



Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: UNI 03/04 - 04/05

Validation date :	08.04.2005*
Extension dates :	15.09.2006 29.03.2007
Renewal date :	24.09.2009*
End of validity :	08.04.2013

* The EN ISO 16140 standard protocol was used in 2005 for preliminary study (initial validation) and in 2009 for the inter-laboratory study (renewal)

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

Listeria Precis™
(detection method)

Protocol reference : OCLA-R3 08/2009

SCOPE

All human food products and environmental samples.

RESTRICTIONS OF USE

None.

REFERENCE METHOD

EN ISO 11290-1 (1997) including amendment A1 (2004): Food Microbiology – horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection Method.

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PRINCIPLE OF THE METHOD

The Listeria Precis™ method is a medium for isolation and presumptive identification of *Listeria monocytogenes*. The method consists of an incubation of a specific selective pre-enrichment broth, followed by an isolation of colonies onto the chromogenic medium Brilliance™ Listeria Agar.

In the context of AFNOR Validation, all samples identified as positive by Listeria Precis™ method must be confirmed by one of the following means:

- According to classical tests described in methods standardized by CEN or ISO (including a purification step) from colonies isolated on a chromogenic media ;
- Implementing the OBIS MONO test from characteristic colonies isolated before onto Brilliance™ Listeria agar.

In the event of discordant results (positive with alternative method, non-confirmed by the options described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

NOTE 1 : Scope of validation

The Listeria Precis™ method is validated for two protocols :

- One protocol for human food products and environmental samples, except meat products
- Another specific protocol suitable for meat products

Different enrichment broths are used for each method (alternative and reference), that can introduce a risk to obtain discrepant results during the validation study.

NOTE 2 : Validation history

1/ Content of validation extension of 2006

Supplementary tests were carried out in 2006 to extend the scope of validation to the analysis of environmental samples. The following parameters were tested for this new category in accordance with the EN ISO 16140 standard protocol: relative accuracy/specificity/sensitivity and relative detection level. The results are available in this certificate.

2/ Content of validation extension of 2007

The extension study conducted in 2007 permitted to validate a new test of confirmation: the OBIS MONO method.

Assays were performed on strains grown in a nutritious broth and isolated in parallel onto Brilliance™ Listeria and TSA-YE agars :

- 150 strains of *Listeria monocytogenes* from different serotypes and origins were tested
- 100 strains not belonging to the genus *Listeria monocytogenes* were tested

The results were in accordance with those expected.

3/ Modification of the trademark reference (February, 2008)

The alternative method formerly **OCLA** (Oxoid Chromogenic Listeria Agar) is now named **Listeria Precis™**. The name of the **chromogenic media OCLA** was replaced by **Brilliance™ Listeria**.

4/ Renewal validation (2009)

The inter-laboratory study was entirely redone according to the EN ISO 16140 standard protocol. The results are available in this certificate.

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY Comparison of performances of the alternative method and the reference method

Tests were carried out in 2004 on 337 product samples, of which 99 were naturally contaminated, 68 artificially contaminated, and 170 non-contaminated, belonging to the following principal food product categories:

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

Supplementary tests were carried out in 2006 on 69 environmental samples (siphons, surfaces, résidus et poussières) of which 25 were naturally contaminated, 14 artificially contaminated, and 30 non-contaminated.

All samples were analysed in **single** by the **two methods**.

Table of results (Cf. Table 1 of the EN ISO 16140 standard):

	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement A+ / R+ PA = 174 ⁽¹⁾	Positive deviation A+ / R- PD = 14 ⁽¹⁾
Alternative method negative (A-)	Negative deviation A- / R+ ND = 18 ⁽²⁾	Negative agreement A- / R- NA = 200 ⁽³⁾

(1) Confirmed positives

(2) (3) Of which no samples, presumed positive by the alternative method, were found to be negative after confirmation

Percentages obtained compared to the reference method are as follows:

- Relative accuracy : **92.1%**
- Relative specificity : **93.4%**

NB : **relative specificity** below 100% results from a number of confirmed supplementary positives and not from false positives.

- Relative sensitivity : **90.6%**

Sensitivity was also recalculated taking into account all confirmed positives (including supplementary positives by alternative method):

$$\begin{aligned} &\text{Alternative method :} \\ &(PA + PD) / (PA + PD + ND) = \mathbf{91.2\%} \end{aligned}$$

$$\begin{aligned} &\text{Reference method :} \\ &(PA + ND) / (PA + PD + ND) = \mathbf{93.2\%} \end{aligned}$$

Analysis of discrepant results (according to appendix F of standard EN ISO 16140) :

$$PD = 14, \quad ND = 18, \quad Y = PD + ND = 32, \quad Y > 22, \quad d = PD - ND = 4, \quad d^2/Y = 16/32 = 0,5 < 3,841$$

Conclusion

The two methods are not statistically different.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2004, on the first 5 combinations of food products/strains described in the table below. These products belong to the following principal food product categories: meat products, seafood, vegetables, dairy products and egg products.

Supplementary tests were carried out in 2006, on a sixth category (environmental samples) on the combination of food product/strain presented in the table below.

Products were analysed 6 times by the 2 methods at 4 levels of contamination.

Results obtained are as follows:

Matrix	Strain	Relative detection level (CFU/25g or 25 ml) With confidence interval (3) LOD ₅₀	
		Alternative method	Reference method
Potted meat	<i>L. monocytogenes</i> 4e	0.9 [0.5–1.7]	0.2 [0.05–0.7]
Smoked salmon	<i>L. monocytogenes</i> 1/2b	0.5 [0.2–1.3]	0.1 [0.03–0.5]
Lettuce	<i>L. monocytogenes</i> 1/2a	0.3 [0.1–1.2]	0.2 [0.03–0.8]
Raw milk	<i>L. monocytogenes</i> 1/2b	0.4 [0.1–1.4]	0.6 [0.6–0.6]
Egg custard	<i>L. monocytogenes</i> 1/2a	0.3 [0.1–0.9]	1.1 [0.7–1.6]
Water process	<i>L. monocytogenes</i> 1/2a	0.8 [0.2–3.0]	0.4 [0.1–1.4]

(3) LOD₅₀ : estimation of level of contamination enabling positive detection by alternative method in 50% of cases.

"Hitchins A. Proposed Use of a 50% Limit of detection Value in Defining Uncertainty Limits in the Validation of Presence-Absence Microbial detection Methods, Draft 10th December, 2003"

Conclusion

The detection limit of the alternative method is between 0.1 and 3.0 CFU/gram.
The detection limit of the reference method is between 0.03 and 1,6 CFU/gram.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 48 strains of *Listeria monocytogenes* were detected out of 50 tested, before 24 hours of incubation of the enrichment broth. The 2 strains which gave a negative results were strains of collection from serotype 3a and 4e.
- The study of 30 strains not belonging to the genus *Listeria monocytogenes* did not detect the presence of any cross-reaction.

PRACTICABILITY

Implementation of alternative method only

Response time:

- **Positive** results are obtained in 4 to 8 days or in 5 to 9 days (case of meat products) using the alternative method, against 7 to 11 days using the reference method.
- If no characteristic colonies are observed onto the selective agar medias, **negative** results are obtained in 2 or to 3 days (meat products) using the alternative method against 3 to 5 days using the reference method.
- If characteristic colonies are observed onto the selective agar medias, **negative** results are obtained in 2 to 4 days or 2 to 5 days (meat products) using the alternative method against 5 to 11 days using the reference method.
- In the case of results presumed positive using the alternative method, but rendered negative following confirmation, these negative results are obtained in 4 to 8 days or in 5 to 9 days (meat products).

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2009 with 13 participating laboratories. The analysis were carried out on samples of pasteurized milk, artificially contaminated with a *Listeria monocytogenes* 4b 153 strain at the 4 following 3 levels of contamination:

- 0 UFC/25 ml
- 1 – 10 UFC/ 25 ml
- 5 – 50 UFC/25 ml

The laboratories tested, using **both methods**, **8 replicate samples** for **each level** of contamination, giving a total of 24 analysis for each participating laboratory.

The following results were obtained:

Contamin- ation level	Total number of samples	Number of samples analysed *	Number of results processed	Number of negative results		Number of positive results	
				REF	ALT	REF	ALT
0	104	96	96	96	0	0	
1	104	96	96	1	0	95	96
2	104	96	96	0	0	96	96

* 1 laboratory, which did not receive the samples in time, did not realize the analysis.

Calculations

- Relative accuracy = **99.7%**
- Specificity = **100%**
- Sensitivity = **100%**

Interpretation

Results of the collaborative study are comparable to those obtained during the preliminary study.

Sensitivity was also recalculated taking into account all confirmed positive results (this includes supplementary positives with alternative method):

Alternative method:

$$(PA + PD) / (PA + PD + ND) = 100\%$$

Reference method:

$$(PA + ND) / (PA + PD + ND) = 99.5\%$$

Accordance, concordance and concordance odds ratio :

Accordance: percentage chance of finding the same result (i.e. both negative or both positive) from two identical test portions analysed in the same laboratory, under repeatability conditions (i.e. one operator using the same apparatus and same reagents within the shortest feasible time interval). The accordance is the average (mean) of the probabilities that two replicates give the same result for each laboratory

Concordance: percentage chance of finding the same result for two identical samples analysed in two different laboratories. The concordance is the percentage of all pairings of duplicates giving the same result

Concordance odds ratio (COR) : defined by the following formula:

$$COR = \text{accordance} \times (100 - \text{concordance}) / \text{concordance} \times (100 - \text{accordance})$$

The following table indicates values for the **alternative method**:

Contamination level	Accordance	Concordance	COR
L0	100	100	1.00
L1	100	100	1.00
L2	100	100	1.00

The following table indicates values for the **reference method**:

Contamination level	Accordance	Concordance	COR
L0	100	100	1.00
L1	97.8	97.9	1.00
L2	100	100	1.00

Conclusion

Variability of the alternative method (accordance, concordance, concordance odds ratio) is equivalent to that of 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