



Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: BIO-12/11-03/04

Validation date :	12.03.2004*
Extension dates :	02.12.2004*
	14.12.2006*
Renewal date :	17.01.2008
End of validity :	12.03.2012

* EN ISO 16140 protocol was used in 2004 for the preliminary study and in 2006 for the inter-laboratory study

The company
(head office, distribution, and 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:

**VIDAS Listeria monocytogenes II (VIDAS LMO2) – Ref. 30704
Enrichment stage at 37 °C**

Protocol reference: 11600 versions (J) and (K)

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

A handwritten signature in black ink, appearing to be "JBESLIN", written over a horizontal line.

Deputy General Manager
Jacques BESLIN

PRINCIPLE OF THE METHOD

The VIDAS LMO2 test is an enzyme immunoassay test which detects *Listeria monocytogenes* antigens using the ELFA (Enzyme Linked Fluorescent Assay) method on the VIDAS or mini VIDAS analyzers.

Each test is composed of two parts:

- The disposable SPR, which serves both as the solid phase and the pipetting device for the test. The SPR is coated with anti *Listeria monocytogenes* antibodies adsorbed on its surface.
- The strip, which contains all ready-to-use reagents necessary for the test: washing solution, alkaline phosphatase-labeled anti *Listeria monocytogenes* antibodies and substrate.

The VIDAS LMO2 method consists of an enrichment of half-Fraser incubated at 30°C ±1°C for 24h to 26h, after which 0.1 ml of the suspension is transferred into Fraser broth, incubated at 37°C ± 1°C for 24h to 26h. The VIDAS LMO2 test is performed with an aliquot of Fraser broth.

In the context of AFNOR VALIDATION, all samples identified positive by the VIDAS LMO2 test must be confirmed as follows:

- From enrichment broth according to classical tests described in the standardized methods by CEN or ISO (including the purification step).
- Using a chromogenic medium, according to the specified conditions of the package insert referenced above.

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

NOTE: Validation history

The first validation was granted on March 2004 and the study compared VIDAS LMO2 with reference method EN ISO 11290-1 (1997)

As VIDAS LMO2 method with enrichment at 30 °C (same protocol as VIDAS Listeria) had been AFNOR validated in July 2002 under certificate BIO 12/09 – 07/02, some results of the validation study made in 2002 have been kept for the validation of the method with enrichment at 37°C: inclusivity / exclusivity study and practicability study. The remainder of preliminary study was carried out according to the protocol described in the EN ISO 16140 standard.

In December 2004 an extension of validation dealt with the comparison of the VIDAS LMO2 method to the reference standard EN ISO 11290-1 including **amendment A1** (2004).

The amendment A1 modifies the plating media used after enrichment steps in Half Fraser and Fraser broths: OXFORD and PALCAM media have been replaced by media "Agar Listeria according to Ottaviani and Agosti" with a second medium of choice.

Assays of extension of validation have been carried out with three media: "Agar Listeria according to Ottaviani and Agosti", OXFORD and PALCAM.

In December 2006 a new extension of validation dealt with the inter-laboratory study which was completely repeated in order to comply with the EN ISO 16140 standard.

In December 2007, the Validation renewal was granted without any other assay, as neither VIDAS LMO2 method, nor the reference method, nor the validation protocol have been modified since the last extension.

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY

Comparison of performances of the alternative method and the reference method

In 2004 tests were carried out on 327 product samples, of which 101 were naturally contaminated, 58 artificially contaminated, and 168 non-contaminated, belonging to the following principal food product categories :

- meat products,
- dairy products,
- vegetables,
- seafood products,
- environmental samples

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

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

These results take into account the ALOA and PALCAM agar plates used in the reference method.

	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement A+ / R+ PA = 155 ⁽¹⁾	Positive deviation A+ / R- PD = 2 ⁽¹⁾
Alternative method negative (A-)	Negative deviation A- / R+ ND = 1 ⁽²⁾	Negative agreement A- / R- NA = 169 ⁽³⁾

(1) Confirmed positives

(2) and (3) Of which none sample presumed positive by the alternative method was negative after confirmation

Percentages obtained compared to the reference method are as follows:

- Relative accuracy: **99.1%**
- Relative specificity: **98.8%**

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

- Relative sensitivity: **99.4 %**

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

Reference method :

$$(PA + PD) / (PA + PD + ND) = 98.7\%$$

Alternative method :

$$(PA + ND) / (PA + PD + ND) = 99.4\%$$

Conclusion

Performances of VIDAS LMO2 method appear similar to those of the reference method.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2004, on the following combinations of food products/strains and with the following categories: dairy products, meat products, vegetables, seafood products, environment samples.

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 ₆₀	
		Alternative method	Reference method
Raw milk	L monocytogenes 1/2b	0.4 [0.3 – 0.6]	0.4 [0.3 – 0.6]
Potted minced (rillettes)	L monocytogenes 1/2c	0.5 [0.3 - 0.7]	0.4 [0.3 – 0.7]
Red cabbage	L monocytogenes 4b	0.6 [0.4 – 1.1]	0.6 [0.4 – 1.1]
Smoked salmon	L monocytogenes 1/2a	0.4 [0.3 – 0.6]	0.4 [0.3 – 0.6]
Processed water	L monocytogenes 1/2c	0.8 [0.4 – 1.8]	0.8 [0.4 – 1.8]

(3) LOD₆₀: 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 levels obtained by the alternative method are similar as those obtained by the reference method.

The detection level of the alternative method is between 0.3 and 1.8 CFU/ 25g.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 50 strains of *L. monocytogenes* were detected out of 50 tested.
- The study of 44 strains non *Listeria monocytogenes* did not detect the presence of any cross-reaction.

PRACTICABILITY (study realized in 2002)

Implementation of alternative method only

- **Response time:**
 - **Positive** results are obtained in 9 to 10 days using VIDAS LMO2 method (including confirmation according to classical tests of the reference method, with purification step included) or in 3 to 4 days (confirmation with a chromogenic agar) against 5 to 11 days using the reference method.
 - **Negative** results are obtained in 2 days using the alternative method against 5 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 9 to 10 days.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2006 with 16 participating laboratories. The analysis were carried out on samples of pasteurized milk artificially contaminated with a *Listeria monocytogenes* strain at the 3 following levels of contamination:

- Level 0: 0 CFU / 25 ml,
- Level 1: 3 CFU / 25 ml,
- Level 2: 30 CFU / 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:

Contami- nation level	Total number of samples	Number of samples analysed*	Number of results exploited **	Number of negative results		Number of positive results	
				REF	ALT	REF	ALT
0	128	112	104	0	0	104	104
1	128	108	104	0	0	104	104
2	128	112	104	0	0	104	104

* 2 laboratories received the samples lately and did not realize the analysis. Another laboratory did not realize the analysis of 4 samples because of a leakage.

** Finally 3 laboratories were excluded.

Calculations

- Relative accuracy = 100 %
- 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 :

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

Reference method :

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

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 concordance 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:

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

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

Contamination level	Accordance	Concordance	COR
L0	100 %	100 %	1.0
L1	100 %	100 %	1.0
L2	100 %	100 %	1.0

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

Contamination level	Accordance	Concordance	COR
L0	100 %	100 %	1.0
L1	100 %	100 %	1.0
L2	100 %	100 %	1.0

Conclusion

Variability of the alternative method (accordance, concordance, concordance odds ratio) is identical 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