

# NordVal International Certificate

Issued for:	HyServe Compact Dry ETB Method for the Enumeration of <i>Enterobacteriaceae</i>
NordVal No:	034
First approval date:	1 December 2008
Renewal date:	1 December 2016
Valid until:	1 December 2018

## HyServe Compact Dry ETB

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 21528-2:2004: "Microbiology of foods and animal feeding stuffs. Horizontal method for the detection and enumeration of *Enterobacteriaceae* - part 2: Colony Count Method".

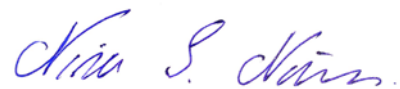
The validation studies have been conducted by CCFRA Technology Limited, Chipping Campden, UK, according to the design of ISO 16140:2003. NordVal International has recalculated the data according to the ISO 16140-2:2016, and concludes that Compact Dry ETB provide equivalent results to ISO 21528-2:2004.

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 ETB is a ready-to-use selective plate containing glucose for the enumeration of *Enterobacteriaceae*. An aliquot of 1 ml of an appropriate dilution is plated onto Compact Dry ETB plate. The plate is incubated at  $37 \pm 1^\circ\text{C}$  and colonies (red/purple) were counted after  $24 \pm 2\text{h}$ .

## FIELD OF APPLICATION

The method has been tested on enumeration of *Enterobacteriaceae* 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

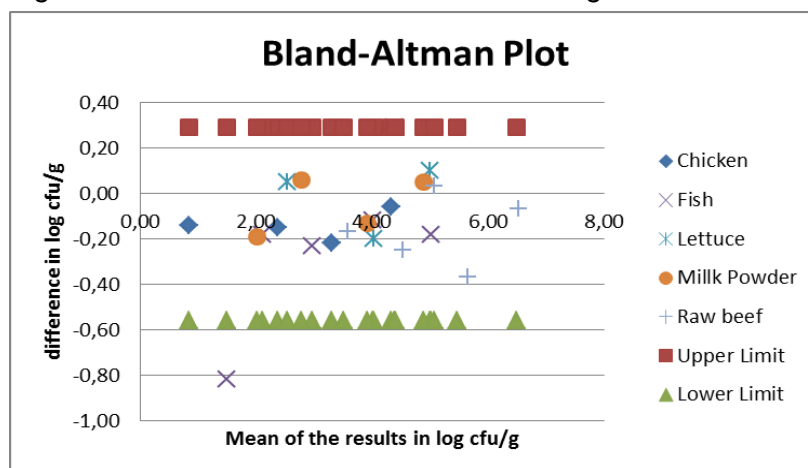
The comparison study was carried out by CCFRA Technology Limited in 2007 on cooked chicken, frozen fish, lettuce, milk powder and raw beef. Five levels of contamination were used for each food matrix. For all foods, except milk powder, naturally contaminated samples were tested. Five replicates were 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  $\pm 2$  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



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. As the results are based on data obtained according to ISO 16140:2003, the mean of the replicates are used rather than the median. The five food categories, including five levels each category and five replicates each level were analysed by using the Compact Dry ETB and ISO 21528-2:2004.

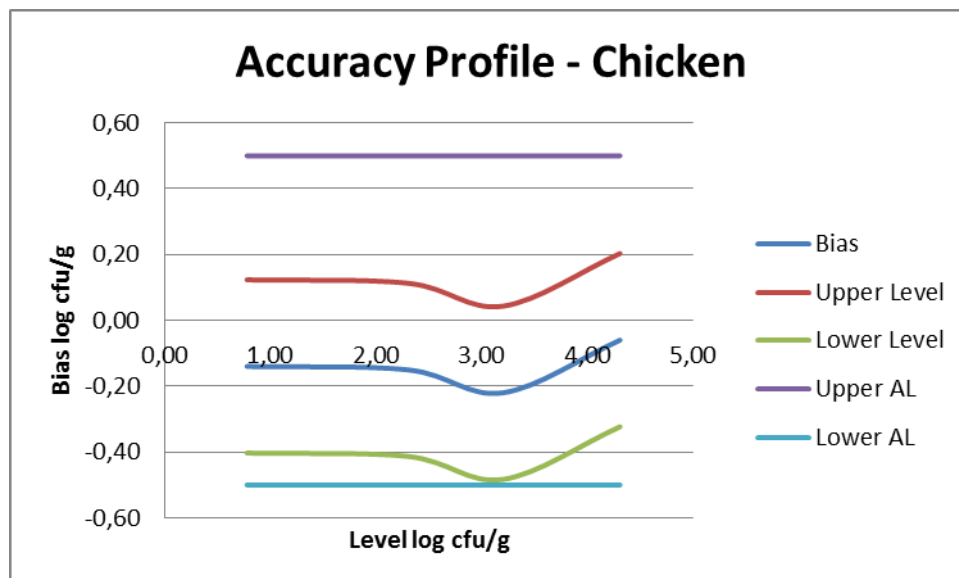
#### Cooked chicken

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

Table 1 Results in log cfu/g of the enumeration of *Enterobacteriaceae* in chicken

	Alternative method		Reference method					
Level	Mean	SD	Mean	SD	Bias	Upper Level	Lower Level	±AL
2	0,78	0,16	0,92	0,24	-0,14	0,12	-0,40	0,50
3	2,3	0,16	2,45	0,15	-0,15	0,11	-0,41	0,50
4	3,2	0,11	3,42	0,19	-0,22	0,04	-0,48	0,50
5	4,31	0,11	4,37	0,08	-0,06	0,20	-0,32	0,50
Combined SD		0,137		0,175				

Figure 2 Accuracy Profile for the results of the enumeration of *Enterobacteriaceae* in chicken



Whenever no biases exist, the results would be on  $y=0$ . In the figure above, the acceptability limits ( $AL = \pm 0,5$ ) 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

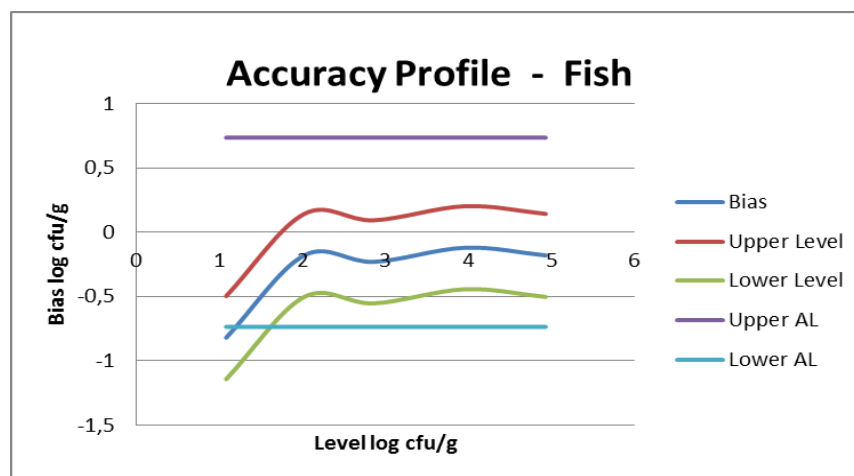
All five levels were enumerated, however, for the alternative method, only 3 of the 5 replicates for the lowest level were countable.

The results for the enumeration of *Enterobacteriaceae* in frozen fish are given in Table 2, and illustrated by an Accuracy Profile in Figure 3.

Table 2 Results in log cfu/g of the enumeration of *Enterobacteriaceae* in frozen fish

Level	Alternative method		Reference method		Bias	Upper Level	Lower Level	± AL
	Mean	SD	Mean	SD				
1	1,08	0,10	1,90	0,15	-0,82	-0,50	-1,14	0,74
2	2,02	0,20	2,20	0,19	-0,18	0,14	-0,50	0,74
3	2,86	0,13	3,09	0,12	-0,23	0,09	-0,55	0,74
4	3,96	0,15	4,08	0,15	-0,12	0,20	-0,44	0,74
5	4,93	0,24	5,11	0,20	-0,18	0,14	-0,50	0,74
Combined SD		0,171		0,165				

Figure 3 Accuracy Profile for the results of the enumeration of *Enterobacteriaceae* in frozen fish



The accuracy profile shows that the results are satisfactory except for the lowest level, with a bias of -0,82 log cfu/g, and where only 3 of the 5 samples were countable.

### Lettuce

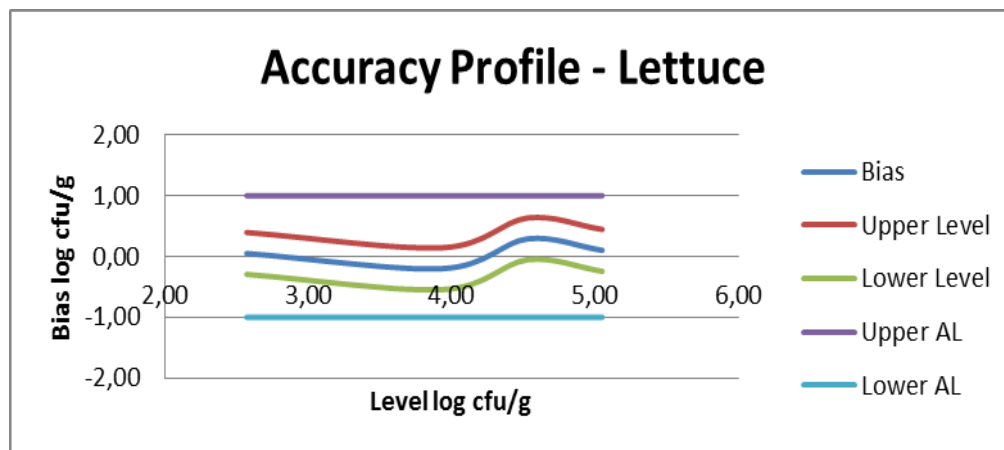
Four of the five levels were enumerated (the lowest level was not countable). The results for the enumeration of *Enterobacteriaceae* in lettuce are given in Table 3, and illustrated by an Accuracy Profile in Figure 4.

Table 3 Results in log cfu/g of the enumeration of Enterobacteriaceae in lettuce

Level	Alternative method		Reference method		Bias	Upper Level	Lower Level	± AL
	Mean	SD	Mean	SD				
2	2,57	0,91	2,52	0,49	0,05	0,39	-0,29	1,0
3	3,94	0,22	4,14	0,16	-0,20	0,14	-0,54	1,0
4	4,53	0,14	4,24	0,36	0,29	0,63	-0,05	1,0
5	5,05	0,17	4,95	0,18	0,10	0,44	-0,24	1,0
Combined SD		0,180		0,250				

The precision is not satisfactory for level 2, and is omitted from the calculation of combined standard deviation. The standard deviation is relatively high for the reference method, yielding an acceptance level, AL of ± 1.

Figure 4 Accuracy Profile for the results of the enumeration of Enterobacteriaceae in lettuce



All results falls within the acceptance levels.

### Milk powder

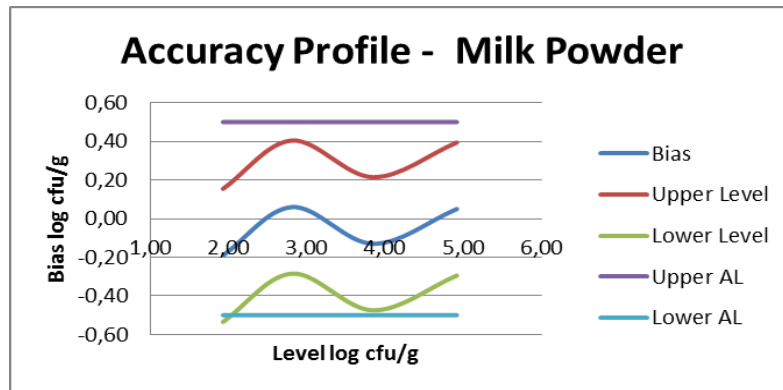
Four of the five levels were enumerated (the lowest level was not countable). The results for the enumeration of *Enterobacteriaceae* in milk powder are given in Table 4, and illustrated by an Accuracy Profile in Figure 5.

Table 4 Results in log cfu/g of the enumeration of Enterobacteriaceae in milk powder

Level	Alternative method		Reference method		Bias	Upper Level	Lower Level	± AL
	Mean	SD	Mean	SD				
2	1,93	0,91	2,12	0,83	-0,19	0,15	-0,53	0,50
3	2,81	0,22	2,75	0,24	0,06	0,40	-0,28	0,50
4	3,85	0,14	3,98	0,07	-0,13	0,21	-0,47	0,50
5	4,92	0,17	4,87	0,17	0,05	0,39	-0,29	0,50
Combined SD		0,180		0,174				

The precision is not satisfactory for level 2, and is omitted from the calculation of combined standard deviation for both methods, or else the acceptance level would be very high.

Figure 5 Accuracy Profile for the results of the enumeration of *Enterobacteriaceae* in milk powder



All results falls within the acceptance levels.

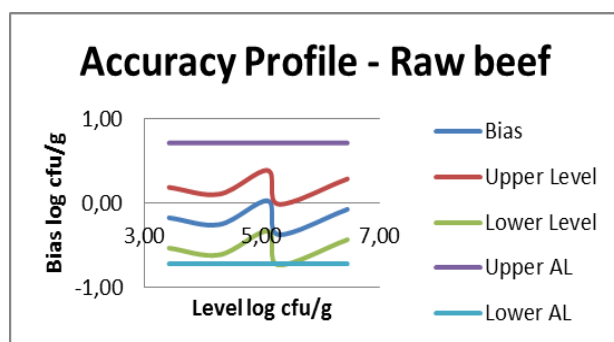
### Raw beef

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

Table 5 Results in log cfu/g of the enumeration of *Enterobacteriaceae* in raw beef

Level	Alternative method		Reference method		Bias	Upper Level	Lower Level	± AL
	Mean	SD	Mean	SD				
1	3,42	0,03	3,59	0,10	-0,17	0,19	-0,53	0,50
2	4,28	0,06	4,53	0,10	-0,25	0,11	-0,61	0,50
3	5,10	0,22	5,07	0,19	0,03	0,39	-0,33	0,50
4	5,29	0,09	5,66	0,09	-0,37	-0,01	-0,73	0,50
5	6,46	0,34	6,53	0,31	-0,07	0,29	-0,43	0,50
Combined SD		0,188		0,179				

Figure 6 Accuracy Profile for the results of the enumeration of *Enterobacteriaceae* in raw beef



All results falls within the acceptance levels.

### **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. One of the 32 strains failed to grow on Compact Dry ETB.

**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. 18 of the 20 strains did not interfere. One strain interfered at both the alternative and the reference method. One strain gave very little growth on the reference method and atypical growth at the Compact Dry ETB.

### CONCLUSION OF THE COMPARISON STUDY

The results of the method comparison study showed that the Compact Dry ETB provide equivalent results to the reference method ISO 21528-2:2004. The lowest validated level with satisfactory precision varies from 2,0 – 3,4 log cfu/g depending on the matrix.

### INTERLABORATORY STUDY

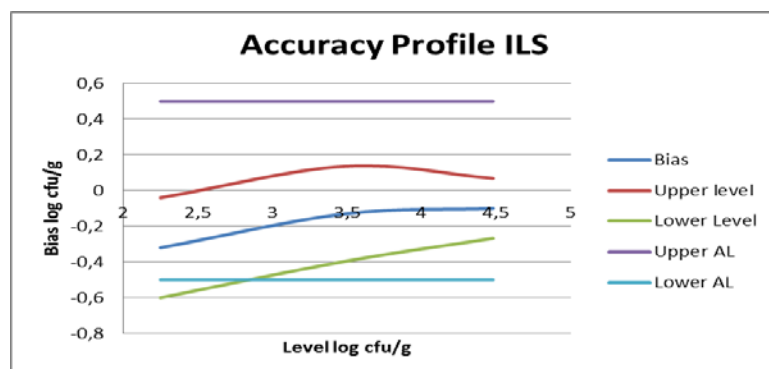
The interlaboratory study was conducted in November 2007. Ten laboratories analysed samples of pasteurised milk artificially contaminated with defined numbers of *Esheria coli* and *Enterobacter aerogenes* according to ISO 21528-2:2004 and Compact Dry ETB respectively.

The obtained results (log cfu/g) is given in Table 6, and illustrated by an Accuracy Profile in Figure 7.

Table 6 The interlaboratory study results in log cfu/g

	Reference method		Alternative method			Upper	Lower	
Level	Median	S <sub>R</sub>	Median	S <sub>R</sub>	Bias	Level	Level	±AL
1	2,57	0,12	2,25	0,20	-0,32	-0,04	-0,60	0,50
2	3,62	0,13	3,49	0,19	-0,13	0,14	-0,40	0,50
3	4,58	0,069	4,48	0,12	-0,1	0,07	-0,27	0,50

Figure 7 Accuracy Profile of the interlaboratory study



The lowest level has a rather high negative bias, and thus the lower level is below –AL.

### CONCLUSION

According to the comparison and the interlaboratory study no substantial differences were found between the HyServe Compact Dry ETB method and the reference method ISO 21528-2:2004 for the enumeration of *Enterobacteriaceae*.