



**Alternative methods for agribusiness  
Analytical performances certified**

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

**Certificate No.: BIO-12/24-03/08**

**Validation date : 27.03.2008**  
**End of validity : 27.03.2012**

**The company**  
(head office, Distribution, production site)

**BIOMERIEUX**  
**Chemin de l'Orme**  
**69280 MARCY L'ETOILE**  
**FRANCE**

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

**chromID™ Ottaviani Agosti Agar**  
enumeration of *Listeria monocytogenes*

Protocol reference : 12695 G

**SCOPE**

All human food products.

**RESTRICTIONS OF USE**

None.

**REFERENCE METHOD**

**EN ISO 11290-2** (1998) including **amendment A1** (2004) : Food Microbiology – horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2 : Enumeration method.

**Deputy General Manager**  
**Jacques BESLIN**

**AFNOR Certification**

## PRINCIPLE OF THE METHOD

ChromID™ Ottaviani Agosti (OAA) agar is a chromogenic medium which allow selective isolation of *Listeria monocytogenes* contains a nutritive base combining different peptones and two substrates, including a chromogenic one.

## Relative LINEARITY and ACCURACY

### Comparison of performances of the alternative method and the reference method

#### Linearity study :

Assays were carried out in 2007 on 5 combinations of food product/strain belonging to the food product categories mentioned in the table below.

All samples were analysed **in duplicate** by each of the **two methods** at the five level of contamination artificially contaminated :

- 0
- 100
- 500
- 5 000
- 50 000

Results obtained are as follows :

| Food category  | Food Matrix/Strain combinaison                | Regression line         |
|----------------|---|-------------------------|
| Meat products  | Rillettes / <i>Listeria monocytogenes</i> Ad  | $Y = 0.9746 X + 0.0956$ |
| Dairy products | Raw milk / <i>Listeria monocytogenes</i> 4b   | $Y = 0.9838 X + 0.1116$ |
| Seafood        | Smoked fish / <i>Listeria monocytogenes</i>   | $Y = 0.9801 X + 0.0836$ |
| Vegetables     | White cabbage / <i>Listeria monocytogenes</i> | $Y = 1.0122 X - 0.031$  |
| Egg products   | Raw egg / <i>Listeria monocytogenes</i> Ad    | $Y = 1.0157 X - 0.0729$ |

Y = log (N alternative method)

X = log (N reference method)

#### Study of accuracy :

Tests were carried out in 2007. Statistical analysis was conducted on 77 interpretable results from 8 samples naturally contaminated and 69 artificially contaminated, belonging to the following main food categories:

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

Samples were analyzed **in duplicate** by each of the **two methods**.

As an indication, the contamination scales (concentration) were as follow:

| Food category    | Contamination scale (log) |
|------------------|---------------------------|
| Meat products    | 1.51 to 6.72              |
| Dairy products   | 1.93 to 4.99              |
| Seafood products | 2.20 to 5.61              |
| Vegetables       | 2.41 to 4.90              |
| Egg products     | 1.96 to 4.86              |

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

$$\text{Equation of the regression line : } Y = 0.9878 X + 0.0436$$

Y = log (N alternative method)

X = log (N reference method)

The repeatability for the two methods and the bias between the two methods were determined according to the method of calculation used for the collaborative study (Cf. § 6.3.5 and § 6.3.6 of standard NF EN ISO 16140). These results provide additional information for the accuracy criterion.

The limit of repeatability (in log) obtained for the alternative method is 0.264.  
The limit of repeatability (in log) obtained for the reference method is 0.205.

The bias (in log) between the two methods (alternative – reference) is as follows :  
D = -0.005 for the average of individual biases.

#### **Conclusion for linearity and relative accuracy :**

The linearity study and accuracy studies show that the results obtained with the alternative method are comparable to the results obtained with the reference method.

### **SELECTIVITY (INCLUSIVITY/EXCLUSIVITY) Implementation of the alternative method only.**

- 50 *Listeria monocytogenes* strains were detected out of 50 tested.
- The study of 18 non-*Listeria* strains did not detect the presence of cross-reactions.
- 16 non-*Listeria monocytogenes* strains were all developed giving blue colonies without halo, except 3 *Listeria ivanovii* strains (on 3 tested) gave characteristic colonies with halo.  
These 3 strains also gave characteristic colonies on medium of the reference method.

### **PRACTICABILITY Implementation of the alternative method only**

- **Response time :**
  - Positive results are obtained in 2 to 3 days with the alternative method against 4 to 7 day with the reference method.
  - Negative results are obtained in 2 days with the alternative and the reference method.

### **INTER-LABORATORY STUDY**

The inter-laboratory study was performed in 2008 with 12 participating laboratories. The analysis were carried out on samples of pasteurized milk products, artificially contaminated with a *Listeria monocytogenes* serotype at the 4 following level :

- Level 0 : 0 UFC/ml
- Level 1 : 100 UCF/ml
- Level 2 : 1 000 UFC/ml
- Level 3 : 10 000 UFC/ml

The laboratories tested, using both methods, 2 replicates by contamination level.

The following results were obtained :

| Level of contamination | Number of laboratories giving exploitable results* | Reference method |                   | Alternative method |                   |       |
|------------------------|--|------------------|-------------------|--------------------|-------------------|-------|
|                        |  | Repeatability r  | Reproducibility R | Repeatability r    | Reproducibility R | Bias  |
| Level 1                | 96   | 0.213            | 0.333             | 0.359              | 0.402             | -0.02 |
| Level 2                | 96   | 0.065            | 0.175             | 0.231              | 0.312             | 0.00  |
| Level 3                | 96   | 0.122            | 0.187             | 0.247              | 0.282             | 0.01  |

### **Conclusion**

The inter-laboratory study shows that the results obtained with the alternative method are similar to those 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](http://www.afnor-validation.com)