



Alternative methods for agribusiness
Analytical performances certified

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

Certificate N° : 3M 01/08– 06/01

Validation date : 06.14.2001
Renewal dates : 04.07.2005*
05.18.2009
End of validity : 06.14.2013

**The EN ISO 16140 protocol was implemented
for the renewal application in 2005*

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

3M™ Petrifilm™ Select E.coli Count Plate
(Petrifilm Select E.coli – SEC)

Protocol reference : 34-8704-9517-2

SCOPE

All human food products

RESTRICTIONS FOR USE

None

REFERENCE METHOD

ISO 16649-2 (July 2001) : Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β -glucuronidase positive *E.coli* – Part 2 : colony count technique at 44°C using 5-bromo-4-chloro-3-indolyl β -D-glucuronide

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PRINCIPLE OF THE METHOD

The Petrifilm Select *E.coli* count plate (SEC) is a sample-ready culture medium system which allows selective enumeration of β -glucuronidase producing *E.coli*. This enzyme reacts with the BCIG (5-bromo-4-chloro-3-indolyl- β -D-glucuronide) in the Petrifilm SEC plates to produce dark green to blue-green colonies.

Incubation for $24\text{h} \pm 2\text{h}$ at $42^\circ\text{C} \pm 1^\circ\text{C}$ is validated by this certificate.

NOTE (Validation history)

1/ In addition to the initial 2001 validation, a study for the renewal of validation was conducted in 2005 according to the protocol described in the new EN ISO 16140 standard.

- The results obtained in 2001 have been retained : accuracy (with a new statistical analysis according to EN ISO 16140 standard), specificity/selectivity and practicability .
- The new linearity and collaborative studies were conducted in 2005 in compliance with EN ISO 16140 standard.

2/ In May 2009, Petrifilm Select *E.coli* plate AFNOR VALIDATION certification was renewed, 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:

Tests were performed in 2005 on the 5 product/strain combinations and for the food categories given in the table below.

The samples were analysed **in duplicate** with each of the **two methods**, at the 5 following artificial contamination levels : 10 to 50, 50 to 100, 100 to 500, 500 to 1 000, 1 000 to 10 000 UFC/g.

The following results were obtained:

Food categories	food product /strain pair	regression line
Meat products	Raw ground beef / <i>E. coli</i> 13	$X = 0.10 + 0.97 Y$
Poultry	Chicken escalope / <i>E. coli</i> 96	$Y = - 0.08 + 1.03 X$
Dairy products	Milk / <i>E. coli</i> 14	$Y = - 0.25 + 1.07 X$
Fish and seafood products	Raw frozen fish / <i>E. coli</i> Ad 228	$X = 0.39 + 0.89 Y$
Fruit and Vegetable based products	Shredded carrots / <i>E. coli</i> 19	$X = - 0.02 + 1,02 Y$

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

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

Accuracy :

Tests were performed in 2001. The statistical interpretation was conducted on 118 countable results from samples, which were all naturally contaminated, belonging to the following major food categories : meat products, poultry, dairy products, seafood products, vegetables, egg products and others.

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

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

Food categories	Contamination range (log)
Meat products	0.66 to 2.97
Poultry	0.66 to 4.76
Dairy products	- 0.04 to 5.12
Seafood products and vegetables	0.66 to 4.54
Egg products and others	0.70 to 3.56

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

$$Y = 1.00 X + 0.23$$

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 limit of repeatability (in log) is **0.250** for the alternative method

The limit of repeatability (in log) is **0.308** for the reference method.

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

p = **0.21** calculated from the median, or

D = **0.24** calculated from the average individual bias.

Conclusion for linearity and relative accuracy :

There is a bias between the alternative method and the reference method which reflects a better recovery with the alternative method.

SELECTIVITY (INCLUSIVITY / EXCLUSIVITY)

Use of alternative method only

2001 study (AFNOR validation) and 2003 study (NordVal validation)

- 28 strains of *E.coli* were detected out of 31 tested. 3 strains gave non characteristic white colonies and were for one of them *E.coli* O157:H7 (ATCC 43888) and for two of them *E.coli* (*E.coli* 12 and *E.coli* 91). All three strains also gave white colonies on TBX.
- The study of 21 non-*E. coli* strains did not show presence of false positives. *Shigella flexneri* CIP 8248 gave white colonies (on TBX as well).

PRACTICALITY

Use of alternative method only

- **Response time :**
 - **Positive** results are obtained within **one day (24 hours)** with the alternative method as with the reference method.
 - **Negative** results are obtained within **one day (24 hours)** with the alternative method as with the reference method.

- **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
- Reading of plates inoculated with products presenting a high background flora is easier with the alternative method than with the reference method, since there are a limited number of non characteristic colonies.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2005 with 14 participating laboratories. The analyses were carried out on samples of half-skimmed pasteurized milk, artificially contaminated with a strain of *E. coli* 94, isolated from raw milk, at 4 contamination levels :

- Level 0
- Level 1: 10 – 100 CFU/ml
- Level 2: 100 – 1 000 CFU/ml
- Level 3: 1 000 – 10 000 CFU/ml

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

The following results were obtained:

Contamination level	Number of samples	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
Level 1	28	0.117	0.157	0.176	0.150	- 0.02
Level 2	28	0.250	0.675	0.161	0.223	0.03
Level 3	28	0.250	0.124	0.235	0.235	0.26

Conclusion

The bias between the reference method and the Petrifilm SEC plate method is not significant for levels 1 and 2. The Petrifilm SEC plate allowing a better recovery compared to the reference method, a bias is then observed at the level 3, in favour of alternative method (higher counts).

The repeatability of Petrifilm SEC plate is comparable to the one of the reference method, for all tested levels.

The reproducibility of Petrifilm SEC plate is better than the one of the reference method for all tested levels.

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