NMKL - NordVal International
c/o National Food Institute, Technical University of Denmark
Kemitorvet, Building 201, 2800 Kgs. Lyngby, Denmark
www.nmkl.org

NordVal International Certificate

Issued for:	HyServe Compact Dry TC Method for the Enumeration of Total Count
NordVal No.:	033
First approval date:	1 December 2008
Renewal date:	1 December 2019
Valid until:	1 December 2021

HyServe Compact Dry TC

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

Supplied by:
HyServe GmbH & Co. KG,
Hechenrainerstr 24,
82449 Uffing,
Germany

The principle of this method is based on enumeration on a rehydratable media plate.

The performance of this method has been compared to the reference method ISO 4833-1:2013: “Microbiology of foods and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony count techniques at 30ºC”.

The validation studies have been conducted by Campden BRI, UK, according to ISO 16140-2:2016 and NordVal International Protocol 1.

NordVal International concludes that Compact Dry TC provides equivalent results to ISO 4833-1:2013 for a broad range of foods feed and environmental samples.

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

Date: 1/12 2019

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary-General

PRINCIPLE OF THE METHOD

HyServe Compact Dry TC is a ready-to-use dry chromogenic plate for enumeration of total count. An aliquot of 1 ml of an appropriate dilution is plated onto Compact Dry TC plate. The incubation conditions tested in the study were 30 ± 1°C for 48 ± 3h.

FIELD OF APPLICATION

The method has been tested on enumeration of total viable organisms in a broad range of foods, pet food and environmental samples. Lowest levels tested for the categories can be found in Table 2.

HISTORY

In 2007, the method was validated according to the ISO 16140:2003.

June 2016, a new edition of ISO 16140 for validation of alternative methods was published, which included new validation design and statistical evaluation of the results. NordVal International had a transition period of two years for additional studies required according to the new protocol.

In 2019, a new comparison study is performed according to ISO 16140-2 and NordVal protocol 1. The study includes more samples and the additional categories feed and environmental samples. The results of the interlaboratory study conducted in November 2007 were recalculated in 2019.

COMPARISON STUDY

COMPLIANCE BETWEEN COMPACT DRY TC METHOD AND THE REFENCE METHOD

The comparison study was carried out by Campden BRI in 2007 on cooked chicken, frozen fish, lettuce, milk powder and raw beef. Five levels of contamination were used for each food matrix. For all foods, except milk powder, naturally contaminated samples were tested. Five replicates were analysed at each level.

For all matrices, there were no statistical difference between the results obtained after 48 h and 72 h, hence the results in this certificate are reproduced for the shortest incubation time only.

In the renewal and extension study of 2019, the comparison study was carried out on five food categories, one petfood and one environmental sample each with three types. In the accuracy profile, new matrices were tested; dessert powder, chilled tuna steak, spinach, chicken breast fillets, pork liver pate, dog pate, cat pate, wash water and cooling water. Artificially contaminated samples were used. Results were obtained after 72±3 h for the reference method and 48±3 h for the Compact Dry TC.

RELATIVE TRUENESS

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. Table 1 shows the different categories, types and items tested. The Bland-Altman Plot in Figure 1, illustrates the difference obtained in the enumeration of total viable organisms in foods by the alternative and the reference method, respectively.

Table 1: Categories and types tested

Category	Types	No. of samples
Dairy products (combined category; raw milk and heat processed)	Dry	5
	Pasteurised dairy products	5
	Pasteurised milk	6
Fishery products (combined category: raw, RTE, RTRH, RTC)	Raw	5
	RTE fish	5
	Acidified and marinated	5
Produce and fruits (combined category fresh and processed)	Cut RTE	5
	Heat processed	5
	Vegetable and fruit juices	5
Raw and RTC meat and poultry (combined category)	Cuts unprocessed	5
	Mince unprocessed	5
	RTC	5
RTE and RTRH meat and poultry (combined category)	RTE cooked	5
	Fermented or dried	5
	Cured smoked	5
Pet food and animal feed	Dry Food	5
	Wet food (raw and canned)	5
	Animal feeds (poultry and fish)	5
Environmental samples (food or feed production)	Surfaces (wipes, swabs)	5
	Process water	5
	Dusts	5

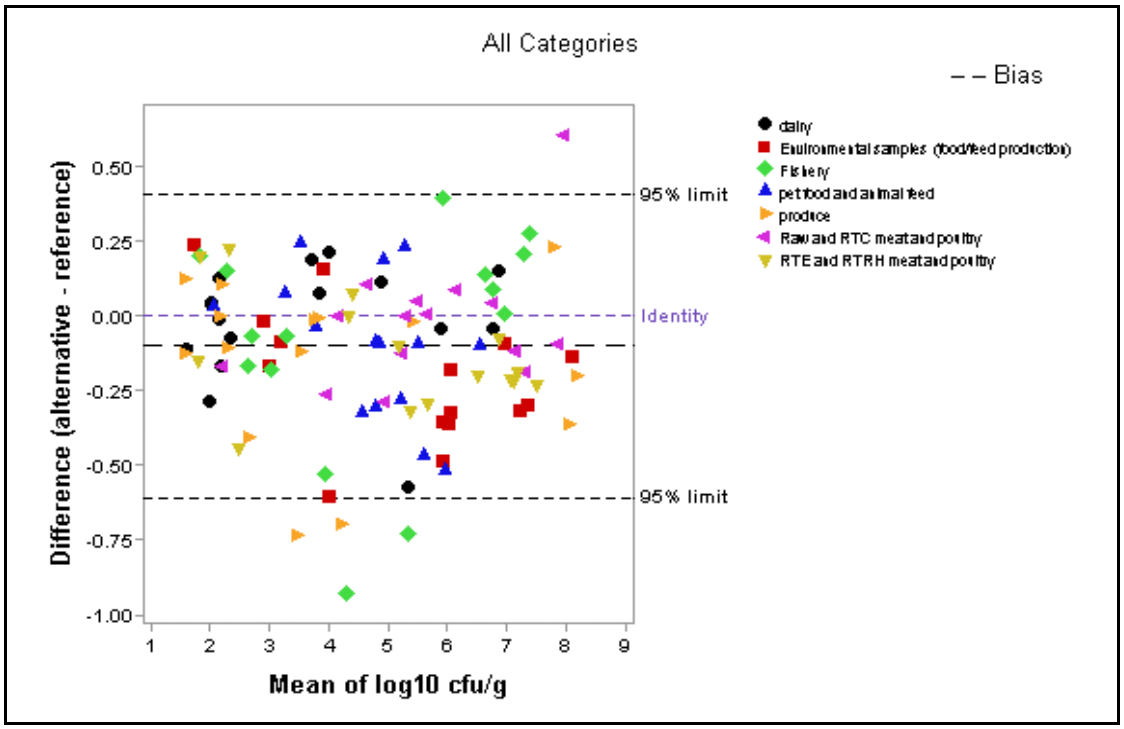


Figure 1 Bland-Altman Plot of the food categories tested

It is expected that no more than 1 in 20 data values will lie outside the 95% confidence levels (upper limit and lower limits). In this, study 5 data points of 106 in total were outside the accepted limits. Four with a negative bias. The points were from 3 different categories and different food types. The Bland-Altman plot shows that there is a small negative bias the bias-line is below zero. The results obtained are in accordance with the expectations.

ACCURACY PROFILE

The accuracy profile study is a comparative study between the results obtained by the reference method and the results of the alternative method.

The tested categories, types, items and inoculated strains are provided in the Table 2.

Table 2: Categories, types and food items

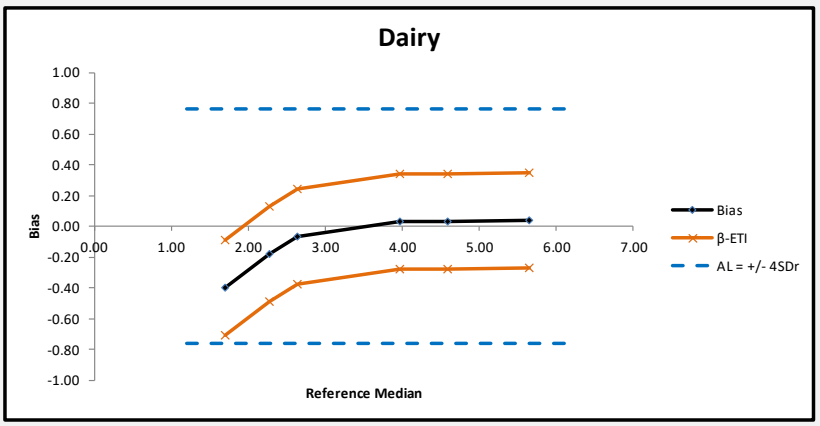
Category	Types	Strain	Item	Level
Dairy products (combined category; raw milk and heat processed)	Dry dairy products	*E.faecalis* NCIMB 1993	Milk powder	10^2 cfu /g
				10^3 cfu/g
				10^5 cfu /g
		Bacillus cereus CRA 1724 Dried milk	Dessert powder	10^2 cfu /g
				10^3 cfu/g
				10^4 cfu/g
Fishery products Combined category: raw, RTE, RTRH, RTC	RTC	natural	Frozen white fish	10^3 cfu /g
				10^4 cfu/g
				10^6cfu /g
		Pseudomonas fragi CRA7222 spoiled fish	Chilled tuna steak	10^2 cfu /g
				10^3 cfu/g
				10^5 cfu/g
Produce and fruits (combined category fresh and processed)	Cut ready to eat	natural	Lettuce	10^2 cfu /g
				10^3 cfu/g
				10^5-cfu/g
		E.coli CRA3379 Spinach	Spinach	10^2 cfu/g
				10^3 cfu/g
				10^4 cfu/g
Raw and RTC meat and poultry (Combined category)	Fresh meats	natural	Raw ground beef	10^3 cfu/g
				10^6 cfu/g
				10^7 cfu /g
		Citrobacter freundii CRA403 chicken	Chicken breast fillets	10^3 cfu/g
				10^5 cfu/g
				10^6 cfu/g
RTE and RTRH meat and poultry (Combined category)	Cooked products	natural	Cooked chicken	10^3 cfu/g
				10^5 cfu/g
				10^6 cfu/g
		Hafnia alvei CRA7417 (from pate)	Pork liver pate	10^2 cfu/g
				10^3 cfu/g
				10^5 cfu/g

Category	Types	Strain	Item	Level
Pet food and animal feed	Wet food (cooked)	*Staph aureus* CRA 1246 (from pork sausage)	Dog pate	10^2 cfu/g
				10^3 cfu/g
				10^5 cfu/g
			Cat pate	10^2 cfu/g
				10^3 cfu/g
				10^4 cfu/g
Environmental samples	Process water	*Pseudomonas fluorescens CRA 7774* (from wash house)	Wash water	10^2 cfu/g
				10^3 cfu/g
				10^5 cfu/g
			Cooling water	10^2 cfu/g
				10^3 cfu/g
				10^5 cfu/g

The samples in the renewal study was bulk inoculated and five replicate test portions examined from the bulk sample. Data from the original study, marked with darker background, contained naturally present organisms, except for except for milk powder.

The statistical results and the accuracy profiles are provided in the Figures 2 to 8.

(Food) Category	Dairy
(Food) Type	Dairy

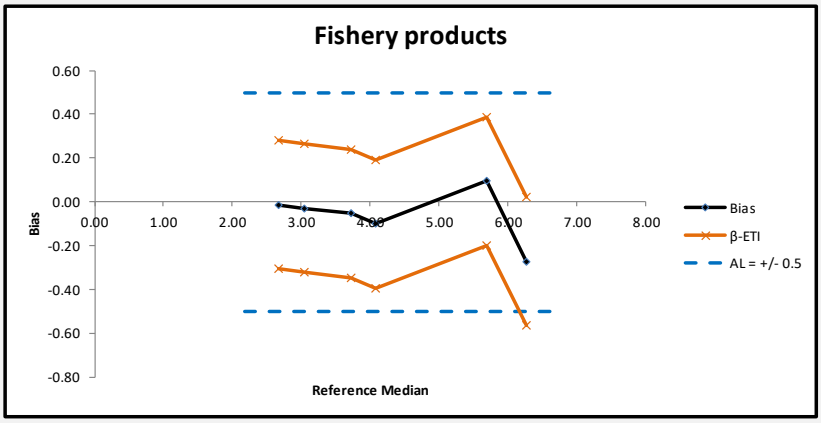


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
1a - e	1.70	-0.398	-0.707	-0.089	NO	YES
141-145	2.28	-0.182	-0.491	0.127	YES	YES
2a - e	2.64	-0.068	-0.377	0.241	YES	YES
171-175	3.97	0.032	-0.277	0.340	YES	YES
3a - 3e	4.59	0.032	-0.277	0.341	YES	YES
161 -165	5.64	0.038	-0.271	0.347	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.190	0.214	NO	+/- 0.760

Figure 2 Dairy products

(Food) Category	Fishery products
(Food) Type	Fishery products

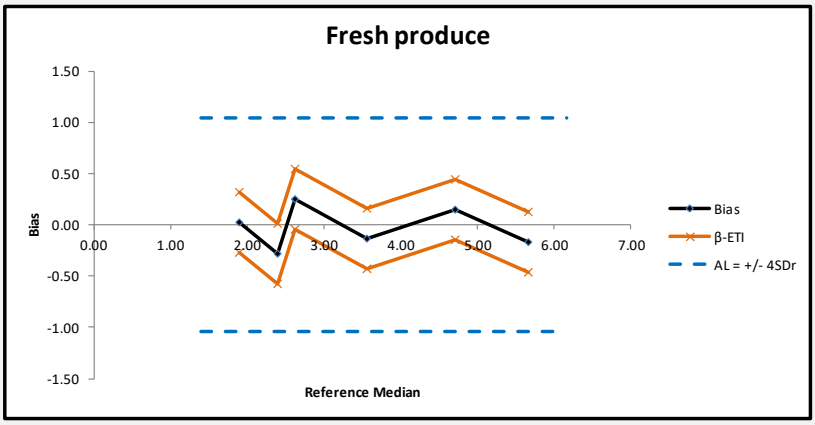


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
4 a-e	2.67	-0.013	-0.306	0.280	YES	YES
31 - 35	3.04	-0.029	-0.322	0.264	YES	YES
5 a-e	3.72	-0.053	-0.346	0.240	YES	YES
131-135	4.08	-0.101	-0.395	0.192	YES	YES
6a-e	5.69	0.095	-0.198	0.388	YES	YES
101-105	6.27	-0.272	-0.565	0.022	NO	NO

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.100	0.203	YES	+/- 0.500

Figure 3 Fishery products

(Food) Category	Produce
(Food) Type	Fresh produce

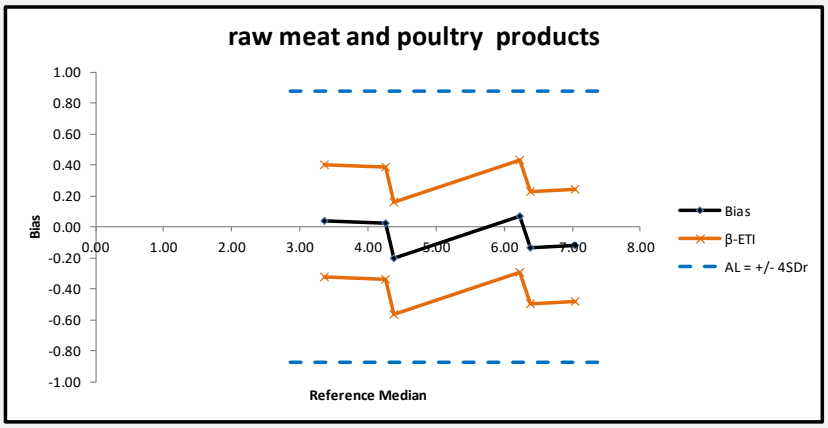


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
7a-e	1.90	0.026	-0.268	0.321	YES	YES
96-100	2.40	-0.284	-0.578	0.011	NO	YES
8a-e	2.63	0.253	-0.042	0.547	NO	YES
61-65	3.56	-0.131	-0.425	0.164	YES	YES
9a-e	4.72	0.153	-0.141	0.448	YES	YES
76-80	5.66	-0.171	-0.466	0.123	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.261	0.204	NO	+/- 1.044

Figure 4 Fresh produce

(Food) Category	Raw RTC meat and poultry
(Food) Type	raw meat and poultry products

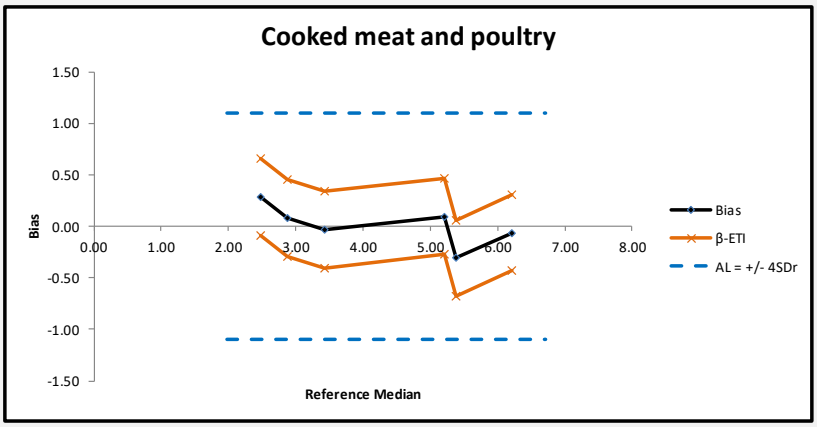


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
10 a-e	3.36	0.036	-0.328	0.400	YES	YES
11 a-e	4.26	0.023	-0.340	0.387	YES	YES
6, 7, 8,9 , 10	4.38	-0.204	-0.568	0.160	NO	YES
12 a-e	6.23	0.071	-0.293	0.434	YES	YES
41 - 45	6.38	-0.137	-0.501	0.227	NO	YES
66 - 70	7.05	-0.122	-0.486	0.241	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.219	0.252	NO	+/- 0.876

Figure 5 Raw and RTC meat and poultry products

(Food) Category	RTE and RTRH meat and
(Food) Type	Cooked meat and poultry

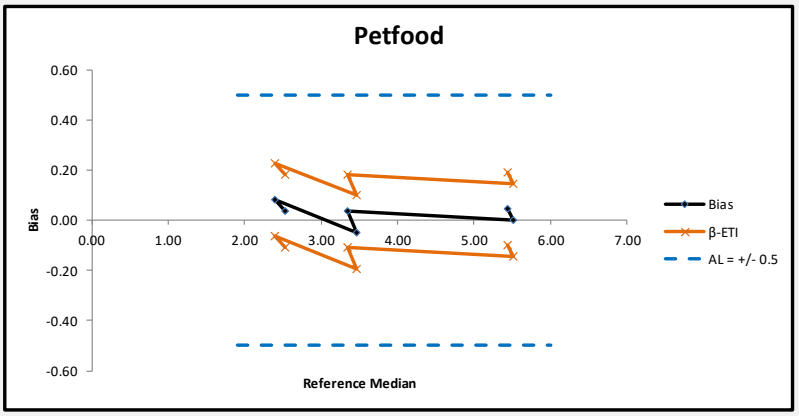


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
13a -13e	2.48	0.287	-0.086	0.659	NO	YES
14a - 14e	2.87	0.082	-0.290	0.455	YES	YES
41 - 50	3.43	-0.033	-0.406	0.339	YES	YES
15a - e	5.20	0.097	-0.276	0.469	YES	YES
151 - 155	5.37	-0.310	-0.683	0.062	NO	YES
106 110	6.22	-0.062	-0.434	0.311	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.275	0.258	NO	+/- 1.100

Figure 6 RTE and RTRH meat and poultry products

(Food) Category	Petfood
(Food) Type	Petfood

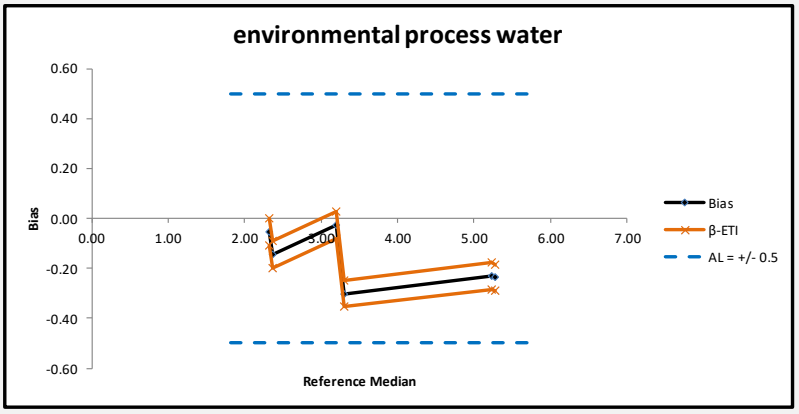


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
19 a - e	2.52	0.036	-0.110	0.182	YES	YES
16 a - e	2.40	0.083	-0.063	0.229	YES	YES
20 a - e	3.46	-0.047	-0.193	0.098	YES	YES
17 a- e	3.34	0.038	-0.108	0.184	YES	YES
21 a- e	5.51	0.000	-0.146	0.146	YES	YES
18 a - e	5.43	0.046	-0.100	0.192	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.143	0.101	NO	+/- 0.500

Figure 7 Pet food and animal feed products

(Food) Category	environmental process water
(Food) Type	environmental process water



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
25 a - e	2.32	-0.055	-0.108	-0.002	YES	YES
22 a - e	2.36	-0.145	-0.198	-0.092	YES	YES
26 a - e	3.20	-0.028	-0.081	0.025	YES	YES
23 a - e	3.30	-0.301	-0.354	-0.248	YES	YES
24 a- e	5.23	-0.230	-0.284	-0.177	YES	YES
27 a-e	5.28	-0.237	-0.291	-0.184	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.088	0.037	YES	+/- 0.500

Figure 8 Environmental samples

If any of the upper or lower limits exceeded the 0.5log Acceptance Limits (AL) and the standard deviation of the reference method was >0.125 log cfu/g, a new AL can be calculated as 4xSD. This was done for the four categories: Dairy products (AL ± 0.76 log cfu/g), Fresh produce (AL ± 1.04 log cfu/g), Raw and RTC meat and poultry products (AL ± 0.88 log cfu/g) and RTE and RTRH meat and poultry products (AL ± 1.1 cfu/g).

For one category, Fishery Products, a new AL could not be calculated as the SD was below 0.125 log cfu/g but it should be noted that one item (high level for white fish) was just outside the AL ± 0.50 log cfu/g.

Thus, the accuracy of the alternative method met the AL of 0.50 log cfu/g or the re-calculated AL, except for one fishery product.

CONCLUSION OF THE COMPARISON STUDY

The results of the method comparison study showed that the Compact Dry TC provide equivalent results to the reference method ISO 4833:2003. The lowest validated level with satisfactory precision varies from 2 - 3 log cfu/g depending on the matrix.

INTERLABORATORY STUDY

Thirteen laboratories analysed samples of pasteurised milk artificially contaminated with defined numbers of *E.coli.* The laboratories performed the analyses according to ISO 4833 after 72 h and Compact Dry TC after 48 h. The results are given in Table 3 and the accuracy profile is shown in figure 9.

Table 6 Results (log cfu/g) of the interlaboratory study

	Alternative method			**Reference method**		
Levels	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	**2.70**	**3.84**	**4.85**			
Number of participants	12	12	12	12	12	12
Average for alternative method	2.28	3.70	4.77	2.70	.3.84	4.85
Repeatability standard deviation (sr)	0.08	0.04	0.06	0.05	0.04	0.05
Between-labs standard deviation (sL)	0.00	0.15	0.16	0.08	0.08	0.10
Reproducibility standard deviation (sR)	0.08	0.16	0.17	0.10	0.09	0.10
Bias	0.08	-0.14	-0.08			
Relative Lower TI limit (beta = 80%)	-0.02	-0.36	-0.33			
Relative Upper TI limit (beta = 80%)	0.18	0.08	0.16			
Lower Acceptability Limit	-0.5	-0.5	-0.5			
Upper Acceptability Limit	0.5	0.5	0.5			

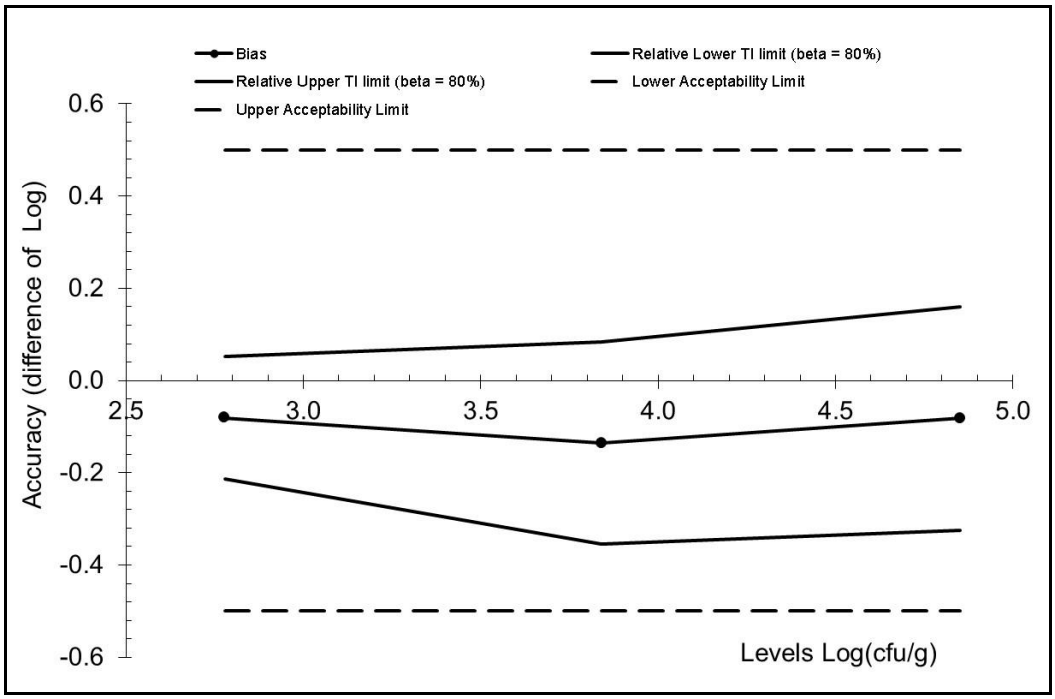


Figure 9 The Accuracy Profile for the interlaboratory study, ILS

CONCLUSION

According to the comparison and the interlaboratory study no substantial differences were found between the HyServe Compact Dry TC method and the reference method (ISO 4833-1:2013) for the enumeration of total viable microorganisms at 30˚C.