NordVal / NMKL
c/o National Veterinary Institute
PB 750 Sentrum, 0106 Oslo, Norway
www.nmkl.org

NordVal Certificate

Issued for:	3M™Petrifilm™ Aerobic Count Plate
NordVal No:	012
First approval date:	5 May 2003
Renewal date:	1 June 2011
Valid until:	1 June 2013

3M™Petrifilm™ Aerobic Count Plate

Manufactured by:
3M Health Care,
Microbiology Products,
St. Paul,
Minnesota 551144-1000
USA

Supplied by:
3M MEDICA,
Hammfelddamm 11,
D-41453 Neuss,
Germany

fulfils the requirements of the NordVal validation protocol. 3M™Petrifilm™ Aerobic Count Plate has been validated by Afnor according to ISO 16140 against the reference method ISO 4833, 2003: Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony count technique at 30 degrees C. The results document no statistical difference in the performances between the methods.

Date: 01.06.2011

Yours sincerely

Sven Qvist
Chair of NordVal

Hilde Skår Norli
NMKL Secretary General

PRINCIPLE OF THE METHOD

The 3M Petrifilm Aerobic Count Plate is a sample-ready-culture-medium system which contains Standard Methods nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration.

Undiluted or diluted samples are added to plates at a rate of 1 mL/plate. Sample is spread over a surface of 20 cm^2. Gelling agent is allowed to solidify, plates are incubated, and colonies are counted.

On Petrifilm Aerobic Count Plates, aerobic bacteria colonies will appear as red colonies.

FIELD OF APPLICATION

The method has been tested on foods.

COMPARISON STUDY
COMPLIANCE BETWEEN 3M PETRIFILM AEROBIC COUNT PLATE AND THE REFERENCE METHOD:

Comparison studies have been performed twice, the latest one in 2007, for extension to a 48 hours incubation in addition to the 72 hours incubation test. The following results were obtained:

Relative accuracy

48 hours test
66 naturally contaminated samples, belonging to the following major food categories, were analyzed in duplicates with each of the two methods.
As an indication, the contamination ranges were as follows:

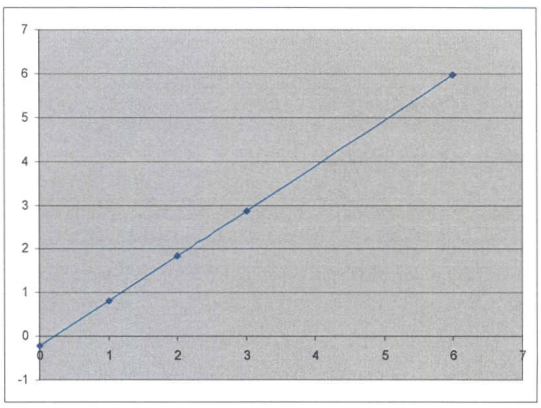
Food category	Contamination range (in log cfu/g)
Meat products	3.45 to 7.86
Egg products and Pastries	1.3 to 6.79
Vegetables	1.6 to 7.26
Fishes and Sea-Foods	2.90 to 7.42

The equation of the regression line between the alternative method and the reference method, for all categories combined, is as follows:

$y = -0.214 + 1.030x$

y = log cfu/g (alternative method)
x = log cfu/g (reference method)

The regression coefficient is close to 1.000. The intersection of -0.214 indicates that the reference method obtains about 0.2 log cfu/g more bacteria than the alternative method, however, that would be considered to be within acceptable level.



72 hours incubation

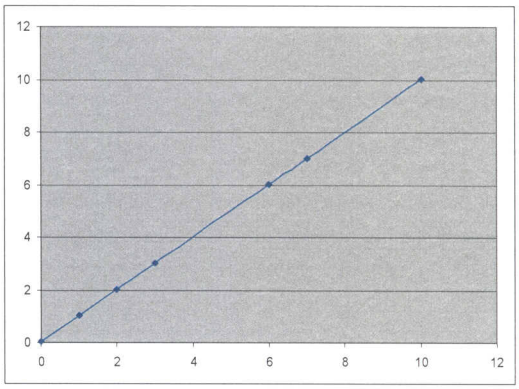
219 naturally contaminated samples, belonging to the following major food categories, were analyzed in duplicates with each of the two methods.
As an indication, the contamination ranges were as follows:

Food category	Contamination range (in log cfu/g)
Meat	From 2 to 7.85 log UFC/g
Dairy	3.74 to 5.81
Vegetables	1.95 to 10.48
Fish & Seafood	3.73 to 8.12
Egg	1.45 to 5.30

The equation of the regression line between the alternative method and the reference method, for all categories combined, is as follows:

y = 0.029 + 0.999x
x = log cfu/g (reference method)
y = log cfu/g (alternative method)

The regression coefficient is close to 1.000 and the point of intersection is close to zero, i.e. there are no significant difference between the results obtained by the alternative method and the reference method.



Precision

The repeatability, the closeness of agreement between successive and independent results obtained by the same method on identical test material under the same conditions, is estimated for the 42 hours and 72 hours incubations for the 3M method and the reference method.

	Alternative method Repeatability limit (log cfu/g)	Reference method Repeatability limit (log cfu/g)
42 hours incubation	0,16	0,12
72 hours incubation	0,15	0,18

The repeatability limit is the value less than or equal to which the absolute difference between two tests results obtained under repeatability conditions is expected to be with a probability of 95%.

Specificity

20 strains among the most common species (strictly aerobic and anaerobic, lactic acid bacteria) were inoculated in duplicate on Petrifilm Aerobic Count Plate and on PCA agar (poured plate). Most strains grow on both the Petrifilm Aerobic Count Plate and on the PCA agar, the deviations were as follows:

- √ Two strains (*Shewanella putrefaciens* and *Photobacterium phosphoreum*) grow on Petrifilm but not on PCA.
- √ One *Micrococcus luteus* strain grow on PCA but not on Petrifilm.
- √ One *Lactobacillus casei rhamnosus* grow giving counts on Petrifilm one log lower

than on PCA.

COLLABORATIVE STUDY

The collaborative study was conducted in 2001.
Number of laboratories: 15
Matrix: Raw milk samples naturally contaminated, diluted in a UHT milk in order to obtain 4 levels of contamination.

The laboratories analysed duplicates for each spiking level.
The following results in were obtained:

Level of contamination log cfu/g	Number of samples	**Reference method**		**Alternative method**	
		Repeatability r log cfu/g	Reproducibility R log cfu/g	Repeatability r log cfu/g	Reproducibility R log cfu/g
3 - 4	30	0.13	0.77	0.16	0.57
4 – 5	30	0.19	0.83	0.31	0.85
5 – 6	30	0.32	0.61	0.24	0.53
6 - 7	30	0.26	0.71	0.22	0.48

The repeatability limit is the value less than or equal to which the absolute difference between two tests results obtained under repeatability conditions is expected to be with a probability of 95%.

The reproducibility is the closeness of agreement between single test results on identical test material using the same method and obtained by operators in different laboratories using different equipment.
The reproducibility limit is the value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions is expected to be with a probability of 95%.

CONCLUSION:

According to the comparison and the collaborative study no statistical differences were found between the 3M™Petrifilm™ Aerobic Count Plate and the reference method ISO 4833 for the enumeration of aerobic microorganisms.