



NordVal International Certificate

Issued for:	HyServe Compact Dry CF Method for the Enumeration of Total Coliforms
NordVal No:	035
First approval date:	1 December 2008
Renewal date:	1 December 2016
Valid until:	1 December 2018

HyServe Compact Dry CF

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 reference method used is 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 CCFRA Technology Limited, Chipping Campden, UK, according to the design of ISO 16140:2003. NordVal International has recalculated the data according to the ISO 16140-2:2016, and concludes that results document no statistical difference in the performances between Compact Dry TC and the ISO 4832:2006.

Date: 1/12 2016

Yours sincerely

A handwritten signature in blue ink, appearing to read "Hilde Skår Norli".

Hilde Skår Norli
Chair of NordVal International

A handwritten signature in blue ink, appearing to read "Nina Skall Nielsen".

Nina Skall Nielsen
NMKL Secretary General

PRINCIPLE OF THE METHOD

HyServe 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 HyServe 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 foods.

HISTORY

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

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 has a transition period of two years for additional studies required according to the new protocol. In this certificate the data obtained according to ISO 16140 has been recalculated using statistical models given in the new standard.

COMPARISON STUDY

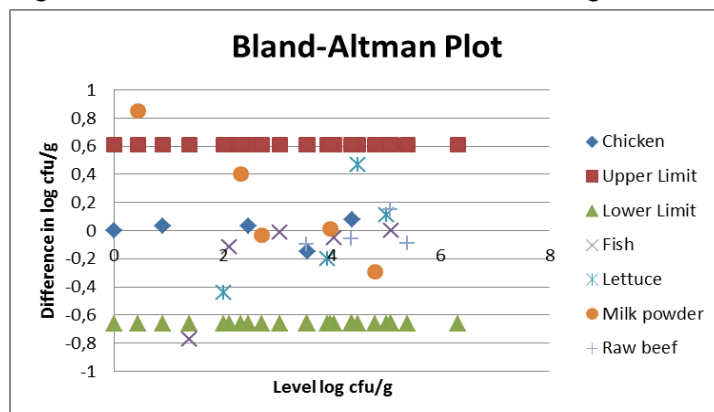
COMPLIANCE BETWEEN COMPACT DRY TC METHOD AND THE REFERENCE METHOD

The comparison study was carried out by CCFRA Technology Limited 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.

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. The Bland-Altman Plot in Figure 1, illustrates the difference obtained in the enumeration of total coliforms in foods by the alternative and the reference method, respectively.

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). 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.

Cooked chicken

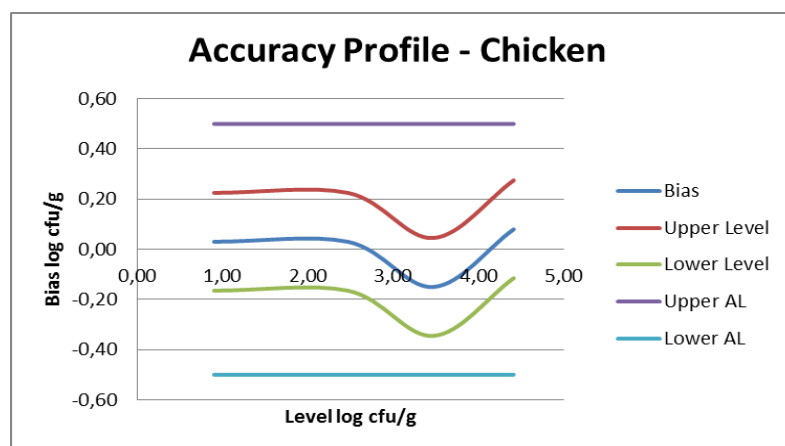
Four of the five levels were enumerated (the lowest level was not countable). The results for the enumeration of total coliforms in cooked chicken are given in Table 1, and illustrated by an Accuracy Profile in Figure 2.

Table 1 The results for cooked chicken in log cfu/g

Level	Alternative method		Reference method		Bias	Upper level	Lower level	± AL
	Mean	SD	Mean	SD				
2	0,90	0,13	0,87	0,21	0,03	0,23	-0,17	0,50
3	2,47	0,10	2,44	0,13	0,03	0,23	-0,17	0,50
4	3,47	0,10	3,62	0,38	-0,15	0,05	-0,35	0,50
5	4,41	0,07	4,33	0,14	0,08	0,28	-0,12	0,50
	Combined SD	0,102		0,237				

The lowest validated level with satisfactory precision: 0.9 log cfu/g.

Figure 2 Accuracy Profile for the results of the enumeration of total coliforms in chicken



Whenever no biases exist, the results would be on $y=0$. In the figure above, the acceptability limits (AL) are represented by the purple and the light blue lines. The levels where the results might be expected to vary between (upper and lower levels) are given as red and green lines. The bias (the difference obtained by the results obtained by the alternative method and the reference method) is given as the blue line.

As the upper level (red line) is below the upper AL (purple line), and the lower level (green line) is above the lower AL (light blue line) the alternative method is accepted as being equivalent to the reference method.

Frozen fish

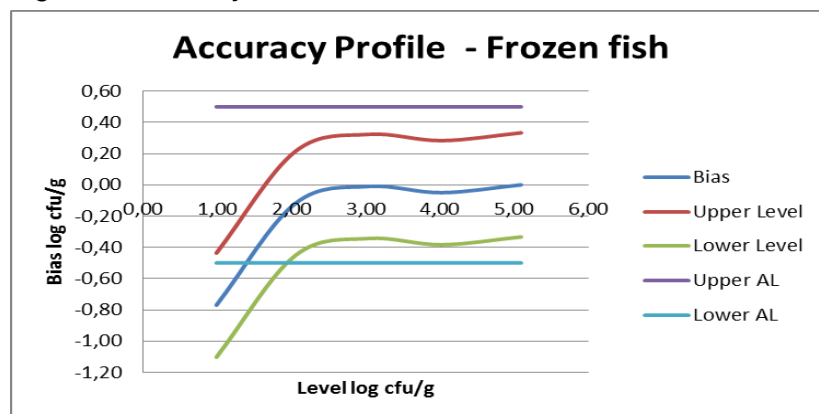
All five levels were enumerated. The results for the enumeration of total coliforms in frozen fish are given in Table 2, and illustrated by an Accuracy Profile in Figure 3.

Table 2 The results in log cfu/g for frozen fish

Level	Alternative method		Reference method		Bias	Upper Level	Lower Level	±AL
	Mean	SD	Mean	SD				
1	0,99	0,23	1,76	0,23	-0,77	-0,44	-1,10	0,50
2	2,05	0,17	2,17	0,18	-0,12	0,21	-0,45	0,50
3	3,04	0,11	3,05	0,08	-0,01	0,32	-0,34	0,50
4	4,02	0,20	4,07	0,10	-0,05	0,28	-0,38	0,50
5	5,09	0,15	5,09	0,21	0,00	0,33	-0,33	0,50
	Combined SD	0,177		0,171				

The lowest validated level with satisfactory precision: 1,2 log cfu/g.

Figure 2 Accuracy Profile for the results of the enumeration of total coliforms in fish



The results are within the upper and lower Acceptance Levels.

Lettuce

Four of the five levels were enumerated (the lowest level was not countable). For level 2, four of the five replicates were enumerated by the alternative method.

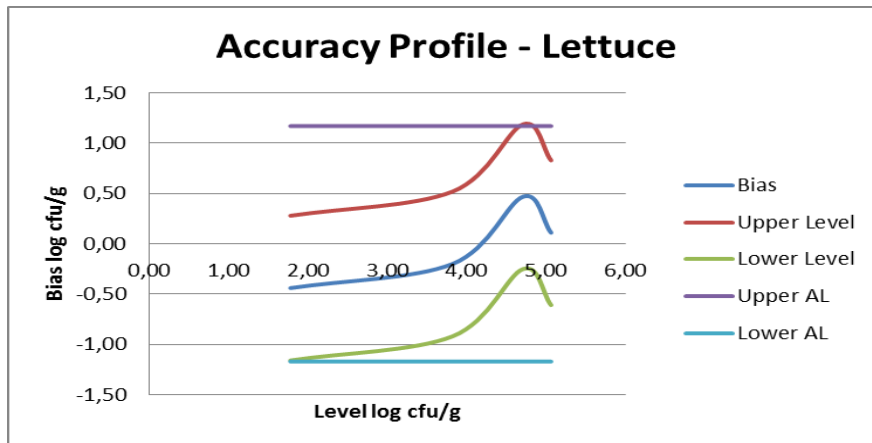
The results for the enumeration of total coliforms in lettuce are given in Table 3, and illustrated by an Accuracy Profile in Figure 4.

Table 3 The results in log cfu/g for lettuce

Level	Alternative method		Reference method		Bias	Upper Level	Lower Level	±AL
	Mean	SD	Mean	SD				
2	1,78	0,65	2,22	0,53	-0,44	0,28	-1,16	1,2
3	3,82	0,22	4,02	0,15	-0,20	0,52	-0,92	1,2
4	4,71	0,29	4,24	0,15	0,47	1,19	-0,25	1,2
5	5,06	0,09	4,95	0,13	0,11	0,83	-0,61	1,2
	Combined SD	0,38		0,29				

The lowest validated level with satisfactory precision: 3.9 log cfu/g.

Figure 4 Accuracy Profile for the results of the enumeration of total coliforms in lettuce



The results are within the upper and lower Acceptance Levels.

Milk powder

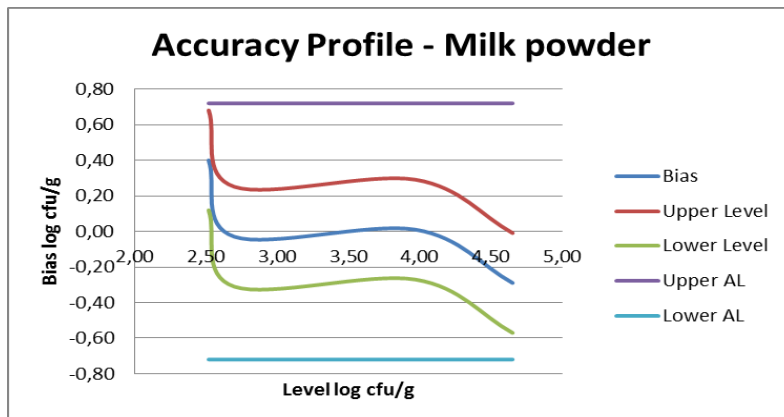
Four of the 5 levels were enumerated (for the alternative method, 3 of the 5 replicates were counted at level 1). For level 2 (around 2 log cfu/g), the standard deviations are high (precision is poor) for both methods. The high standard deviation for this level with the reference method would yield a high acceptance level (of 1,6 log cfu/g). In the accuracy profile, the standard deviation for level 2 is therefore omitted in the calculation of the combined standard deviation and calculation of the levels.

The results for the enumeration of total coliforms in milk powder are given in Table 4, and illustrated by an Accuracy Profile in Figure 5.

Table 4 The results in log cfu/g for milk powder

Level	Alternative method		Reference method		Bias	Upper Level	Lower Level	AL
	Mean	SD	Mean	SD				
1	0,85	0,24						
2	2,52	0,61	2,12	0,71	0,40	0,68	0,12	0,72
3	2,70	0,11	2,73	0,24	-0,03	0,25	-0,31	0,72
4	3,97	0,08	3,96	0,07	0,01	0,29	-0,27	0,72
5	4,65	0,22	4,94	0,19	-0,29	-0,01	-0,57	0,72
Combined SD (1-5)		0,32		0,39				
Combined SD (3-5)		0,15		0,18				

Figure 5 Accuracy Profile for the results of the enumeration of total coliforms in milk powder



The results are within the upper and lower Acceptance Levels.

Raw beef

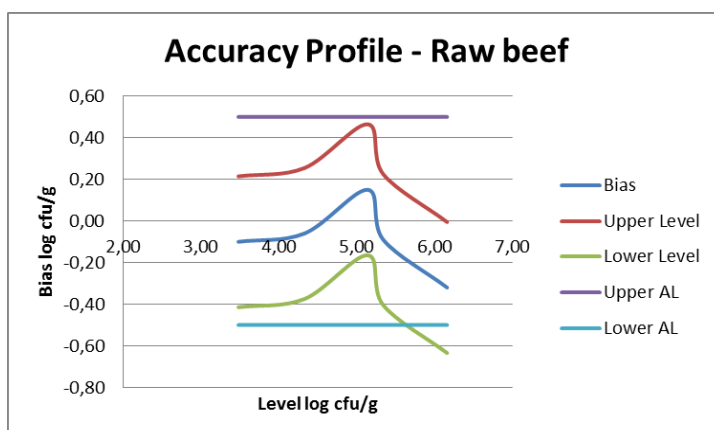
All five levels were detected and enumerated. The results for the enumeration of total coliforms in raw beef are given in Table 5, and illustrated by an Accuracy Profile in Figure 6.

Table 5 The results in log cfu/g for raw beef

Level	Alternative method		Reference method		Bias	Upper Level	Lower Level	± AL
	Mean	SD	Mean	SD				
1	3,48	0,10	3,58	0,08	-0,10	0,21	-0,41	0,50
2	4,33	0,03	4,39	0,08	-0,06	0,25	-0,37	0,50
3	5,14	0,23	4,99	0,14	0,15	0,46	-0,16	0,50
4	5,34	0,09	5,43	0,15	-0,09	0,22	-0,40	0,50
5	6,16	0,26	6,48	0,23	-0,32	-0,01	-0,63	0,50
Combined SD		0,17		0,16				

The lowest validated level with satisfactory precision: 3,4 log cfu/g.

Figure 6 Accuracy Profile for the results of the enumeration of total coliforms in raw beef



The results are considered satisfactory.

THE SELECTIVITY OF THE METHOD (INCLUSIVITY/EXCLUSIVITY)

The selectivity study was performed according to ISO 16140:2003.

Inclusivity is the ability of an alternative method to detect the target analyte from a wide range of strains. 32 strains (at 2-3 log cfu/ml) were studied. Two of the 32 strains gave atypical growth on Compact Dry CF.

Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method. 20 strains (at 2-3 log cfu/ml) were studied. At the Compact Dry CF, 3 strains gave typical colonies (will interfere), 8 strains gave atypical growth. With the ISO 4832 method, 9 strains gave typical growth and 4 strains gave atypical. This shows that for the strains tested, Compact Dry CF is more selective than ISO 4832.

CONCLUSION OF THE INTERLABORATORY 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 with satisfactory precision varies from 0,9 – 3,4 log cfu/g depending on the matrix.

INTERLABORATORY STUDY

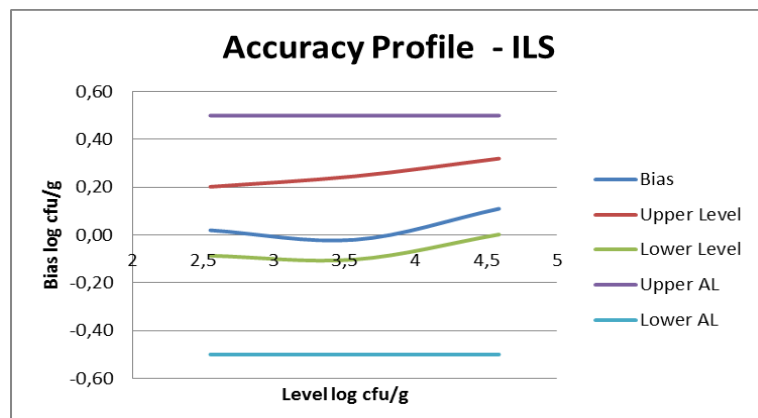
The interlaboratory study was conducted in November 2007. Eleven laboratories analysed samples of pasteurised milk artificially contaminated with defined numbers of *Esherichia coli* and *Enterobacter aerogenes*. The laboratories performed the analyses according to ISO 4832 and Compact Dry CF.

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

	Reference 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





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