



**Alternative methods for agribusiness
Analytical performances certified**

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

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

RAPID'Staph
Coagulase-positive staphylococcus enumeration

Protocol reference : RAPID'Staph/Agar (356-4704 / 355-3960) – V3

SCOPE

All food products for human and animal consumption and environmental samples.

RESTRICTIONS FOR USE

Some non-characteristic *Staphylococcus aureus* strains exist, which do not reduce tellurite and/or do not induce proteolysis in egg yolks.

REFERENCE METHOD

Standard NF EN ISO 6888-1 - Microbiology of food - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) Part 1: Technique using Baird-Parker agar medium.

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PRINCIPLE OF THE METHOD

RAPID'*Staph* consists in a culture medium based on a Baird-Parker formula optimized to make it possible to obtain results in 24 hours. Its principle is based on the ability of *Staphylococcus aureus* to reduce tellurite (black colonies) and to induce proteolysis in egg yolks (light halo around colonies). The RAPID'*Staph* test is used to enumerate coagulase-positive staphylococci (*Staphylococcus aureus* and other species) at 37°C in 24 hours. The confirmation is performed using a PASTOREX® STAPH-PLUS latex test or on pre-poured Baird-Parker + RPF agar.

LINEARITY AND relative ACCURACY

Comparison of performances of the alternative method and the reference method

Linearity study:

Tests were performed in 2004 on the 6 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 to 50, 50 to 100, 100 to 500, 500 to 1000, 1000 to 10,000 CFU/g.

The following results were obtained:

Food category	Food product/strain pair	Regression line
Meat products	Hamburger patty / <i>S. aureus</i> Ad 160	$y = -0.34 + 1.12 x$
Animal feeds	Sausage for dogs / <i>S. aureus</i> Ad 162	$y = 0.07 + 0.97 x$
Dairy products	Raw milk / <i>S. aureus</i> Ad 501	$y = -0.04 + 1.01 x$
Seafood products	Frozen raw fish / <i>S. aureus</i> Ad 154	$y = -0.22 + 1.06 x$
Miscellaneous	Liquid egg product / <i>S. aureus</i> Ad 159	$y = -0.18 + 0.97 x$
Environmental samples	Rinsing water / <i>S. aureus</i> A00M071	$y = -0.23 + 1.06 x$

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

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

Accuracy study:

Tests were performed in 2004. The statistical interpretation was conducted on 50 results, including 35 naturally contaminated samples and 15 artificially contaminated samples, belonging to the following major food categories: meat products, animal feeds, dairy products, seafood products, miscellaneous, environmental samples.

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	1.19 log to 4.76 log
Animal feeds	1.54 log to 3.95 log
Dairy products	1.24 log to 5.76 log
Seafood products	0.70 log to 3.88 log
Miscellaneous	0.94 log to 4.10 log
Environmental samples	1.30 log to 4.95 log

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

$$y = -0.21 + 1.03 x$$

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

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

The repeatability for both methods and the bias between the two methods were determined according to the method of calculation used for the 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
$r = 0.294$	$r = 0.250$

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

$$D = 0.05 \log \text{ CFU/g}$$

Conclusion for linearity and relative accuracy:

The linearity and accuracy studies demonstrate that the results obtained with the RAPID'*Staph* method are comparable to the results obtained with the reference method.

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)

Use of alternative method only

- 28 coagulase-positive *Staphylococcus* strains were detected out of 30 tested. The 2 strains which showed small colonies that were difficult to enumerate are *Staphylococcus aureus* and *Staphylococcus intermedius* strains. Only the latter did not produce a positive confirmation test using the confirmation methods. These two strains also only show a light halo with the reference method.
- The study of 20 coagulase-negative strains did not detect the presence of cross-reactions.

PRACTICABILITY

Use of alternative method only

- **Positive** results are obtained with the alternative method in 1 day with confirmation using the PASTOREX test or in 2 days with confirmation with the coagulase test on BP + RPF medium, 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, provided that no characteristic or non-characteristic colonies are enumerated with the reference method, otherwise the time required is increased to 4 days.
- The RAPID'*Staph* alternative method reduces the processing time considerably, particularly for colony confirmation. Overall, two to three times less time is required to conduct the RAPID'*Staph* method with respect to the reference method.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2004 with 12 participating laboratories. The analyses were carried out on samples of semi-skimmed pasteurized milk, artificially contaminated with a *Staphylococcus aureus* strain at the 4 following levels:

- level 0
- level 1: 10 to 100 CFU/ml
- level 2: 100 to 1000 CFU/ml
- level 3: 1000 to 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
10 to 100	22*	0.352	0.365	0.881	0.881	0.13
100 to 1000	24	0.264	0.268	0.338	0.377	-0.01
1000 to 10,000	24	0.117	0.280	0.323	0.461	-0.03

* The samples from two laboratories were not taken into account for the calculations for the following reasons:

- one laboratory produced an enumeration of less than 10 for one of the level 1 duplicates;
- another laboratory enumerated confirmed characteristic coagulase-positive colonies for level zero probably due to an accidental contamination during the analytical procedure.

Conclusion

The collaborative study demonstrates that the results obtained with the alternative method are comparable to the results obtained with 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