NordVal International / NMKL
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NordVal Certificate

Issued for:	HyServe Compact Dry EC Method for the Enumeration of *Escherichia coli* and coliforms
NordVal No:	036
First approval date:	1 December 2008
Renewal date:	13 November 2014
Valid until:	1 December 2016

HyServe Compact Dry EC

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 methods were:

- ISO 16649-2:2001: “Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.”
- ISO 4832:2006: “Microbiology of food 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 EC and the reference methods.

Date: 13 November 2014

Yours sincerely,

Sven Qvist
Chair of NordVal International

Hilde Skår Norli
NMKL Secretary General

PRINCIPLE OF THE METHOD:

HyServe Compact Dry EC is a ready-to-use dry chromogenic plate for enumeration of *E.coli*. An aliquot of 1 ml of an appropriate dilution is plated onto Compact Dry EC plate. The incubation conditions tested in the study were 37 ± 1°C for 24 ± 2h.

FIELD OF APPLICATION:

The method has been tested on enumeration of *Escherichia coli* and coliforms in foods.

COMPARISON STUDIES

COMPLIANCE BETWEEN COMPACT DRY EC METHOD AND THE REFENCE METHODS:

The comparison studies were 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 the respective levels. When the results obtained by the alternative method fall within the confidence level, there is no significant difference between the methods.

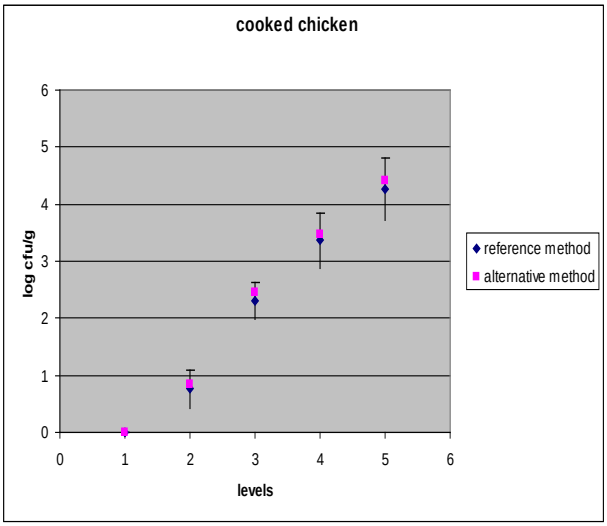
Cooked chicken

Four of the five levels were enumerated for *E.coli* and coliforms. The lowest level was not countable in neither of the methods.

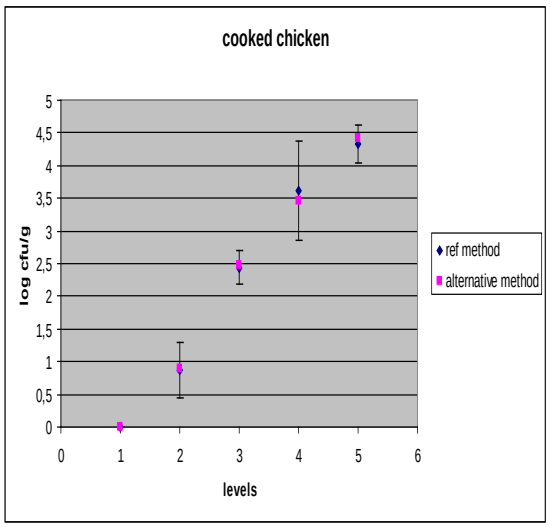
The following results were obtained:

	E.coli		**coliforms**	
Methods	**ISO 16649**	**Dry EC**	**ISO 4832**	**Dry EC**
concentration range (mean) cfu/g:	0.76 - 4.27	0.84 - 4.41	0.87 - 4.33	0.90 - 4.41
precision; standard deviation, cfu/g:	0.16 - 0.27	0.071 - 0.16	0.13 - 0.38	0.070 - 0.12
The lowest validated level with satisfactory precision (cfu/g):		0.8		0.9

Results E.coli:



Results coliforms:



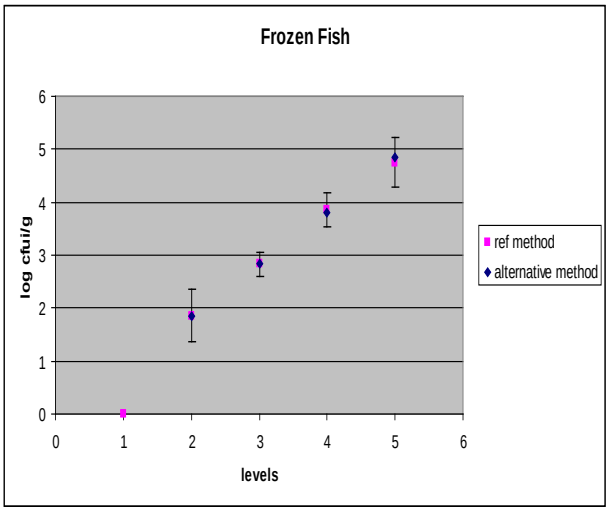
Frozen fish

Four of the five levels were enumerated for *E.coli*. The lowest level was not countable for *E.coli* in neither of the methods. All five levels were enumerated for coliforms.

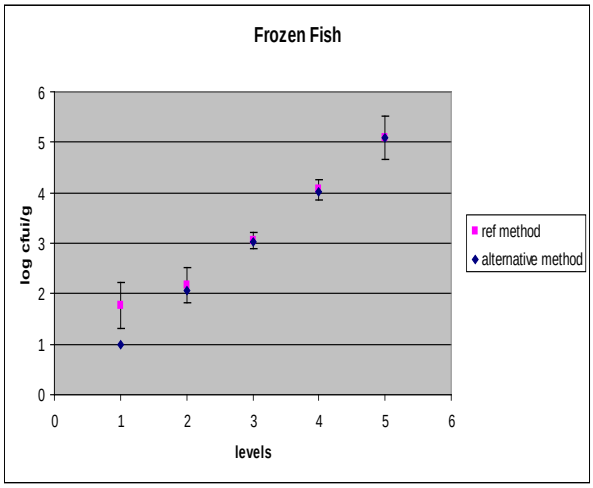
The following results were obtained:

	E.coli		**coliforms**	
Methods	**ISO 16649**	**Dry EC**	**ISO 4832**	**Dry EC**
concentration range (mean) cfu/g:	1.86 – 4.75	1.85 – 4.84	1.76 – 5.09	0.99 – 5.09
precision; standard deviation, cfu/g:	0.11 - 0.16	0.12 - 0.22	0.081 - 0.23	0.11 - 0.23
The lowest validated level with satisfactory precision (cfu/g):		1.9		1.0

Results E.coli:



Results coliforms:



Lettuce

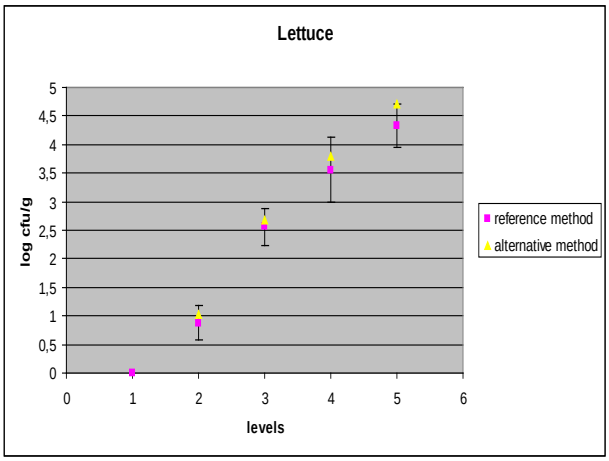
Four of the five levels were enumerated for *E.coli* and coliforms, respectively. The lowest level was not countable in neither of the methods.

The following results were obtained:

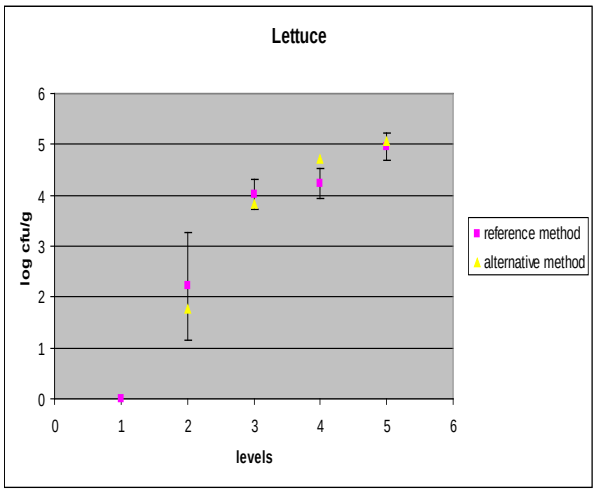
	E.coli		**coliforms**	
Methods	**ISO 16649**	**Dry EC**	**ISO 4832**	**Dry EC**
concentration range (mean) cfu/g:	0.88 – 4.34	1.03 – 4.71	2.22 – 4.95	1.78 – 5.06
precision; standard deviation, cfu/g:	0.15 - 0.28	0.29 - 0.31	0.13 - 0.55	0.094 - 0.65
The lowest validated level with satisfactory precision (cfu/g):		1.0		3.8*

* For level 2, the standard deviations were too high for both methods.

Results E.coli:



Results coliforms:



Milk powder

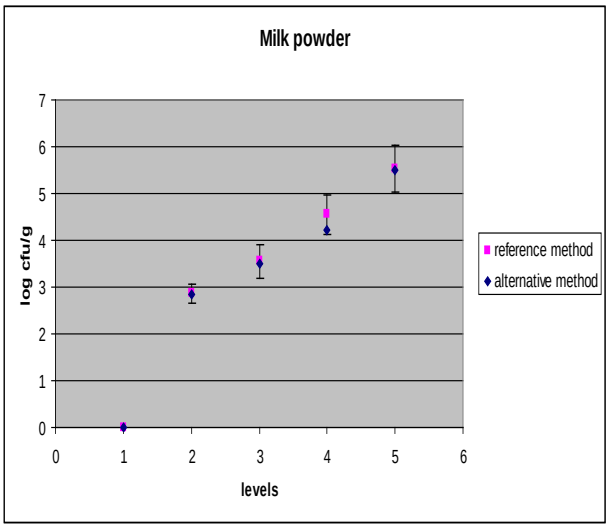
Four of the five levels were enumerated for *E.coli* and coliforms, respectively. The lowest level was not countable in neither of the methods.

The following results were obtained:

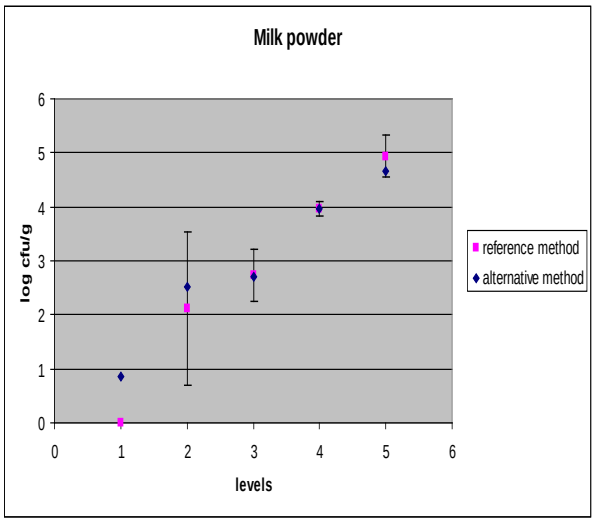
	E.coli		**coliforms**	
Methods	**ISO 16649**	**Dry EC**	**ISO 4832**	**Dry EC**
concentration range (mean) cfu/g:	2.86 – 5.52	2.84 - 5.49	2.12 – 4.94	0.85 – 4.65
precision; standard deviation, cfu/g:	0.10 - 0.25	0.076 - 0.28	0.066 - 0.71	0.078 - 0.61
The lowest validated level with satisfactory precision (cfu/g):		2.8		2.7 *

* For level 2, the standard deviations were too high for both methods.

Results E.coli:



Results coliforms:



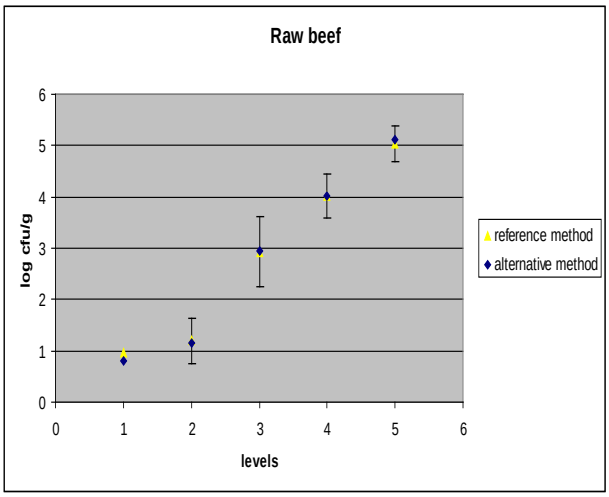
Raw beef:

Four of the five levels were enumerated for *E.coli.* The lowest level was not countable in neither of the methods. All the five levels were enumerated for coliforms.

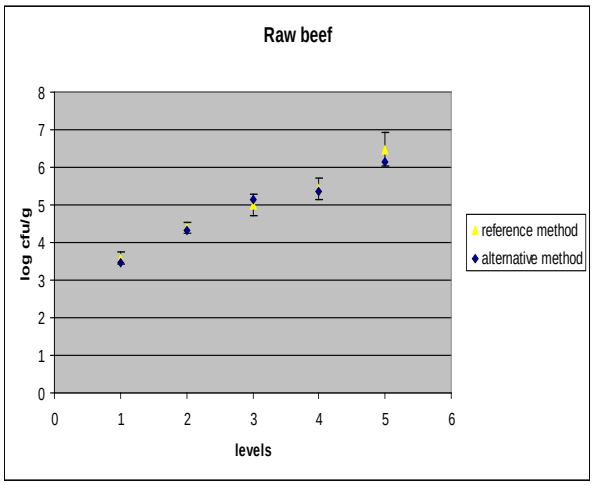
The following results were obtained:

	E.coli		**coliforms**	
Methods	**ISO 16649**	**Dry EC**	**ISO 4832**	**Dry EC**
concentration range (mean) cfu/g:	1.20 – 5.04	1.16 – 5.11	3,58 – 6,48	3.48 – 6.16
precision; standard deviation, cfu/g:	0.17 - 0.34	0.22 – 0.36	0.077 - 0.23	0.029 – 0.26
The lowest validated level with satisfactory precision (cfu/g):		1.2		3.5

Results E.coli:



Results coliforms:



SELECTIVITY (INCLUSIVITY/EXCLUSIVITY):

Inclusivity is the ability of an alternative method to detect the target analyte from a wide range of strains.

For E.coli: 31 strains (at 2-3 log cfu/ml) were studied. All 31 strains grew and produced typical colonies on the Compact Dry EC medium. By comparison, 5 strains failed to grow in the TBX medium (ISO16649-2:2001) and one strain yielded atypical colonies. This could be attributed to the composition of this medium or the sensitivity of these strains to the temperature of the molten medium, which was not investigated further.

For coliforms: 31 strains (at 2-3 log cfu/ml) were studied. All 32 strains grew and produced typical colonies in VRBGA (ISO 4832) and on Compact Dry EC medium.

Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

For E.coli: 21 strains (at 2-3 log cfu/ml) were studied. 18 of the 20 strains did not interfere. Two strains of *Shigella* did yield typical colonies by both methods which are not surprising, because strains of *Shigella* have E-glucuronidase activity which would give rise to typical conies with chromogenic media developed to show this activity.

For coliforms: 20 strains (at 2-3 log cfu/ml) were studied. 10 strains did not grow on the EC medium, 8 were atypical and 2 (both *Shigella sonnei*) appeared typical. By comparison, 7 stains failed to grow in VRBA (ISO 4832), 4 strains were atypical and 8 were typical in appearance.

CONCLUSION OF THE COMPARISON STUDIES:

For E.coli: The results of the method comparison study clearly showed that the Compact Dry EC is equivalent to the reference method ISO 16649-2:2001. The lowest validated level with satisfactory precision varies from 0.8 – 2.8 log cfu/g depending on the matrix.

For coliforms: The results of the method comparison study clearly showed that the Compact Dry EC is equivalent to the reference method ISO 4832:2006. The lowest validated level with satisfactory precision varies from 0.9 – 3.6 log cfu/g depending on the matrix.

COLLABORATIVE STUDY OF *E.COLI*:

The collaborative study was conducted in November 2007.

Number of laboratories: 9 [13 labs participated. 2 were excluded as the analysis were not performed on the agreed date and further 2 labs failed to test their samples for *E.coli* by the reference method.]

Samples: Pasteurised milk artificially contaminated with defined numbers of *E.coli.* The laboratories performed the analyses according to ISO 16649-2:2001 and Compact Dry EC method.

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

Method	Level	Median	Repeatability, sr	Reproducibility, SR
ISO 16649-2	Control	0	-	-
	Low	2,40	0,071	0,17
	Middle	3,50	0,11	0,17
	High	4,38	0,12	0,34
EC	Control	0	-	-
E.Coli	Low	2,45	0,065	0,18
	Middle	3,46	0,092	0,17
	High	4,50	0,081	0,20

CONCLUSION OF THE COLLABORATIVE STUDY OF *E.COLI*:

According to the comparison and the collaborative study no substantial differences were found between the Compact Dry EC method and the reference method (ISO 16649-2:2001) for the enumeration of *Escherichia coli*.

COLLABORATIVE STUDY OF COLIFORMS:

The collaborative study was conducted in November 2007.
Number of laboratories: 11
Samples: Pasteurised milk artificially contaminated with defined numbers of *E.coli.* The laboratories performed the analyses according to ISO 4832:2006 and Compact Dry EC method.

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

Method	Level	Median	Repeatability sr	Reproducibility SR
ISO 4832	Control	-	-	-
	Low	2.53	0.072	0.16
	Middle	3.59	0.088	0.11
	High	4.48	0.075	0.28
EC	Control	-	-	-
- coliforms	Low	2.55	0.076	0.13
	Middle	3.57	0.059	0.19
	High	4.59	0.077	0.15

CONCLUSION OF THE COLLABORATIVE STUDY OF COLIFORMS:

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