Method Comparison Study Report for the ISO 16140-2:2016 validation of Compact Dry EC, for the enumeration of coliforms

MicroVal study number: 2007LR04

Method/Kit name: Compact Dry EC

Report version:MCS ILS Summary report created 03/09/2019

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Foreword

This report is prepared in accordance with ISO 16140-2:2016 and MicroVal Technical Committee interpretation of ISO 16140-2 v.1.0

Company: Nissui Pharmaceutical Co. Ltd

Expert Laboratory: Campden BRI

Method/Kit name: Compact Dry EC coliforms

Validation standard: ISO 16140-2:2016 Microbiology of the food chain —Method validation —Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

Reference methods ISO 4832:2006 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms —Colony-count technique

Scope of validation: A broad range of foods based on categories

- Milk and dairy products
- Fresh produce and fruits
- Raw poultry and meats
- Ready to eat foods
- Multi component foods or meal components

Certification organisation: Lloyd's Register

List of abbreviations

- AL Acceptability Limit
- AP Accuracy Profile
- Art. Cont. Artificial contamination
- CFU Colony Forming Units
- CL confidence limit (usually 95%)
- EL Expert Laboratory
- $\bar{D}$ Average difference
- g Gram
- h Hour
- ILS Interlaboratory Study
- Inc/Ex Inclusivity and Exclusivity
- LOQ Level of Quantification
- MCS Method Comparison Study
- min minute
- ml Millilitre
- MR (MicroVal) Method Reviewer
- MVTC MicroVal Technical Committee
- EL Expert Laboratory
- n number of samples
- na not applicable
- neg negative (target not detected)
- NG no growth
- nt not tested
- RT Relative Trueness
- SD standard deviation of differences
- 10^{-1} dilution 10-fold dilution of original food
- 10^{-2} dilution 100-fold dilution of original food
- PSD Peptone salt diluent

Contents

1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of alternative method(s) for the enumeration of coliforms in five different food categories was carried out by Campden BRI as the MicroVal Expert Laboratory.

The alternative method used was:

- Enumeration of coliforms on Compact Dry EC, incubated at 37°C±1°C for 24±2h. The minimum time of 22h was used.

The reference method used was:

- ISO 4832:2006 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms —Colony-count technique

Categories included :

- Milk and dairy products
- Fresh produce and fruits
- Raw poultry and meats
- Ready to eat foods
- Multi component foods or meal components

Criteria evaluated during the study have been:

- Relative trueness study;
- Accuracy profiles;
- Limits of quantification (LOQ);
- Inclusivity and exclusivity
- Interlaboratory Study

The final conclusion on the Method Comparison Study and ILS is summarised below:

The alternative method Compact Dry EC shows comparable performance to the reference method (ISO 4832:2006 for the enumeration of coliforms in a broad range of foods.

2 Method protocols

The Method Comparison Study was carried out using 10g gram portions of sample material.

According to ISO 16140-2 the reference method and alternative methods were performed with the same sample. The study was therefore a paired study design.

2.1 Reference method

The reference method was ISO 4832:2006 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms —Colony-count technique.

See the flow diagram in Annex A.

Sample preparations used in the reference method were done according to ISO 6887-series parts 1, 2, 3, 4 and 5. Plating was done according to ISO 7218:2007+A1:2013 section 10.2.2 which says at least one plate per dilution shall be used with at least two successive dilutions. Two plates per dilution may also be used to improve reliability. If only one dilution is used, then two plates of this dilution shall be used to improve reliability of the results. Depending on the sample being tested and the expected contamination level, single or multiple dilutions were used with single or duplicate plates if considered necessary to improve the reliability of the calculated result and ensure at least two relevant plates were available for use in calculations.

2.2 Alternative method

See the flow diagram of the alternative method in Annex A.

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. This is a ready to use, selective plate chromogenic plate for the enumeration of *E.coli* and coliforms. These organisms are differentiated by the colony morphology. *E.coli* colonies form blue colonies and other coliforms form red colonies after the required incubation period. The total coliform count will therefore be based on a total count of all red and blue colonies after 24±hr incubation at 37±1ºC.

Samples of product containing the target organism were diluted 1 in 10 with an appropriate diluent according to ISO 6887 and homogenised in a stomacher.

Appropriate serial dilutions were made, and all relevant dilutions were analysed using the reference method and alternative method.

3 Method comparison study

3.1 Relative trueness study

The 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 for this.

A total of 5 categories were included in this validation study. A minimum of 15 items for each category were tested by both the reference method and the alternative method in the relative trueness study, with a minimum of 15 interpretable results per category.

Each category was made up of 3 types, with at least 5 items representative for each type.

3.1.1 Number of samples
The categories, the types and the number of samples analyzed are presented in Table 1.

Table 1 – Categories, types and number of samples analyzed

Category	Types	Number of samples
Milk and dairy products	Dry milk product e.g. milk powder, powder for milk based desserts, dried infant formula	5
	Dairy products e.g. ice-cream, yogurts, cream, hard cheese, soft cheese, raw milk cheese	5
	Pasteurised milk products e.g. skimmed, semi-skimmed, full fat and flavoured milks	5
Fresh produce and fruits	Cut ready to eat fruit e.g. fruit mixes, fruit juices	5
	Cut ready to eat vegetables e.g. Bagged pre-cut salads and shredded carrot, cabbage, vegetable juices	5
	Leafy greens/Sprouts e.g. soy, mung, alfalfa,	5
Raw poultry and meats (Combined category raw/ RTC meats and poultry)	Fresh poultry cuts e.g. turkey breast, turkey fillet	5
	Fresh mince e.g. lamb, beef, pork	5
	Processed ready to cook e.g. frozen patties, marinated kebabs, seasoned chicken breasts	5
Ready to eat foods (Combined category RTE/RTRH meats and poultry)	Ready to eat poultry e.g. turkey fillet, chicken sausage, pate	5
	Cooked fish products e.g. prawns, terrine, pate, smoked fish	5
	Cooked meat e.g. ham, salami, pate, corned beef	5
Multi component foods or meal components	Ready to re-heat refrigerated food e.g. cooked chilled foods, rice and pasta, products	5
	Ready to re-heat food frozen e.g. fries, pizza	5
	Composite foods with substantial raw ingredients e.g. .pasta salads, sandwiches, deli-salads	5

75 samples were analysed, leading to 75 interpretable results

3.1.2 Test sample preparation

It is preferable to have naturally contaminated samples where possible, however, it is also necessary to artificially inoculate some samples where naturally contamianted samples cannot be sourced. Artificial contamination was carried out by spiking or seeding protocols. Injury efficiency was evaluated by enumerating the pure culture on selective and non-selective agars.

The observed injury measurements varied from 0.60 to 1.02 log cfu/g difference between non-selective and selective plates.

All the samples were artificially contaminated in this study because it was not possible to find naturally contaminated samples. In total 44 samples were screened for naturally present coliforms, but none were found.

3.1.3 Protocols applied during the validation study

A single protocol was applied for the study.

Reference method plates were incubated at 37±1ºC for a total of 24±2h. Compact Dry EC plates were incubated at 37±1ºC for 24±2h. In all cases the minimum incubation times were used.

3.1.4 Test results

The samples were analysed by the reference and the alternative methods in order to have at least 15 interpretable results per category, and at least 5 interpretable results per tested type by the two methods.

3.1.5 Calculation and interpretation of relative trueness study

The obtained data were analysed using the scatter plot. The graphs are provided with the line of identity (y = x). Figures 1 to 5 shows the data plotted per category and Figure 6 summarises all the data.

Figure 1 : Dairy products

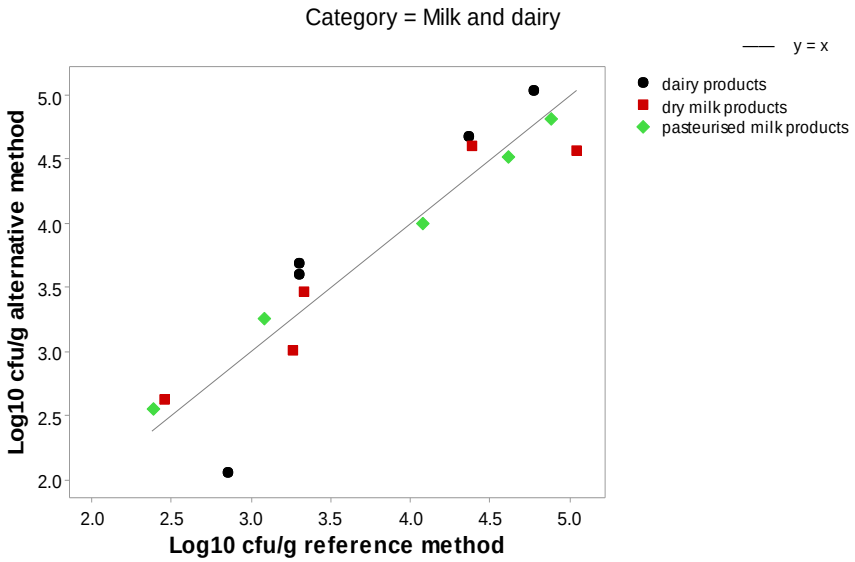


Figure 2: Fresh produce and fruits

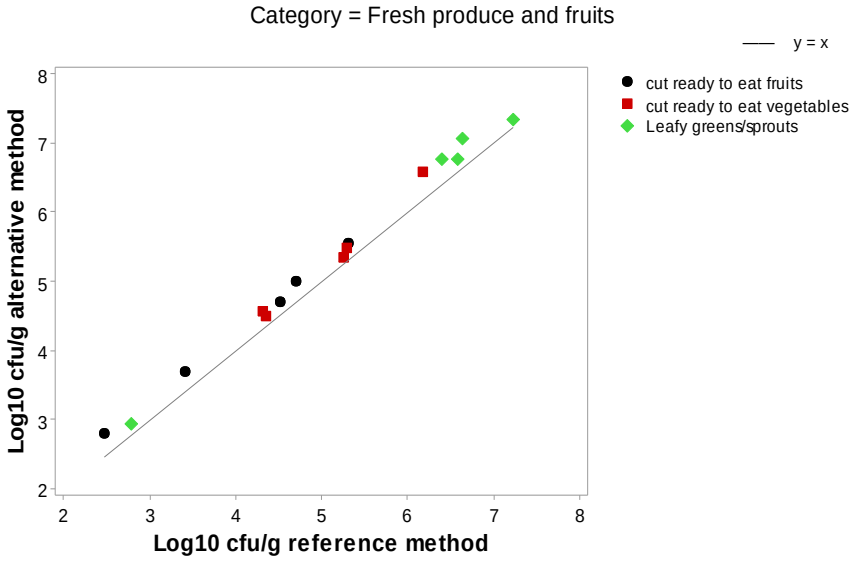


Figure 3: Raw Meat and poultry

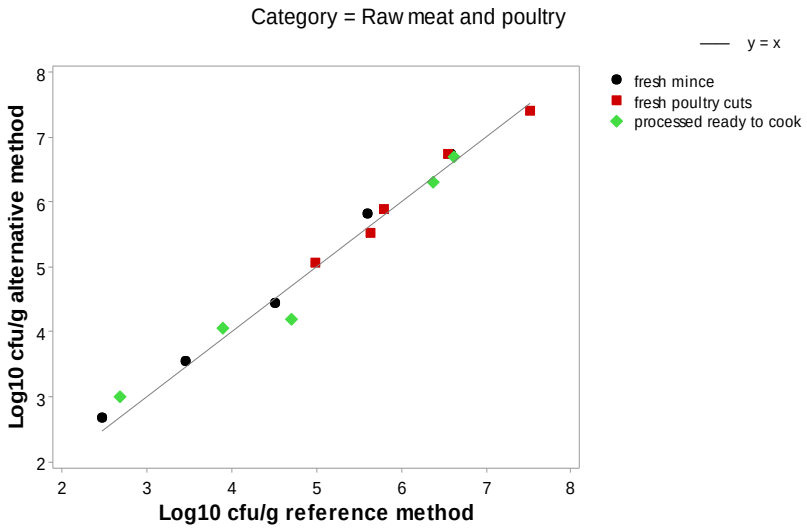


Figure 4: Ready to eat Foods

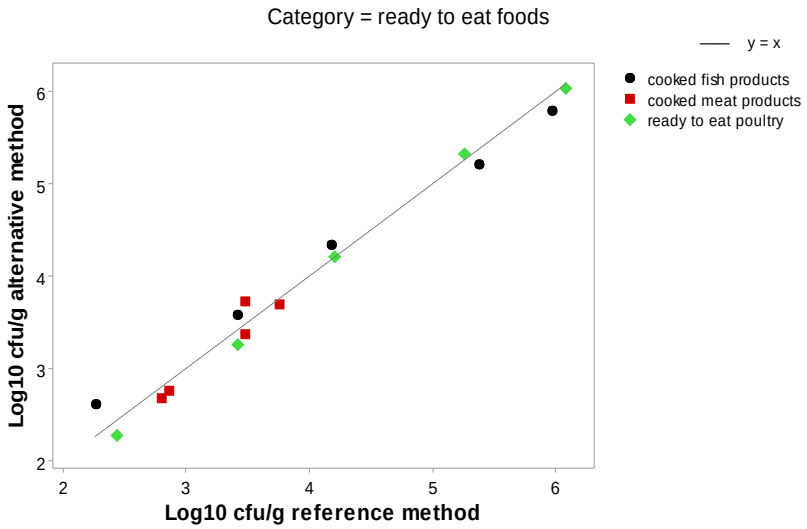


Figure 5: Multi-component Foods

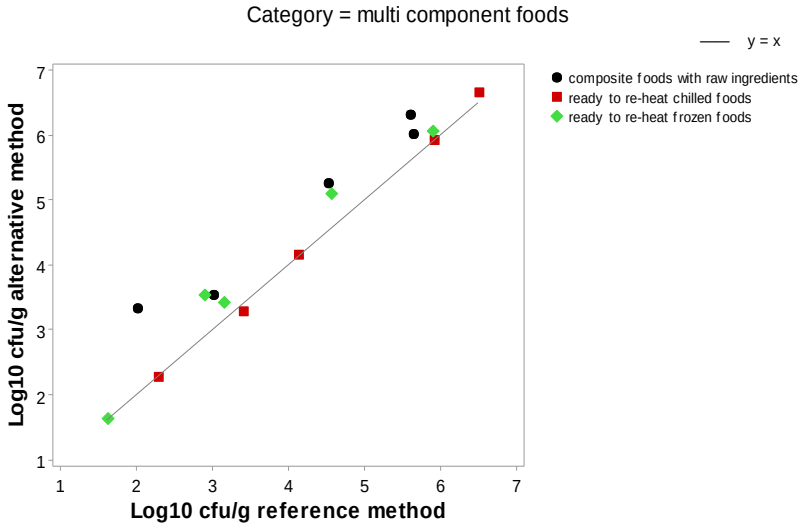
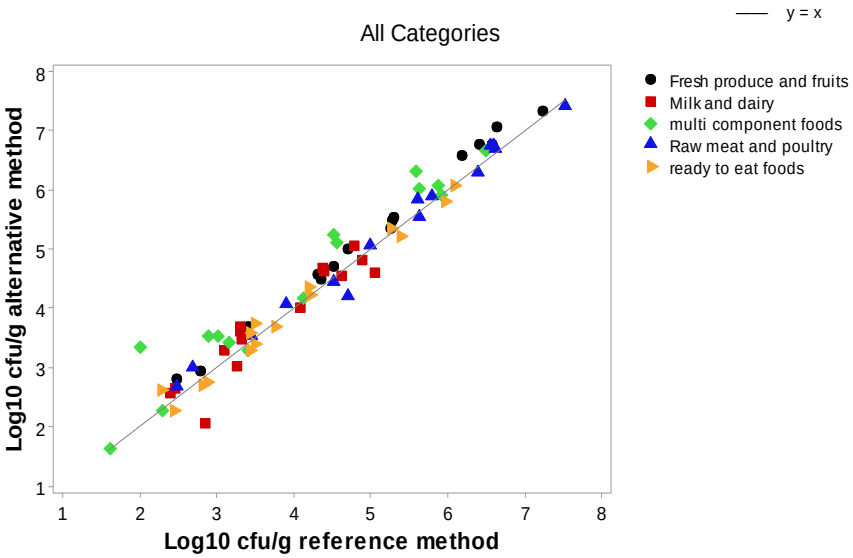


Figure 6: All categories plot



According to ISO 16140-2:2016 6.1.2.3 the results of the scatter plot are interpreted based on a visual observation on the amount of bias and extreme results. The data appears acceptable overall but there is some evidence of a positive bias for the alternate method for multicomponent foods, particularly the products containing raw ingredients and also for the fresh produce category. This can be seen from the individual product Figures (2 and 5) and from the all categories Figure (6). These products were spiked with strains of *Klebsiella*. These maybe under recovered on the reference method.

A summary of the calculated values per category is provided in Table 2. There was a very slight overall positive bias for the 'all categories' data and a more positive bias for the multi-component foods which supports the visual observations from Figures 2 and 5.

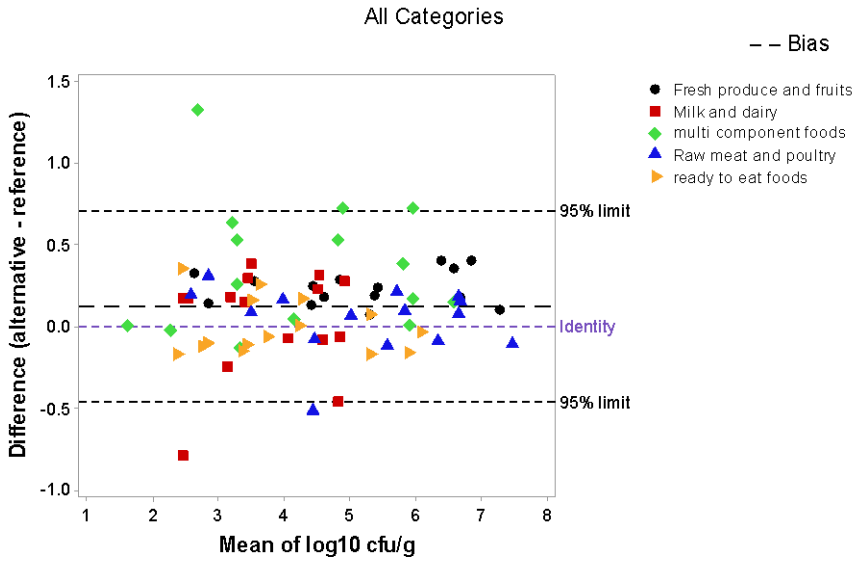
The Bland-Altman difference plot for all the samples is given Figure 7

Table 2 - Summary of the calculated values per category

Category.	n	$\overline{D}$	s_D	95% Lower limit	95% Upper limit
Fresh produce and	15	0.22874	0.105686	-0.00537	0.462848
Milk and dairy	15	0.022567	0.327377	-0.70261	0.747749
Multi component	15	0.347944	0.394301	-0.52548	1.22137
Raw meat and poultry	15	0.031564	0.201279	-0.41429	0.477421
ready to eat foods	15	-0.01184	0.169736	-0.38783	0.364144
All Categories	75	0.123794	0.291318	-0.46053	0.708115

$\overline{D}$: Average difference SD: standard deviation of differences n: number of samples

Figure 7 – Bland-Altman difference plot for all the samples



Samples for which the difference between the result observed with the reference and the alternative methods is above or lower than the limits are listed in Table 3.

Table 3 - Data which are outside of the accepted limits –

Food Category	Food type	Sample code	Food item	Strain	Spiking/seeding protocol	Difference log cfu/g (alternative – reference)
Milk and dairy	dairy products	6B	Strawberry yogurt	*Citrobacter braakii* 16279	Chill 2-3 days	-0.80371
Milk and dairy	dry milk products	1C	dried skimmed milk	*E.coli* 1253	Ambient 2 weeks	-0.47319
Raw meat and poultry	processed ready to cook	43	southern fried chicken goujons	*Escherchia fergusonii* CRA 7522	frozen 2 weeks	-0.52288
multi component foods	composite foods with raw ingredients	73B	bacon, lettuce, tomato sandwich	*Klebsiella ozaenae* 4273	chill 2-3 days	0.711935
multi component foods	composite foods with raw ingredients	75B	minted bean salad	*Klebsiella ozaenae* 4273	chill 2-3 days	0.721246
multi component foods	composite foods with raw ingredients	72	cheese and onion sandwich	*Klebsiella ozaenae* 4273	chill 2-3 days	1.322219

Comments

It is expected that not more than one in 20 data values will lie outside the CLs. Any disagreements with the expectation should be recorded.

For 'All Categories' there are six in 75 values which lie outside the CLs. This is a little more than the expectation of less than one in 20.

The six points which were outside of the CLs are shown below in Table 3. The data covered 3 different food categories, and 3 different inoculated strains. It is worth noting that the 3 data points above the upper CL were all inoculated with *Klebsiella ozanae* in multi-component foods containing raw ingredients which were seeded and stored chilled. This strain gave a similar response on both the alternate and reference methods in the inclusivity studies, but it appears that after chill storage in foods with raw ingredients, the alternate methods give a higher recovery than the reference method.

3.1.6 Conclusion (RT study)

The relative trueness of the Alternative method (Compact Dry EC) for coliforms is satisfied.

3.2 Accuracy profile study

The accuracy profile study is a comparative study between the results obtained by the reference method and the results of the alternative method. This study is conducted using artificially contaminated samples, using one type per category.

3.2.1 Categories, sample types and strains

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 tested categories, types, items and inoculated strains are provided in the Table 4.

Table 4 - Categories, types, items, strains and inoculation levels for accuracy profile study

Category	Types	Strain	Item	Target Level*	Test portions
Dairy products	Pasteurised dairy products	*E. coli* CRA 1476 from dried milk	Pasteurised cream	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
		Enterobacter agglomerans CRA 5613 from milk powder	Cream cheese	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
Fruits and vegetables	Fresh produce	*E.hermanii* CRA 7477 from sesame seeds	Ready to cook Vegetable preparation	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
		Citrobacter amalonaticus CRA 7458 from beansprouts	Vegetable juice	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
Raw poultry and meats (Combined category raw/ RTC meats and poultry)	Fresh meat	*Enterobacter aerogenes* NCTC 10006	Pork mince	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
		Citrobacter freundii NCTC 9750	Raw bacon	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
Ready to eat foods (Combined category RTE/RTRH	Cooked fish products e.g. prawns	*E.coli* CRA 2003 from fish	Fresh prawns	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
		Klebsiella oxytoca ATCC 15926	Fish pate	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5

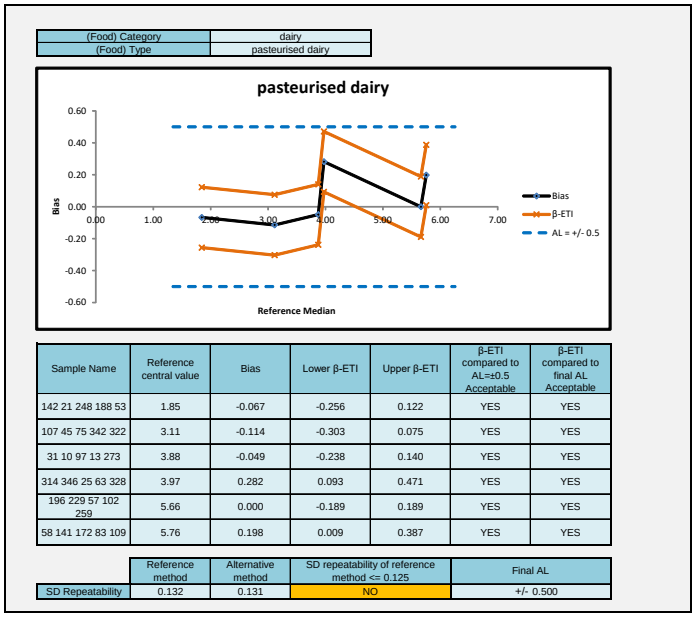
meats and poultry)					
Multi component foods	Composite foods with raw ingredients	*Enterobacter agglomerans* CRA 5513 from skimmed milk powder	Sandwiches	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5
		E. adecarboxylata CRA 5501 from skimmed milk powder	Cooked chilled rice	Low 10^2 cfu/g	5
				Medium : 10^4 cfu/g	5
				High : 10^6 cfu/g	5

Total number of samples tested= 150

3.2.2 Calculations and interpretation of accuracy profile study
The statistical results and the accuracy profiles are provided in Figures 8 to 12.

The calculations were done using the AP Calculation Tool MCS (Clause 6-1-3-3 calculation and interpretation of accuracy profile study) available on http://standards.iso.org/iso/16140

Figure 8: Dairy products

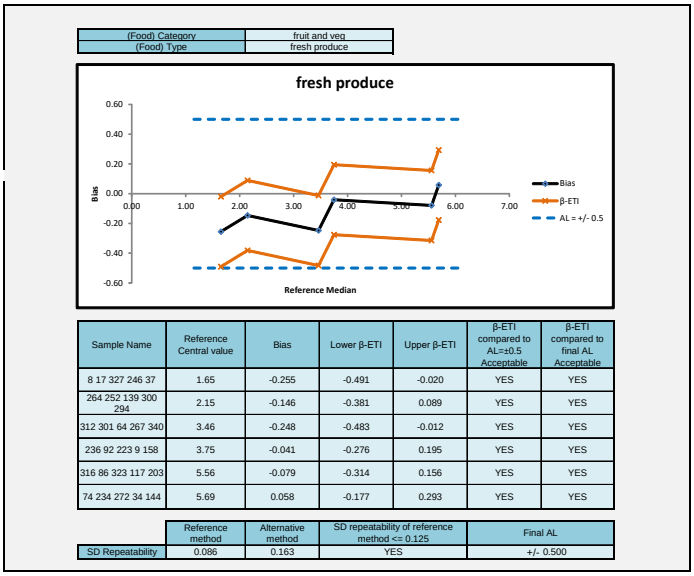


(Food) Category	dairy
(Food) Type	pasteurised dairy

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
142 21 248 188 53	1.85	-0.067	-0.256	0.122	YES	YES
107 45 75 342 322	3.11	-0.114	-0.303	0.075	YES	YES
31 10 97 13 273	3.88	-0.049	-0.238	0.140	YES	YES
314 346 25 63 328	3.97	0.282	0.093	0.471	YES	YES
196 229 57 102 259	5.66	0.000	-0.189	0.189	YES	YES
58 141 172 83 109	5.76	0.198	0.009	0.387	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.132	0.131	NO	+/- 0.500

Figure 9: Fruit and vegetable products

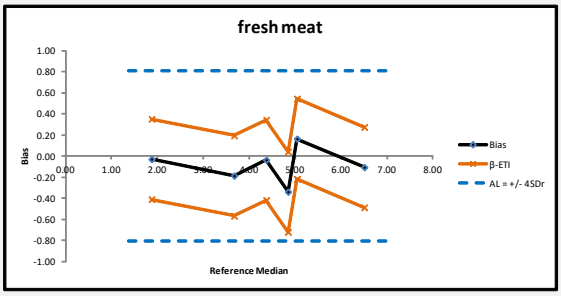


(Food) Category	fruit and veg
(Food) Type	fresh produce

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
8 17 327 246 37	1.65	-0.255	-0.491	-0.020	YES	YES
264 252 139 300 294	2.15	-0.146	-0.381	0.089	YES	YES
312 301 64 267 340	3.46	-0.248	-0.483	-0.012	YES	YES
236 92 223 9 158	3.75	-0.041	-0.276	0.195	YES	YES
316 86 323 117 203	5.56	-0.079	-0.314	0.156	YES	YES
74 234 272 34 144	5.69	0.058	-0.177	0.293	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.086	0.163	YES	+/- 0.500

Figure 10: Meat and poultry

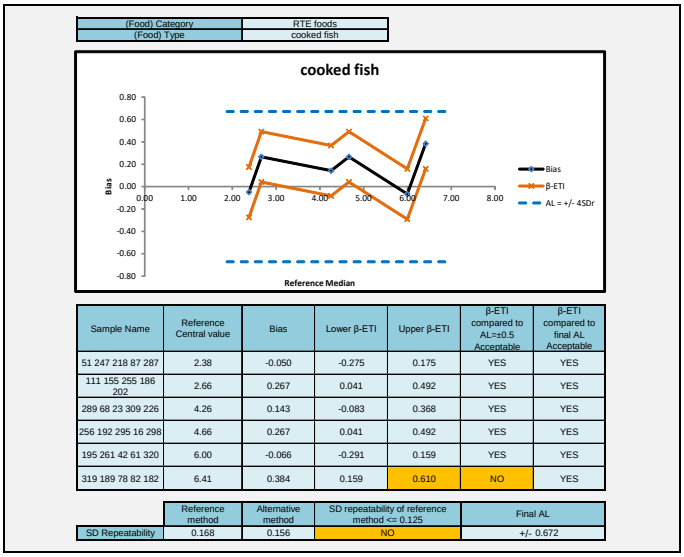


(Food) Category	raw poultry and meat
(Food) Type	fresh meat

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
168 135 283 214 47	1.88	-0.030	-0.411	0.351	YES	YES
204 253 207 76 156	3.67	-0.188	-0.569	0.193	NO	YES
284 225 85 257 96	4.37	-0.038	-0.419	0.343	YES	YES
348 171 65 281 151	4.85	-0.340	-0.721	0.041	NO	YES
167 36 324 216 133	5.04	0.163	-0.218	0.544	NO	YES
165 69 310 118 349	6.51	-0.107	-0.488	0.274	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.202	0.264	NO	+/- 0.808

Figure 11: Ready to eat foods

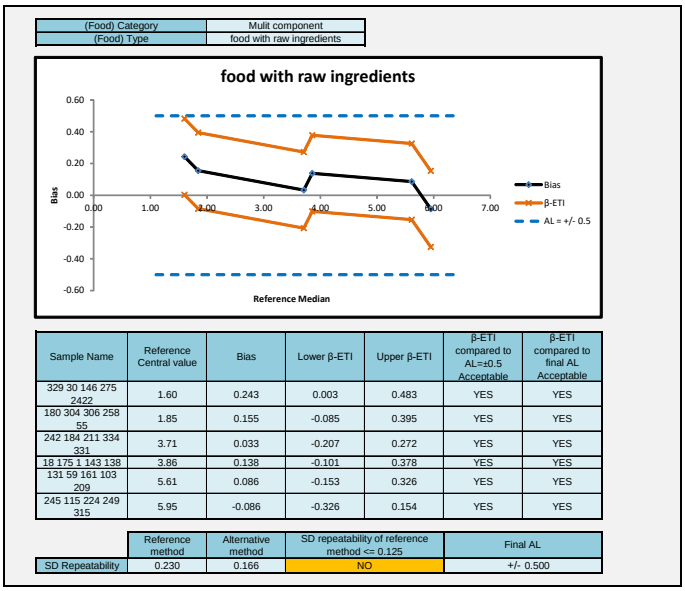


(Food) Category	RTE foods
(Food) Type	cooked fish

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
51 247 218 87 287	2.38	-0.050	-0.275	0.175	YES	YES
111 155 255 186 202	2.66	0.267	0.041	0.492	YES	YES
289 68 23 309 226	4.26	0.143	-0.083	0.368	YES	YES
256 192 295 16 298	4.66	0.267	0.041	0.492	YES	YES
195 261 42 61 320	6.00	-0.066	-0.291	0.159	YES	YES
319 189 78 82 182	6.41	0.384	0.159	0.610	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.168	0.156	NO	+/- 0.672

Figure 12: Multi component foods



(Food) Category	Mulit component
(Food) Type	food with raw ingredients

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
329 30 146 275 2422	1.60	0.243	0.003	0.483	YES	YES
180 304 306 258 55	1.85	0.155	-0.085	0.395	YES	YES
242 184 211 334 331	3.71	0.033	-0.207	0.272	YES	YES
18 175 1 143 138	3.86	0.138	-0.101	0.378	YES	YES
131 59 161 103 209	5.61	0.086	-0.153	0.326	YES	YES
245 115 224 249 315	5.95	-0.086	-0.326	0.154	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.230	0.166	NO	+/- 0.500

According to ISO 16140, if any of the upper or lower limits for the six samples exceeds the 0.5log Acceptability Limits (ALs) and the standard deviation, *S*ref > 0,125, then an additional evaluation procedure is followed: New ALs are calculated as a function of the standard deviation: AL *s* = 4_ *s*ref. If for all *i* in the accuracy profile *Ui* ≤ AL*s* and *Li* _ −AL*s* , the alternative method is accepted as being equivalent to the reference method for the given combination category and type.

For some of the food categories the additional AL calculation was required. This was for the meat and RTE products. For the meat category the lower level for ground beef and the medium level for pork mince showed a negative bias and the high level for pork mince showed positive bias. For RTE foods, the high level for fish pate showed a positive bias. Newly calculated AL's were 0.808 for the meat category and 0.672 for the RTE category.

3.3 Inclusivity / exclusivity

The inclusivity study is a study involving pure target strains to be detected or enumerated by the alternative method

3.3.1Protocol

After being grown according to appropriate conditions, decimal dilutions were made, and the 53 target strains and 30 non-target strains were enumerated by the alternative method, the reference method and a non selective agar.

3.3.2 Results

Inclusivity

Of the 53 inclusivity strains tested 51 strains were detected using both methods. Two strains were not detected by either method. These were *Shimwellia blattae* NCTC 12127 and *Klebsiella rhinoscleromatis* CRA 4272.

Exclusivity
The results from the 30 strains of non-target organisms used to determine the exclusivity of the EC method showed that 16 strains did not grow on either the EC medium or reference medium (VRBA). One strain of strain of *Yersinia enterocolitica* did not grow on the Compact Dry EC medium but did grow on VRBA.

The other 13 strains were able to grow on both the reference media and alternative medium showing equivalent performance

3.4 Limit of quantification (LOQ)

The limit of Quantification (LOQ) is only required for instrumental measurements. It was not done in this study

3.5 Conclusion (MCS)

Overall, the conclusions for the Method Comparison are:

- The Compact Dry EC for enumeration of coliforms in foods method shows satisfying trueness
- The Compact Dry EC for enumeration of coliforms in foods method shows satisfactory and accuracy profile.
- The Compact Dry EC for enumeration of coliforms *s* in foods method was shown to be specific and selective. Compared to the Reference method it was able to detect more inclusivity cultures covering a wider range of species.

4 Interlaboratory study

The inter-laboratory study is a study performed by multiple laboratories testing identical samples at the same time, the results of which are used to estimate alternative-method performance parameters.

4.1 Study organization

There were10 organisations used in this study representing 5 different countries.

4.2 Matrix and strain used

Pasteurised milk was used as the food matrix for the interlaboratory study and the samples of milk were artificially contaminated with a single strain of *E. coli* (CCFRA code 11017, NCTC 12241) and a single strain of *Enterobacter aerogenes* (CCFRA 15736, NCTC 10006). Each strain was cultured in 10 ml NB incubated overnight at 37 ± 1°C. Both cultures were serially diluted in MRD to give the desired levels of inoculum for the contamination of the samples and were mixed together in equal concentrations.

A set of 8 x 25 ml samples of pasteurised milk were prepared for each laboratory, including the organising laboratory. Two samples remained uninoculated, whereas the other six samples were inoculated at 3 different contamination levels (low, medium and high). Appropriate dilutions of the mixed culture cocktail were used to individually inoculate 2 x 25ml samples at the low (10^1 – 10^2 CFU/ml), medium (10^2 – 10^3 CFU/ml) and high (10^3 – 10^4 CFU/ml) contamination levels. The samples were blind coded and stored at 2 – 8°C prior to despatch to the collaborative laboratories. Before despatch, each set of eight samples was packed into a suitable container with cool packs. Also, an additional vial containing water was packed with each set of samples. This enabled the laboratories to take a temperature measurement upon receipt.

Upon receipt, each collaborative laboratory tested a 10 ml test portion from each of the eight milk samples by the ISO 4832:2006 method and the Compact Dry EC method. In addition, the organising laboratory tested a set of eight milk samples at the same time as the collaborative laboratories to confirm the presence of the target organism and the contamination levels. This data was not used in the analyses.

4.3 Calculation and interpretation of data

The data from the collaborative trial were calculated and interpreted according to section 6.2.3 of ISO 16140-2:2016 using the freely available Excel® spreadsheet (http://standards.iso.org/iso/16140). Version 14-03-2016 was used for these calculations.

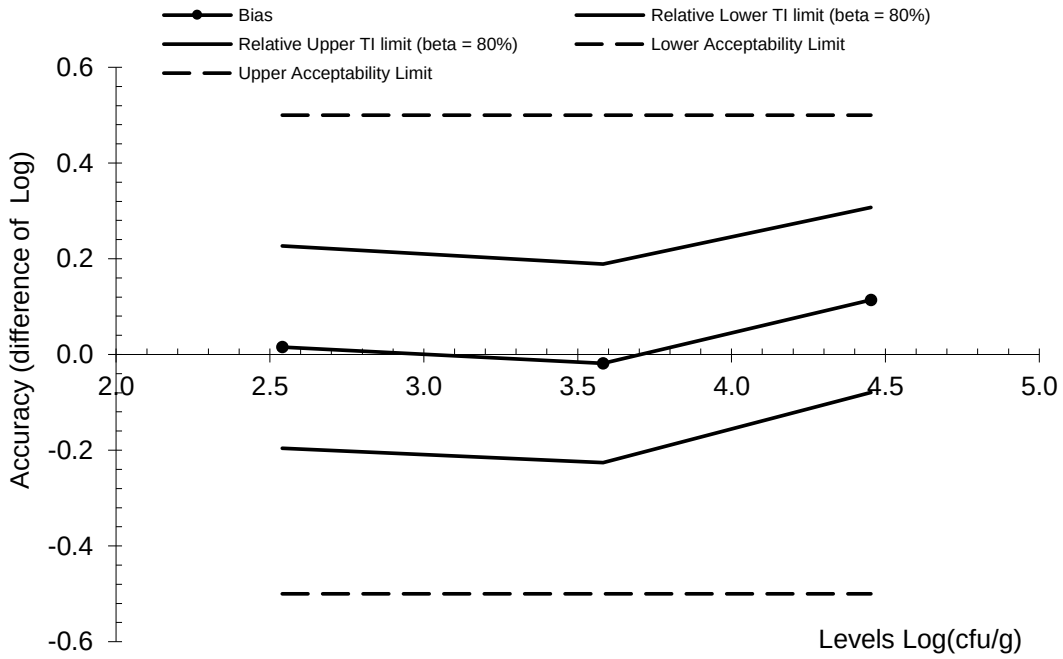
The results obtained by the collaborators are shown in Tables 5. The accuracy profile plot is shown in Figures 13 and the statistical analysis of the data is shown in Tables 6.

Table 5: Summary of the results of the interlaboratory study per analyte level

		Reference method x $_{ijk}$		**Alternative method k $_{ijk}$**	
Collaborators (i)	**Level (k)**				
1	Blank	<10	<10	<10	<10
2	Blank	<10	<10	<10	<10
3	Blank	<10	<10	<10	<10
4	Blank	<10	<10	<10	<10
5	Blank	<10	<10	<10	<10
6	Blank	<10	<10	<10	<10
7	Blank	<10	<10	<10	<10
8	Blank	<10	<10	<10	<10
9	Blank	<10	<10	<10	<10
10	Blank	<10	<10	<10	<10
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	Low	2.70	2.63	2.70	2.57
2	Low	2.74	2.63	2.54	2.53
3	Low	2.51	2.57	2.47	2.59
4	Low	2.46	2.53	2.30	2.54
5	Low	2.46	2.26	2.48	2.23
6	Low	2.50	2.44	2.70	2.63
7	Low	2.48	2.57	2.57	2.56
8	Low	2.65	2.61	2.65	2.61
9	Low	2.48	2.56	2.53	2.57
10	Low	2.49	2.86	2.59	3.02
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	Medium	3.65	3.58	3.62	3.76
2	Medium	3.67	3.71	3.75	3.71
3	Medium	3.69	3.55	3.60	3.55
4	Medium	3.47	3.55	3.41	3.41
5	Medium	3.62	3.53	3.53	3.47
6	Medium	3.49	3.68	3.64	3.82

		Reference method x_{ijk}		Alternative method k_{ijk}	
Collaborators (i)	**Level (k)**				
7	Medium	3.81	3.66	3.75	3.60
8	Medium	3.78	3.60	3.63	3.62
9	Medium	3.57	3.58	3.49	3.47
10	Medium	3.60	3.50	3.61	3.39
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	High	4.32	4.25	4.74	4.67
2	High	4.61	4.79	4.73	4.77
3	High	4.47	4.46	4.58	4.59
4	High	4.61	4.54	4.53	4.52
5	High	4.49	3.98	4.53	4.25
6	High	4.50	4.44	4.71	4.68
7	High	4.68	4.73	4.53	4.66
8	High	4.72	4.68	4.57	4.68
9	High	4.36	4.02	4.57	4.50
10	High	4.34	4.50	4.51	4.44

Figure 13. Accuracy profile of Compact Dry EC from the ILS



The statistical analysis of the existing ILS data is shown in Table 6 below. It can be seen that the repeatability standard deviation (S_r) was very similar for the alternate method and the reference method ranging from 0.080 to 0.132 for the compact dry EC and 0.084 to 0.150 for the reference method.

The between-labs standard deviation (S_L) was slightly better for the alternative method (0.086 to 0.101) and the reference method (0.074 to 0.164) as was the reproducibility standard deviation (S_R) with alternative method values of 0.125 to 0.157 and reference method values of (0.92 to 0.222).

According to the ISO 16140-2:2016 standard, if any of the values of the β-ETI fall outside of the ±0.5log AL then a further calculation is done to calculate the pooled average S_R of the reference method. This was not required as all values were within the required limits. The data are plotted in Figure 4 and it can be seen that no values lie outside of these AL_S values and therefore the alternative method is accepted as being equivalent to the reference method.

Table 6. Statistical analysis of the ILS data according to the ISO spreadsheet

Accuracy profile							
Study Name	Compact Dry EC coliuforms						
Date	Study done 11/2007 nd re-calculated 06/2017						
Coordinator	Campden BRI						
Tolerance probability (beta)	80%	80%	80%				
Acceptability limit in log (lambda)	0.50	0.50	0.50				
	Alternative method				**Reference method**		
Levels	**Low**	**Medium**	**High**		**Low**	**Medium**	**High**
Target value	**2.556**	**3.614**	**4.474**				
Number of participants (K)	10	10	10		10	10	10
Average for alternative method	2.567	3.589	4.587		2.556	3.614	4.474
Repeatability standard deviation (sr)	0.132	0.080	0.080		0.106	0.084	0.150
Between-labs standard deviation (sL)	0.086	0.101	0.096		0.074	0.038	0.164
Reproducibility standard deviation (sR)	0.157	0.129	0.125		0.130	0.092	0.222
Corrected number of dof	16.928	13.124	13.429		16.593	18.104	14.013
Coverage factor	1.376	1.403	1.401				
Interpolated Student t	1.334	1.350	1.348				
Tolerance interval standard deviation	0.1622	0.1336	0.1294				
Lower TI limit	2.351	3.409	4.413				
Upper TI limit	2.783	3.770	4.762				
Bias	**0.011**	**-0.025**	**0.113**				
Relative Lower TI limit (beta = 80%)	**-0.205**	**-0.205**	**-0.061**	FALSE			
Relative Upper TI limit (beta = 80%)	**0.228**	**0.156**	**0.288**	FALSE			
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	0.158						

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.

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

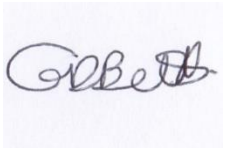
5 Overall conclusions of the validation study

Based on the results of the Methods comparison study (MCS) and the Inter-laboratory study (ILS):

- The Compact Dry EC for enumeration of coliforms in foods method shows satisfying trueness from the MCS
- The Compact Dry EC for enumeration of coliforms in foods method shows satisfactory accuracy profile from the MCS
- The Compact Dry EC for enumeration of coliforms in foods method was shown to be specific and selective from the MCS. Compared to the Reference method it was able to detect more inclusivity cultures covering a wider range of species.
- From the ILS it would appear that in the hands of the ten collaborators, the performance of Compact Dry EC was comparable to the Reference method

The alternative Compact Dry EC shows comparable performance to the reference method: ISO 4832:2006 for enumeration of coliforms in a broad range of foods

Date : 03/03/2019

Signature: 

Annexes A: Flow diagram of the reference and alternative method.

ANNEX A: Flow diagram of the alternative method and reference methods

Comparison of Reference method (ISO 4832:2006) and Alternative Method: HyServe Compact Dry EC for enumeration of coliforms