AFNOR Validation

Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: BIO 12/27 – 02/10	**Validation date:** 04.02.2010 **End of validity:** 04.02.2014

The company **BIOMERIEUX**
Chemin de l'Orme
F-69280 MARCY L'ETOILE

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

VIDAS® *Listeria monocytogenes* Xpress (VIDAS LMX)

Ref. 30 123

Protocol reference: 14226 **C**

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.

Deputy General Manager
Jacques BESLIN

AFNOR Certification
11, rue Francis de Pressensé – 93571 La Plaine Saint-Denis Cedex - France
Phone +33 (0)1 41 62 80 00 – Fax +33 (0)1 49 17 90 00
certification@afnor.com - www.afnor-validation.com

PRINCIPLE OF THE METHOD

VIDAS® LMX is an enzyme immunoassay which detects *Listeria monocytogenes* antigens using the ELFA (Enzyme Linked Fluorescent Assay) method on the VIDAS® automated system.

Each test is composed of a 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. A strip contains all the others ready-to-use reagents necessary for the immunological test.

In the context of AFNOR VALIDATION, the two following protocols are validated:

- General protocol for the analysis of all human food products, excepted raw milk cheeses, and environmental samples
- Specific protocol for the analysis of raw milk cheeses.

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

- Isolate on a chromogenic medium. Incubate the agar according to the instructions in the package insert. Identify 1 to 5 isolated characteristical colonies by classical tests described in methods standardized by CEN or ISO (including a purification step).
- Using any others chromogenic agar media being part of a method certified AFNOR Validation, without identification of characteristical colonies. The presence of typical *Listeria monocytogenes* colonies after isolation of LMX broth is sufficient to confirm the positive result.
- On chromogenic agar media Ottaviani & Agosti. The confirmation of typical *Listeria monocytogenes* colonies using an API® strip can be performed without prior purification, if the colony is sufficiently isolated.

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 (see the user guide for recommended protocols).

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY

Comparison of performances of the alternative method and the reference method

In 2009 tests were carried out on 384 product samples, of which 110 were naturally contaminated, 80 artificially contaminated, and 194 non-contaminated, belonging to the following principal food product categories : Meat products, dairy products (including raw milk cheese), vegetables, seafood products and environmental 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 = **153** (1)	Positive deviation A+ / R- PD = **19** (1)
Alternative method negative (A-)	Negative deviation A- / R+ ND = **18** (1) (2)	Negative agreement A- / R- NA = **194** (3)

(1) Confirmed positives

(2) (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 = 90.4%**
- Relative specificity: **SP = 91.1%**

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

- Relative sensitivity: **SE = 89.5%**

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

Alternative method :	Reference method :
(PA + PD) / (PA + PD + ND) = 90.5%	(PA + ND) / (PA + PD + ND) = 90.0%

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

Y = PD + ND = 37; Y > 22; Test of Mc Nemar: d = | PD-ND | =1 ; d minimal = 12 ; d<d minimal

Conclusion

The two methods are considered equivalent.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2009, on the 6 combinations of food products/strains, described in the table below.

These products represent the following principal food product categories: Meat products, dairy products (including raw milk cheese), vegetables, seafood products and environmental 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**
Potted meat	*L.monocytogenes* 1/2b	1.1 [0.6 – 1.8]	0.7 [0.4 – 1.3]
Raw milk	*L.monocytogenes* 1/2b	0.4 [0.2 – 0.7]	0.5 [0.3 – 0.9]
Raw milk cheese	*L.monocytogenes* 1/2a	0.6 [0.4 – 1.0]	0.7 [0.4 – 1.3]
Grated red cabbage	*L.monocytogenes* 4b	0.6 [0.4 – 1.0]	0.5 [0.3 – 0.8]
Smoked salmon	*L.monocytogenes* 1/2a	0.4 [0.2 – 0.7]	0.5 [0.2 – 0.9]
Water process	*L.monocytogenes* 1/2c	0.3 [0.2 – 0.6]	0.4 [0.2 – 0.7]

(3) **LOD_{50}**: estimation of level of contamination enabling positive detection by alternative method in 50% of cases.
"Hitchins A. Proposed Used 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 relative detection level of the alternative method is between 0.2 and 1.8 CFU/25g.
The relative detection level of the reference method is between 0.2 and 1.3 CFU/25g.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 60 strains of *Listeria monocytogenes* were detected out of the 60 tested.
- The study of 31 non *Listeria monocytogenes* strains showed cross reactions with one strain of *Bacillus sphaericus* and two strains of *Staphylococcus* (*S. intermedius* and *S. aureus*) after enrichment in the non selective enrichment broth. For these 3 non target strains, VT values were just above the threshold and inoculation levels were high. Negative results were obtained after enrichment in the selective broth of the method.

PRACTICABILITY

Implementation of alternative method only

- **Response time**:

- **Positive** results are obtained in 2 to 8 days using the alternative method against 4 to 11 days using the reference method.
- **Negative** results are obtained in 1 day using the alternative method against 4 to 5 days using the reference method.
- In the case of results presumed positive using the alternative method, but given negative following confirmation, these negative results are obtained in 2 days.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2009 with 16 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a *Listeria monocytogenes* strain at the 4 following 3 levels of contamination:

- 0 CFU/25ml
- 3 CFU/25ml
- 22 CFU/25ml

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

The following results were obtained:

Contamination 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	128	120	96	96	96	0	0
1	128	120	96	0	0	96	96
2	128	120	96	1	5	95	91

** A laboratory did not do the assays.*
***The results of three laboratories were not taken into account in the interpretation because they received the samples after the deadline.*

Calculations

- Relative accuracy = **96.6%**
- % specificity = **100%**
- % sensitivity = **97.4%**

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:	Reference method:
(PA + PD) / (PA + PD + ND) = 97.9%	(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:
COR= accordance x (100 - concordance) / concordance x (100 – 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	94%	89.8%	1.79

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

Contamination level	Accordance	Concordance	COR
L0	100%	100%	1.00
L1	100%	100%	1.00
L2	98%	97.9%	1.15

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