



# 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 2016
Valid until:	1 December 2018

## 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 reference method used is ISO 4833:2003: "Microbiology of foods and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony count techniques at 30°C".


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 4833:2003.

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 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  $30 \pm 1^\circ\text{C}$  for  $48 \pm 3\text{h}$  and  $72 \pm 3\text{h}$ , respectively.

## **FIELD OF APPLICATION**

The method has been tested on enumeration of total viable organisms 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**

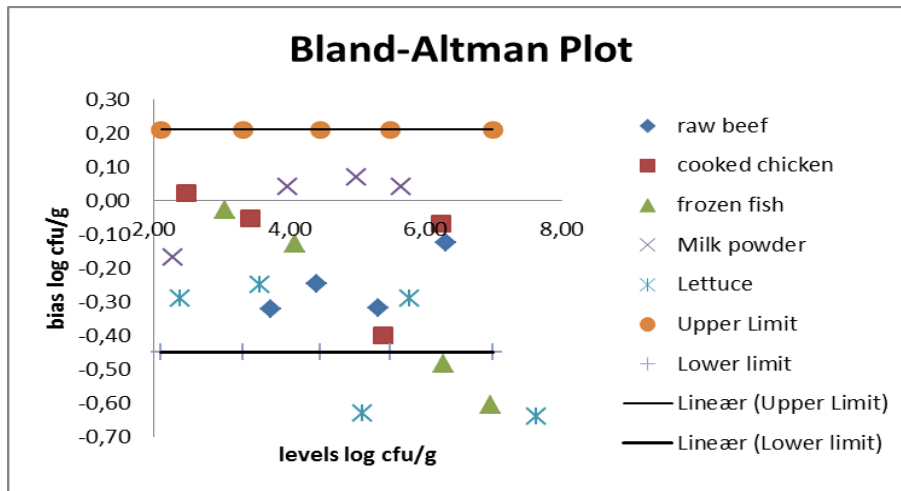
The comparison study was carried out by CCFRA Technology Limited in 2007 on cooked chicken, frozen fish, lettuce, milk powder and raw beef. Five levels of contamination were used for each food matrix. For all foods, except milk powder, naturally contaminated samples were tested. Five replicates were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

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.

## **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 total viable organisms in foods by the alternative and the reference method, respectively.

Figure 1 Bland-Altman Plot of the food categories tested



The relative trueness study also shows that most of the results have a negative bias, i.e. the results are generally lower with the alternative method than with the reference method, however the difference is not necessarily statistically significant. Most of the samples fall within the limits, only a couple of samples for lettuce and frozen fish are below the lower limit.

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

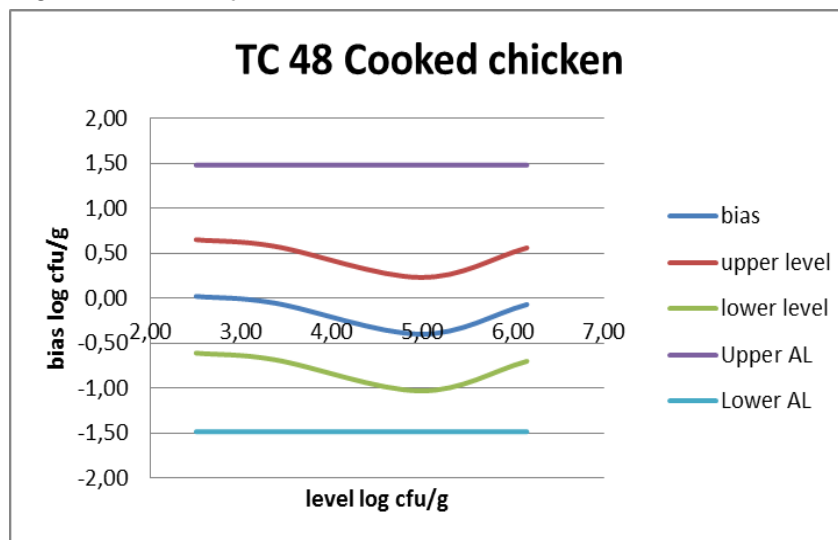
Four of the five levels were enumerated. (The lowest level was not countable in neither of the methods.) The results for the enumeration of total count in cooked chicken are given in Table 1, and illustrated by an Accuracy Profile in Figure 2.

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

Alternative method		Reference method		Bias	Upper level	Lower level	$\pm AL(4 \cdot SD_{ref})$
Median	SD	Median	SD				
2,51	0,44	2,48	0,59	0,02	0,65	-0,61	1,5
3,37	0,38	3,42	0,37	-0,05	0,58	-0,68	1,5
4,98	0,17	5,37	0,22	-0,40	0,23	-1,03	1,5
6,15	0,26	6,22	0,13	-0,07	0,56	-0,70	1,5
Combined SD 0,330		Combined SD 0,371					

As the precision for the lowest level by both methods are high, the combined standard deviation is high for the reference method, yielding a higher acceptance level than the usual  $\pm 0,5$  log units.

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



As the  $s_{ref} > 0,125$ , the  $AL = 4 \cdot s_{ref}$ .

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

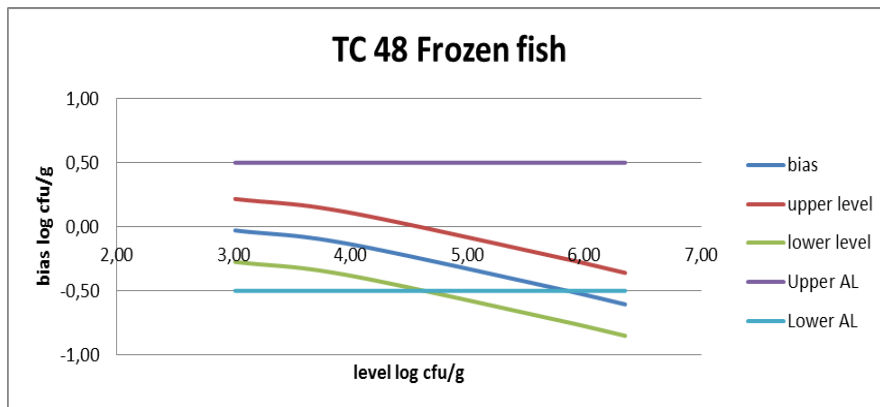
Four of the five levels were enumerated. The results for the enumeration of total count in frozen fish are given in Table 2, and illustrated by an Accuracy Profile in Figure 3.

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

Alternative method		Reference method					
Median	SD	Median	SD	Bias	Upper level	Lower level	$\pm AL$
3,01	0,18	3,04	0,17	-0,03	0,22	-0,27	0,50
3,95	0,12	4,08	0,08	-0,13	0,12	-0,37	0,50
5,78	0,13	6,27	0,09	-0,49	-0,24	-0,73	0,50
6,35	0,05	6,96	0,06	-0,61	-0,36	-0,85	0,50
Comb. SD	0,128		0,107				

The results obtained by the ISO method are higher than the results by Compact Dry TC, however, the standard deviations obtained are small (the precision is very good) and hence the accompanying confidence level are narrow.

Figure 3 The Accuracy Profile for frozen fish



As the standard deviation is narrow, the biases at high levels have to be small in order to be within the given ALs.

### Lettuce

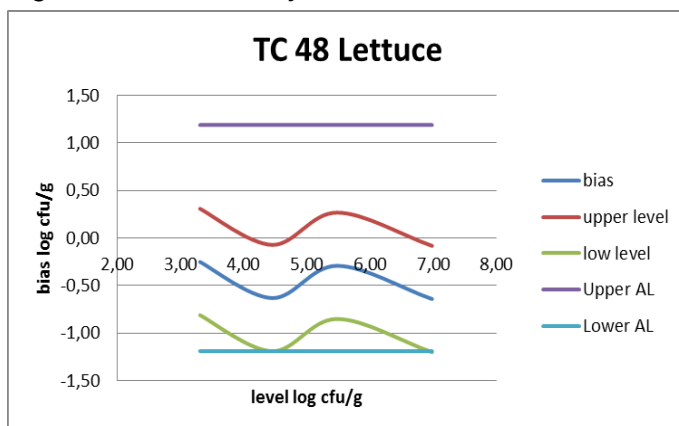
All five levels were enumerated, however, the lowest level around 2 log cfu/g had a very high standard deviation and is omitted for calculation in the combined standard deviation. The results for the enumeration of total count in lettuce are given in Table 3, and illustrated by an Accuracy Profile in Figure 4.

Table 3: The results for lettuce in log cfu/g

Alternative method		Reference method					
Median	SD	Median	SD	Bias	Upper level	Lower level	$\pm$ AL
2,11	0,70	2,40	0,73	-0,29			
3,31	0,45	3,56	0,34	-0,25	0,31	-0,81	1,2
4,45	0,32	5,08	0,31	-0,63	-0,07	-1,2	1,2
5,48	0,17	5,77	0,35	-0,29	0,27	-0,85	1,2
6,98	0,13	7,62	0,14	-0,64	-0,08	-1,2	1,2
Combined SD	0,296		0,297				

As the  $s_{ref} > 0,125$ , the  $AL = 4 \cdot s_{ref}$ .

Figure 4 The Accuracy Profile for lettuce



The results are within the upper and lower Acceptance Levels.

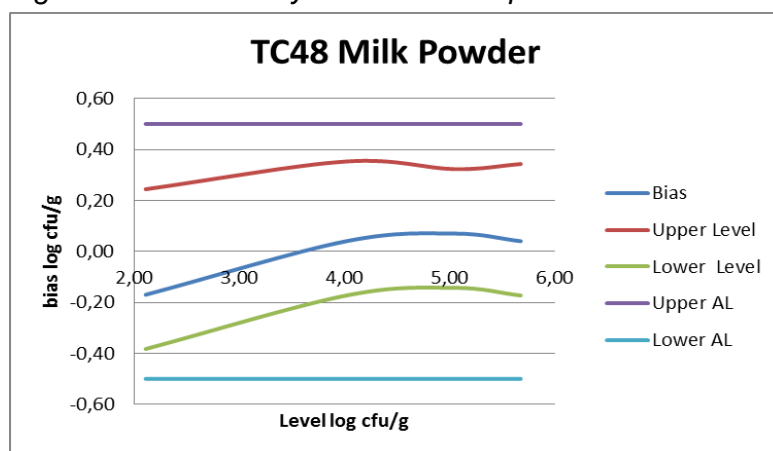
## Milk powder

Four of the five levels were enumerated. The results for the enumeration of total count in milk powder are given in Table 4, and illustrated by an Accuracy Profile in Figure 5.

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

Alternative method		Reference method		Bias	Lower Level	Upper Level	± AL
Median	SD	Median	SD				
2,11	0,03	2,28	0,15	-0,17	-0,38	0,24	0,50
4,02	0,14	3,98	0,08	0,04	-0,17	0,35	0,50
5,05	0,11	4,98	0,11	0,07	-0,14	0,32	0,50
5,68	0,13	5,64	0,17	0,04	-0,17	0,34	0,50
Comb. SD	0,111		0,133				

Figure 5 The Accuracy Profile for milk powder



The results are within the upper and lower Acceptance Levels.

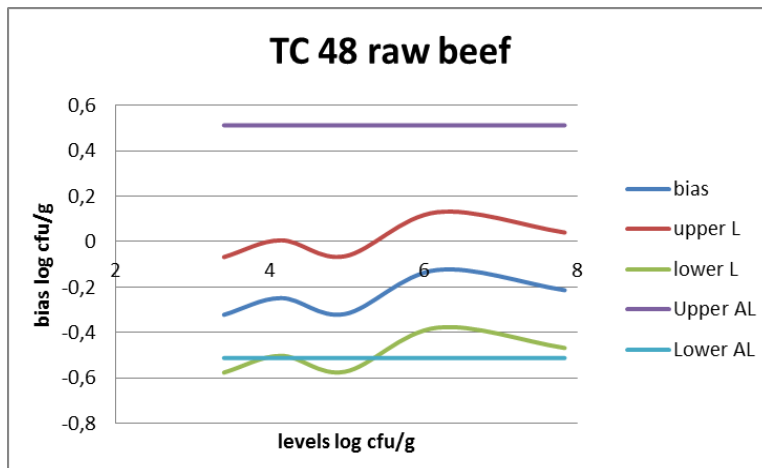
## Raw beef

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

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

Alternative method		Reference method		Bias	Upper Level	Lower Level	± AL
Median	SD	Median	SD				
3,41	0,09	3,73	0,06	-0,32	-0,07	-0,58	0,50
4,15	0,09	4,40	0,08	-0,25	0,01	-0,50	0,50
4,98	0,27	5,29	0,14	-0,32	-0,06	-0,57	0,50
6,18	0,04	6,30	0,22	-0,12	0,13	-0,38	0,50
7,83	0,04	8,05	0,06	-0,21	0,04	-0,47	0,50
Combined SD	<b>0,13</b>		<b>0,13</b>				

Figure 6 The Accuracy Profile for raw beef



The results obtained by the ISO method is somewhat higher than the alternative method, however their confidence intervals are overlapping.

### 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. There were no difference in the results between 48 and 72h. The lowest validated level with satisfactory precision varies from 2,1 – 3,4 log cfu/g depending on the matrix.

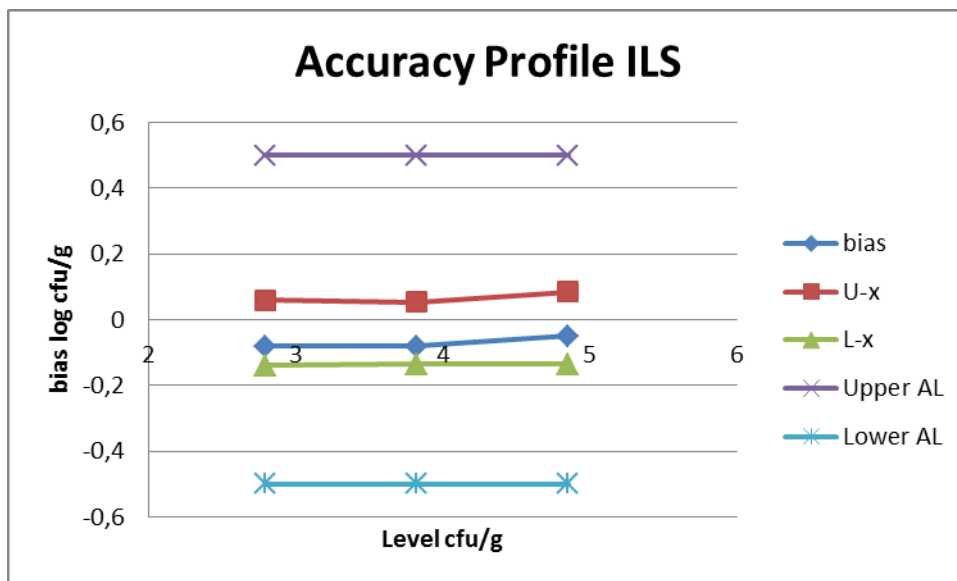
### INTERLABORATORY STUDY

The interlaboratory study was conducted in November 2007. Thirteen laboratories analysed samples of pasteurised milk artificially contaminated with defined numbers of *E.coli*. The laboratories performed the analyses according to ISO 4833 and Compact Dry TC after 48 h and 72h. The results are given in Table 6 and the accuracy profile is shown in figure 7.

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

Method	Level	Median	Repeatability	Reproducibility
ISO 4833	Control	-	-	-
	Low	2,79	0,056	0,068
	Middle	3,82	0,041	0,095
	High	4,85	0,076	0,096
TC after 48h	Control	-	-	-
	Low	2,71	0,030	0,10
	Middle	3,74	0,040	0,096
	High	4,80	0,041	0,097
TC after 72h	Control	-	-	-
	Low	2,71	0,032	0,096
	Middle	3,74	0,040	0,080
	High	4,81	0,058	0,097

Figure 7 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 4833:2003) for the enumeration of total viable microorganisms at 30°C.