



**Alternative methods for agribusiness  
Analytical performances certified**

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

**Certificate N°: 3M 01/5 – 03/97 B**

**Validation date: 18.03.1997**  
**1<sup>st</sup> Renewal date\*: 13.12.2001**  
**2<sup>nd</sup> Renewal date\*: 19.09.2005**  
**3<sup>rd</sup> Renewal date : 28.11.2008**  
**End of validity : 18.03.2013**

*\* The EN ISO 16140 protocol has been implemented for the 2<sup>nd</sup> renewal in 2006*

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

**3M™ Petrifilm™ Rapid Coliform Count Plate**  
 Reading after 24 hours : gas and non gas producing colonies

Protocol reference : 34-8702-8710-8

**SCOPE**

All human food products.

**RESTRICTIONS OF USE**

Insufficient dilution.

**REFERENCE METHOD**

**NF EN ISO 4832 (2006)** – Horizontal method for the enumeration of coliforms – Colony-count technique (VRBL).

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## PRINCIPLE OF THE METHOD

3M™ Petrifilm™ Rapid Coliform Count plate consists of a medium for coliforms containing a pH indicator which allows one to detect acid production, and a tetrazolium red indicator which facilitates the count.

During metabolism, coliforms produce acid and / or gas from lactose. While colonies grow and produce acid, pH indicator turns from orange-red to yellow. Acidification zone may appear before the colonies. Gas is trapped around coliform colonies thus allows detecting gas producing coliforms. The reading after 24 hours of all coliforms is done by counting all red colonies with or without gas.

## HISTORY OF THE VALIDATION

**NOTE 1 :** The protocol described in EN ISO 16140 standard has been conducted during 2005 renewal. Some results obtained at the previous studies have been maintained for a further use. Here they are :

- Relative accuracy : results obtained in 1997 from the expert laboratory and results obtained in 1996 from an external laboratory (taken into account within the AFNOR validation)
- Practicability : 1997 data
- Selectivity : 1997 data
- Inter-laboratory study : re-use of results obtained in 1997 on milk food type.

The validation concerned the 2 following protocols :

- Processed pork products and sea-foods (incubation at 30°C)
- Other food products (incubation at 35°C)

**NOTE 2 :** In November 2008, The validation of 3M™ Petrifilm™ Rapid Coliform Count Plate was renewed. As the method has not been modified since previous validation, no additional test was performed.

## LINEARITY and RELATIVE ACCURACY

### Comparison of performances of the alternative method and the reference method

#### Linearity study (1996/97 and 2005 studies) :

Tests were performed in 1996 on 3 food categories (dairy products, vegetables, ready meals) artificially contaminated with 3 strains (*Escherichia coli*, *Enterobacter cloacae*, *Klebsiella oxytoca*) ; 4 contamination levels were tested : 0, 100 to 1 000, 1 000 to 10 000, 10 000 to 100 000 CFU/g.

Samples were analysed in duplicate with alternative method and with no duplication with reference method ; 5 artificial contamination levels were tested : 100, 500, 1 000, 5 000, 10 000 CFU/g.

The statistical interpretation gives following results :

Food category	Regression line
Dairy products	$Y = 0.96X + 0.19$
Ready meals	$Y = 0.98X + 0.11$
Vegetables	$Y = 0.95X + 0.19$

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

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

A complementary study was conducted in 2005 on the 3 combinations food samples/strains as described in the table hereunder. The processed pork products and sea-foods products categories were separately tested in order to apply the related specific protocol (incubation at 30°C).

The samples were analysed **in duplicate** with **both methods**, 5 artificial contamination levels were tested : 100, 500, 1 000, 5 000, 10 000 CFU/g.

The following results were obtained :

Food categories	Food samples/strains	Regression line
Processed pork products	Cooked ham / <i>Enterobacter cloacae</i>	$Y = 1.03X - 0.24$
Meat products	Raw ground beef / <i>Citrobacter freundii</i>	$Y = 1.05X - 0.16$
Seafood products	Fish / <i>Escherichia coli</i>	$Y = 0.98X + 0.12$

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

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

### Accuracy study (1996/97 and 2006 studies) :

Tests were performed in 1996 and 2005. The statistical interpretation was conducted on 108 countable results from samples which were all naturally contaminated, belonging to the major food categories : Meat products, dairy products, seafood products, vegetables, egg products.

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

For information, the levels of contamination (concentration) ranged as follows :

Food categories	Contamination range (log)
Meat products	2.30 to 5.56
Dairy products	1.30 to 8.15
Seafood products	1.88 to 5.32
Vegetables	2.40 to 7.94
Egg products	2.78 to 5.66

Regression line between the alternative method and the reference method, for all food categories, is as follows :

$$Y = 0.96 X + 0.18$$

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

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

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

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

Alternative method	Reference method
$r = 0.210$	$r = 0.235$

The bias (in log) between both methods (alternative – reference) is :

$$D = -0.025$$

### Conclusion for linearity and relative accuracy :

Linearity and accuracy tests show comparable results between alternative method and reference method.

## SELECTIVITY (INCLUSIVITY / EXCLUSIVITY) – 1997 study

### Use of alternative method only

- 20 strains of coliforms from 20 tested were detected.
- from 17 non coliform strains, 8 were developed on Petrifilm. They belong to following species : *Aeromonas hydrophila*, *Proteus mirabilis*, *Proteus vulgaris*, *Shigella sonnei*, *Salmonella* spp (4 strains). Besides, 5 strains were developed on VRBL media with NF EN ISO 4832 reference method ; they belong to the following species : *Aeromonas hydrophila*, *Aeromonas sobria*, *Proteus mirabilis*, *Proteus vulgaris*, *Shigella sonnei*.

## PRACTICABILITY (1997 study)

### Use of alternative method only

- **Response time** : **Positive** and **negative** results are obtained within 24 hours with the alternative method as well as with the reference method.
- **Other criteria** : space saving, time saving for preparation of media, easier waste management.

## INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 1997 with 12 participating laboratories. Analyses were done with half-skimmed pasteurized milk samples, artificially contaminated with a strain of *Enterobacter cloacae*, at the 4 following levels :

- Level 0
- Level 10 to 100 log CFU/g
- Level 100 to 1 000 log CFU/g
- Level 1 000 to 10 000 log CFU/g

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

The following results were obtained :

Contamination level log CFU/g	Number of laboratories with countable results*	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
10 to 100	11	0.117	0.217	0.264	0.220	-0.04
100 to 1000	11	0.235	0.300	0.205	0.221	-0.02
1000 to 10 000	11	0.294	0.332	0.235	0.230	0.01

\* 1 laboratory has received samples at a temperature higher than 8°C and his results have not been used.

### Conclusion

The inter-laboratory study shows comparable results between 3M™ Petrifilm™ Rapid Coliform Count Plate and the 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)