



# NordVal 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:	13 November 2014
Valid until:	1 December 2016

## 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

fulfils the requirements of the NordVal validation protocol. The reference method was ISO 4832:2006: "Microbiology of foods and animal feeding stuffs. Horizontal method for the enumeration of coliforms. Colony-count technique".

NordVal International has reviewed the method and the validation studies conducted by CCFRA Technology Limited, Chipping Campden, UK. The studies have been conducted according to ISO 16140:2003. The results document no statistical difference in the performances between Compact Dry CF and the ISO 4832:2006.

Date: 13 November 2014

Yours sincerely

A handwritten signature in blue ink that reads 'Sven Qvist'.

Sven Qvist  
Chair of NordVal International

A handwritten signature in blue ink that reads 'Hilde Skår Norli'.

Hilde Skår Norli  
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.

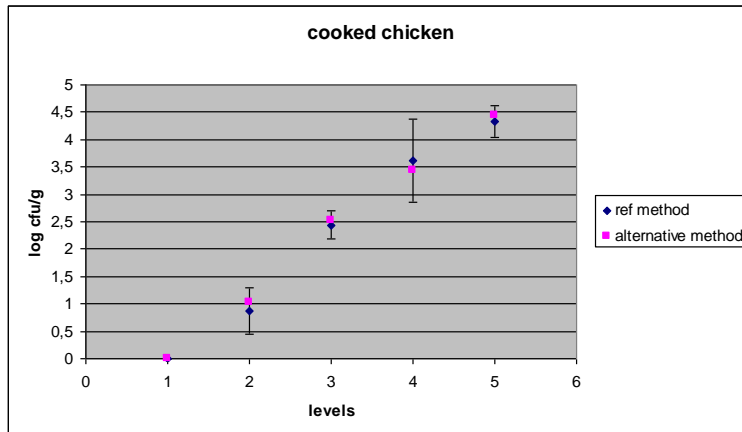
## COMPARISON STUDY

### 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.

The graphs below show the means of the results obtained by the reference and the alternative method, respectively, along with the confidence level ( $\pm 2$  times the standard deviation) of the reference method at different levels. When the results obtained by the alternative method fall within the confidence level, there are no significant difference between the methods.

#### Cooked chicken



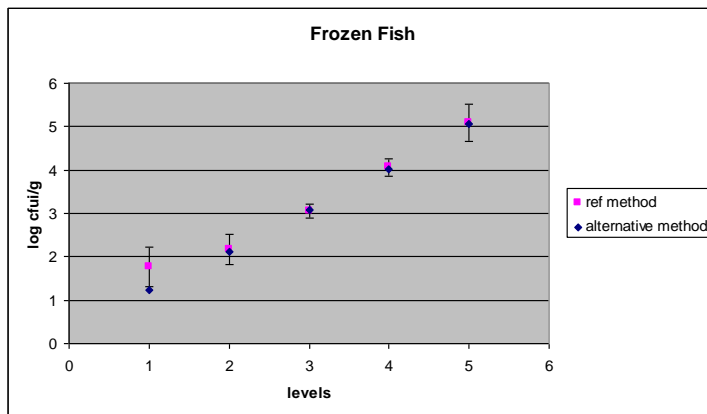
Four of the 5 levels were enumerated (the lowest level was not countable). The following results were obtained:

by the reference method: concentration range (mean): 0.87 – 4.33 log cfu/g  
precision; standard deviation: 0.13 - 0.38 log cfu/g

by Compact Dry CF: concentration range (mean): 1.03 – 4.44 log cfu/g  
precision; standard deviation: 0.056 - 0.23 log cfu/g

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

## Frozen fish

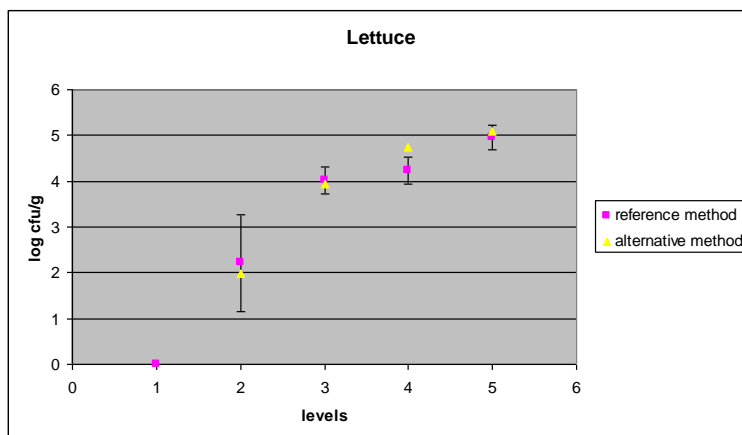


All five levels were enumerated. The following results were obtained:  
 by the reference method: concentration range (mean): 1.76 – 5.09 log cfu/g  
 precision; standard deviation: 0.081 - 0.23 log cfu/g

by Compact Dry CF: concentration range (mean): 1.24 – 5.05 log cfu/g  
 precision; standard deviation: 0.081 - 0.27 log cfu/g

The lowest validated level with satisfactory precision: 1.2 log cfu/g.

## Lettuce



Four of the 5 levels were enumerated (the lowest level was not countable). Four of the 5 replicates were enumerated at level 2 for the alternative method. The following results were obtained:

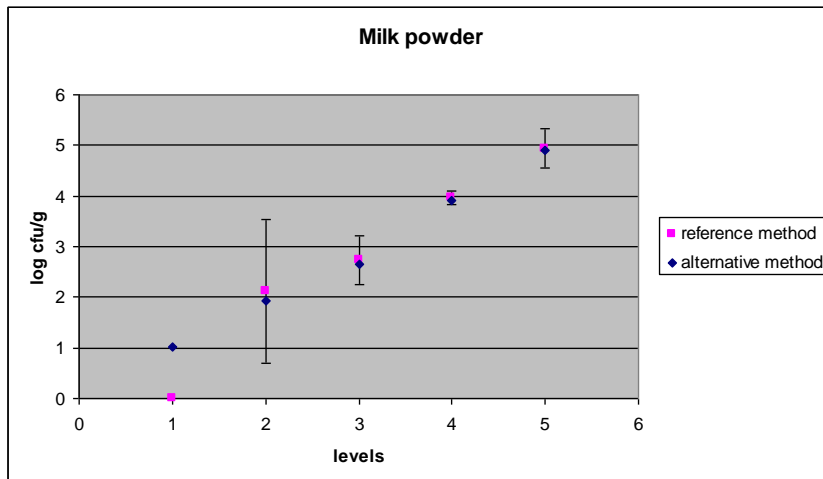
by the reference method: concentration range (mean): 2.22 – 4.95 log cfu/g  
 precision; standard deviation: 0.13- 0.53 log cfu/g

by Compact Dry CF: concentration range (mean): 1.99 – 5.09 log cfu/g  
 precision; standard deviation: 0.28 - 0.31 log cfu/g

The precision was not satisfactory for level 2 for the reference method.

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

Milk powder



Four of the 5 levels were enumerated (for the alternative method, 3 of the 5 replicates were counted at level 1). The following results were obtained:

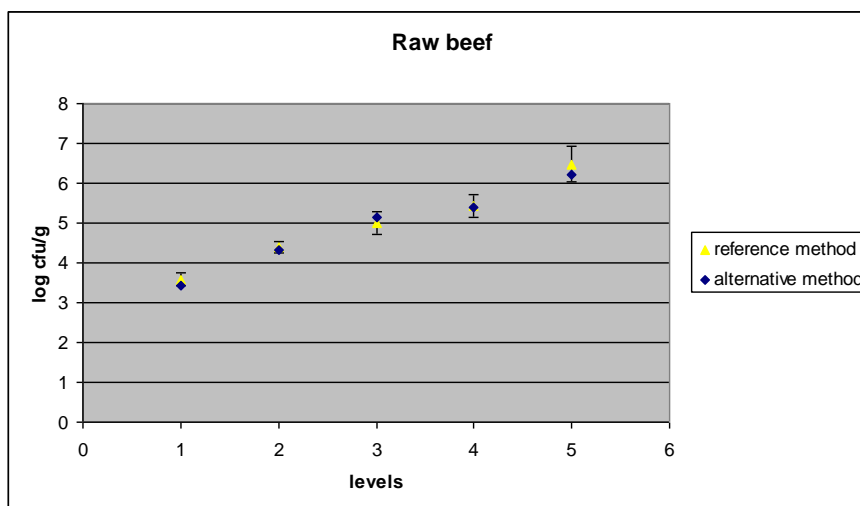
by the reference method: concentration range (mean): 2.12 – 4.94 log cfu/g  
precision; standard deviation: 0.066 - 0.71 log cfu/g

by Compact Dry CF: concentration range (mean): 1.94 – 4.89 log cfu/g  
precision; standard deviation: 0.050- 0.87 log cfu/g

The precision was not satisfactory for level 2 for either of the methods.

The lowest validated level with satisfactory precision: 2.0 log cfu/g.

Raw beef:



All 5 levels were detected and enumerated. The following results were obtained:

by the reference method: concentration range (mean): 3.58 – 6.48 log cfu/g  
precision; standard deviation: 0.077 - 0.23log cfu/g

by Compact Dry CF: concentration range (mean): 3.44 – 6.20 log cfu/g  
precision; standard deviation: 0.064 - 0.32 log cfu/g

The lowest validated level with satisfactory precision: 3.4 log cfu/g.

### 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. 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 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 with satisfactory precision varies from 0.9 – 3.4 log cfu/g depending on the matrix.

### COLLABORATIVE STUDY:

The collaborative 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.

Results (log cfu/g) of the collaborative study:

Method	Level	Median	Repeatability sr	Reproducibility SR
ISO 4832	Control	0	-	-
	Low	2.53	0.072	0.16
	Middle	3.59	0.088	0.11
	High	4.48	0.075	0.28
CF	Control	0	-	-
	Low	2.53	0.073	0.14
	Middle	3.54	0.12	0.15
	High	4.57	0.057	0.16

### CONCLUSION:

The collaborative study showed that there are no statistical differences between the results obtained by the Compact Dry CF Method and the ISO 4832:2006 for the enumeration of coliforms.