



NordVal International / NMKL
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NordVal International Certificate

Issued for:	Compact Dry CF Method for the Enumeration of Total Coliforms
NordVal No:	035
First approval date:	1 December 2008
Renewal date:	1 December 2018
Valid until:	1 December 2020

Manufactured by:
Nissui Pharmaceutical Co.Ltd,
3-23-9 Ueno,
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Supplied by:
HyServe GmbH & Co. KG,
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Compact Dry CF

The principle of this method is growth of dedicated bacteria on specific chromogen media.

The performance of this method has been compared to the reference method ISO 4832:2006: "Microbiology of foods and animal feeding stuffs. Horizontal method for the enumeration of coliforms. Colony-count technique".

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 4832:2006 for a broad range of foods. The production of Compact Dry TC is certified according to ISO 9001 and ISO 13485.

Date: 1/12 2018

Yours sincerely

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary General



PRINCIPLE OF THE METHOD

Compact Dry CF is a ready-to-use chromogenic plate containing for the enumeration of coliforms. An aliquot of 1ml of an appropriate dilution is plated onto Compact Dry CF plate. The plate is inverted and incubated at $37 \pm 1^\circ\text{C}$ and colonies (blue/blue green) were counted after $24 \pm 2\text{h}$.

FIELD OF APPLICATION

The method has been tested on enumeration of total 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.

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

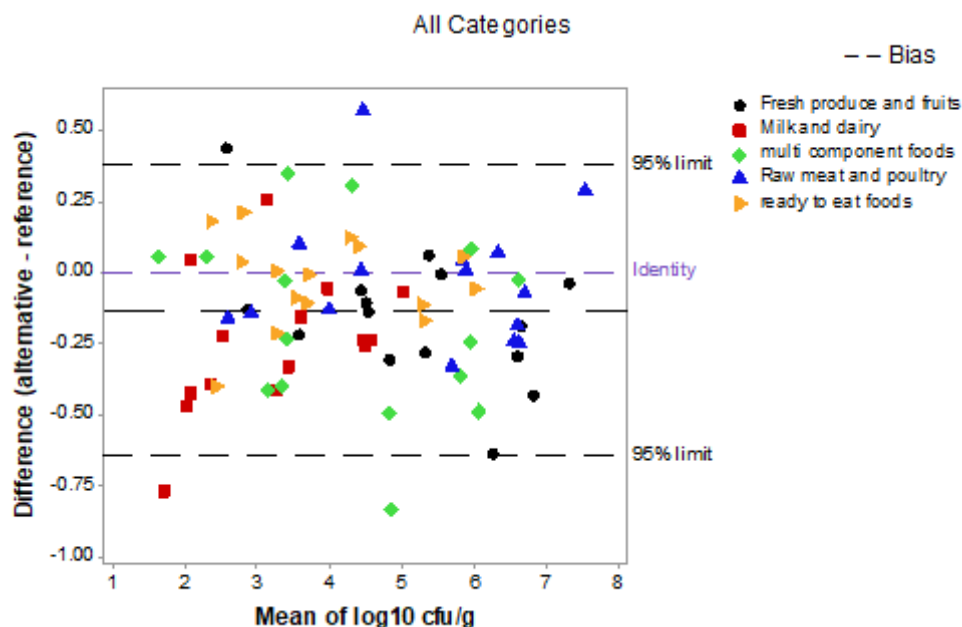
Category	Types	No. of samples
Heat processed milk and dairy products	Dry milk product e.g. milk powder, powder for milk based desserts, dried infant formula	5
	Dairy products e.g. ice-cream, yogurts, cream, hard cheese, soft cheese, raw milk cheese	5
	Pasteurised milk products e.g. skimmed, semi-skimmed, 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 *Enterobacteriaceae* in foods by the alternative and the reference method, respectively.

Figure 1 Bland-Altman Plot of the food categories tested



For 'All Categories' there are four in 75 values which lie outside the CLs. The results obtained are in accordance with the expectations of no more than one of 20 results outside the CLs.

For the four points which were outside of the CLs, there were no identifiable trends in the data and they covered 4 different food categories, 3 different inoculated strains and a range of stresses

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	Strain	Item
Dairy products	Pasteurised dairy products	<i>E. coli</i> CRA 1476 from dried milk	Pasteurised cream
		<i>Enterobacter agglomerans</i> CRA 5613 from milk powder	Cream cheese
Fruits and vegetables	Fresh produce	<i>E. hermanii</i> CRA 7477 from sesame seeds	Ready to cook Vegetable preparation
		<i>Citrobacter amalonaticus</i> CRA 7458 from beansprouts	Vegetable juice
Raw poultry and meats (Combined category raw/ RTC meats and poultry)	Fresh meat	<i>Enterobacter aerogenes</i> NCTC 10006	Pork mince
		<i>Citrobacter freundii</i> NCTC 9750	Raw bacon
Ready to eat foods (Combined category RTE/RTRH meats and poultry)	Cooked fish products e.g. prawns	<i>E. coli</i> CRA 2003 from fish	Fresh prawns
		<i>Klebsiella oxytoca</i> ATCC 15926	Fish pate
Multi component foods	Composite foods with raw ingredients	<i>Enterobacter agglomerans</i> CRA 5513 from skimmed milk powder	Sandwiches
		<i>E. adecarboxylata</i> CRA 5501 from skimmed milk powder	Cooked chilled rice

The statistical results and the accuracy profiles are provided in the Figures 2 to 6.

Figure 2 Dairy Products

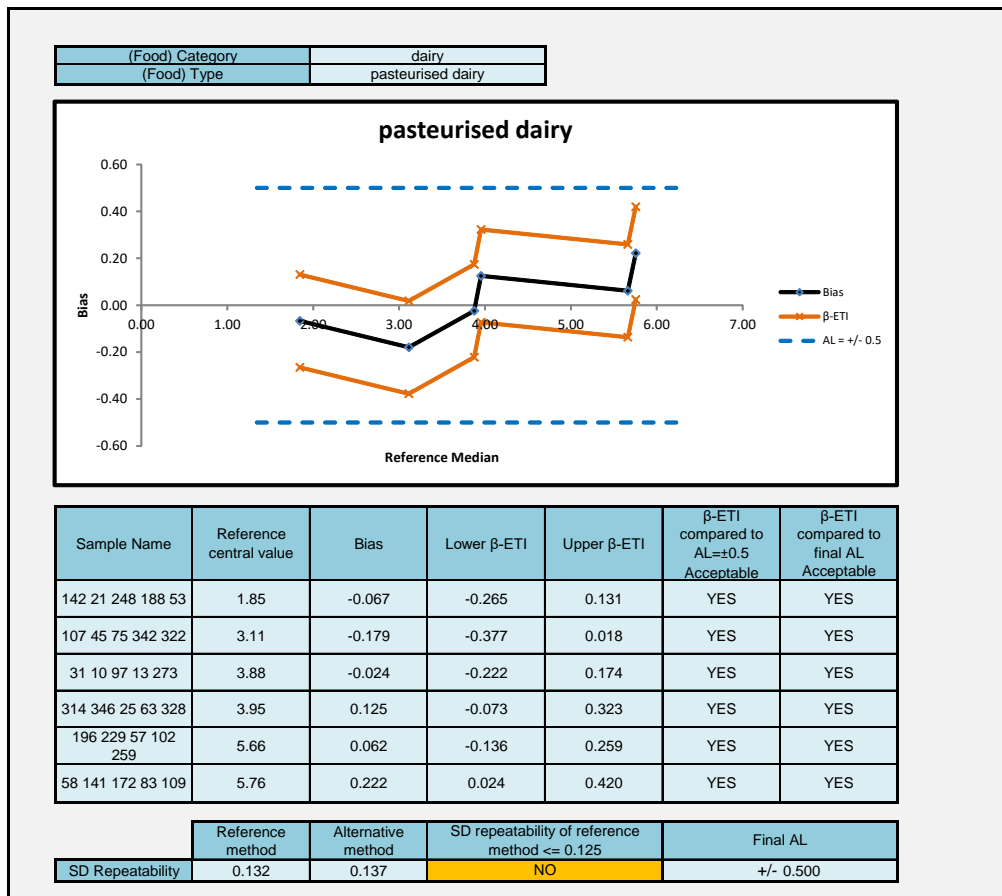


Figure 3 Fruit and vegetable products

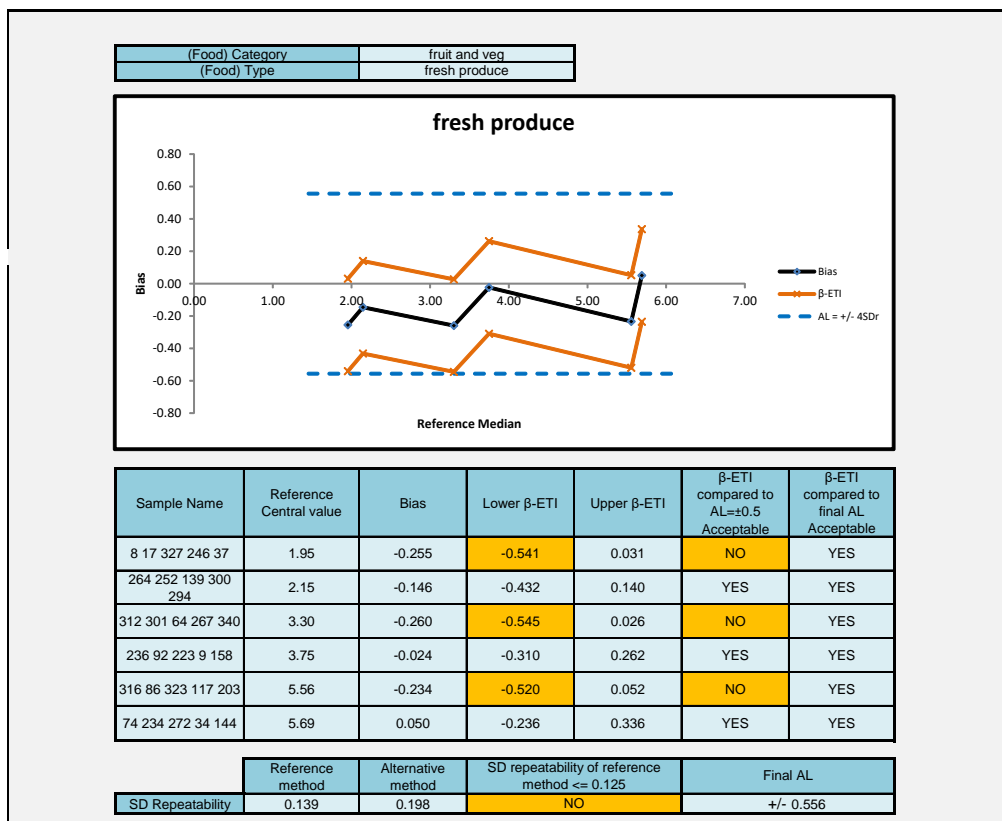


Figure 4 Meat and poultry

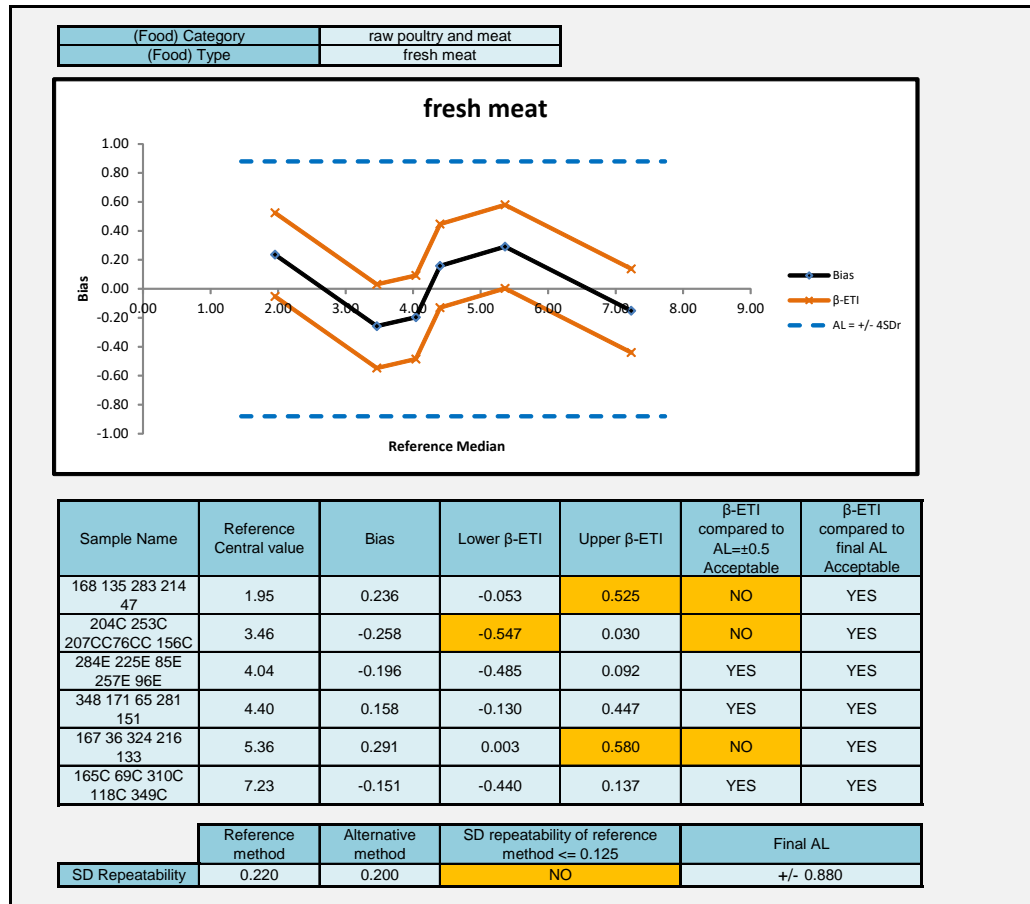


Figure 5 Ready to eat foods

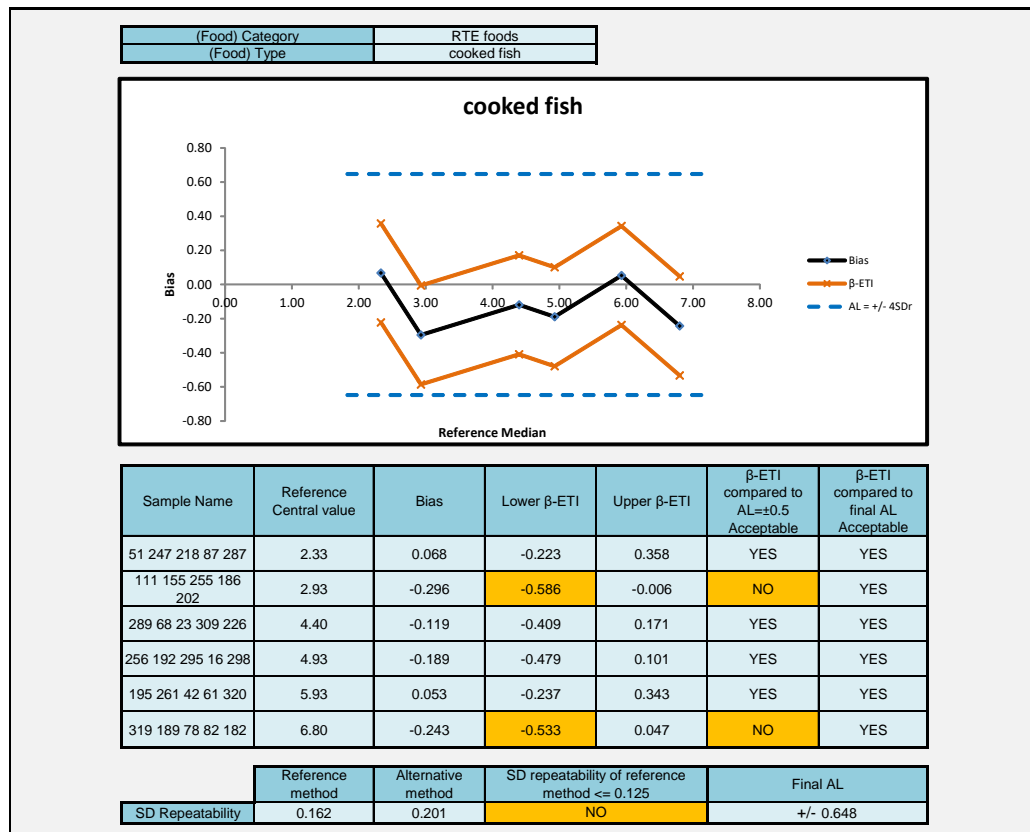
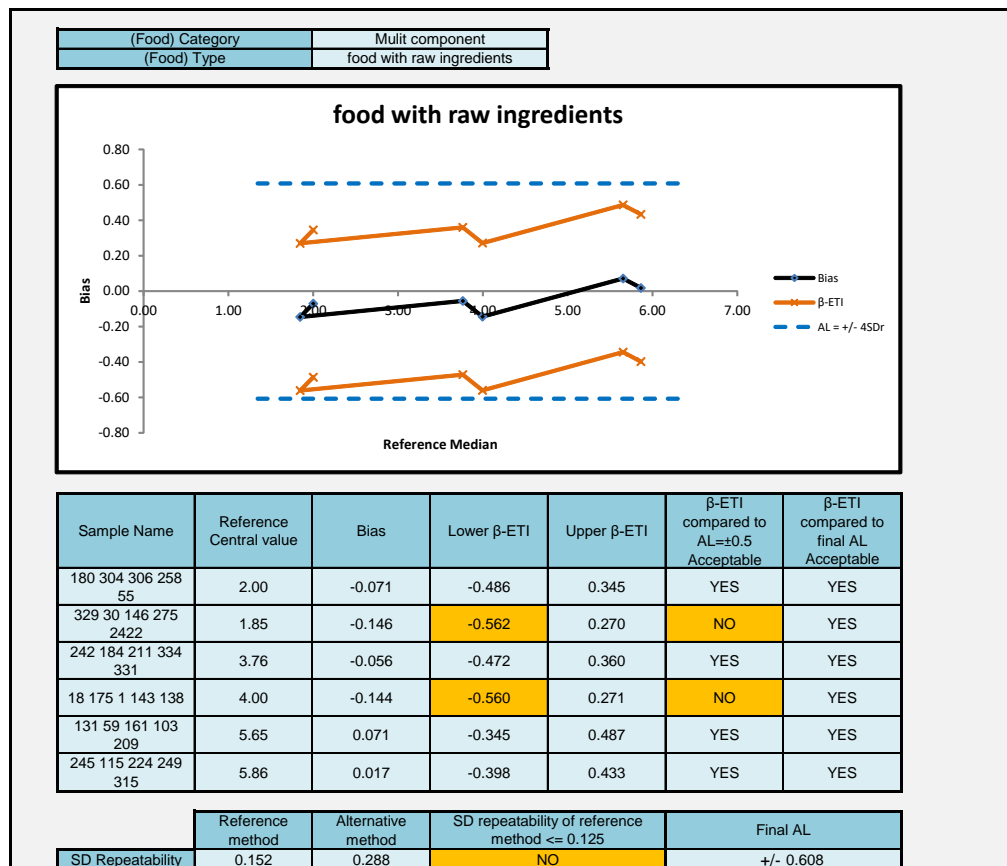


Figure 6 Multi component foods:



The observed profiles are within the 0.5 log AL (for dairy products) or the recalculated AL limit calculated according to ISO16140-2:2015 section 6.1.3.3 (other products).

All the accuracy profiles fulfil the performance criteria after the permitted recalculation and the alternative method is accepted as being equivalent to the reference method.

THE SELECTIVITY OF THE METHOD (INCLUSIVITY/EXCLUSIVITY)

Inclusivity is the ability of an alternative method to detect the target analyte from a wide range of strains. In the original study, all 33 strains studied revealed typical colonies. Of the 20 inclusivity strains tested in the current study, 18 strains were detected using the alternative and reference methods. Those not detected by the alternative 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. In the original study, 20 were studied. 10 strains did not grow on the CF medium and 8 were atypical in appearance. Two strains of *Shigella sonnei* appeared typical owing to the presence of galactosidase activity. By comparison, 9 stains failed to grow in VRBA, 5 strains were atypical and 6 were typical in appearance.

In the recent study, 10 non-target strains were tested. Of these strains, three strains (*A. hydrophila* CRA 4111, *A. sobria* CRA 8390 and *S. Fonticola* CRA 4613) were detected by both methods. An additional two strains (*Serratia liquefaciens* CRA 10670 and *Serratia proteamaculans* 1643) were detected by the reference method but not the alternate method.

The alternate method is more selective than the reference method.

CONCLUSION OF THE COMPARISON STUDY

The results of the method comparison study clearly showed that the Compact Dry ETB is equivalent to or better (regarding the selectivity) than the reference method ISO 4832:2006. The lowest validated level is about 2 log cfu/g.

INTERLABORATORY STUDY

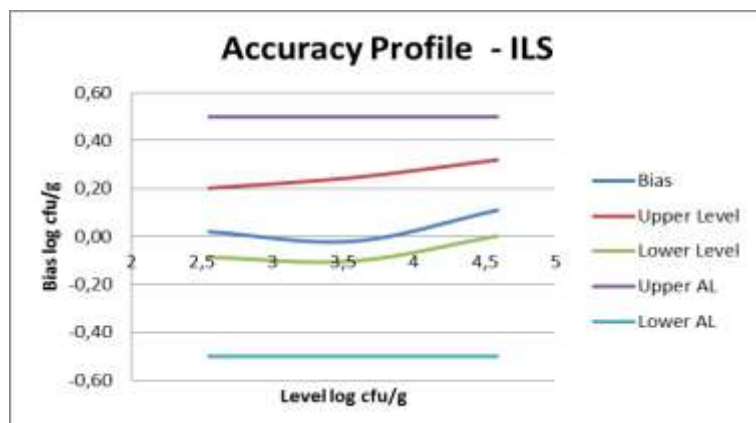
The interlaboratory study was conducted in November 2007. Eleven laboratories analysed samples of pasteurised milk artificially contaminated with defined numbers of *Escherichia coli* and *Enterobacter aerogenes*. The laboratories performed the analyses according to ISO 4832 and Compact Dry CF.

Table 3 Results (log cfu/g) of the collaborative study

Level	Reference method		Alternative method		Bias	Upper	Lower	Upper	Lower
	Median	S _R	Alt method	S _R		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. All the results fall within the acceptance levels (AL).

Figure 7 Accuracy Profile of the interlaboratory study for coliforms



CONCLUSION

According to the comparison and the interlaboratory study no substantial differences were found between the Compact Dry CF method and the reference method (ISO 4832:2006) for the enumeration of coliforms.