



Alternative methods for agribusiness
Analytical performances certified

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

Certificate N° : 3M – 01/09 – 04/03 A

**Validation date : 02.04.2003
1st Renewal date* : 29.03.2007
End of validity : 02.04.2011**

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

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

3M™ Petrifilm™ Staph Express System

Protocol reference : 34-8702-1081-1

SCOPE

All human food products.

RESTRICTIONS FOR USE

None.

REFERENCE METHOD

NF EN ISO 6888-1 : 1999 and NF EN ISO 6888-1/A1 : 2004 - Microbiology - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium / Amendment 1 : inclusion of precision data.

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AFNOR Certification

PRINCIPLE OF THE METHOD

The 3M™ Petrifilm™ Staph Express System consists of a Petrifilm Staph Express count plate and a Petrifilm Staph Express disk. The Staph Express plate is a sample-ready culture medium system which contains a cold-water-soluble gelling agent. The chromogenic, modified Baird-Parker medium in the plate is selective and differential for coagulase positive *Staphylococci*. The Staph Express disk contains toluidine blue-O that facilitates the visualization of desoxyribonuclease (DNase) reactions. Incubation of the plate at 37°C for 24h +/- 2 h and incubation of the disk at 37°C for 3 hours are validated by this certificate.

LINEARITY AND relative ACCURACY

Comparison of performances of the alternative method and the reference method

Linearity study :

Tests were performed in 2003 and 2007 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 :

In 2003, for minced meat, raw fish filet, shredded carrots :

- 50 CFU/g.
- 100 CFU/g
- 500 CFU/g
- 1 000 CFU/g
- 10 000 CFU/g

In 2007, for raw milk, and feed patty :

- 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
- 10 000 to 100 000 CFU/g

The following results were obtained :

Food category	Food product/strain pair	Regression line
Meat products	Minced meat / <i>Staphylococcus aureus</i>	$y = 0.9621 x + 0.0526$
Dairy products	Raw milk / <i>Staphylococcus aureus</i>	$y = 0.9719 x - 0.1634$
Fish and Sea-Food	Raw fish filet / <i>Staphylococcus aureus</i>	$y = 0.9624 x + 0.0626$
Vegetables	Shredded carrots / <i>Staphylococcus aureus</i>	$y = 0.9548 x + 0.00441$
feed	Patty for dog / <i>Staphylococcus aureus</i>	$y = 1.0569 x - 0.4343$

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

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

Accuracy study:

Tests were performed in 2003 and interpreted again in 2007. The statistical analysis was conducted on 106 interpretable results, including 88 naturally contaminated samples and 18 artificially contaminated samples, belonging to the following major food categories :

Meat products, dairy products, pastry, egg products, sea-food, vegetable and feed.

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 products	1.00 - 4.58
Dairy products	1.48 - 4.90
Sea-food	1.00 - 3.90
Vegetables	1.00 - 5.76
Pastry- egg products	2.10 - 4.48
Feed	1.00 - 4.63

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

$$Y = 0.9503 X - 0.0114$$

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 = 0.23	r = 0.21

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

P = - 0.17 (if median is considered)

Or D = - 0.16 log if individual bias average is considered

Conclusion for linearity and relative accuracy :

Linearity and accuracy study show that results obtained with alternative method are comparable with results obtained with reference method.

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)

Use of alternative method only

In 2003, specificity study was run using 22 *Staphylococcus aureus* strains and 11 strains other than *Staphylococcus aureus*.

In 2007, specificity study has been completed with *S.aureus* strains and non *S.aureus* strains.

Finally (if both 2003 and 2007 results are taken into account)

- On 35 coagulase positive *Staphylococcus* strains tested, 35 have been detected :
 - 22 *Staphylococcus aureus* in 2003 and 6 *Staphylococcus aureus* in 2007
 - 1 *Staphylococcus hyicus* in 2003 and 4 *Staphylococcus hyicus* in 2007
 - *Staphylococcus intermedius* in 2003 and 1 *Staphylococcus hyicus* in 2007
- The study of 26 non- targeted strains (15 strains other than coagulase positive *Staphylococcus* , and 11 non *Staphylococcus* strains has not shown any cross-reaction.

PRACTICABILITY

Use of alternative method only

- **Response time :**

- **Positive** results are obtained with the alternative method in 1 day, as opposed to 4 days with the reference method.
- **Negative** results are obtained in 1 day with the alternative method as opposed to 2 days with the reference method.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2007 with 13 participating laboratories. The analyses were carried out on samples of pasteurized milk artificially contaminated with a *Staphylococcus aureus* serotype strain at the 4 following levels:

- level 0
- level 1: 100 CFU/ml
- level 2: 1000 CFU/ml
- level 3: 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 taken into account*	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
Level 1	11	0,972	0,972	0,367	0,482	0,091
Level 2	11	0,125	0,398	0,125	0,161	- 0,050
Level 3	11	0,265	0,372	0,158	0,393	-0,064

**results from one laboratory have not been taken into account , because the temperature of reception was higher than 8°C, and a laboratory has not followed reference method protocol for one of the replicate at the level 2.*

Conclusion

Interlaboratory study shows that results obtained with alternative method are comparable with those 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