Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: BIO 12/18 - 03/06

Validation date :	**09.03.2006**
Renewal date :	**03.12.2009**
End of validity :	**09.03.2014**

The company
(head office, distributor and production site)

BIOMERIEUX
69280 MARCY L'ETOILE
FRANCE

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

VIDAS LDUO

Protocol references: **13282** versions **I** and **J**

SCOPE

All human food products and environmental samples

RESTRICTIONS

None

REFERENCE METHOD

EN ISO 11290-1 (1997) including **amendment A1** (2004): 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
www.afnor.org - www.afnor-validation.com

PRINCIPLE OF THE METHOD

The VIDAS LDUO test is an enzyme immuno assay test for the simultaneous detection and differentiation of *Listeria monocytogenes* and *Listeria* antigens using the ELFA technique (Enzyme Linked Fluorescent Assay) on the automated VIDAS system.

The disposable SPR serves both as the solid phase and as the pipetting device for the test. The SPR is coated with anti-*Listeria monocytogenes* and anti-*Listeria* antibodies adsorbed on its surface. The other reagents of the immunological reaction are ready to use and pre-dispersed in the sealed reagent strip. All the steps are performed automatically by the instrument. At the final step of reading, the fluorescence is measured by the instrument, which supplies 2 test values per sample.

In the context of NF VALIDATION, all samples identified as positive by the alternative method must be confirmed from the non-heated LX broth stored at 2-8 °C by one of the following means:

- According to classical tests described in methods standardized by CEN, ISO or AFNOR (including a purification step), and to the protocol indicated by the supplier,

  It is possible to perform an API strip without previous purification if the colony is well isolated on the selective agar plate (tested in the validation study),

- If a *Listeria monocytogenes* is detected: by using a chromogenic agar plate issued from a method certified NF VALIDATION. The presence of typical *Listeria monocytogenes* colonies after the streaking of the LX broth allows to confirm the presence of *Listeria monocytogenes*.

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

NOTE 1

As a VIDAS LDUO test allows the simultaneous detection of both *Listeria monocytogenes* and *Listeria spp*, two answers are supplied by the test: presence or absence of *Listeria monocytogenes* (DLMO) and presence or absence of *Listeria spp* (DLIS).

The protocol described in EN ISO 16140 standard was adapted to the double detection of the VIDAS LDUO test in the validation study.

NOTE 2 (History of validation)

In December 2009, the renewal of the validation has been pronounced without performing complementary assays since neither the VIDAS LDUO method, nor the reference method, nor the protocol validated have been modified.

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY

Comparison of performances of the alternative method and the reference method

In 2005, tests were carried out on 466 product samples, of which 171 were naturally contaminated, 109 artificially contaminated, and 189 were non-contaminated. All samples belonged to the following principal food product categories:

Meat products, vegetables, dairy products, seafood products, and environmental products

Out of 280 positive samples (naturally or artificially contaminated), 113 samples contained just *Listeria monocytogenes*, 65 contained *Listeria monocytogenes* in a mixture, and 102 samples contained *Listeria* non-*monocytogenes* strains.

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

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

***Listeria spp* (DLIS) answer**

	Reference method positive (R+)	**Reference method negative (R-)**
Alternative method positive (A+)	Positive agreement A+ / R+ PA = **239** (1)	Positive deviation A+ / R- PD = **26** (1)
Alternative method negative (A-)	Negative deviation A- / R+ ND = **15** 2)	Negative agreement A- / R- NA = **186** (3)

(1) Confirmed positives

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

***Listeria monocytogenes* (DLMO) answer**

	Reference method positive (R+)	**Reference method negative (R-)**
Alternative method positive (A+)	Positive agreement A+ / R+ PA = **155** (1)	Positive deviation A+ / R- PD = **16** (1)
Alternative method negative (A-)	Negative deviation A- / R+ ND = **7** 2)	Negative agreement A- / R- a) NA = **288** (3) b) NA = **186** (3)

a) stating the samples contaminated with *Listeria* non-*monocytogenes* as negatives

b) eliminating all the results issued from samples contaminated with *Listeria* non *monocytogenes* in order to restore the balance between positive and negative samples

(1) Confirmed positives

(2) Of which none sample presumed positive by the VIDAS LDUO (DLMO positive), negative after confirmation

(3) Of which one sample was negative by the reference method and according to the DLMO answer of the VIDAS LDUO test, but positive according to the DLIS answer and *Listeria monocytogenes* positive by streaking of the LX broth.

Percentages obtained compared to the reference method are as follows:

	L. spp (**DLIS**) answer	*L. monocytogenes* (**DLMO**) answer
Relative accuracy : AC %	91.2	93.7
Relative specificity : SP %	87.7	92.1
Relative sensitivity : SE %	94.1	95.7

Note: **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):

	Alternative method (**SE** %)	Reference method (**SE** %)
DLIS answer *L. spp*	(PA + **PD**) / (PA + **PD** + ND) = **94.6**	(PA + **ND**) / (PA + **PD** + ND) = **90.7**
DLMO answer *L. monocytogenes*	(PA + **PD**) / (PA + **PD** + ND) = **96.1**	PA + **ND**) / (PA + **PD** + ND) = **91.0**

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

	Y = PD + ND	d minimum	D = [PD – ND]	Conclusion
DLIS answer *L. spp*	Y = 41 so Y > 22	13	11	equivalence
DLMO answer *L. monocytogenes*	Y = 23 so Y > 22	10	9	equivalence

Conclusion

Both methods are not different in statistical term.

The number of discrepant samples is linked to the first enrichment broths that are different for the reference method and the VIDAS LDUO test.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2005, on 6 combinations of food products/strains.

These products represent the following food matrices:

Meat products, vegetables, dairy products, seafood products, environmental samples

Products were analysed **6 times** by **both 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**
Raw milk	*L. monocytogenes* 1/2b	0.6 [0.4 - 0.9]	0.5 [0.3 – 0.8]
Raw milk	*L. innocua*	1.4 [0.8 – 2.5]	1.3 [0.7 – 2.5]
Potted minced (rillettes)	*L. welshimeri*	0.6 [0.3 – 1.0]	0.5 [0.3 – 0.9]
Smoked salmon	L. monocytogenes 1/2a	0.7 [0.4 – 1.3]	0.7 [0.4 – 1.3]
Red cabbage	*L. monocytogenes* 4b	0.4 [0.3 – 0.7]	0.5 [0.3 – 1.0]
Processed water	*L. monocytogenes* 1/2c	0.8 [0.5 – 1.3]	0.6 [0.5 – 0.8]

(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

As a whole, the detection level of the alternative method is identical that of the reference method. It is assessed between 0.3 and 2.5 cells/25g.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 50 strains of *Listeria monocytogenes* were detected by the VIDAS LDUO test (DLMO answer) out of 50 tested.
- 30 strains of *Listeria* non-*monocytogenes* were detected by the VIDAS LDUO test (DLIS answer) out of 30 tested and showed a negative result for DLMO.

  The study of 31 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 in *Listeria spp* are obtained in 4 to 5 days when using the alternative method (including confirmation with API strip) and up to 10 days (if confirmation with classical test) compared to 7 to 11 days when using the reference method.
- **Positive** result in *Listeria monocytogenes* are obtained in 3 to 4 days when using the alternative method (if confirmation by streaking onto chromogenic agar plate) and up to 10 days (if confirmed by classical tests), compared to 7 to 11 days with the reference method.
- **Negative** results are obtained in 2 days when using the alternative method compared to 5 days when using the reference method.
- In the case of results presumed positive when using the alternative method, but rendered negative following confirmation, these negative results are obtained in 3 to 4 days.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2006 with 17 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:

- 0,
- 3 cells/ml (level1),
- 30 cells/ml (level 2).

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

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	136	120	120	120	120	0	0
1	136	120	120	5	4	115	116
2	136	120	120	0	0	120	120

REF: reference method
ALT: alternative method

** One laboratory received the samples after the dead line and did not performed the analysis*
*** Another laboratory did not perform the analysis due to a problem of update of the VIDAS software.*

Calculations

- Relative accuracy is **97.5 %**
- % specificity is **100 % for both reference and alternative methods**
- % sensitivity is **98.3 % for the alternative method** and **97.9 % for the reference method.**

Interpretation

Results of the inter-laboratory study are comparable to those obtained during the preliminary study.

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	94 %	94 %	1,01
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	93 %	92 %	1,01
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