



## VALIDATION CERTIFICATE FOR ALTERNATIVE ANALYSIS METHOD ACCORDING TO THE NF V 03-100 STANDARD

*The French version of this certificate is to be used as a reference*

Certificate N°: 3M – 01/9 – 04/03

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is authorized to use this AFNOR validation certificate as a reference document for the following alternative analysis method :

### 3M™ PETRIFILM™ Staph Express Count System

Protocol reference: 38-9018-3218-8

#### METHOD PRINCIPLE

The 3M™ Petrifilm™ Staph Express Count System (Staph Express) 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 *Staphylococcus aureus*. The Staph Express disk contains toluidine blue-O that facilitates the visualization of deoxyribonuclease (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.

#### SCOPE

All human food products.

#### RESTRICTIONS OF USE

None

#### REFERENCE METHOD

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

Executive Director  
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## PRACTICABILITY

- Results are obtained after 24h instead of from 48 to 96 hrs with the reference method,
- Operator training time shorter than one day for a microbiologist trained to classical methods.

## SPECIFICITY

- 20 *Staphylococcus aureus* strains were detected on 20 tested strains.
- Among 7 non *S.aureus* staphylococci tested strains , two strains (*S. hyicus* and *S. intermedius*) gave typical *S.aureus* colonies.
- The 4 non staphylococci tested strains did not give typical colonies.

## LINEARITY

A study was conducted in 2003 on 4 food categories (raw ground meat, raw milk, shredded carrots, raw fish filet), each artificially contaminated with 4 *S. aureus* strains (*S. aureus* ATCC 25923, *S. aureus* ATCC 6538, *S. aureus* from dairy origin and *S. aureus* from meat origin) at 5 contamination levels: 50, 10<sup>2</sup>, 500, 10<sup>3</sup>, 10<sup>4</sup> CFU/g.

Regression line equations are the following:

- Raw milk  $\log(\text{Petrifilm}) = 0.14 + 0.95 \log(\text{inoculum})$   
R = 0.98                      n = 20
- Raw ground meat  $\log(\text{Petrifilm}) = -0.05 + 1.00 \log(\text{inoculum})$   
R = 0.97                      n = 20
- Shredded carrots  $\log(\text{Petrifilm}) = 0.08 + 0.97 \log(\text{inoculum})$   
R = 0.99                      n = 20
- Raw fish  $\log(\text{Petrifilm}) = -0.06 + 1.03 \log(\text{inoculum})$   
R = 0.98                      n = 20

### Conclusion

Whatever the tested matrix, correlation coefficients and slopes of regression lines are close to 1. Linearity is good.

## ACCURACY

### Comparison of alternative method performance to reference method performance

Tests were conducted in 2003 on 205 samples and 4 food categories (meat products, dairy products, seafood, vegetables and miscellaneous). Statistical analysis was performed on 126 positive products including 42% of artificially contaminated samples.

### Accuracy of calibration

Calibration line between results obtained with the reference method and those obtained with the alternative method is as follows:

$$\log(\text{Petrifilm}) = 0.03 + 0.94 \log(\text{reference method})$$

$$R = 0.92 \quad n = 126$$

Repeatability of 3M<sup>TM</sup> Petrifilm<sup>TM</sup> Staph Express System in expert laboratory ( r = 0.36 log) was similar to that of the reference method.

### Conclusion

3M<sup>TM</sup> Petrifilm<sup>TM</sup> Staph Express System is accurate when compared to the reference method.

## PRECISION

Precision data were determined during a test conducted in 2003 comprising 14 laboratories. Analyses were done with pasteurised milk samples artificially contaminated by a strain of coagulase positive *S. aureus* at 4 following levels of contamination: 0, 10 to 100, 100 to 1000 and 1000 to 10000 CFU/ml.

Laboratories tested 2 samples for each contamination level.

### Collaborative study summary table

|                          | Low level<br>1.7 log/ml | Middle level<br>2.7 log/ml | High level<br>3.7 log/ml |
|--------------------------|-------------------------|----------------------------|--------------------------|
| Number of laboratories * | 11                      | 11                         | 11                       |
| Mean m                   | 1.69                    | 2.68                       | 3.68                     |
| Repeatability r          | 0.14                    | 0.33                       | 0.29                     |
| Reproducibility R        | 0.20                    | 0.37                       | 0.33                     |

*\* eleven laboratories among the fourteen laboratories have received their samples within 24 hours after shipment, at the requested temperature (between 0°C and 8 °C).*

### **Conclusion**

Repeatability and reproducibility of the alternative method are satisfactory.

**Please send any queries concerning the performance of the validated method to AFNOR CERTIFICATION**

**On request, AFNOR CERTIFICATION will send you a summary document (in French) on the preliminary and collaborative studies.**