

RESULTS OF A MICROVAL EN ISO 16140 VALIDATION OF THE COMPACT DRY TOTAL COUNT PLATE METHOD FOR THE ENUMERATION OF TOTAL VIABLE MICROORGANISMS IN FOODS

C.L.Baylis, R.A. Green, R. Limburn, K. Jewell and R.P. Betts

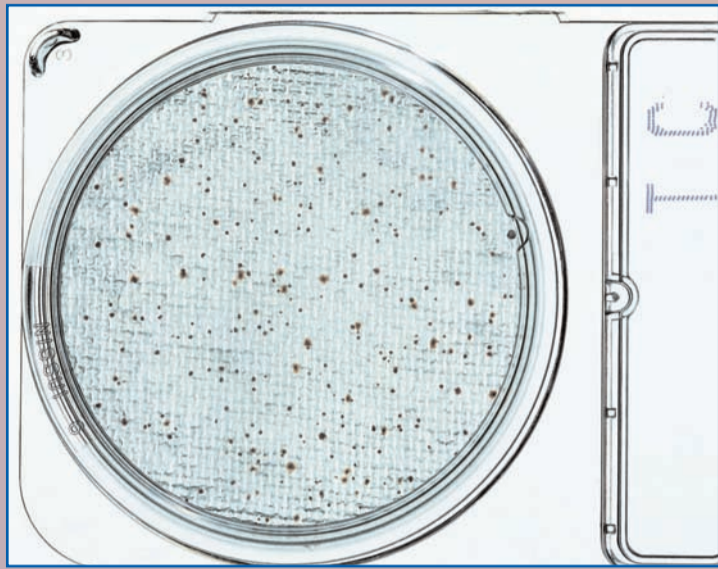


Fig 1: Compact Dry TC

Introduction

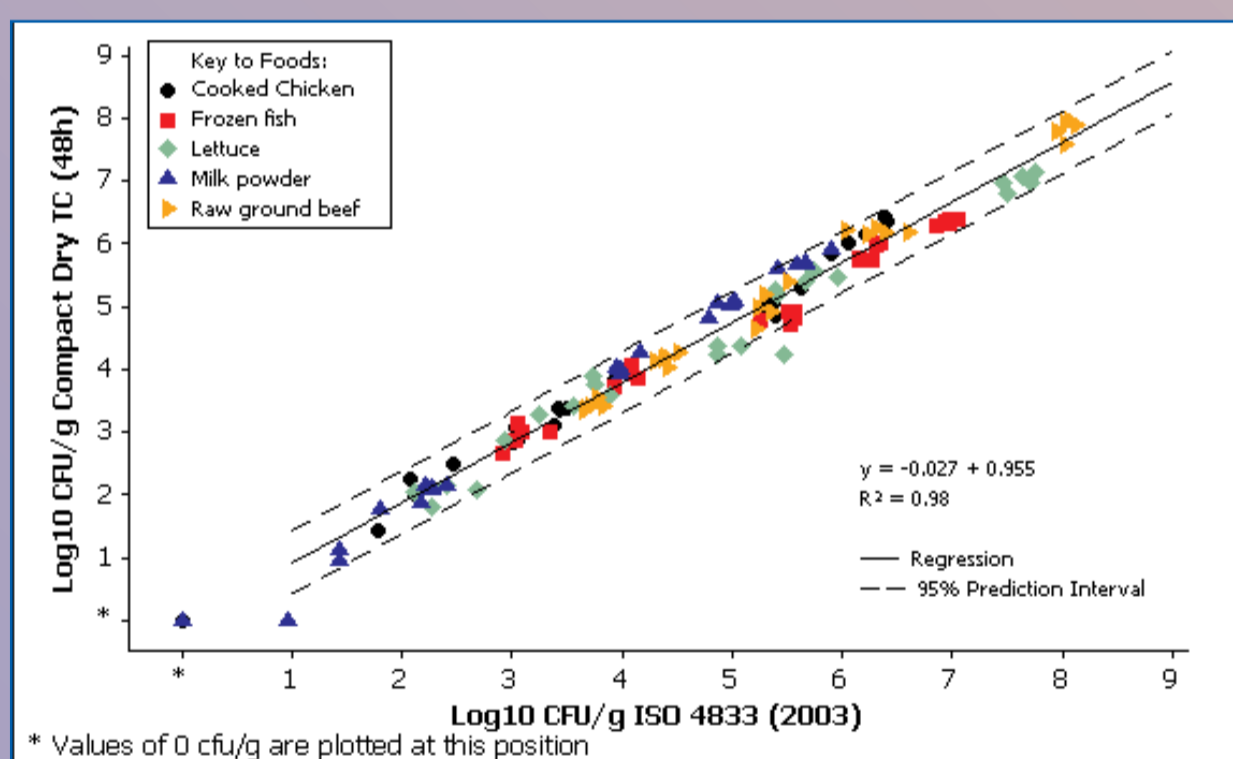
Compact Dry (Nissui Pharmaceutical Co. Ltd; supplied by Hyserve GmbH & Co. KG) are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent, rehydrated by inoculating 1 ml of diluted sample into the centre of the self-diffusible medium. The Compact Dry TC (Total Count) method contains the redox indicator

tetrazolium salt and is an alternative method to the standard plate count, enabling determination of aerobic colony counts in foods after 48h incubation (Fig 1). This MicroVal validation study compared the Compact Dry TC method against a standard method (EN ISO 4833; 2003) using validation protocol EN ISO 16140.

Methods

The Compact Dry TC method (30°C/48h) was performed in accordance with the manufacturer's instructions and ISO 4833 was a 1ml pour plate (30°C/72h). In addition, results from the Compact Dry TC tests were also recorded at 72h. The method comparison study compared methods for the enumeration of total aerobic microorganisms in naturally and artificially contaminated raw ground beef, cooked chicken, lettuce, milk powder and frozen fish. The interlaboratory study involved CCFRA and 13 laboratories from 5 countries (UK, Belgium, Netherlands, Poland, Switzerland), each testing artificially contaminated milk samples by both methods. Results were analysed using the principles of EN ISO 16140 and calculations/data transformations were performed with Minitab statistical analysis software (v14, Minitab Inc).

Fig 2: Plot showing global assessment (all food types) of results (log₁₀ cfu/ml) of Compact Dry TC test after 48h against ISO 4833 (2003)



Results

The method comparison results clearly showed the Compact Dry TC method to be equivalent to the reference method for a range of foods with a calculated correlation coefficient (R^2) of 0.98 (Fig 2). There appeared to be no significant difference between the results obtained with the method after 48h and 72h ($R^2=1.00$). The statistical results and line equations for the Compact Dry TC test are shown in Table 1 and Table 2, respectively. Results from the interlaboratory study (analysing 13 data sets) revealed no evidence of differences in reproducibility between the methods (Table 3).

Conclusions

The Compact Dry TC plate method is an easy-to-use alternative to the conventional culture method. This validation study found no substantial differences between the Compact Dry TC plate method and the reference method ISO 4833 (2003) for the enumeration of total viable microorganisms at 30°C. This method was granted MicroVal Certification following this validation against EN ISO 16140 in March 2007.

Table 1 - Summary of results obtained using ordinary least-squares linear regression (OLS) for Compact Dry TC test after 48h against ISO 4833 (2003)

Food category	EN 16140 reference	Correlation coefficient (r)	Intercept (a)	Slope (b)	Residual standard deviation (Sy:x)	Standard deviation of intercept (Sa)	P{a=0}	Standard deviation of slope b (Sb)	P{b=1}
All foods		R4.2	R 4.3	R 4.3	R 4.4	R4.5	R 4.5	R 4.6	R 4.6
Meat products		97.7%	-0.027	0.955	0.247	0.069	0.694	0.014	<0.001
Poultry products		99.1%	-0.370	1.025	0.158	0.120	0.005	0.021	<0.001
Fish and seafood products		98.8%	-0.067	0.980	0.179	0.114	0.562	0.025	<0.001
Fruit and vegetable based products		98.4%	0.255	0.875	0.168	0.126	0.056	0.024	<0.001
Dairy products		97.4%	0.039	0.914	0.281	0.166	0.818	0.032	<0.001
		99.6%	-0.371	1.086	0.103	0.060	<0.001	0.014	<0.001

Table 2 - Summary of linearity results for Compact Dry TC test after 48h against ISO 4833 (2003)

Food category	Food product	Regression line	R ²
Meat products	Raw ground beef	y = -0.370 + 1.03 x	0.99
Poultry products	Cooked chicken	y = -0.067 + 0.980 x	0.99
Fish and seafood products	Frozen fish	y = 0.225 + 0.875 x	0.98
Fruit and vegetable based products	Lettuce	y = 0.039 + 0.914 x	0.97
Dairy products	Milk powder	y = -0.371 + 1.09 x	1.00
All foods	NA	y = -0.027 + 0.955 x	0.98

y = log (N alternative method) x = log (N reference method)

Table 3 - Method reproducibility for ISO 4833 method and Compact Dry CT plate method after 48h incubation (CDTC48)

Method	Level	R	RSD _R (%)	F	p(F)	1/F	p(1/F)
ISO 4833	Control	0.000	*				
	Lower	0.190	2.425				
	Middle	0.266	2.479				
	Upper	0.268	1.972				
CDTC48	Control	0.000	*				
	Lower	0.284	3.755	2.252	0.087	0.444	0.913
	Middle	0.268	2.588	1.015	0.490	0.985	0.510
	Upper	0.271	2.016	1.024	0.484	0.977	0.516

* RSD_R is undefined because value is zero

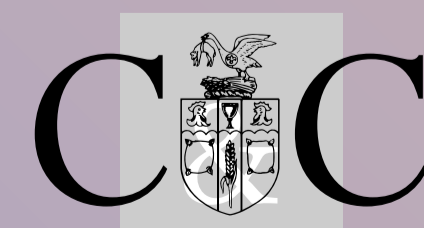
+ Control level in cfu/ml, other levels in log₁₀ cfu/ml

Key R Reproducibility limit
RSD_R Relative standard deviation of reproducibility
F Ratio^R between reproducibility of alternative and reference methods

References

EN ISO 4833 (2003) Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30°C. International Organization for Standardization, Geneva.

EN ISO 16140 (2003) Microbiology of food and animal feeding stuffs - protocol for the validation of alternative methods. International Organization for Standardization, Geneva.



Campden & Chorleywood Food
Research Association Group

Chipping Campden, Gloucestershire, GL55 6LD, U.K.
Tel: +44 (0)1386 842000 Fax: +44 (0)1386 842100
www.campden.co.uk