



NordVal 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:	13 November 2014
Valid until:	1 December 2016

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

fulfils the requirements of the NordVal validation protocol. The reference method was ISO 4833:2003: "Microbiology of foods and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony count techniques at 30°C".

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 TC and the ISO 4833:2003.

Date: 13 November 2014

Yours sincerely,

A handwritten signature in blue ink, appearing to read 'Sven Qvist'.

Sven Qvist
Chair of NordVal International

A handwritten signature in blue ink, appearing to read 'Hilde Skår Norli'.

Hilde Skår Norli
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.

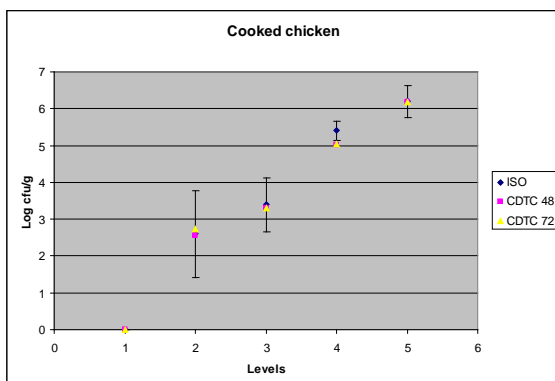
COMPARISON STUDY

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 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



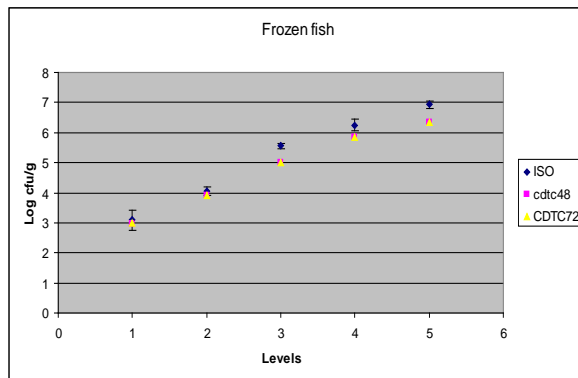
Four of the 5 levels were enumerated. (The lowest level was not countable in neither of the methods.) The following results were obtained:

by the reference method: concentration range (mean): 2.60 – 6.20 log cfu/g
precision; standard deviation: 0.13 - 0.59 log cfu/g

by Compact Dry TC: concentration range (mean): 2.55 - 6.18 log cfu/g
precision; standard deviation: 0.17- 0.44 log cfu/g

In this study, the precision was not satisfactory for level 2 for either of the methods. The lowest validated level with satisfactory precision: 3.9 log cfu/g.

Frozen fish



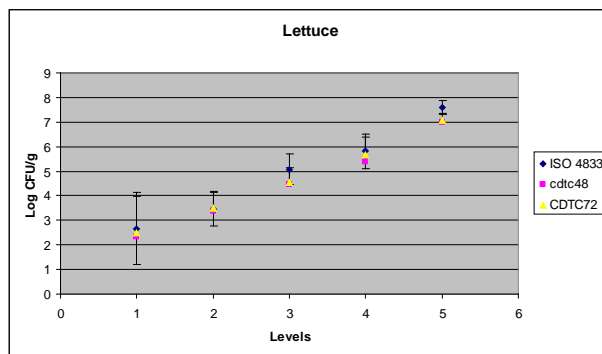
All five levels were enumerated. The following results were obtained:
 by the reference method: concentration range (mean): 3.09 - 6.93 log cfu/g
 precision; standard deviation: 0.046 - 0.17 log cfu/g

by Compact Dry TC: concentration range (mean): 2.96 - 6.35 log cfu/g
 precision; standard deviation: 0.048 - 0.18 log cfu/g

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

The lowest validated level with satisfactory precision: 3.0 log cfu/g.

Lettuce

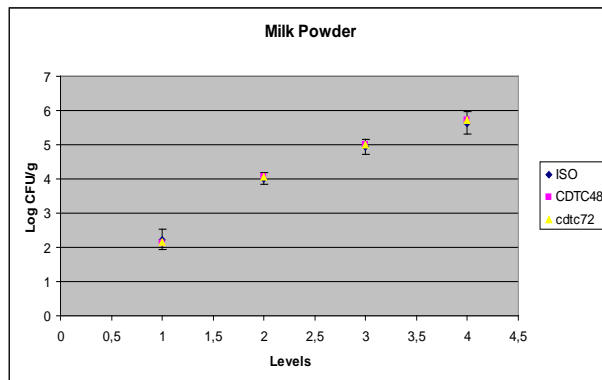


All five levels were enumerated. The following results were obtained:
 by the reference method: concentration range (mean): 2.66 – 7.61 log cfu/g
 precision; standard deviation: 0.14 - 0.73 log cfu/g

by Compact Dry TC: concentration range (mean): 2.34 - 6.99 log cfu/g
 precision; standard deviation: 0.13 - 0.70 log cfu/g

The precision is not satisfactory for the lowest level of either of the methods.
 The lowest validated level with satisfactory precision: 3.4 log cfu/g.

Milk powder

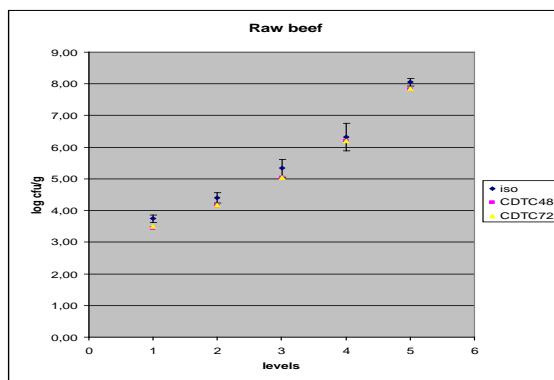


Four of the 5 levels were enumerated. The following results were obtained:
 by the reference method: concentration range (mean): 2.23 – 5.64 log cfu/g
 precision; standard deviation: 0.083 - 0.17 log cfu/g

by Compact Dry TC: concentration range (mean): 2.12 – 5.71 log cfu/g
 precision; standard deviation: 0.031 - 0.14 log cfu/g

The lowest validated level with satisfactory precision: 2.1 log cfu/g.

Raw beef



All 5 levels were enumerated. The following results were obtained:
 by the reference method: concentration range (mean): 3.74 – 8.05 log cfu/g
 precision; standard deviation: 0.057 - 0.22 log cfu/g

by Compact Dry TC: concentration range (mean): 3.44 – 7.83 log cfu/g
 precision; standard deviation: 0.038 - 0.27 log cfu/g

All the results on raw beef are higher for the ISO method than for the alternative method, however, the confidence intervals of these two methods are overlapping and hence there is no statistical significance.

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

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY):

Not relevant as this method is for total viable organisms.

CONCLUSION OF THE COMPARISON STUDY:

The results of the method comparison study clearly showed that the Compact Dry TC is equivalent 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.

COLLABORATIVE STUDY:

The collaborative 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.

Results (log cfu/g) of the collaborative 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

CONCLUSION:

According to the comparison and the collaborative 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.