



**Alternative methods for agribusiness
Analytical performances certified**

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

Certificate No: 3M 01/07 – 03/99

Validation date:	03.23.1999
Renewal dates *:	04.02.2003
	05.24.2007*
	02.03.2011
End of validity:	03.23.2015

** The EN ISO 16140 protocol has been implemented for the renewal in 2007*

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is hereby authorized to use this **NF VALIDATION** certificate as a reference document for the following alternative quantitative analysis method :

3M™ Petrifilm™ High Sensitivity Coliform Count Plate
Application to enumeration of gas forming colonies after 24 hours

Protocol reference : 34-8704-4239-8

SCOPE: All human food products

RESTRICTIONS OF USE: None

REFERENCE METHODS

NF ISO 4831(2006) - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms - Most probable number technique

NF ISO 4832 (2006) - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony count technique

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METHOD PRINCIPLE

Petrifilm HSCC plates contain a ready-to-use Violet Red Bile (VRB) selective culture medium, a cold-water-soluble gelling agent and a tetrazolium indicator that colours in red all colonies growing on the plate facilitates colony enumeration. The top film traps gas produced by the lactose fermenting coliforms, this allowing enumeration of gas producing coliforms, as red colonies surrounding with gas bubbles. 5ml of inoculum are plated on Petrifilm HSCC, instead of 1 mL on the other Petrifilm plates, this provides a better sensitivity.

NOTE

The comparative study and the interlaboratory study done in 2007 were done in accordance with ISO 16140, and in comparison with reference methods NF ISO 4831 and NF ISO 4832 for comparative study, and NF ISO 4832 for interlaboratory study. Results of this study are presented below.

Accuracy study results from 1998 and 1998 and inclusivity / exclusivity study results from 1999 were kept, interpreted following ISO 16140 standard and completed.

The validation of the method was renewed in February 2011. The HSCC method and the reference methods remain unchanged. Interlaboratory study data obtained in 2007 were re-investigated in accordance with the standard EN ISO 16140/prA1 :2009. New results are detailed in this certificate.

LINEARITY and relative ACCURACY

Comparison of alternative method performance to reference method performance

Linearity study :

Tests were performed in 2007 on the 5 combinations food / strains belonging to 5 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 :

50 to 100 CFU/g
 100 to 500 CFU/g
 500 to 1,000 CFU/g
 1,000 to 5,000 CFU/g
 5,000 to 10,000 CFU/g

The following results were obtained :

Food categories	Food product/strain	Regression line / ISO 4831*	Regression line / ISO 4832*
Meat products	Minced beef/ <i>Enterobacter cloacae</i>	$X = 0.860 Y + 0.167$	$Y = 0.934 X + 0.385$
Vegetables and others	Green pepper/ <i>Escherichia coli</i>	$X = 1.075 Y - 0.984$	$Y = 0.938 X + 0.259$
Seafood products	Cod filet / <i>Escherichia coli</i>	$X = 0.918 Y + 0.255$	$Y = 1.004 X + 0.186$
Dairy products	Milk / <i>Cronobacter sakasaki</i>	$X = 0.951 Y + 0.114$	$X = 0.939 Y - 0.072$
Egg products	Liquid egg/ <i>Klebsiella pneumoniae</i>	$X = 0.978 Y - 0.087$	$X = 1.070 Y - 0.212$

Y = log(N alternative method)

X = log(N reference method)s

*Choice between abscissa and ordinate depend on type of regression choosen.

Accuracy study (in comparison with ISO 4831) :

Results obtained during studies done in 1998 and 1999 were interpreted according to ISO 16140 standard.

Additional tests were performed in 2007. Artificially contaminated samples were used for egg products and meat products categories.

The statistical interpretation was conducted on 104 countable results from 83 naturally contaminated samples and 21 artificially contaminated samples, belonging to the following major food categories :

Meat products, vegetables and miscellaneous products, dairy products, seafood products and egg products.

The samples were analysed **in duplicate** with alternative method and reference method ISO 4831.

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

Food categories	Contamination range (log)
Meat products	0.56 to 5.16
Dairy products	0.60 to 3.63
Seafood products	-0.04 to 4.38
Egg products	-0.04 to 4.91
Vegetable and others	0.18 to 3.87

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

$$\text{Regression line : } Y = 0.975 X + 0.291$$

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 inter-laboratory 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 the reference method are as follows :

Alternative method	Reference method (ISO 4831)
r = 0.323	r = 0,895*

*Calculation based on 2007 data obtained with eggs and meat products.

The bias (in log) between both methods (alternative method - reference method) is as follows:
D = 0.255

Conclusion for linearity and relative accuracy :

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

SELECTIVITY (INCLUSIVITY / EXCLUSIVITY)

Alternative method only

1999 data was completed with 2007 data. Finally following data was found :

- 33 strains of gas producing coliforms were detected out of 33 tested. The non-recognized strain is an *Enterobacter cloacae* strain (this strain was used during relative accuracy study to inoculate meat products ; it developed colonies with gas on Petrifilm High sensitivity coliform, and it produced gas by the reference method ISO 4831 on only 1 out of 3 inoculated samples).
- The study of 31 non coliforms strains did not show cross reaction.

PRACTICABILITY (1997 study) Alternative method only

- **Time response :**
- **Positive** results are obtained within 1 day with the alternative method instead of 2 to 4 days with the reference method.
- **Negative** results are obtained within 1 day with the alternative method instead of 2 to 4 days with the reference method, depending on the case.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2007 with 12 participating laboratories. Analysis were done with half-skimmed pasteurized milk samples, artificially contaminated with a strain of *Escherichia coli* at the 4 following levels :

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

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

The results calculated in accordance with the draft amendment 1 to EN ISO 16140 standard (version prA1 :2009) were the following :

Contami- nation level	Number of samples taken into account*	Reference method		Alternative method		Bias
		Repeatability standard deviation S_r	Reproducibility standard deviation S_R	Repeatability standard deviation S_r	Reproducibility standard deviation S_R	
Level 1	11	0.046	0.091	0.090	0.189	- 0.0571
Level 2	11	0.043	0.066	0.069	0.086	- 0.0290
Level 3	11	0.048	0.091	0.054	0.065	- 0.0124

* 1 laboratory has incubated samples at a temperature of 44°C, its data have not been used.

NB: Limit of repeatability $r = 2.8 S_r$, with S_r : repeatability standard deviation
Limit of reproducibility $R = 2.8 S_R$, with S_R : reproducibility standard deviation

Conclusion

The inter-laboratory study shows comparable results between 3M™ Petrifilm™ High Sensitivity 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