



Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: BIO 12/09 – 07/02

Validation date : 03.07.2002
Renewal date* : 15.06.2006
Extension date* : 14.12.2006
End of validity : 03.07.2010

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

The company

(head office, distribution, and production site)

BIOMERIEUX
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 30 °C

Protocol reference: 30704, sheet reference 11600 version I and version J

SCOPE

All human food products (except raw products) and environment samples

RESTRICTIONS OF USE

None

REFERENCE METHOD

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

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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 in an enrichment of half-Fraser incubated at 30°C ±1°C for 24h to 26h, after which 1 ml of the suspension is transferred into 10 ml of Fraser broth, incubated at 30°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, ISO or AFNOR (including the purification step).
- Using a chromogenic medium, according to the specified conditions of the package insert referenced above.

NOTE 1/ Compared to the first validation study carried out in 2002, the preliminary study was completed in order to take into account the new validation protocol described in the EN ISO 16140 standard.

These results obtained during the first validation were maintained :

- the study of relative accuracy, relative specificity and relative sensitivity (partially repeated),
- the study of inclusivity/exclusivity and practicability.

The inter-laboratory study performed in 2002 was not repeated.

NOTE 2/ The extension of validation carried out in December 2006 concerned the inter-laboratory study, completely repeated according the EN ISO 16140 standard, leading to this certificate.

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

The results obtained in 2002 were kept for 60 negative results and 43 positive results from naturally contaminated samples. The study was completed in 2006 to get the required amount of samples, and the results were interpreted according to the EN ISO 16140 standard.

As a whole, 361 product samples were analysed, of which 63 were naturally contaminated, 93 artificially contaminated, and 205 non-contaminated, belonging to the following principal food product categories :

Meat processed products, dairy pasteurised products, seafood cooked products, processed vegetables, environment samples.

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 = 148 ¹⁾ | Positive deviation A+ / R- PD = 3 ¹⁾ |
| Alternative method negative (A-) | Negative deviation A- / R+ ND = 5 ²⁾ | Negative agreement A- / R- NA = 205 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 : **AC = 97.6 %**
- Relative specificity : **SP = 96.7%**

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

- Relative sensitivity : **SE = 98.3 %**

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

Alternative method :

$$(PA + PD) / (PA + PD + ND) = 96.8\%$$

Reference method :

$$(PA + ND) / (PA + PD + ND) = 98.1\%$$

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

$$PD = 3, ND = 5 \text{ so } Y = PD + ND = 8 ; 6 \leq Y \leq 22$$

$$m = 3, M = 0$$

$$\text{so } m > M$$

Conclusion

Both methods are not different in statistical terms.

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 product categories: meat processed products, dairy pasteurised products, seafood cooked products, processed vegetables, environment samples.

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

Results obtained are as follows :

| | | Relative detection level (CFU/25g or 25 ml) With confidence interval (3) LOD ₅₀ | |
|---------------------------|----------------------|---|------------------|
| Matrix | Strain | Alternative method | Reference method |
| Pasteurized milk | L monocytogenes 1/2b | 0.8 [0.3 – 1.3] | 0.8 [0.3 – 1.3] |
| Potted minced (rillettes) | L monocytogenes 1/2c | 0.7 [0.4 – 1.2] | 0.7 [0.4 – 1.2] |
| Cooked vegetables | L monocytogenes 4b | 0.4 [0.3– 0.7] | 0.4 [0.3– 0.7] |
| Baked fish | L monocytogenes 1/2a | 0.6 [0.4– 0.9] | 0.6 [0.4– 0.9] |
| Processed water | L monocytogenes 1/2c | 0.6 [0.3– 1.2] | 0.6 [0.3– 1.2] |

(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 obtained by the alternative method is identical to that obtained by the reference method. It is assessed between 0.3 and 1.3 CFU/ 25g.

INCLUSIVITY / EXCLUSIVITY (study performed in 2002)

Implementation of alternative method only

- 50 strains of *L. monocytogenes* were detected out of 50 tested.
- The study of 43 strains non *Listeria monocytogenes* did not detect the presence of any cross-reaction.

PRACTICABILITY

Implementation of alternative method only

Response time :

- **Negative** results are obtained in 2 days using the VIDAS LMO2 method against 5 days using the reference method.
- **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 (including confirmation with a chromogenic agar) against 5 to 11 days using the reference method.
- Presumed positive results using the VIDAS LMO2 method, but rendered negative, are obtained in 3 days (using a chromogenic agar plates) to 10 days (using the reference method) depending to the confirmation protocol.

INTER-LABORATORY STUDY (according to EN ISO 1640 standard)

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:

| Contamin- ation 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 | 104 | 104 | 0 | 0 |

* 2 laboratories received the samples lately and did not realized the analysis. Another laboratory did not realized 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 :

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

Reference method :

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

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 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 % | 1.0 |
| 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
AFAQ AFNOR Certification.

You may download a summary document on the preliminary and inter-laboratory
studies on www.afnor-validation.com