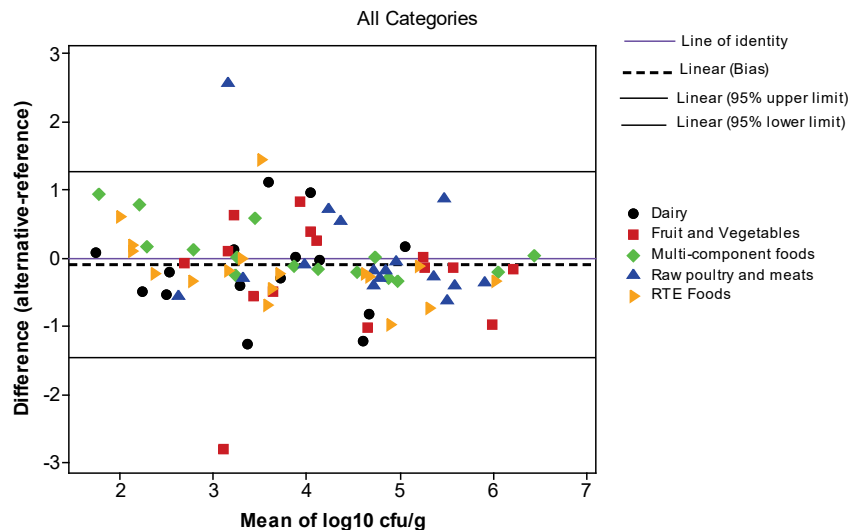
The trueness study is a comparative study between results obtained by the reference method and the results of the alternative method. This study was conducted in 2015 using naturally or artificially contaminated samples. Five food categories were tested. The number of samples per tested category and type is provided in Table 1.

**Table 1. Categories and types to be tested**

Categories	Types
Dairy products	Dairy desserts e.g. chilled custard, trifle
	Soft cheese
	Hard cheese e.g. cheddar
	Total
Fruits and vegetables	Seasonings e.g. spices
	Sprouts e.g. mung beans
	Leafy greens e.g. parsley, lettuce
	Total
Raw poultry and meats	Fresh chicken cuts
	Fresh mince
	Frozen patties
	Total
Ready to eat foods	Ready to eat poultry e.g. turkey fillet
	Cooked fish products e.g. prawns
	Cooked meat e.g. ham
	Total
Multi component foods	Composite foods with raw ingredients e.g. sandwiches, pasta salads.
	Mayonnaise based salads
	Cooked chilled foods e.g. rice products
	Total

108 samples were analysed, leading to 78 interpretable results and 30 negative samples. The difference in enumeration obtained by the reference method and the alternative method is illustrated in a Bland-Altman plot (Figure 1).

**Figure 1. Bland-Altman plot for all categories**



It is expected that not more than one in 20 data values will lie outside the limits. For this data set there are 3 in 78 data values which lie outside the limits, which is in accordance with the expectations, and hence the relative trueness of the alternative method is considered to be satisfactory.

### 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.

### Accuracy profile for Compact Dry ETC method for water analysis

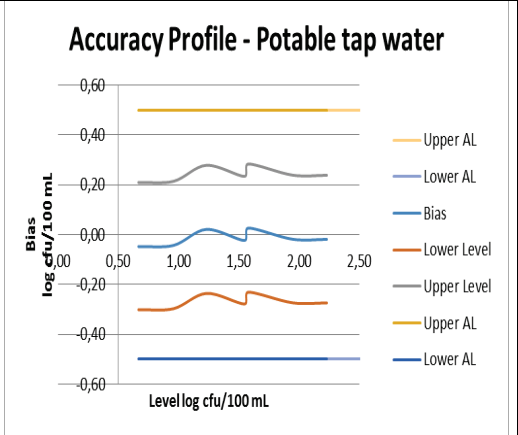
The Compact Dry ETC method was tested against the reference method using five different water types:

- potable tap water,
- bottled mineral still water,
- drinking fountain water,
- food processing water, and
- bottled fizzy water.

For each water type, 5-9 different levels were analysed using 8 replicates. For each level, water type and method (alternative or reference), the mean (log cfu/100mL) and the standard deviation, SD, (log cfu/100mL) were determined. The squares cfu/100 mL of the obtained results was also calculated, as it is recommended by some PT-providers for water analyses. Whether the results are calculated based on log cfu/100 mL or square cfu/100 mL, the conclusions will be the same. In this certificate, only the results of log cfu/ 100 mL are included. The results are given in the tables 2-5. The tables also include the accuracy profiles of the results. Explanation of the accuracy profile and a conclusion of the results are given after the tables.

**Table 2. Potable tap water - 9 levels each with 8 replicates**

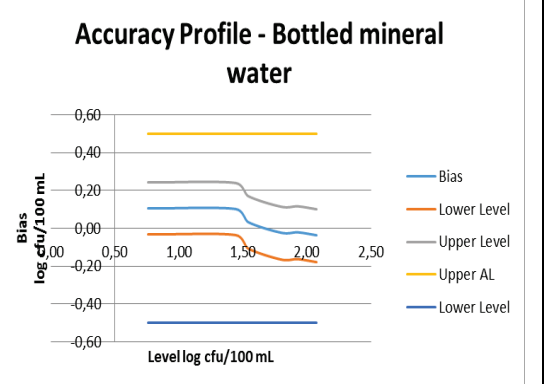
Compact Dry ETC		ISO 7899-2:		Bias	Lower level	Upper level	± AL
Mean	SD	Mean	SD				
0.92	0.17	0.89	0.17	0.02	-0.12	0.16	0.5
1.50	0.06	1.46	0.06	0.04	-0.10	0.18	0.5
1.21	0.12	1.16	0.36	0.04	-0.10	0.18	0.5
1.46	0.07	1.41	0.04	0.06	-0.08	0.20	0.5
1.72	0.04	1.78	0.11	-0.06	-0.20	0.08	0.5
2.16	0.03	2.18	0.04	-0.02	-0.16	0.12	0.5
1.68	0.04	1.68	0.04	0.00	-0.14	0.15	0.5
1.79	0.04	1.85	0.06	-0.06	-0.20	0.08	0.5
2.45	0.03	2.27	0.38	0.18	0.04	0.32	0.5
Comb SD	0.080		0.19				



The graph shows Bias (log cfu/100 ml) on the y-axis (ranging from -0.60 to 0.60) versus Level log cfu/100 ml on the x-axis (ranging from 0.50 to 2.50). It includes lines for Bias, Lower Level, Upper Level, Upper AL, and Lower AL. The bias is generally small, fluctuating around zero, and the levels are well-defined.

**Table 3. Bottled mineral still water - 6 levels each with 8 replicates**

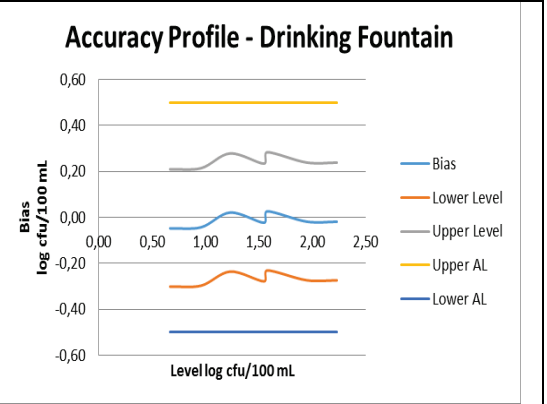
Compact Dry ETC		ISO 7899-2:		Bias	Lower level	Upper level	± AL
Mean	SD	Mean	SD				
0.76	0.14	0.65	0.24	0.11	-0.03	0.24	0.5
1.44	0.06	1.34	0.09	0.10	-0.04	0.24	0.5
1.54	0.06	1.51	0.08	0.03	-0.11	0.17	0.5
1.80	0.06	1.83	0.04	-0.02	-0.16	0.11	0.5
1.93	0.05	1.95	0.06	-0.02	-0.16	0.12	0.5
2.08	0.05	2.12	0.04	-0.04	-0.18	0.10	0.5
Comb SD	0.074		0.112				



The graph shows Bias (log cfu/100 ml) on the y-axis (ranging from -0.60 to 0.60) versus Level log cfu/100 ml on the x-axis (ranging from 0.50 to 2.50). It includes lines for Bias, Lower Level, Upper Level, Upper AL, and Lower AL. The bias is generally small, fluctuating around zero, and the levels are well-defined.

**Table 4. Drinking fountain water - 7 levels each with 8 replicates**

Compact Dry ETC		ISO 7899-2:		Bias	Lower level	Upper level	± AL
Mean	SD	Mean	SD				
0.62	0.21	0.67	0.15	-0.05	-0.30	0.21	0.5
0.92	0.25	0.96	0.15	-0.04	-0.30	0.22	0.5
1.25	0.08	1.23	0.06	0.02	-0.24	0.28	0.5
1.52	0.06	1.55	0.12	-0.02	-0.28	0.23	0.5
1.60	0.08	1.58	0.09	0.03	-0.23	0.28	0.5
1.93	0.04	1.95	0.05	-0.02	-0.27	0.24	0.5
2.21	0.03	2.23	0.05	-0.02	-0.28	0.24	0.5
Comb SD	0.136		0.104				

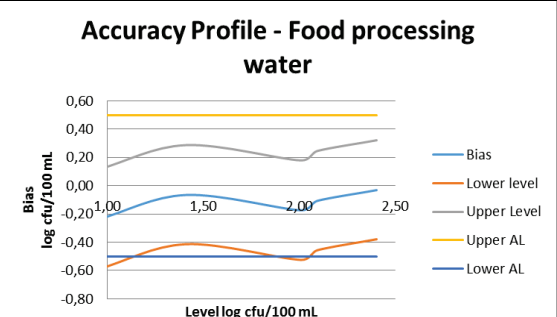


The graph shows Bias (log cfu/100 ml) on the y-axis (ranging from -0.60 to 0.60) versus Level log cfu/100 ml on the x-axis (ranging from 0.50 to 2.50). It includes lines for Bias, Lower Level, Upper Level, Upper AL, and Lower AL. The bias is generally small, fluctuating around zero, and the levels are well-defined.

**Table 5. Food processing water - 5 levels each with 8 replicates**

Compact Dry ETC		ISO 7899-2:		Bias	Lower level	Upper level	± AL
Mean	SD	Mean	SD				
0.71	0.37	0.95	0.16	-0.24	-0.59	0.11	0.5
1.35	0.10	1.41	0.10	-0.06	-0.41	0.29	0.5
1.83	0.07	2.01	0.04	-0.17	-0.52	0.18	0.5
1.99	0.06	2.09	0.07	-0.11	-0.46	0.25	0.5
2.37	0.06	2.40	0.04	-0.03	-0.38	0.32	0.5
Comb SD	0.179		0.092				

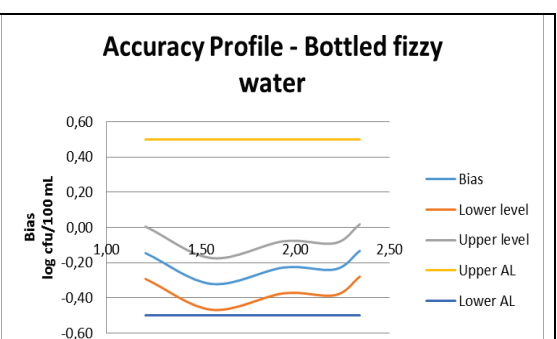
**Accuracy Profile - Food processing water**



**Table 6. Bottled fizzy water - 5 levels each with 8 replicates**

Compact Dry ETC		ISO 7899-2:		Bias	Lower level	Upper level	± AL
Mean	SD	Mean	SD				
1.06	0.11	1.21	0.06	-0.14	-0.29	0.00	0.5
1.24	0.12	1.56	0.07	-0.32	-0.47	-0.17	0.5
1.71	0.04	1.94	0.05	-0.23	-0.37	-0.08	0.5
1.97	0.03	2.21	0.13	-0.24	-0.39	-0.09	0.5
2.21	0.02	2.34	0.05	-0.13	-0.28	0.02	0.5
Comb SD	0.0753		0.078				

**Accuracy Profile - Bottled fizzy water**



Whenever no biases exist, the results would be on  $y=0$ . In the figures above, the acceptability limits (AL) are represented by the yellow and dark blue lines. The levels where the results might be expected to vary between (upper and lower levels) are given as red and grey lines. The bias (the difference obtained by the results obtained by the alternative method and the reference method) are given as blue lines.

When the upper level (grey line) is below the upper AL (yellow line), and the lower level (red line) is above the lower AL (dark blue line), the alternative method is accepted as being equivalent to the reference method. The results show that the Compact Dry ETC method performs equivalent to the ISO 7899-2 standard for water samples.

### Accuracy profile for Compact Dry ETC method for food analysis

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. Five food categories were tested, using 9 samples per category (6 is required according to ISO 16140-2). Of the 9 samples, there were 3 at a low level, 3 at a medium level and 3 at a high level of contamination. For each of the 9 samples per category, 5 replicate test portions were tested. The tested categories, types, items and inoculated strains are provided in the Table 7.

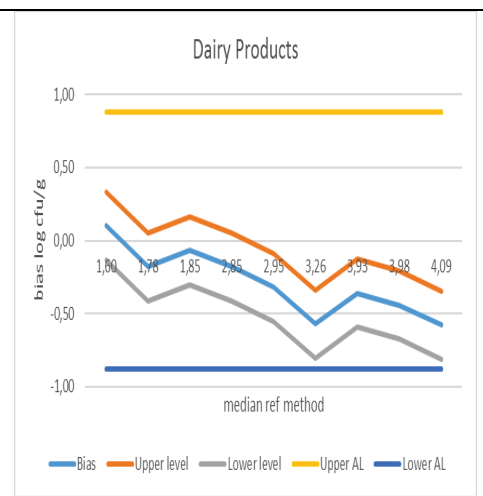
**Table 7. Food categories, types and food items**

Category	Types	Strain	Item	Level
Dairy products	Dairy desserts	<i>E.mundtii</i> CRA 16812	Chilled custard Batch 1	Low:100cfu/g Medium : 1000cfu/g High : 10,000cfu/g
			Chilled custard Batch 2	
			Whipped cream	
Fruits and vegetables	Leafy greens e.g. parsley, lettuce	<i>E.faecium</i> NCIMB 9645	Parsley Batch 1	Low: 50cfu/g Medium : 1000cfu/g High : 50,000cfu/g
			Parsley Batch 2	
			Shredded lettuce	
Raw poultry and meats	Fresh beef	<i>E.avium</i> NCIMB 702366	Fresh steak Batch 1	Low: 50cfu/g Medium : 1000cfu/g High : 50,000cfu/g
			Fresh steak Batch 2	
			Patties	
Ready to eat foods	Cooked fish products e.g. prawns	<i>E. casseliflavus</i> CRA 16811	Tuna pate Batch 1	Low: 50cfu/g Medium : 100cfu/g High : 1000cfu/g
			Tuna pate Batch 2	
			Fresh cooked prawns	
Multi component foods	Composite foods with raw ingredients	<i>E.hirae</i> CRA 15939	Pasta salad Batch 1	Low 500cf/g Medium : 5000cfu/g High : 50,000cfu/g
			Pasta salad Batch 1	
			Sandwiches	

The results and the accuracy products for the samples tested are given in the tables 8 -12. A conclusion of the results is given after table 8.

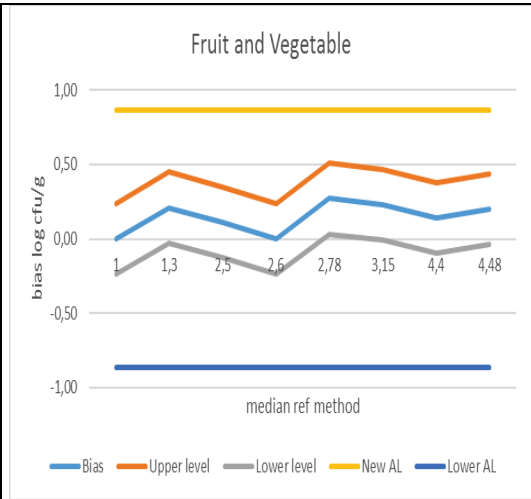
**Table 8. Dairy products – 3 types, 3 levels each with 5 replicates**

Compact Dry ETC		NMKL 68		Bias	Lower level	Upper level	± AL
Median	SD	Median	SD				
1.78	0.16	1.85	0.28	-0.07	0.16	-0.30	0.88
1.60	0.22	1.78	0.21	-0.18	0.05	-0.41	0.88
1.70	0.22	1.60	0.15	0.10	0.33	-0.13	0.88
2.69	0.09	3.26	0.19	-0.57	-0.34	-0.80	0.88
2.63	0.07	2.95	0.05	-0.32	-0.09	-0.55	0.88
2.67	0.04	2.85	0.42	-0.18	0.05	-0.41	0.88
3.51	0.04	4.09	0.48	-0.58	-0.35	-0.81	0.88
3.57	0.07	3.93	0.21	-0.36	-0.13	-0.59	0.88
3.54	0.05	3.98	0.18	-0.44	-0.21	-0.67	0.88
Comb SD	0.126		0.221				



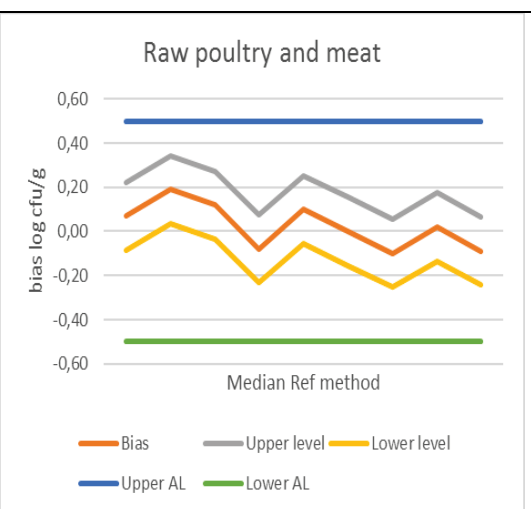
**Table 9. Fruit and vegetables – 3 types, 3 levels each with 5 replicates**

Compact Dry ETC		NMKL 68		Bias	Lower level	Upper level	± AL
Median	SD	Median	SD				
1.30	0.21	1.00	0.13	0.30	0.54	0.06	0.86
1.30	0.27	1.30	0.20	0.00	0.24	-0.24	0.86
2.71	0.06	2.50	0.25	0.21	0.45	-0.03	0.86
2.71	0.07	2.60	0.33	0.11	0.35	-0.13	0.86
2.78	0.08	2.78	0.19	0.00	0.24	-0.24	0.86
3.42	0.08	3.15	0.11	0.27	0.51	0.03	0.86
4.63	0.07	4.40	0.21	0.23	0.47	-0.01	0.86
4.62	0.07	4.48	0.33	0.14	0.38	-0.10	0.86
4.70	0.07	4.50	0.17	0.20	0.44	-0.04	0.86
Comb SD	0.129		0.216				



**Table 10. Raw poultry and meats – 3 types, 3 levels each with 5 replicates**

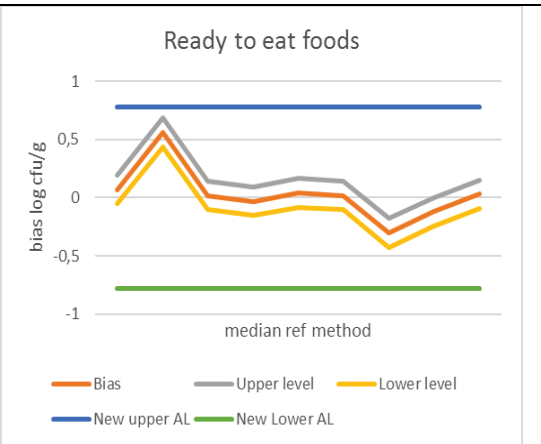
Compact Dry ETC		NMKL 68		Bias	Lower level	Upper level	± AL
Median	SD	Median	SD				
2.78	0.09	2.71	0.22	0.07	0.22	-0.08	0.5
2.79	0.06	2.60	0.14	0.19	0.34	0.04	0.5
2.89	0.16	2.77	0.18	0.12	0.27	-0.03	0.5
3.99	0.04	4.07	0.05	-0.08	0.07	-0.23	0.5
4.06	0.04	3.96	0.14	0.10	0.25	-0.05	0.5
4.04	0.06	4.04	0.04	0.00	0.15	-0.15	0.5
5.77	0.09	5.87	0.18	-0.10	0.05	-0.25	0.5
5.62	0.04	5.60	0.09	0.02	0.17	-0.13	0.5
5.74	0.13	5.83	0.04	-0.09	0.06	-0.24	0.5
Comb SD	0.083		0.122				





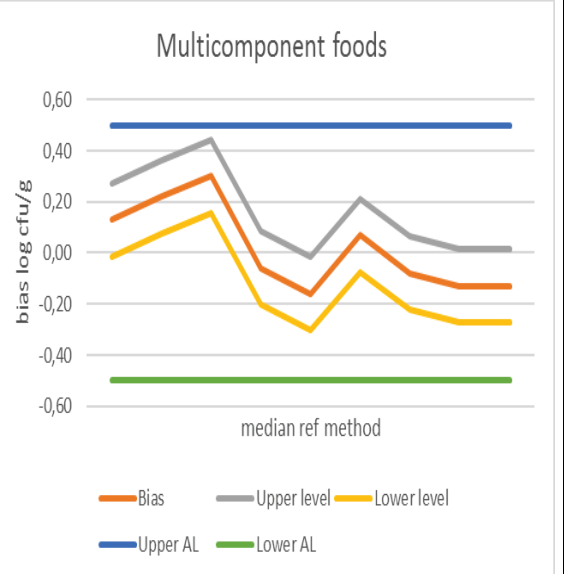
**Table 11. Ready to eat foods – 3 types, 3 levels each with 5 replicates**

Compact Dry ETC		NMKL 68		Bias	Lower level	Upper level	± AL
Median	SD	Median	SD				
1.85	0.09	1.78	0.06	0.07	0.19	-0.05	0.78
2.26	0.10	1.70	0.22	0.56	0.68	0.44	0.78
2.02	0.06	2.00	0.33	0.02	0.14	-0.10	0.78
2.38	0.03	2.41	0.27	-0.03	0.09	-0.15	0.78
2.30	0.09	2.26	0.10	0.04	0.16	-0.08	0.78
2.53	0.07	2.51	0.22	0.02	0.14	-0.10	0.78
2.85	0.02	3.15	0.11	-0.30	-0.18	-0.42	0.78
2.88	0.04	3.00	0.18	-0.12	0.00	-0.24	0.78
2.88	0.04	2.85	0.12	0.03	0.15	-0.09	0.78
Comb SD	0.067		0.194				



**Table 12. Multi component foods – 3 types, 3 levels each with 5 replicates**

Compact Dry ETC		NMKL 68		Bias	Lower level	Upper level	± AL
Median	SD	Median	SD				
2.11	0.03	1.98	0.07	0.13	0.27	-0.01	0.5
2.00	0.06	1.78	0.35	0.22	0.36	0.08	0.5
1.90	0.20	1.60	0.34	0.30	0.44	0.16	0.5
3.89	0.04	3.95	0.07	-0.06	0.08	-0.20	0.5
3.87	0.04	4.03	0.14	-0.16	-0.02	-0.30	0.5
3.85	0.04	3.78	0.26	0.07	0.21	-0.07	0.5
4.87	0.09	4.95	0.03	-0.08	0.06	-0.22	0.5
4.86	0.04	4.99	0.15	-0.13	0.01	-0.27	0.5
4.83	0.07	4.96	0.05	-0.13	0.01	-0.27	0.5
Comb SD	0.078		0.199				



The bias is the difference obtained by the alternative method and the reference method. Whenever no biases exist, the results would be on  $y=0$ . Taking the standard deviation into account, the results might be expected to vary between upper and lower levels.

When the upper level is below the upper Acceptability Limit (AL), and the lower level is above the lower AL, the alternative method is accepted as being equivalent to the reference method. The AL is  $\pm 0.5$  log cfu, however, in cases where the upper or lower levels exceeds the AL and the combined standard deviation, SD, of the reference method is 0.125 or above, new AL is calculated as  $4 \times SD$ . This has been done for dairy products, fruit and vegetables, and ready to eat food. As the results fall within the acceptability limits, the Compact Dry ETC method is accepted as being equivalent to the reference method.

## INTERLABORATORY STUDY, ILS

### Compact Dry ETC method for water analysis

The ILS for Compact Dry ETC method for water was arranged in 2014 by Campden BRI (Chipping Campden) Limited. Eight laboratories were involved. All laboratories analysed eight samples using both the reference and the alternative method. Results from one laboratory were not included as the laboratory did not follow the reference method correctly.

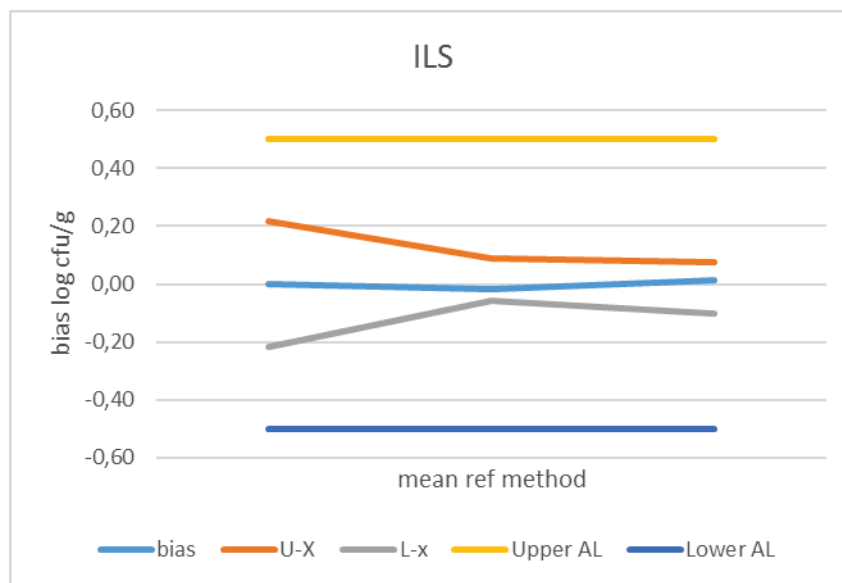
The results of the study are given Table 13 and illustrated in Figure 2.

**Table 13. Results (in log cfu/100 mL) of the interlaboratory study, ILS, for analysis of water**

Levels	Dry ETC Mean	Alt SR	ISO 7899-2 Mean	Ref SR	Bias	Upper level u-x	Lower level L-x	Upper AL	Lower AL
Blind	-		-						
Low	0.98	0.16	0.98	0.16	0.00	0.22	-0.22	0.5	-0.50
Middel	1.63	0.080	1.64	0.051	-0.02	0.09	-0.06	0.5	-0.50
High	2.01	0.059	2.00	0.063	0.02	0.07	-0.10	0.5	-0.50

SR = standard deviation of reproducibility

**Figure 2. Accuracy Profile of the ILS on water analysis.**



The difference obtained by the alternative method and the reference method, the bias, is close to 0, and the levels where the results are expected to be found (U-x and L-x) is within the acceptability limits, AL  $\pm$  0.5 log cfu/100 mL. The alternative method is therefore accepted as being equivalent to the reference method.

### Compact Dry ETC method for food analysis

The ILS for Compact Dry ETC method for food analysis was arranged in 2016 by Campden BRI (Chipping Campden) Limited. There were 5 organisations used in this study representing 3 different countries. The number of collaborators from each organisation varied from 1 to 3 giving a maximum of 11 potential data sets. Three of the data sets were not used in the analysis due to incomplete data for the reference method, even though the alternative method performed well. Thus, finally there were 8 valid data sets from 4 different organisations and 3 different countries.

Each participant in the ILS received 7 x 10g samples of salmon pâté in sterile stomach bags

One sample of pâté remained uninoculated. For the remaining six samples, appropriate dilutions of the *E. faecalis* culture were used to individually inoculate 2 x 10g samples at the low ( $\sim 10^2$  cfu/ml), middle ( $\sim 10^4$  cfu/ml) and high ( $\sim 10^6$  cfu/ml) contamination levels.

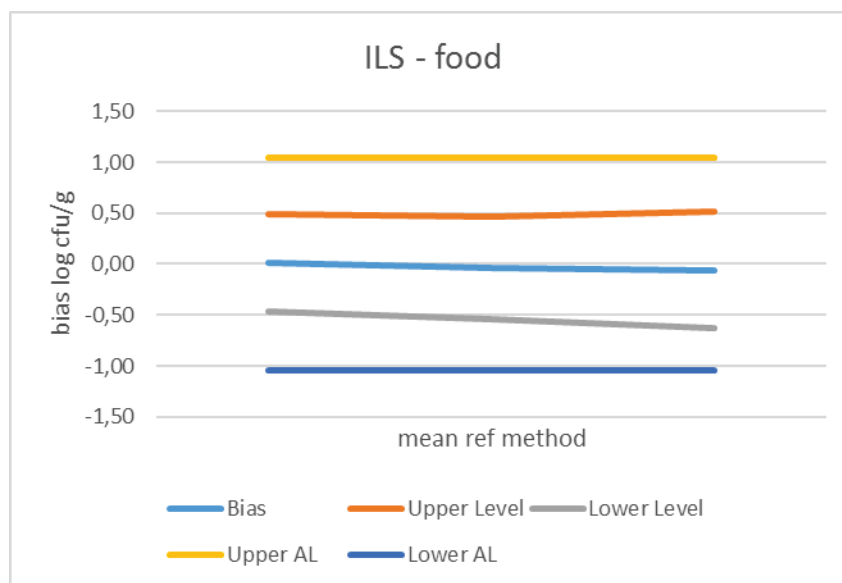
The results of the study are given in Table 14 and illustrated in Figure 3.

**Table 14. Results (in log cfu/25g) of the interlaboratory study, ILS, for analysis of food**

Levels	Dry ETC Mean	Alt SR	ISO 7899-2 Mean	Ref SR	Bias	Upper level u-x	Lower level L-x	Upper AL	Lower AL
Blind	-		-						
Low	2.99	0.32	2.98	0.30	0.01	0.49	-0.47	1.04	-1.04
Middel	4.45	0.34	4.49	0.33	-0.04	0.47	-0.54	1.04	-1.04
High	6.04	0.39	6.10	0.31	-0.06	0.52	-0.63	1.04	-1.04

SR = standard deviation of reproducibility

**Figure 3. Accuracy Profile of the ILS on food analysis.**



## CONCLUSION

The mean difference obtained by the alternative method and the reference method, the bias, is close to 0. The standard deviation of reproducibility is somewhat high both for the alternative method and the reference method, and therefore the acceptability limit, AL, is  $\pm 1.0$  instead of  $\pm 0.5$  log cfu/25g. As expected, levels obtained by the alternative method fall between the ALs, the alternative method is accepted as being equivalent to the reference method.