NMKL - NordVal International
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NordVal International Certificate

Issued for:	Compact Dry YM Method for the Enumeration of Yeasts and Moulds in foods
NordVal No:	043
First approval date:	1 June 2015
Renewal date:	1 June 2023
Valid until:	1 June 2025

Compact Dry YM

Manufactured and supplied by:

Shimadzu Diagnostics Corporation,
20th Floor Ueno Frontier Tower,
3-24-6 Ueno, Taito-ku, Tokyo,
110-8736 JAPAN

fulfils the requirements of the NordVal validation protocol. The reference method was ISO 21527-1:2008: Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds – colony count technique – Part 1: Products with a water activity greater than 0.95.

NordVal International has reviewed the method and the validation studies conducted by Campden BRI, UK. Original studies were conducted according to ISO 16140:2003. A renewal study has been performed according to ISO 16140-2:2016 to test for relative trueness and accuracy profile. The design of the Inter-laboratory study (ILS) is the same for the 2003 and 2016 versions of ISO16140 and therefore the existing ILS data were reanalyse using the new statistical approach outlined in ISO16140-2:2016. The results document no statistical difference in the performances between Compact Dry YM and the ISO 21527-1:2008.

Date: 01 June 2023

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

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 diluted sample into the centre of the self-diffusible medium. The Compact Dry YM (yeasts and moulds) method contains chromogenic medium and selective agents for the detection and enumeration of yeasts, which form blue colonies, and moulds, which form “cottony colonies”. An aliquot of 1 ml of an appropriate dilution is plated onto Compact Dry YM plate. The incubation conditions tested in the study were 25 ± 1°C for 3 and 7 days.

FIELD OF APPLICATION

The method has been tested on the detection and enumeration of yeast and moulds in foods.

HISTORY

Original studies for this certificate were conducted according NordVal protocol from 2010. Selectivity results are from this study. A renewal study was carried out in 2017 according to ISO 16140-2:2016 to test for relative trueness and accuracy profile. The design of the Inter-laboratory study (ILS) is the same for the 2003 and 2016 versions of ISO16140, and therefore the existing ILS data were reanalysed using the new statistical approach outlined in ISO16140-2:2016.

THE COMPARISON STUDIES

Both the reference method and the alternative method give the user the opportunity to record results at two time points. This enables an initial colony count to be made during the early stages of the test. A later colony count is made to enable sufficient time for slower growing fungi to develop into visible colonies that would then be included in the final count.

The selectivity (inclusivity and exclusivity)

Inclusivity: A total of 31 strains comprising 16 moulds and 15 yeasts were analysed using the Compact Dry YM and the ISO 21527-1, respectively.

Inclusivity

Moulds

The inclusivity results for mould showed that 2 strains (one *Monascus bisporus* and one *Chrysosporium farinicola*) of the 15 strains tested failed to grow on the Compact Dry YM or at the media of the reference method. This observation confirms the xerophilic nature of these slow growing mould species which do not normally grow on high water activity media.

Of the 13 remaining strains of moulds, 5 failed to produce visible colonies on Compact Dry YM plates after 3 days but were visible by 7 days. By comparison, the reference method failed to yield visible colonies with 3 strains after 2 days and all except a strain of Aspergillus *echinulatus*, produced visible colonies after 5 days. The colours of the mould colonies carried according to the species.

Yeasts

The inclusivity results for the yeasts revealed 15 strains produced visible colonies after 3 days, and the colony appearance generally conformed to the typically blue coloured colonies on Compact Dry YM plates. However, the manufacturer also acknowledges that some yeast do not produce blue colonies. This was observed with some strains that produced colonies of another colour (green or white) or a mixture of blue with colonies of a different colour.

Exclusivity

None of the 20 non-target organisms grew on Compact Dry YM plates after 3 days and after 7 days only one strain of *Pseduomonas fluorescens* was able to grow on this medium, yielding yeast like colonies, although these were still considered atypical. This strain also produced pink colonies with the reference method after 2 days. In addition the reference method supported the growth of 4 other bacterial strains. This included *Enterobacter aerogenes* and *PS fragi* which produced visible colonies after 2 days and an *E.cloacae, Ps. Aeruginosa,* which produced visible colonies after 5 days.

RESULTS OF THE COMPARISON STUDIES

The study was carried out in 2010 by CCFRA Technology Limited, Chipping Campden, UK.

Figure 1a: Bland-Altman plot for all categories, 3 days (3d)

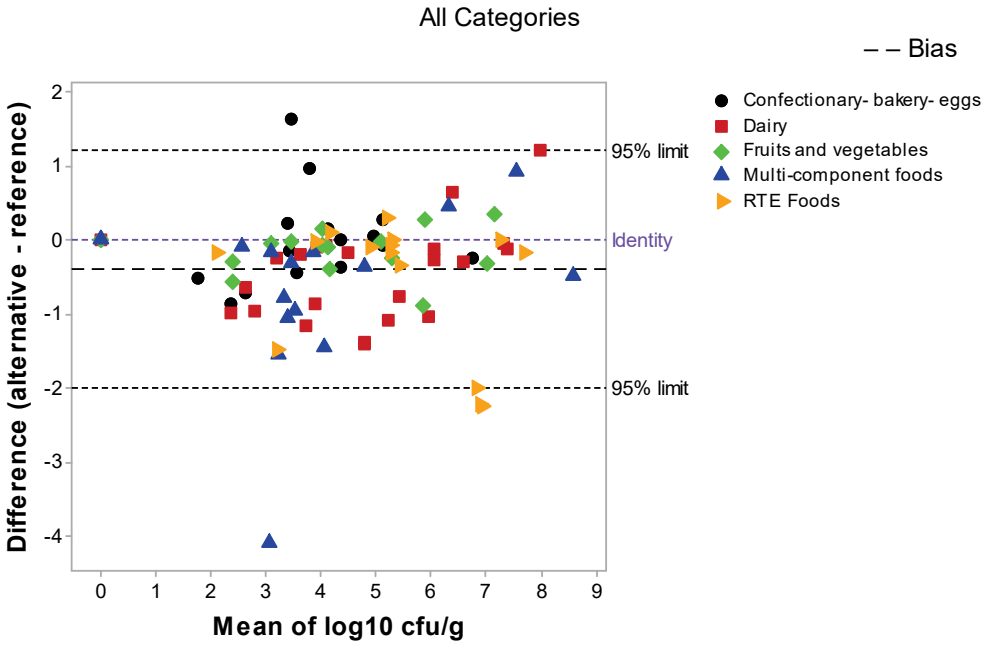
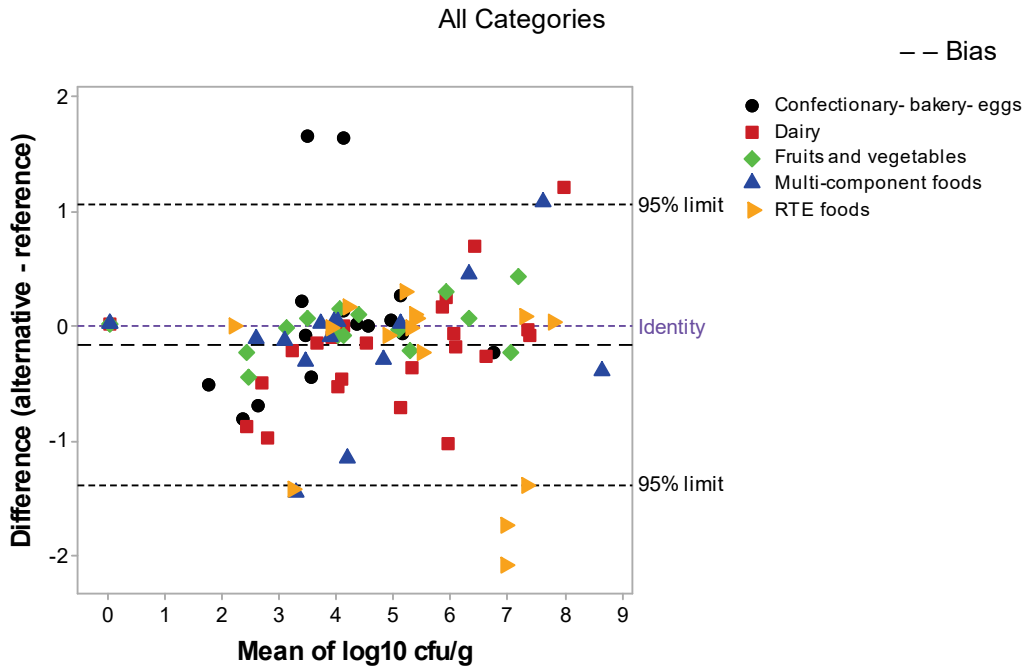


Figure 1b: Bland-Altman plot for all categories, 7 days (7d)



The results of the Bland-Altman plot shows the amount of bias and extreme results. It is expected that not more than one in 20 data values will lie outside the 95% Confidens Limits (CLs).

For 'All Categories' for the 3d data there are four in 85 values which lie outside the CLs. This is in agreement with the expectation of less than one in 20. For 'All Categories' the 7d data there are 7 in 85 values which lie outside the CLs. This is slightly more than the expectation of less than one in 20. There were no identifiable trends in these data and they covered 4 different food categories. For the 7d data, the data points have been examined and there are no obvious reasons for the disagreement, all the colony count data are within the target counting range of the methods and appear accurate. Three of the data points are very close to the lower confidence limit which leaves 4 points which are true outliers. Two of these are above the upper limit with an average difference of 1.635 and two are below the lower limit with an average difference of -1.61. It is concluded that these samples are just individual cases where there is disagreement between the agars with no identifiable explanation. These differences are not unexpected as this data is for a total count of naturally present yeast and moulds which may vary considerably between samples.

ACCURACY PROFILES

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 for this.

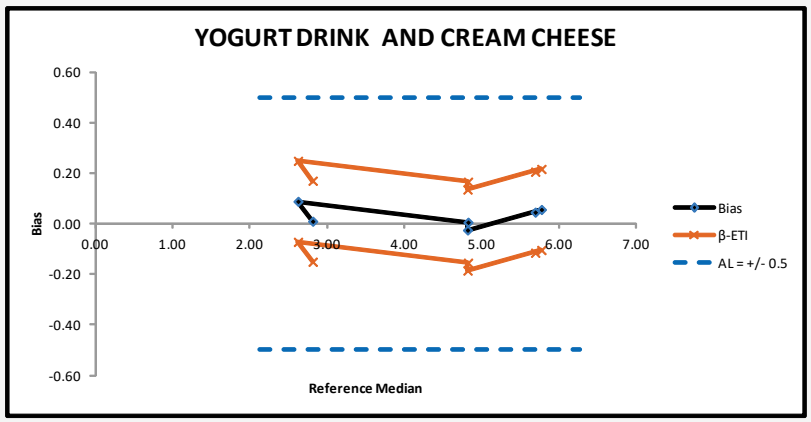
Food matrices

For each of 5 food categories, one type of food was tested using 6 samples per type. Of the 6 samples, there were 2 at a low level, 2 at a medium level and 2 at a high level of contamination. For each of the 6 samples per category, 5 replicate test portions were tested.

The statistical results and the accuracy profiles are provided Figure 2a to e.

Figure 2a: Dairy products

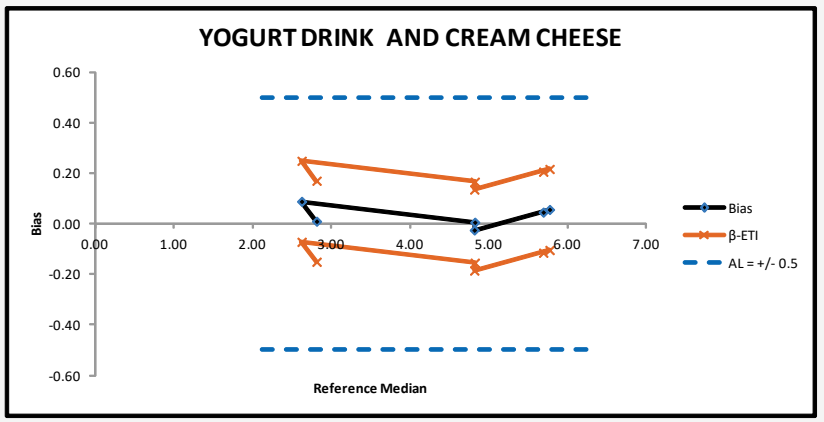
(Food) Category	DAIRY 3-day
(Food) Type	YOGURT DRINK AND CREAM



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
88 a-e	2.81	0.010	-0.150	0.170	YES	YES
147 a-e	2.62	0.089	-0.072	0.249	YES	YES
84 a-e	4.82	0.006	-0.154	0.166	YES	YES
160 a-e	4.82	-0.025	-0.185	0.135	YES	YES
10 a-e	5.77	0.056	-0.104	0.217	YES	YES
15 a-e	5.69	0.046	-0.114	0.206	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.069	0.111	YES	+/- 0.500

(Food) Category	DAIRY 7-day
(Food) Type	YOGURT DRINK AND CREAM

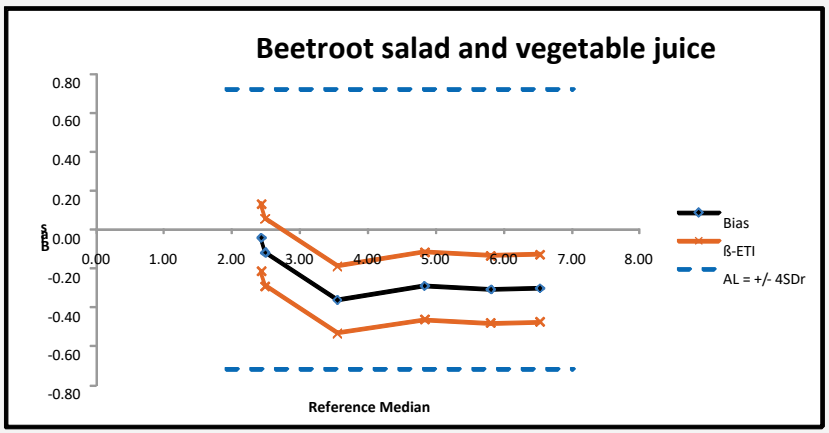


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
88 a-e	2.81	0.010	-0.150	0.170	YES	YES
147 a-e	2.62	0.089	-0.072	0.249	YES	YES
84 a-e	4.82	0.006	-0.154	0.166	YES	YES
160 a-e	4.82	-0.025	-0.185	0.135	YES	YES
10 a-e	5.77	0.056	-0.104	0.217	YES	YES
15 a-e	5.69	0.046	-0.114	0.206	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.069	0.111	YES	+/- 0.500

Figure 2b: Fresh produce

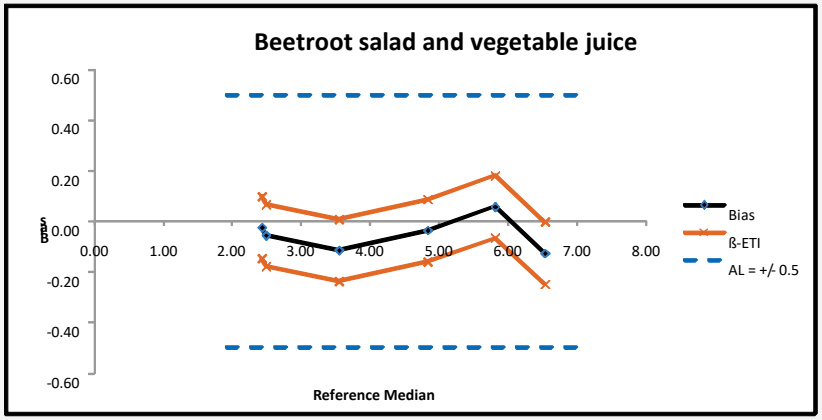
(Food) Category	PRODUCE 3d
(Food) Type	Beetroot salad and vegetable juice



Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Aceeptable	β-ETI compared to final AL Aceeptable
31 a e	2.43	0.042	0.215	0.131	YES	YES
25 a e	2.49	0.118	0.292	0.055	YES	YES
133 a e	3.55	0.361	0.534	0.187	NO	YES
85 a e	4.83	0.290	0.463	0.116	YES	YES
190 a e	5.81	0.307	0.480	0.134	YES	YES
13 a e	6.53	0.301	0.474	0.128	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.180	0.120	NO	+/- 0.720

(Food) Category	PRODUCE 7d
(Food) Type	Beetroot salad and vegetable juice

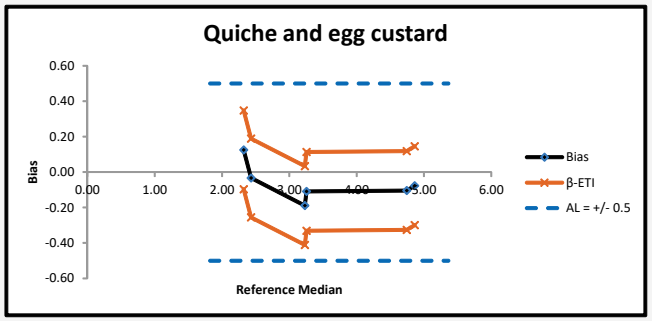


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Aceeptable	β-ETI compared to final AL Aceeptable
31 a e	2.43	0.025	0.148	0.098	YES	YES
25 a e	2.49	0.055	0.178	0.068	YES	YES
133 a e	3.55	0.114	0.237	0.009	YES	YES
85 a e	4.83	0.036	0.159	0.086	YES	YES
190a e	5.81	0.057	-0.065	0.180	YES	YES
13 a e	6.53	0.126	0.248	0.003	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.180	0.085	NO	+/- 0.500

Figure2c: Confectionary, bakery and eggs

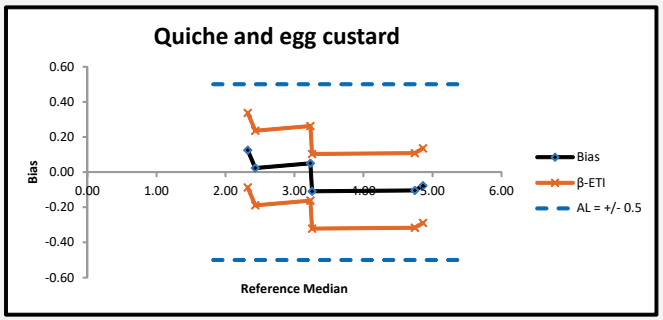
(Food) Category	Confectionary -3 day
(Food) Type	Quiche and egg custard



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
44 a-e	2.32	0.125	-0.097	0.347	YES	YES
134 a-e	2.43	-0.033	-0.256	0.189	YES	YES
2 a-e	3.23	-0.189	-0.411	0.033	YES	YES
124 a-e	3.26	-0.109	-0.331	0.113	YES	YES
15 a-e	4.74	-0.104	-0.326	0.118	YES	YES
165 a-e	4.86	-0.077	-0.299	0.145	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.123	0.154	YES	+/- 0.500

(Food) Category	Confectionary - 7day
(Food) Type	Quiche and egg custard

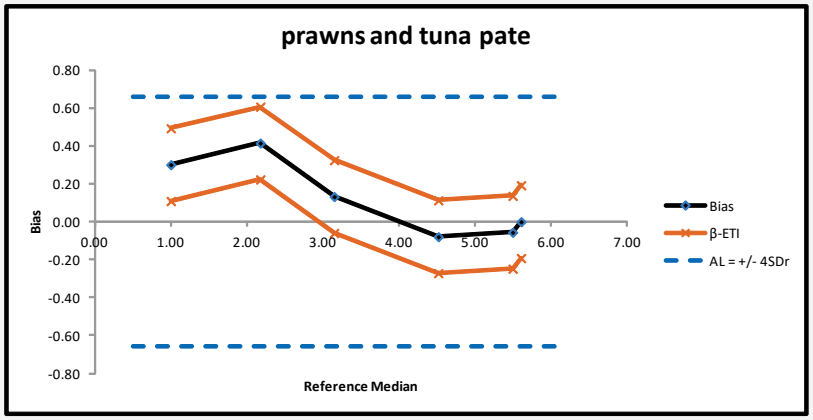


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
44 a-e	2.32	0.125	-0.087	0.337	YES	YES
134 a-e	2.43	0.023	-0.189	0.236	YES	YES
2 a-e	3.23	0.050	-0.162	0.263	YES	YES
124 a-e	3.26	-0.109	-0.321	0.103	YES	YES
15 a-e	4.74	-0.104	-0.316	0.108	YES	YES
165 a-e	4.86	-0.077	-0.289	0.135	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.122	0.147	YES	+/- 0.500

Figure 2d: Ready to eat foods

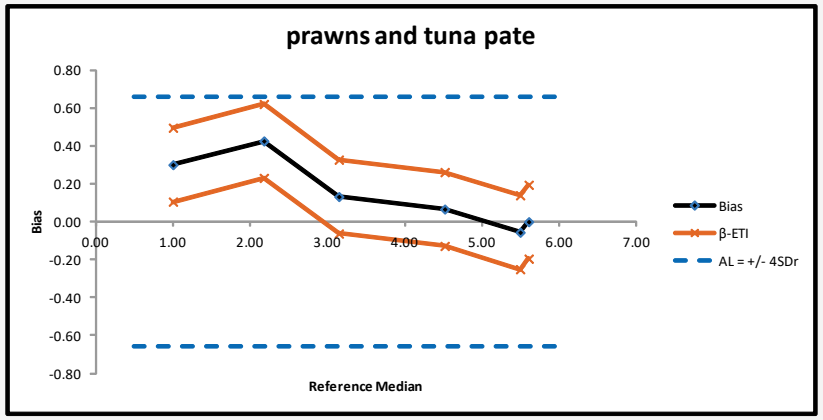
(Food) Category	RTE Foods-3d
(Food) Type	prawns and tuna pate



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
186 a-e	1.00	0.301	0.109	0.493	YES	YES
197 a-e	2.18	0.415	0.223	0.607	NO	YES
68 a-e	3.15	0.133	-0.059	0.325	YES	YES
64 a-e	4.51	-0.079	-0.271	0.113	YES	YES
23 a-e	5.49	-0.056	-0.248	0.136	YES	YES
36 a-e	5.60	0.000	-0.192	0.192	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.164	0.133	NO	+/- 0.656

(Food) Category	RTE Foods- 7d
(Food) Type	prawns and tuna pate

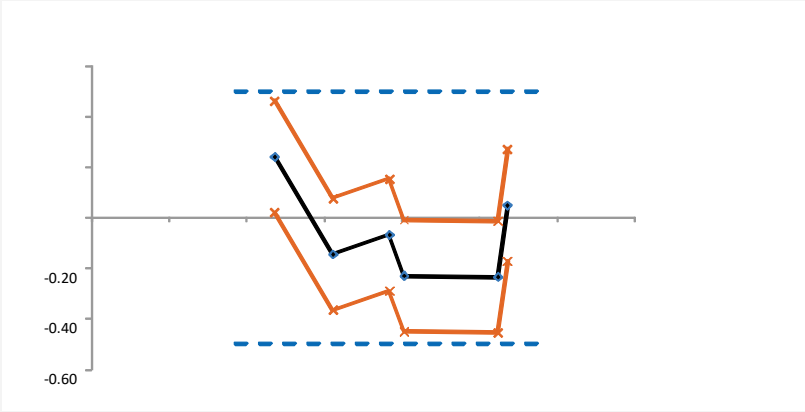


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
186 a-e	1.00	0.301	0.106	0.496	YES	YES
197 a-e	2.18	0.426	0.231	0.621	NO	YES
68 a-e	3.15	0.133	-0.062	0.328	YES	YES
64 a-e	4.51	0.067	-0.128	0.262	YES	YES
23 a-e	5.49	-0.056	-0.251	0.139	YES	YES
36 a-e	5.60	0.000	-0.195	0.195	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.164	0.135	NO	+/- 0.656

Figure 2e: Multicomponent foods

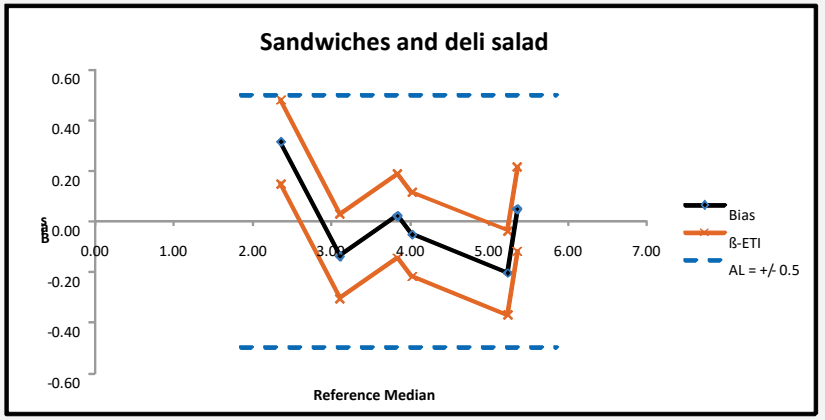
(Food) Category (Food) Type	Multi-component 3d Sandwiches and deli salad



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
174 a-e	2.36	0.240	0.019	0.461	YES	YES
6 a-e	3.11	0.144	0.365	0.076	YES	YES
155 a-e	3.84	0.068	0.289	0.153	YES	YES
200 a-e	4.03	0.229	0.450	0.008	YES	YES
79 a-e	5.24	0.233	0.454	0.013	YES	YES
180 a-e	5.36	0.049	0.172	0.270	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.112	0.153	YES	+/- 0.500

(Food) Category (Food) Type	Multi-component 7d Sandwiches and deli salad



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
174 a-e	2.36	0.315	0.149	0.481	YES	YES
6 a-e	3.11	0.137	0.303	0.029	YES	YES
155 a-e	3.84	0.022	0.144	0.188	YES	YES
200 a-e	4.03	0.051	0.217	0.115	YES	YES
79 a-e	5.24	-0.203	-0.369	-0.037	YES	YES
180 a-e	5.36	0.049	0.117	0.215	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.112	0.115	YES	+/- 0.500

RESULTS OF THE COLLABORATIVE STUDY

The study, organised by CCFRA Technology Limited, Chipping Campden, UK, was carried out in 2011. There were 9 participating laboratories from 6 countries. The results have been re-analysed/re-calculated using the new statistical approach outlined in ISO 16140-2:2016.

A single strain of the yeasts *Debaromyces hansenii* and a single strain of mould *Penicillium chrysogenum* was used in the study. Appropriate dilutions of the yeasts and moulds culture were used to individually inoculate juice samples at lower (10^3 cfu/ml), middle (10^4 cfu/ml) and higher (10^5 cfu/ml) contamination levels. Each laboratory got two samples at each level and two samples of negative control. The organising laboratory participated also in the collaborative study. One laboratory fails to enumerate yeast and moulds on the CD YM after 3 days, so this result was omitted in the calculation for the estimation of the median and the precision (repeatability and reproducibility). The results are shown in Table 1a and b.

Table 1a. Statistical analysis of the ILS data according to the ISO spreadsheet – 3 day data

Accuracy profile	0.5		
Study Name	YM ILS analysis		
Date	03/03/2017		
Coordinator	Campden BRI		
Tolerance probability (beta)	80%	80%	80%
Acceptability limit in log (lambda)	0.50	0.50	0.50

FALSE

Application of clause 6.2.3
Step 8: If any of the values for the β-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.

	Alternative method			**Reference method**		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	**4.011**	**4.811**	**5.743**			
Number of participants (K)	8	8	8	8	8	8
Average for alternative method	3.964	4.745	5.677	4.011	4.811	5.743
Repeatability standard deviation (sr)	0.151	0.084	0.068	0.071	0.114	0.089
Between-labs standard deviation (sL)	0.094	0.171	0.248	0.079	0.109	0.147
Reproducibility standard deviation (sR)	0.178	0.190	0.257	0.106	0.158	0.172
Corrected number of dof	13.368	8.496	7.505	10.856	11.558	9.136
Coverage factor	1.401	1.466	1.488			
Interpolated Student t	1.348	1.390	1.406			
Tolerance interval standard deviation	0.1846	0.2009	0.2724			
Lower TI limit	3.715	4.466	5.295			
Upper TI limit	4.213	5.024	6.060			
Bias	**-0.046**	**-0.066**	**-0.066**			
Relative Lower TI limit (beta = 80%)	**-0.295**	**-0.345**	**-0.449**			
Relative Upper TI limit (beta = 80%)	**0.203**	**0.214**	**0.317**			
Lower Acceptability Limit	**-0.50**	**-0.50**	**-0.50**			
Upper Acceptability Limit	**0.50**	**0.50**	**0.50**			
New acceptability limits may be based on reference method pooled variance						
Pooled repro standard dev of reference	0.148					

FALSE
FALSE

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

Table 1b. Statistical analysis of the ILS data according to the ISO spreadsheet – 7 day data

Accuracy profile	0.5		
Study Name	YM ILS Analysis		
Date	03/03/2017		
Coordinator	Campden BRI		
Tolerance probability (beta)	80%	80%	80%
Acceptability limit in log (lambda)	0.50	0.50	0.50

FALSE

Application of clause 6.2.3
Step 8: If any of the values for the β-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.

	Alternative method			**Reference method**		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	**4.021**	**4.831**	**5.767**			
Number of participants (K)	9	9	9	9	9	9
Average for alternative method	3.979	4.778	5.718	4.021	4.831	5.767
Repeatability standard deviation (sr)	0.140	0.094	0.109	0.067	0.133	0.104
Between-labs standard deviation (sL)	0.087	0.161	0.216	0.080	0.105	0.149
Reproducibility standard deviation (sR)	0.165	0.187	0.242	0.104	0.169	0.182
Corrected number of dof	15.248	10.312	9.794	12.038	14.186	11.049
Coverage factor	1.386	1.434	1.441			
Interpolated Student t	1.340	1.369	1.374			
Tolerance interval standard deviation	0.1707	0.1959	0.2536			
Lower TI limit	3.751	4.509	5.369			
Upper TI limit	4.208	5.046	6.066			
Bias	**-0.042**	**-0.053**	**-0.049**			
Relative Lower TI limit (beta = 80%)	**-0.270**	**-0.322**	**-0.398**			
Relative Upper TI limit (beta = 80%)	**0.187**	**0.215**	**0.299**			
Lower Acceptability Limit	**-0.50**	**-0.50**	**-0.50**			
Upper Acceptability Limit	**0.50**	**0.50**	**0.50**			

FALSE
FALSE

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

New acceptability limits may be based on reference method pooled variance

The repeatability standard deviation (Sr), the between-labs standard deviation (SL) and the reproducibility standard deviation (SR) was similar for the alternate method and the reference method for both the 3d and 7d data. There was a very slight negative bias in the data for the alternate method of <0.1log value

None of the β-ETI values lie outside of the ±0.5log AL values and therefore the alternative method is accepted as being equivalent to the reference method from the Inter laboratory study analysis.

CONCLUSION

The observed profiles are within the 0.5 log AL or the recalculated AL limit calculated according to NordVal International Protocol /ISO16140-2:2016, and hence the alternative method is accepted as being equivalent to the reference method.