



NordVal 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	13 November 2014
Valid until:	1 December 2016

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

fulfils the requirements of the NordVal validation protocol. The reference method was 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".

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 ETB and the ISO 21528-2:2004.

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 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.

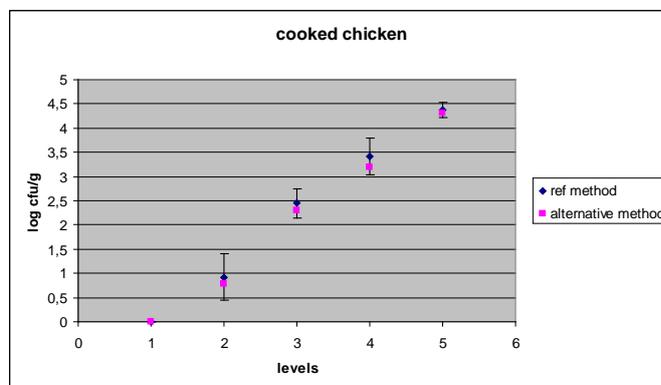
COMPARISON STUDY

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 (± 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 is no significant difference between the methods.

Cooked chicken



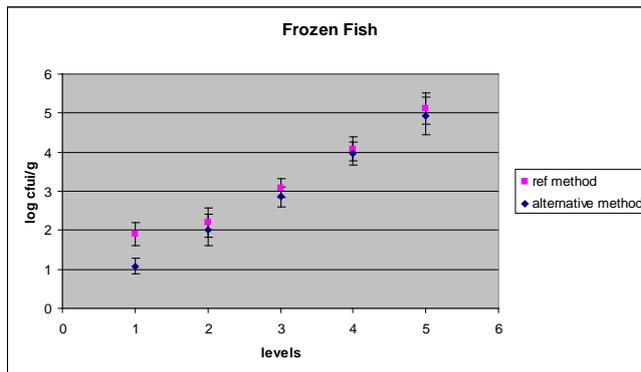
Four of the 5 levels were enumerated (the lowest level was not countable). The following results were obtained:

by the reference method: concentration range (mean): 0.92 – 4.37 log cfu/g
precision; standard deviation: 0.08 – 0.24 log cfu/g

by Compact Dry ETB: concentration range (mean): 0.78 – 4.31 log cfu/g
precision; standard deviation: 0.11 – 0.16 log cfu/g

The lowest validated level with satisfactory precision: 0.8 log cfu/g.

Frozen fish



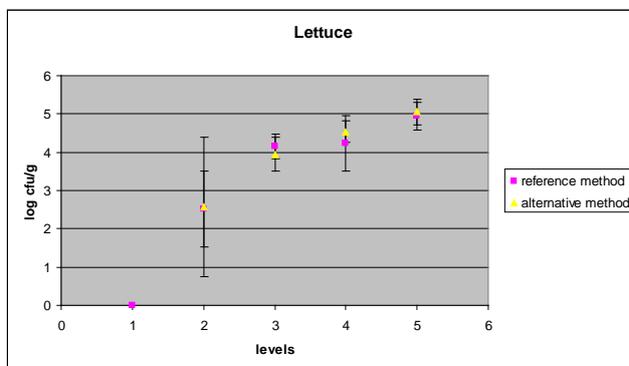
All five levels were enumerated, however, for the alternative method, only 3 of the 5 replicates for the lowest level were countable and hence the lowest level has to be omitted. The following results were obtained:

by the reference method: concentration range (mean): 2.20 – 5.11 log cfu/g
precision; standard deviation, : 0.12 – 0.20 log cfu/g

by Compact Dry ETB: concentration range (mean): 2.02 – 4.93 log cfu/g
precision; standard deviation: 0.13 – 0.24 log cfu/g

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

Lettuce



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

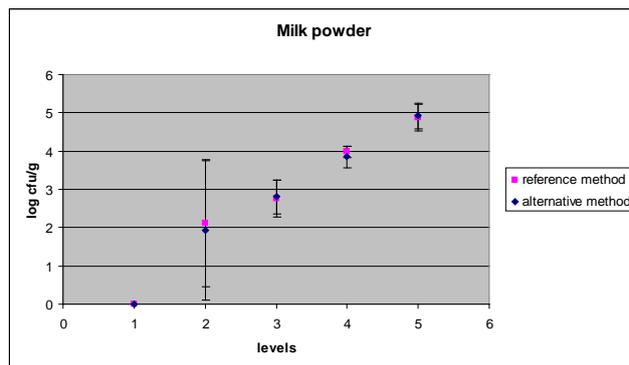
by the reference method: concentration range (mean): 2.52 – 4.95 log cfu/g
precision; standard deviation: 0.16- 0.49 log cfu/g

by Compact Dry ETB: concentration range (mean): 2.57 – 5.05 log cfu/g
precision; standard deviation: 0.14 – 0.91 log cfu/g

The precision is not satisfactory for level 2.

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

Milk powder



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

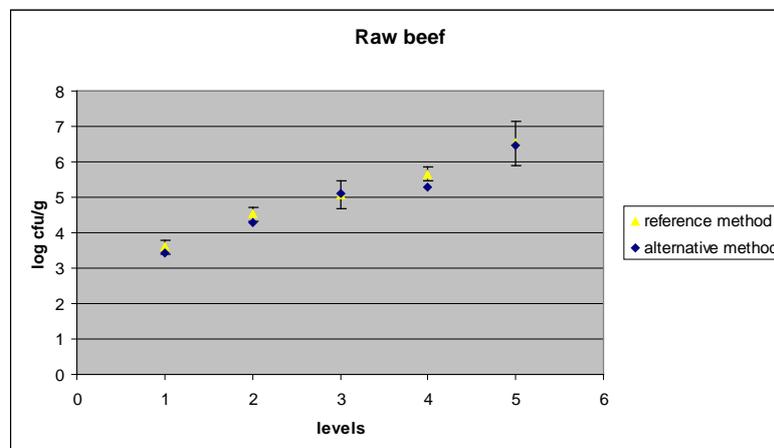
by the reference method: concentration range (mean): 2.12 – 4.87 log cfu/g
precision; standard deviation: 0.069- 0.83 log cfu/g

by Compact Dry ETB: concentration range (mean): 1.93 – 4.92 log cfu/g
precision; standard deviation: 0.14- 0.91log cfu/g

The precision is not satisfactory for the level 2.

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

Raw beef



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

by the reference method: concentration range (mean): 3.59 – 6.53 log cfu/g
precision; standard deviation: 0.093 – 0.31log cfu/g

by Compact Dry ETB: concentration range (mean): 3.42 – 6.46 log cfu/g
precision; standard deviation: 0.034 – 0.34 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. 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 clearly showed that the Compact Dry ETB is equivalent 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.

COLLABORATIVE STUDY:

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

The obtained results (log cfu/g) of the collaborative study:

Method	Level	Median	Repeatability sr	Reproducibility SR
ISO 21528-2	Control	0	-	-
	Low	2.57	0.040	0.12
	Middle	3.62	0.11	0.13
	High	4.58	0.069	0.069
ETB	Control	0	-	-
	Low	2.25	0.13	0.20
	Middle	3.49	0.097	0.19
	High	4.48	0.091	0.12

CONCLUSION:

According to the comparison and the collaborative 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*.