BY AFNOR CERTIFICATION
NF VALIDATION EN ISO 16140

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**
Renewal date:	**02.02.2012**
End of validity:	**27.03.2016**

The company
(Head office, distributor and production site)

bioMérieux
Chemin de l'Orme
69280 MARCY L'ETOILE
FRANCE

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

ChromID™ Ottaviani Agosti Agar (OAA)

For the enumeration of *Listeria monocytogenes*

Protocol reference: Ref. 43 641 and 43 649 - 12695 **version H**

SCOPE

All human food products.

RESTRICTIONS

None.

REFERENCE METHOD

EN ISO 11290-2 (August 1998) including **amendment A1** (February 2005): Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: enumeration method.

Managing Director
Florence MÉAUX

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CERTIFICATION DE PRODUITS INDUSTRIELS
ACCRÉDITATION N° 5-0030 PORTÉE DISPONIBLE SUR WWW.COFRAC.FR

PRINCIPLE OF THE METHOD

ChromID™ Ottaviani Agosti Agar (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.

NOTE (Validation history)

For the renewal study validated in February 2012, the data obtained in 2008 during the inter-laboratory study were re-investigated in accordance with the EN ISO 16140/A1 standard. The new results are detailed in this certificate, the conclusion of the study being the same. The alternative method and the reference method remain unchanged.

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/*Listeria monocytogenes Ad* 669	Y = 0.9746 X + 0.0956
Dairy products	Raw milk/*Listeria monocytogenes* 4b 153	Y = 0.9838 X + 0.1116
Seafood	Smoked fish/*Listeria monocytogenes* 850/109	Y = 0.9801 X + 0.0836
Vegetables	White cabbage/*Listeria monocytogenes* 1/2 1011/1410	Y = 1.0122 X - 0.0310
Egg products	Raw egg/*Listeria monocytogenes* Ad 551	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:

Equation of the regression line: **Y = 0.9878 X + 0.0436**

Y = log (N alternative method)
X = log (N reference method)

The repeatability standard deviation for the two methods and the bias between the two methods were determined according to the method of calculation used for the inter-laboratory study (Cf. § 6.3.5 and § 6.3.6 of EN ISO 16140/A1 standard). These results provide additional information for the accuracy criterion.

The repeatability standard deviation (in log) obtained for the alternative method is 0.094.
The repeatability standard deviation (in log) obtained for the reference method is 0.073.

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

D = - 0.005 log UFC/g, 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.
- 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.
- The study of 18 non-*Listeria* strains did not detect the presence of cross-reactions.

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 analyses were carried out on samples of pasteurized milk products, artificially contaminated with a *Listeria monocytogenes* strain at the 4 following level:

- Level 0 : 0 CFU/mL
- Level 1 : 100 CFU/mL
- Level 2 : 1,000 CFU/mL
- Level 3 : 10,000 CFU/mL

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

The results calculated in accordance with the EN ISO 16140/A1 standard were the following:

Contamination level	Number of samples taken into account	Reference method		Alternative method		Bias
		Repeatability standard deviation S_r	Reproducibility standard deviation S_R	Repeatability standard deviation S_r	Reproducibility standard deviation S_R	
Level 1	12	0.076	0.107	0.143	0.150	0.009
Level 2	12	0.030	0.072	0.078	0.107	0.000
Level 3	12	0.056	0.075	0.094	0.094	0.011

NB: Limit of repeatability $r = 2.8\ S_r$, with S_r: repeatability standard deviation
Limit of reproducibility $R = 2.8\ S_R$, with S_R: reproducibility standard deviation

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