



**Alternative methods for agribusiness
Analytical performances certified**

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

Certificate No.: BIO 12/2 – 06/94

Validation date :	19.06.1994
Renewal dates:	09.06.1998 03.07.2002 04.05.2006*
End of validity :	09.06.2014

The company
(head office, distribution,
and production site)

**BIOMERIEUX
69280 MARCY L'ETOILE
FRANCE**

** EN ISO 16140 protocol was used for the 3rd
renewal in 2006*

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

VIDAS LISTERIA (VIDAS LIS) – Ref. 30 700

Protocol references: **06745** versions **R** and **S**

SCOPE

All human food product and environmental samples.

RESTRICTIONS

None.

REFERENCE METHOD

EN ISO 11290-1 (1997) including **amendment A1** (2004): Microbiology of food and animal feeding stuff – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method.

**Deputy General Manager
Jacques BESLIN**

A handwritten signature in black ink, appearing to read 'Jacques Beslin', with a long horizontal stroke extending to the right.

PRINCIPLE OF THE METHOD

The VIDAS LIS test is an enzyme immunoassay test which detects *Listeria* 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* 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* antibodies and substrate.

The VIDAS LIS method consists in Half-Fraser enrichment broth incubated **20 to 26 hours** at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and sub-culturing of 1 ml in 10 ml of complete Fraser broth incubated for **20 to 26 hours** at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The VIDAS LIS test is performed from a complete aliquot of Fraser heated 15 ± 1 minute at $95 - 100^{\circ}\text{C}$.

In the context of NF VALIDATION, all positive samples after the VIDAS LIS test must be confirmed from the enrichment broth according to classical tests described in the CEN or ISO standardized methods (including the purification step).

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

NOTE: history of validation

For the renewal study of 2006, the alternative method was not modified since the last validation renewal granted in 2002, which was followed by an extension in 2003. The protocol of validation changed (new ISO 16140 protocol for the renewal) and an amendment was included in the reference method EN ISO 11290-1. The validation study was repeated in 2006, without the practicability study carried out in 1998. The relative accuracy, relative specificity and relative sensitivity data obtained in 2003, were also maintained and complemented.

The validation of VIDAS LIS was renewed in May 2010 without additional assays, as the alternative method was not modified, and neither the reference method nor the protocol of validation did change since the previous validation.

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY (Study in 2003 and 2006)

Comparison of performances of the alternative method and the reference method

In 2003 tests were carried out on 135 product samples, in comparison with the reference method using Palcam and Oxford agar plates. Among these 135 samples 73 were naturally contaminated and 62 non-contaminated, belonging to the following principal food product categories: meat products, dairy products, vegetables, seafood products and environment samples.

Trials were carried out in 2006 on 202 samples, of which 55 were naturally contaminated 37 were artificially contaminated and 110 were non contaminated, belonging to the following principal food product categories: meat product, dairy products, vegetables, seafood products, environment samples.

All samples were analysed **in single** by **both methods**.

The table below displays the results of both studies.

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 = 162 ⁽¹⁾	Positive deviation A+ / R- PD = 1 ⁽¹⁾
Alternative method negative (A-)	Negative deviation A- / R+ ND = 2 ⁽²⁾	Negative agreement A- / R- NA = 172 ⁽³⁾

(1) Confirmed positives

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

(3) Of which one 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 : **94.4 %**
- Relative sensitivity : **98.8 %**

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

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

$$\begin{array}{ll} \text{Alternative method :} & \text{Reference method :} \\ (PA + PD) / (PA + PD + ND) = \mathbf{98.8 \%} & (PA + ND) / (PA + PD + ND) = \mathbf{99.4 \%} \end{array}$$

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2006 on the 5 combinations of food products/strains described in the table below.

These products represent the following food products categories: dairy products, meat products, seafood products, vegetables, environment samples.

Products were analysed **6 times** by both **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
Raw milk	<i>L. innocua</i>	1.1 [0.6 - 1.9]	1.1 [0.6 - 1.9]
Potted minced (rillettes)	<i>L. welshimeri</i>	0.7 [0.4 - 1.4]	0.7 [0.4 - 1.4]
Smoked Salmon	<i>L. monocytogenes</i> 1/2a	0.9 [0.6 - 1.5]	0.9 [0.6 - 1.5]
Red cabbage	<i>L. monocytogenes</i> 4b	0.7 [0.4 - 1.4]	0.7 [0.4 - 1.4]
Processed water	<i>L. monocytogenes</i> 1/2c	1.0 [0.8 - 1.3]	1.0 [0.8 - 1.3]

(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 level of the alternative method is equivalent to the reference method's one. It is assessed between 0.4 and 1.9 CFU/25G.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 51 strains of *Listeria* (25 *Listeria monocytogenes* strains and 26 *Listeria non monocytogenes*) were detected out of 51 tested.
- The study of 30 strains not belonging to the genus *Listeria* did not detect the presence of any cross-reaction.

PRACTICABILITY

Implementation of alternative method only

- **Response time :**

- **Positive** results are obtained in 5 days using the alternative method (including confirmation with API strips after purification) or 10 days (if the confirmation is performed with classical tests) against 9 to 12 days using the reference method.

Note: Gram staining and catalase test are sufficient to confirm the *Listeria* genera. The identification of *Listeria genera* is obtained in 4 days, without identification of the species.

- **Negative** results are obtained in 2 days using the alternative method against 5 days using the reference method.
- In the case of results presumed positives by using the alternative method, but rendered negative following confirmation, 3 to 5 days are required.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2006 with 15 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a *Listeria innocua* strain at the 3 following levels of contamination:

- 0
- slightly superior to relative detection level (3 CFU/ml)
- 10 times superior to previous level (30 CFU/ml)

The laboratories tested, using **both methods**, **8 replicate samples** for **each level** of contamination.

The following results were obtained:

Contamination 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	120	112	112	112	112 ⁽¹⁾	0	0
1	120	112	112	13	13	99	99
2	120	112	112	0	0	112	112

* One laboratory did not receive the samples in time and did not realize the analysis.

(1) Of which one sample was positive when using the VIDAS *Listeria* test and negative after confirmation.

Calculations

- Relative accuracy = **100 %**
- % specificity = **100 %**
- % sensitivity = **94.2 %**

Interpretation

The results of inter-laboratory study are comparable to those obtained in the preliminary study regarding the relative accuracy and specificity. The sensitivity value obtained in the inter-laboratory study is lower than the value obtained in the preliminary study. It is due to the calculation of the sensitivity with the expected results and to the expected contaminated samples of the level 1 that were not contaminated (the results issued from the VIDAS *Listeria* method and the reference method were in agreement).

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

$$\begin{aligned} & \text{Alternative method :} \\ & (PA + PD) / (PA + PD + ND) = \mathbf{100 \%} \end{aligned}$$

$$\begin{aligned} & \text{Reference method :} \\ & (PA + ND) / (PA + PD + ND) = \mathbf{100 \%} \end{aligned}$$

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:

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

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

Contamination level	Accordance	Concordance	COR
L0	100 %	100 %	1,00
L1	82 %	79 %	1,03
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	82 %	79 %	1,03
L2	100 %	100 %	1,00

The accordance and concordance percentages of level 1 are due to some samples finally stated as non contaminated (these results issued from the VIDAS Listeria method and the reference method were in agreement).

Conclusion

Variability of the alternative method (accordance, concordance, concordance odds ratio) is equivalent to the reference method's one.

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