



**Alternative methods for agribusiness  
Analytical performances certified**

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

**Certificate No.: 3M-01/6 - 09/97**

**Validation date :** 09/10/1997  
**Renewal dates :** 12/13/2001  
 06/14/2005\*  
 07/03/2009  
**End of validity :** 09/10/2013

*\* EN ISO 16140 protocol was used in 2005 during the 2<sup>nd</sup> renewal study*

**Company**           **3M Health Care**  
 (headquarter)   Microbiology products  
 2501 Hudson Road  
 Building 275 5W 05  
 MN 55144 – IWO – St Paul – USA

**Distributor**   **Laboratoires 3M Santé**  
 Département Microbiologie  
 Boulevard de l'Oise  
 95029 Cergy-Pontoise Cedex  
 France

**Production site**   **3M Health Care**  
 P.O. Box 227 – South Dakota, 57006 – Brookings – USA

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

**3M™ PETRIFILM™ Enterobacteriaceae Count Plate**

Protocol reference : **34-8703-7876-6**

**SCOPE**

All human food products.

**RESTRICTIONS FOR USE**

In the scope of AFNOR validation mark, numeration must be done on plates containing maximum 100 colonies.

**REFERENCE METHOD**

**ISO 21528 (2004)** : Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of *Enterobacteriaceae*.

**Deputy General Manager  
Jacques BESLIN**

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**AFNOR Certification**

11, rue Francis de Pressensé – 93571 La Plaine Saint-Denis Cedex - France  
 Phone +33 (0)1 41 62 80 00 – Fax +33 (0)1 49 17 90 00  
[certification@afnor.com](mailto:certification@afnor.com) - [www.afnor-validation.com](http://www.afnor-validation.com)

## PRINCIPLE OF THE METHOD

The 3M™ Petrifilm™ Enterobacteriaceae Count (EB) Plate is a sample-ready culture medium system which contains modified Violet Red Bile Glucose (VRBG) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. *Enterobacteriaceae* which are Glucose fermenting bacteria, on Petrifilm EB plates will appear as red colonies with yellow zones and/or red colonies with gas bubbles with or without yellow zones.

This validation certificate has been delivered for a 24h +/- 2h plate incubation time. This AFNOR validation is granted for a method use at 30°C, 35°C and 37°C although the validation study has been run only at 37°C.

### NOTE (Validation history)

**1/ 2005 renewal study** : Since the last renewal in 2001, reference method has changed and protocol EN ISO 16140 has been put in place.

- 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 collaborative study, 2001 previous data has been kept and re-investigated following EN ISO 16140.

**2/ In July 2009**, 3M™ Petrifilm™ Enterobacteriaceae Count (EB) Plate AFNOR Validation certification was renewed without any additional test, since 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:

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.

The following results were obtained :

Food category	Food product/strain pair	Regression line
Meat	Pork "Pâté" / <i>Enterobacter agglomerans</i>	$Y = 0.96 X + 0.16$
Dairy	Milk / <i>Hafnia alvei</i>	$Y = 0.87 X + 0.31$
Vegetables	Green beans / <i>Citrobacter freundii</i>	$X = 1.01 Y - 0.04$
Fish & Seafood	Salmon / <i>Klebsiella oxytoca</i>	$Y = 1.04 X - 0.23$
Others	Whole liquid egg / <i>Serratia liquefaciens</i>	$Y = 1.03 X - 0.14$

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

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

### Accuracy study :

Tests were performed in 1997. The statistical interpretation was conducted on 86 results (all from naturally contaminated samples), belonging to the following major food categories : Meat, Dairy, Vegetable, Fish, Others.

The samples were analyzed **in duplicate** with each of the **two methods**.

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

Food category	Contamination range* (in log CFU/g)
Meat	From 1.50 to 5.82
Dairy	From 1.15 to 7.91
Vegetables	From 1.57 to 4.80
Fish & Seafood	From 1.00 to 5.39
Others	From 1.00 to 5.00

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

$$Y = 0.961 X + 0.412$$

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 = 0.250	r = 0.308

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

**P = 0.230** if we take the median, or

**D = 0.288** if we take the individual average bias.

#### **Conclusion for linearity and relative accuracy :**

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 *Enterobacteriaceae* by the alternative method.

### **SELECTIVITY (INCLUSIVITY/EXCLUSIVITY) – 1997 study**

#### **Use of alternative method only**

- A first study was realised with 64 targeted strains and 45 non targeted strains, by comparing the alternative and the ISO 7402 reference methods . All the results were in agreement between both methods. Note that two *Yersinia pseudotuberculosis* strains were not able to growth with both methods, i.e. PEB, and VRBG.
- The study was completed in 1997 :
  - 22 *Enterobacteriaceae* strains were detected out of 23 tested strains. The strain not detected by Petrifilm is a strain of *Erwinia carotovora* CIP 103762, which was not detected by the reference method NF ISO 7402 : 1993. (Another *Erwinia carotovora* strain was detected by Petrifilm and not by the reference method NF ISO 7402 : 1993).
  - On 13 non *Enterobacteriaceae* strains, 2 strains of *Aeromonas* and one strain of *Xanthomonas* developed and produced characteristic colonies on Petrifilm and VRBG.

## PRACTICABILITY (1997study)

### Use of alternative method only

- **Time for obtaining result** : Positive and negative results are obtained in 24 hours with the alternative method, contrary to the reference method , which required 72 hours
- **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
  - Main advantages : Time saving, space saving during incubation, easy to use, waste treatment reduction

## INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2001 with 15 participating laboratories. The analyses were carried out on samples of pasteurised semi skimmed milk, artificially contaminated with a strain of *E.coli* at 4 following levels of contamination : 0,  $10^2$  to  $10^3$ ,  $10^3$  to  $10^4$  and  $10^4$  to  $10^5$  UFC/g.

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

The following results were obtained :

Contamination level	Number of samples taken into account*	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
$10^2 - 10^3$	28	0.205	0.411	0.294	0.294	-0.06
$10^3 - 10^4$	28	0.117	0.393	0.325	0.353	0.04
$10^4 - 10^5$	28	0.176	0.325	0.235	0.329	0.03

\* Late sample reception of one laboratory not allowed us to take in consideration his results

### Conclusion

The inter-laboratory study shows that results obtained with alternative method are comparable to the one obtained with reference 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](http://www.afnor-validation.com)