



**Alternative methods for agribusiness
Analytical performances certified**

**VALIDATION CERTIFICATE FOR ALTERNATIVE ANALYTICAL METHOD
ACCORDING TO STANDARD EN ISO 16140: 2003**

Certificate No.: 3M 01/1 – 09/89

Validation date :	09.29.1989
Renewal dates :	09.06.1993 09.10.1997 12.13.2001 06.14.2005*
	07.03.2009
Extension date :	09.27.2007
End of validity :	09.10.2013

** EN ISO 16140 protocol was used in 2005 during the fourth renewal study*

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is hereby authorized to refer to this **AFNOR Validation certificate** for the following alternative **quantitative** analysis method :

3M™ PETRIFILM™ Aerobic Count Plate

Protocol reference : **34-8703-7875-8**

SCOPE

1. All human food products (reading after 72 hours).
2. All human food products, except dairy products and raw shellfish (reading after 48 hours)

RESTRICTIONS FOR USE

Some strains (such as some lactic acid bacteria or some micrococci) may not be detected on Petrifilm AC plates.

REFERENCE METHOD

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

**Deputy General Manager
Jacques BESLIN**

PRINCIPLE OF THE METHOD

The 3M™ PETRIFILM™ Aerobic Count (AC) Plate is a sample-ready culture medium system which contains standard method nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration.

NOTE (Validation history)

1/ **2005 renewal Study** : Since the last renewal (2001), the reference method has changed, involving now the use of PCA media supplemented with milk, for dairy products analysis. In addition, the validation protocol EN ISO 16140 was used for this renewal study.

- Comparative study has been realized following the protocol EN ISO 16140 (new linearity study and 1997 accuracy data re-investigated)
- Previous data has been kept for specificity and practicability study
- Concerning inter-laboratory study, 2001 previous data has been kept and re-investigated following EN ISO 16140.

2/ **The extension study of 2007**, whose results are presented in this certificate, permitted to validate a protocol in 48 hours of incubation for the analysis of human food products, except dairy products and raw shellfish products.

3/ **In July 2009**, 3M™ PETRIFILM™ Aerobic Count Plate AFNOR Validation certification was renewed without any additional test, since (i) the alternative method was not modified (ii) the reference method and the EN ISO 16140 standard remain unchanged.

LINEARITY AND relative ACCURACY

Comparison of performances of the alternative method and the reference method

Linearity study :

After a 72 hours incubation time, tests were performed in 2005 on the 5 food product/strain combinations and for the food categories given in the table below.

The samples were analyzed **in duplicate** with each of the **two methods**, at the five following artificial contamination levels :

- 10, 50, 100, 500, 1 000 UFC/g for "dairy products"
- 100, 500, 1 000, 5 000, 10 000 UFC/g for others products.

The following results were obtained :

Food category	Food product/strain pair	Regression line
Meat	Pork "Pâté" / <i>Enterobacter agglomerans</i>	$Y = 1.20 X - 0.67$
Dairy	Milk / <i>Staphylococcus aureus</i>	$Y = 1.03 X - 0.09$
Vegetables	Green beans / <i>Xanthomonas maltophilia</i>	$Y = 1.03 X - 0.15$
Fish & Seafood	Salmon / <i>Listeria seeligeri</i>	$X = 1.05 Y - 0.21$
Others	Whole liquid egg / <i>Serratia liquefaciens</i>	$Y = 1.00 X + 0.02$

$y = \log(N \text{ alternative method})$

$x = \log(N \text{ reference method})$

For the 2005 renewal study, the reading of the 3M™ Petrifilm™ count plate tests were realized after 48 hours and 72 hours incubation time. For the extension study, the results obtained after 48 hours incubation were interpreted.

Tests were performed on the 4 food product/strain pairs and for the food categories given in the table below.

The samples were analyzed **in duplicate** with each of the **two methods**, at the five following artificial contamination levels :

- 100, 500, 1 000, 5 000, 10 000 UFC/g

The following results were obtained :

Food category	Food product/strain pair	Regression line
Meat	Pork "Pâté" / <i>Enterobacter agglomerans</i>	$Y = 1.20 X - 0.70$
Fish & Seafood	Salmon / <i>Listeria seeligeri</i>	$X = 1.05 Y - 0.20$
Vegetables	Green beans / <i>Xanthomonas maltophilia</i>	$Y = 1.03 X - 0.15$
Others	Whole liquid egg / <i>Serratia liquefaciens</i>	$Y = 1.04 X - 0.14$

$y = \log(N \text{ alternative method})$

$x = \log(N \text{ reference method})$

Accuracy study :

After 72 hours incubation time, tests were performed in 1992 on 73 vegetable products and 59 meat products.

Tests were performed in 1998 on 21 meat products, 16 fish products, 13 egg and pastries and 11 vegetable products.

In Addition tests were performed in 2005 on 3 fish products and 3 egg products.

Comparative study on 20 dairy products was based on external results obtained by Pitton and Grappin in 1991 (J. Assoc. Ana. Chem., 74, 92-103) with a protocol based on the FIL 100B which is equivalent to the ISO 4833 for dairy products.

The statistical interpretation was conducted in total on 219 results, obtained from naturally contaminated samples, belonging to the following major food categories : Meat products, Dairy products, Vegetable, fish and seafood products, miscellaneous (egg products and pastry).

The samples were analyzed **in duplicate** with both **methods**.

As an indication, the contamination (concentration) ranges were as follows :

Food category	Contamination range* (in log CFU/g)
Meat	From 2 to 7.855
Dairy	From 3.74 to 5.81
Vegetables	From 1.95 to 10.48
Fish & Seafood	From 3.73 to 8.12
Egg	From 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 :

$$Y = 0.999 X + 0.029$$

$y = \log(N \text{ alternative method})$

$x = \log(N \text{ reference method})$

The repeatability for both methods and the bias between the two methods were determined according to the method of calculation used for the interlaboratory study (see sections 6.3.5 and 6.3.6 of the standard EN ISO 16140). These results provide additional information for the accuracy criterion.

The limits of repeatability (in log) obtained for the alternative method and the reference method are as follows :

Alternative method	Reference method
r = 0.147	r = 0.176

The bias (in log) between the two methods (alternative method - reference method) is as follows:

P = 0.026 if we take the median, or
D = 0.026 if we take the individual average bias.

After 48 hours incubation time, tests were performed in 2007. The statistical interpretation was conducted on 66 results, from naturally contaminated samples, belonging to the following major food categories : Meat products, egg products and pastry, vegetable and fish and seafood products.

The samples were analyzed **in duplicate** with both **methods**.

The contamination (concentration) ranges were the following :

Food category	Contamination range* (in log CFU/g)
Meat	From 3.45 to 7.86
Egg and pastry	From 1.30 to 6.79
Vegetables	From 1.60 to 7.26
Fish & Seafood	From 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 :

$$Y = 1.030 X - 0.242$$

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

The repeatability for both methods and the bias between the two methods were determined according to the method of calculation used for the interlaboratory study (see sections 6.3.5 and 6.3.6 of the standard EN ISO 16140). These results provide additional information for the accuracy criterion.

The limits of repeatability (in log) obtained for the alternative method and the reference method are as follows :

Alternative method	Reference method
r = 0.161	r = 0.117

The bias (in log) between the two methods (alternative method - reference method) is as follows:

P = 0.005 if we take the median

Conclusion for linearity and relative accuracy y:

After 72 hours and after 48 hours incubation time, linearity and accuracy studies show that results obtained with alternative method are comparable to the ones obtained with reference method, with a better recovery of the alternative method.

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY) – 1997 study

Use of alternative method only

20 strains among the most common species (strictly aerobic and anaerobic, lactics ...) were inoculated in duplicate on Petrifilm and on PCA agar (inclusion) :

- 2 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.

Conclusion :

3M Petrifilm Aerobic count plate specificity is satisfactory.

PRACTICABILITY(1997 study)

Use of alternative method only

- **Time for obtaining result**
 - **Positive** results are obtained with the alternative method in 72 hours as for the reference method.
 - **Negative** results are obtained in 48 hours with the alternative method as opposed to 72 hours with the reference method.
- **Operator training time** : 0.5 days.
- **Other criteria** :
 - Workflow study : Flexibility of the technique whatever the number of samples to analyse, and organisation facilities in a routine lab.
 - Space and time saving, particularly at the media preparation step.
 - Operator qualification : Same technical qualification as the reference method
 - Common steps with the reference method : Blending and dilution

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2001 with 15 participating laboratories. The analysis were carried out on raw milk samples naturally contaminated.

Raw milk was diluted in a UHT milk in order to obtain 4 levels of contamination :

- 3 to 4 log CFU/g
- 4 to 5 log CFU/g
- 5 to 6 log CFU/g
- 6 to 7 log CFU/g

The laboratories tested, using each of the **two methods, two replicates per contamination level**.

The following results were obtained :

Level of contamination	Number of samples	Reference method		Alternative method		
		Repetability r	Reproducibility R	Repetability r	Reproducibility R	Bias
Level 1	30	0.132	0.765	0.161	0.571	0.23
Level 2	30	0.191	0.829	0.308	0.847	0.37
Level 3	30	0.323	0.612	0.235	0.528	0.44
Level 4	30	0.264	0.710	0.220	0.477	0.27

Conclusion

The inter-laboratory study show that results obtained with alternative method are comparable to the one obtained with reference method with a bias in favour of the alternative method.

Please send any queries concerning the performance of the validated method to
AFNOR Certification.

You may download a summary document on the preliminary and inter-laboratory
studies on www.afnor-validation.com