NMKL - NordVal International
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NordVal International Certificate

Issued for:	Compact Dry EC Method for the Enumeration of *Escherichia coli* and coliforms
NordVal No:	036
First approval date:	01 December 2008
Renewal date:	28 November 2024
Valid until:	01 December 2026

Compact Dry EC

Manufactured and supplied by:

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

The performance of this has been compared to the reference methods:

- ISO 16649-2:2001: “Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.”

and

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

The validation studies have been conducted by Campden, UK, according to t ISO 16140-2:2016, and concludes Compact Dry EC provide equivalent results to the reference methods.

The production of Compact Dry EC is certified according to ISO 9001 and ISO 13485.

Date: 28 November 2024

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

Compact Dry EC is a ready-to-use dry chromogenic plate for enumeration of *E.coli* and coliforms. Pre-treat the samples according to ISO 6687 or NMKL 91. The medium contains two kinds of chromogenic enzyme substrates: Magenta-Gal and X-Gluc. *E.coli* forms blue colonies. The total coliform group count is the sum of both the red and blue colonies.

An aliquot of 1 ml of an appropriate dilution is plated onto Compact Dry EC plate. The incubation conditions tested in the study were 37 ± 1°C for 24 ± 2h.

FIELD OF APPLICATION

The method has been tested on enumeration of *Escherichia coli* and coliforms in a broad range of foods.

HISTORY

In 2007, the method was validated according to the ISO 16140:2003. Every two years until 2018 the method has been renewed without any additional studies.

In 2018 a renewal study was performed to comply with the requirements for relative trueness and accuracy profile in the new standard ISO 16140-2:2016. As the design of the Inter-laboratory study (ILS) is the same for the 2003 and 2016 versions of ISO16140, the data from the ILS data of 2007 are re-evaluated using the new statistical approach outlined in ISO16140-2:2016.

METHOD COMPARISON STUDY

Relative trueness study

The trueness study is a comparative study between results obtained by the reference method and the results of the alternative method. Different categories, types and items were tested as shown in Table 1 below.

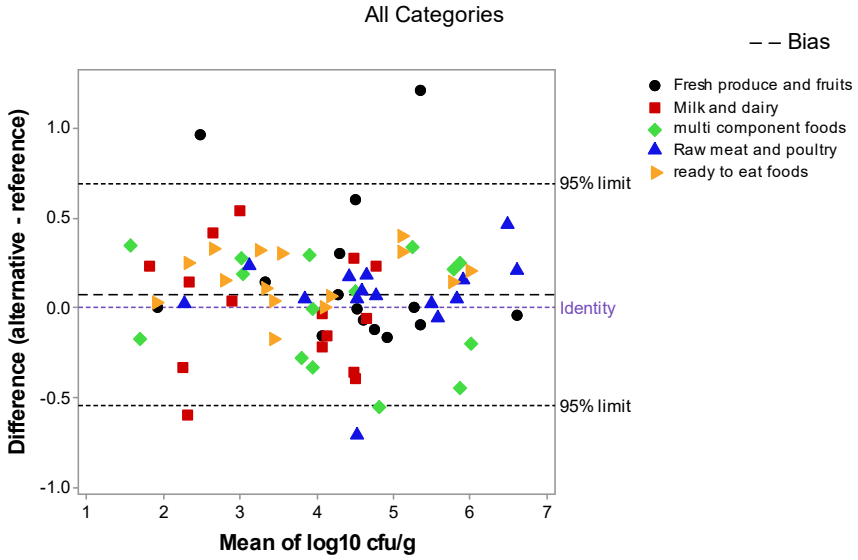
Table 1. Categories and types tested

Category	Types	No. 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

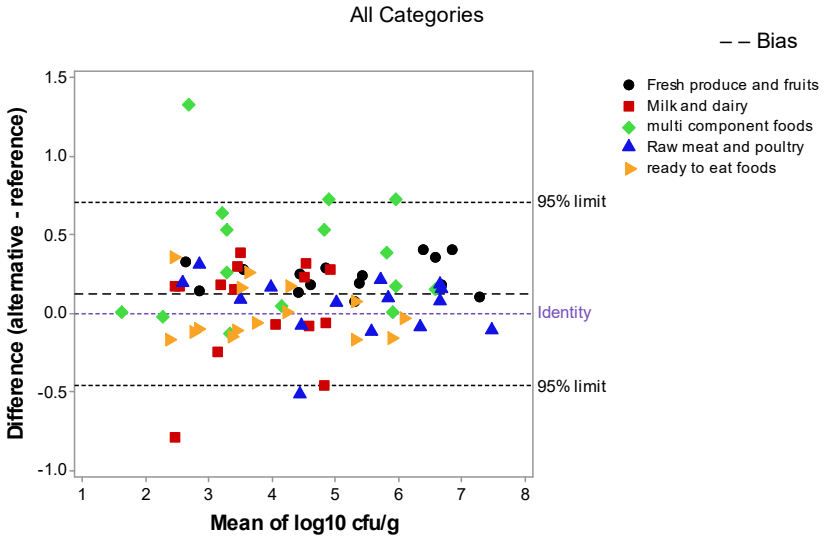
The relative trueness is illustrated by the use of a Bland-Altman plot, i.e. the difference (bias) between paired samples analysed with the reference method and the alternative method respectively, plotted against the mean values obtained by the reference method. In the plot, Upper and Lower limits are included as the bias ± 2 times the standard deviation of the bias. The Bland-Altman Plot in **Figure 1** illustrates the difference obtained in the enumeration of *E. coli* and total count by the alternative and the reference method, respectively.

Figure 1. Bland-Altman Plot of the enumeration of E. coli and total count in foods

E. coli:



Coliforms:



It is expected that no more than 1 in 20 data values will lie outside the 95% confidence levels (upper limit and lower limits).

For *E. coli*:

For 'All Categories' there are five in 75 values which lie outside the CLs. This is a little more than the expectation of less than one in 20. There was no identifiable trend in the data outside the CLs, which covered 4 different food categories, 4 different inoculated strains and 3 different seeding/spiking protocols.

For coliforms:

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. Of the six points outside of the CLs, the data covered 3 different food categories, and 3 different inoculated strains.

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. Each item used were artificially contaminated obtaining three target levels; low (10^2 cfu/g), medium (10^4 cfu/g) and high (10^6 cfu/g). Five test portions of each level of each item were analysed, resulting in 150 samples. The tested categories, types, items and inoculated strains are provided in the Table 2.

Table 2. Categories, types and food items

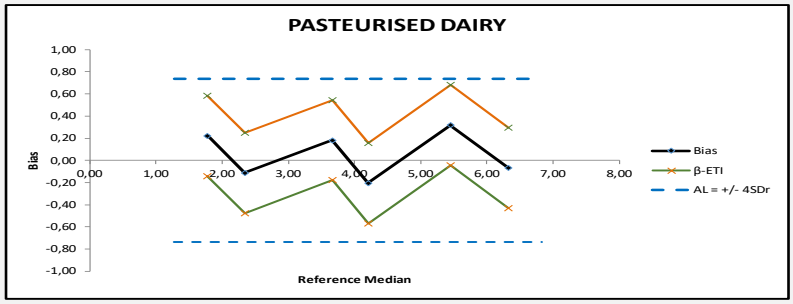
Category	Types	Strains – E.coli	Strains - Coliforms	Items
Dairy products	Pasteurised dairy products	*E. coli* CRA 1476 from dried milk	*E. coli* CRA 1476 from dried milk	Pasteurised cream
		E. coli NCTC 8008	*Enterobacter agglomerans* CRA 5613 from milk powder	Cream cheese
Fruits and vegetables	Fresh produce	*E. coli* ATCC 25922	*E.hermanii* CRA 7477 from sesame seeds	Ready to cook Vegetable preparation
		E. coli NCIMB 700555	*Citrobacter amalonaticus* CRA 7458 from beansprouts	Vegetable juice
Raw poultry and meats (Combined category raw/ RTC meats and poultry)	Fresh meat	*E. coli* CRA 16041 from raw ground mince	*Enterobacter aerogenes* NCTC 10006	Pork mince
		E. coli CRA 1593 from poultry	*Citrobacter freundii* NCTC 9750	Raw bacon
Ready to eat foods (Combined category RTE/RTRH meats and poultry)	Cooked fish products e.g. prawns	*E.coli* CRA 2003 isolated from fish	*E.coli* CRA 2003 from fish	
		E.coli CRA 1968 isolated from lamb	*Klebsiella oxytoca* ATCC 15926	Fish pate
Multi component foods	Composite foods with raw ingredients	*E.coli* CRA 16044 isolated from beef	*Enterobacter agglomerans* CRA 5513 from skimmed milk powder	Sandwiches
		E.coli CRA 1265 dried foods	*E. adecarboxylata* CRA 5501 from skimmed milk powder	Cooked chilled rice

The total number of samples analysed for both *E. coli* and Coliforms with both methods were 150. The statistical results and the accuracy profiles are provided in the **Figures 2 to 6**.

Figure 2. Dairy products

E. coli:

(Food) Category	DAIRY
(Food) Type	PASTEURISED DAIRY

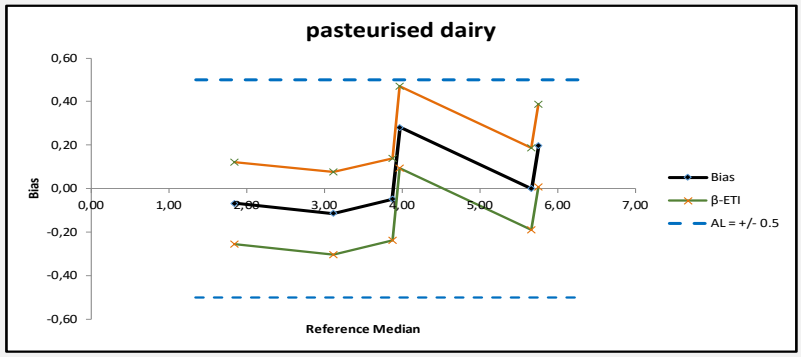


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
107 45 75 342 322	1,78	0,222	-0,140	0,584	NO	YES
299 41 22 277 230	2,34	-0,112	-0,474	0,250	YES	YES
314 346 25 63 328	3,66	0,182	-0,180	0,545	NO	YES
198 154 231 338 254	4,20	-0,204	-0,566	0,158	NO	YES
58 141 172 83 109	5,45	0,316	-0,046	0,679	NO	YES
130 233 94 162 100	6,32	-0,067	-0,429	0,295	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0,184	0,251	NO	+/- 0,736

Coliforms:

(Food) Category	dairy
(Food) Type	pasteurised dairy



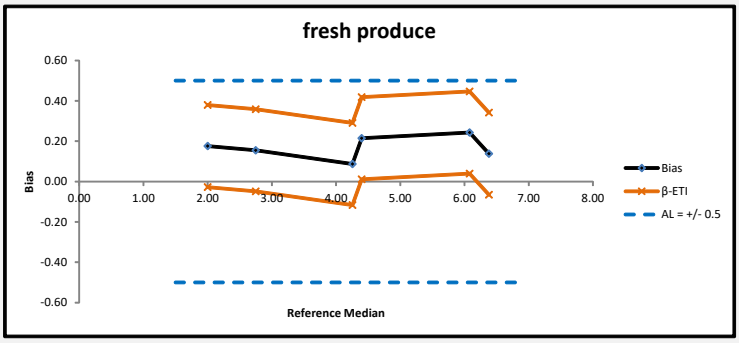
Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
142 21 248 188 53	1,85	-0,067	-0,256	0,122	YES	YES
107 45 75 342 322	3,11	-0,114	-0,303	0,075	YES	YES
31 10 97 13 273	3,88	-0,049	-0,238	0,140	YES	YES
314 346 25 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 3. Fruit and vegetable products

E. coli:

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

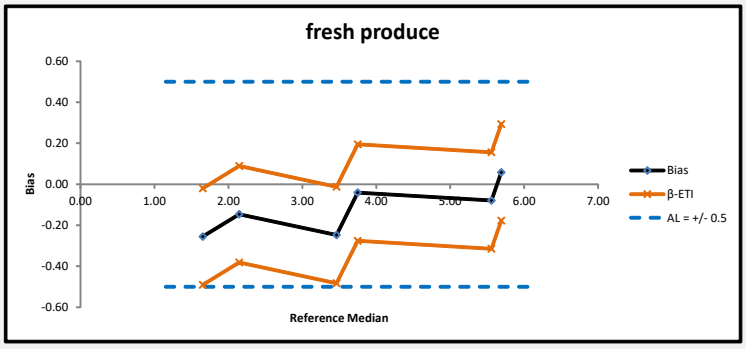


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
164 79 181 213 305	2.00	0.176	-0.027	0.380	YES	YES
119 121 176 150 91	2.75	0.155	-0.049	0.358	YES	YES
276 129 67 3 174	4.26	0.087	-0.116	0.291	YES	YES
116 104 88 201 105	4.40	0.215	0.011	0.418	YES	YES
173 98 44 127 52	6.08	0.243	0.039	0.447	YES	YES
140 326 66 190 343	6.38	0.138	-0.065	0.342	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.132	0.141	NO	+/- 0.500

Coliforms:

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



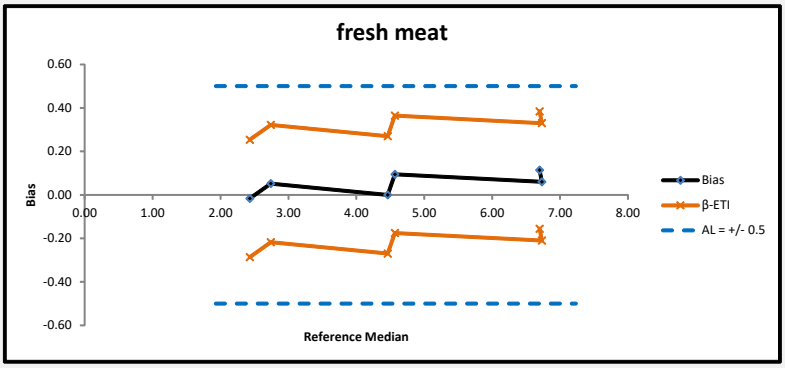
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
8 17 327 246 37	1.65	-0.255	-0.491	-0.020	YES	YES
264 252 139 300 294	2.15	-0.146	-0.381	0.089	YES	YES
312 301 64 267 340	3.46	-0.248	-0.483	-0.012	YES	YES
236 92 223 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 4. Meat and poultry

E. coli:

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

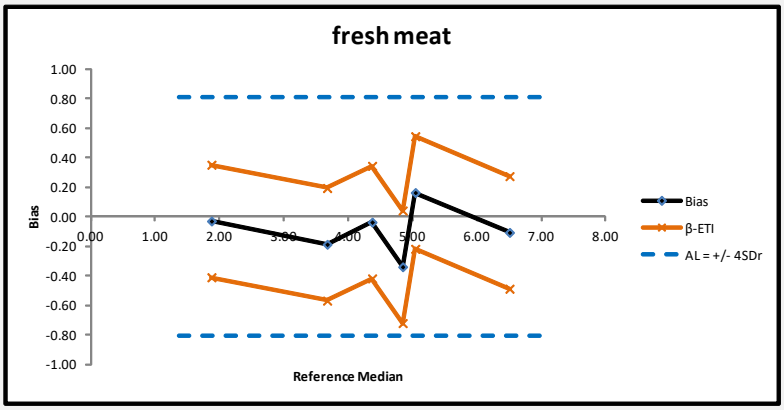


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
206 345 33 210 48	2.43	-0.016	-0.286	0.254	YES	YES
90 81 293 260 286	2.74	0.052	-0.218	0.322	YES	YES
185 199 26 208 178	4.46	0.000	-0.270	0.270	YES	YES
280 220 14 311 50	4.57	0.095	-0.175	0.365	YES	YES
335 191 99 19 193	6.73	0.060	-0.210	0.330	YES	YES
336 237 84 278 221	6.70	0.114	-0.156	0.384	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.159	0.187	NO	+/- 0.500

Coliforms:

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



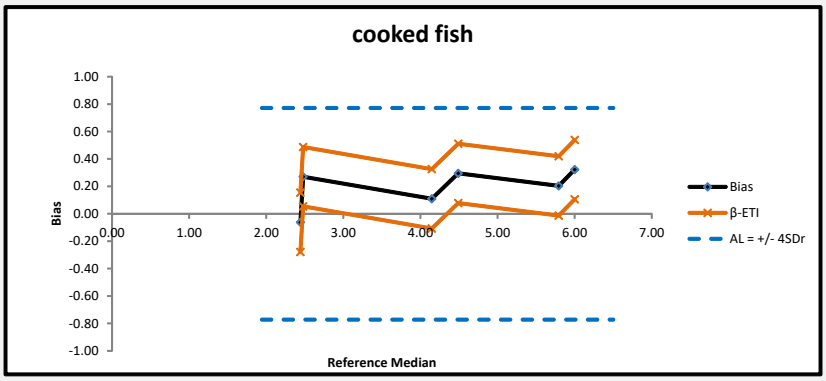
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
168 135 283 214 47	1.88	-0.030	-0.411	0.351	YES	YES
204 253 207 76 156	3.67	-0.188	-0.569	0.193	NO	YES
284 225 85 257 96	4.37	-0.038	-0.419	0.343	YES	YES
348 171 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 5. Ready to eat foods

E. coli:

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

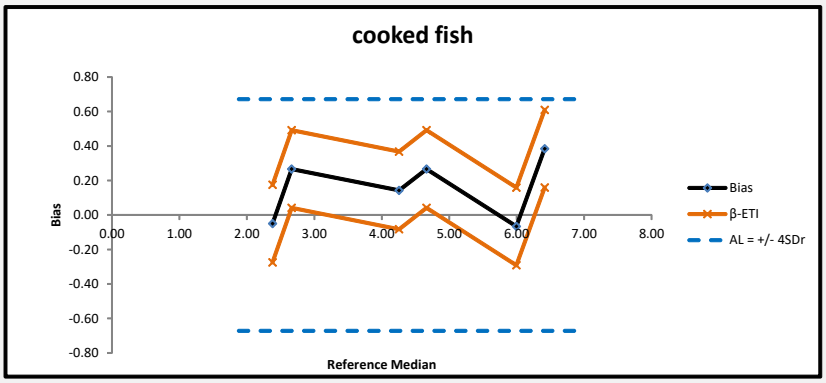


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
51 247 218 87 287	2.44	-0.062	-0.279	0.154	YES	YES
163 241 152 227 125	2.48	0.270	0.054	0.487	YES	YES
289 68 23 309 226	4.15	0.109	-0.107	0.326	YES	YES
24 325 166 70 71	4.49	0.295	0.078	0.511	NO	YES
195 261 42 61 320	5.79	0.203	-0.013	0.420	YES	YES
62 145 266 317 271	6.00	0.322	0.106	0.539	NO	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.193	0.150	NO	+/- 0.772

Coliforms:

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



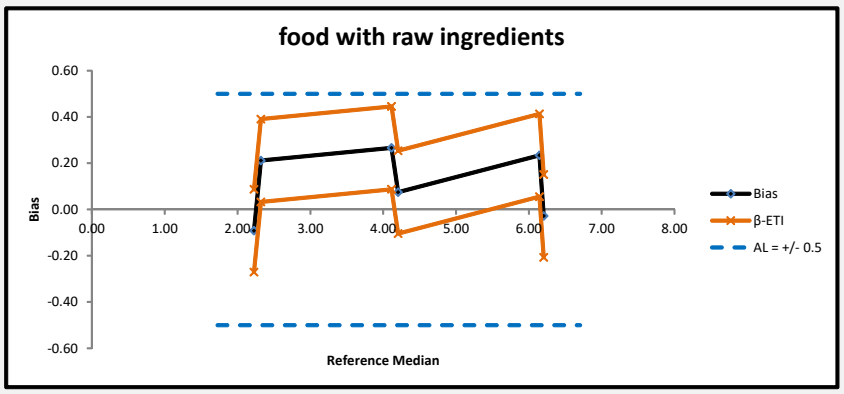
Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
51 247 218 87 287	2.38	-0.050	-0.275	0.175	YES	YES
111 155 255 186 202	2.66	0.267	0.041	0.492	YES	YES
289 68 23 309 226	4.26	0.143	-0.083	0.368	YES	YES
256 192 295 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 6. Multi component foods

E. coli:

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

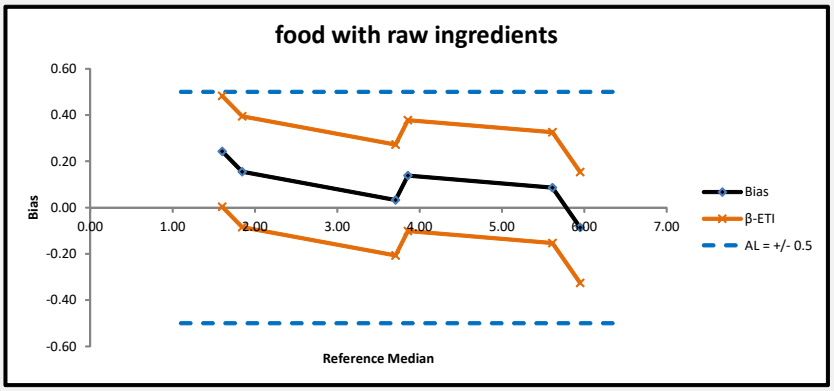


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
37 20 28 148 126	2.23	-0.092	-0.271	0.087	YES	YES
122 169 282 60 159	2.32	0.211	0.032	0.390	YES	YES
308 235 239 179 29	4.11	0.266	0.087	0.445	YES	YES
113 318 112 279 27	4.20	0.075	-0.104	0.254	YES	YES
251 128 329 30 146	6.15	0.234	0.055	0.413	YES	YES
265 93 101 302 114	6.20	-0.028	-0.207	0.151	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.170	0.124	NO	+/- 0.500

Coliforms:

(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

The observed profiles are within the 0.5 log AL or the recalculated AL limit calculated according to ISO16140-2:2015 section 6.1.3.3.

For both E.coli and coliforms, the accuracy profiles fulfil the performance criteria after the permitted recalculation and the alternative method is accepted as being equivalent to the reference methods.

Selectivity (inclusivity/ exclusivity)

Inclusivity is the ability of an alternative method to detect the target analyte from a wide range of strains.

For E.coli:
In the original study: 31 strains were studied. All 31 strains grew and produced typical colonies on the Compact Dry EC medium. By comparison, 5 strains failed to grow in the TBX medium (ISO16649-2:2001) and one strain yielded atypical colonies.
In the renewal study of 2018: Of the 20 inclusivity strains tested all strains were detected using both the alternative and reference method.

For coliforms:
In the original study: All 33 coliform strains produced typical colonies in VRBA (ISO 4832) and Compact Dry EC medium.
In the renewal study from 2018: Of the 20 inclusivity strains tested 18 strains were detected using the alternative and the reference methods. Those not detected by either method were *Shimwellia blattae* NCTC 12127 and *Klebsiella rhinoscleromatis* CRA 4272.

Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

For E.coli:
In the original study: The results from the 21 strains of non-target organisms showed that the majority (19 cultures) failed to grow or produced atypical colonies by both methods. Two strains of *Shigella* did yield typical colonies by both methods, which is not surprising because strains of *Shigella* have β-glucuronidase activity which would give rise to typical conies with chromogenic media developed to show this activity.
In the current study (2018): Of the 10 exclusivity strains tested, none were detected by either the alternate or reference methods.

For coliforms:
In the original study: The results from the 20 strains of non-target organisms used to determine the exclusivity of the EC method showed that 9 strains did not grow on either the EC medium or on VRBA. In addition, one strain of *Yersinia enterocolitica* did not grow on the Compact Dry EC medium but did grow in VRBA. For Compact Dry EC, there were 7 strains giving atypical growth and 3 giving typical growth. For VRBA there were 5 strains giving atypical growth and 6 giving typical growth.
In the recent study (2018): Of the 10 exclusivity strains tested, three were detected by the alternate method and by the reference method these were *A.hydrophila* CRA 4111, *A.sobria* CRA 8390 and *S. fonticola* CRA 4613.

Conclusion of the comparison studies

For E.coli: The results of the method comparison study showed that the Compact Dry EC provide equivalent results to the reference method ISO 16649-2:2001.

For coliforms: The results of the method comparison study showed that the Compact Dry EC provide equivalent results to the reference method ISO 4832:2006.

INTERLABORATORY STUDY

E. coli

The interlaboratory study was conducted in November 2007.

Number of laboratories: 9 [13 labs participated. 2 were excluded as the analysis were not performed on the agreed date and further 2 labs failed to test their samples for *E.coli* by the reference method.]

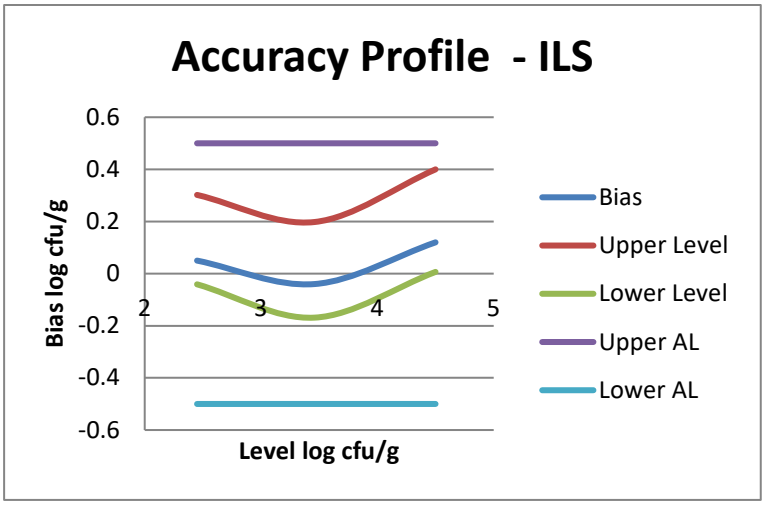
Samples: Pasteurised milk artificially contaminated with defined numbers of *E.coli.* The laboratories performed the analyses according to ISO 16649-2:2001 and Compact Dry EC method.

Table 6. Results (log cfu/g) – interlaboratory study of *E. coli*

	Referance method		Alternative method			Upper	Lower	Upper	Lower
Level	Median	S_R	Alt method	S_R	Bias	Level	Level	AL	AL
1	2.40	0.17	2.45	0.18	0.05	0.30	-0.04	0.50	-0.50
2	3.50	0.17	3.46	0.17	-0.04	0.20	-0.17	0.50	-0.50
3	4.38	0.34	4.50	0.20	0.12	0.40	0.01	0.50	-0.50

The results show that the bias is small and that the precision is satisfactory.

Figure 7. Accuracy Profile of the interlaboratory study for *E. coli*



Coliforms

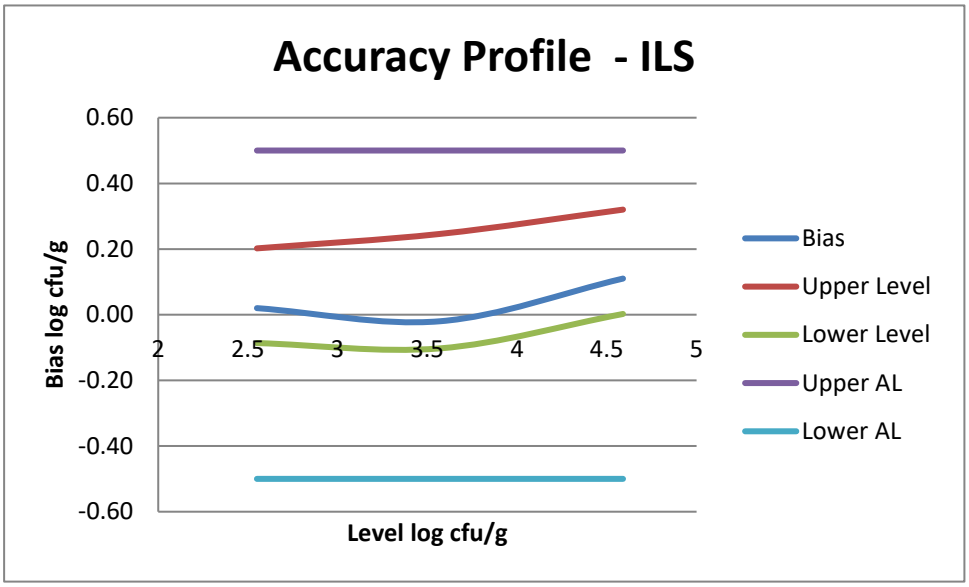
The interlaboratory study was conducted in November 2007.
Number of laboratories: 11
Samples: Pasteurised milk artificially contaminated with defined numbers of *E.coli.* The laboratories performed the analyses according to ISO 4832:2006 and Compact Dry EC method.

Table 7. Results (log cfu/g) – interlaboratory study of coliforms

	Referance method		Alternative method			Upper	Lower	Upper	Lower
Level	Median	S_R	Alt method	S_R	Bias	Level	Level	AL	AL
1	2.53	0.16	2.55	0.13	0.02	0.20	-0.09	0.50	-0.50
2	3.59	0.11	3.57	0.19	-0.02	0.25	-0.10	0.50	-0.50
3	4.48	0.075	4.59	0.15	0.11	0.32	0.00	0.50	-0.50

The results show that the bias is small and that the precision is satisfactory.

Figure 8. Accuracy Profile of the interlaboratory study for coliforms



COLCLUSION

According to the comparison study and the interlaboratory study no substantial differences were found between the Compact Dry EC method and the reference method (ISO 16649-2:2001) for the enumeration of *Escherichia coli*, and no substantial differences were found between the Compact Dry EC method and the reference method (ISO 4832:2006) for the enumeration of coliforms.