AFNOR Validation

Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: CHR-21/1 - 12/01

Validation date :	**13.12.2001**
Renewal date*:	**10.03.2006**
	25.09.2009
End of validity :	**13.12.2013**

**** EN ISO 16140 protocol was used in 2005 during the first renewal study***

The company (Head office and production site)	**CHROMagar** 4, place du 18 Juin 1940 F-75006 Paris	**Retailer**	**Laboratoires Humeau** 4, rue Képler ZA de Gesvrine – BP4125 F- 44241 La Chapelle/Erdre Cedex

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

CHROMagar™ Listeria

Protocol reference: NT-EXT-009 (Version 4)
NT-EXT-009-Annexe (Version 1)
NT-EXT-026 (Version 3)

SCOPE

All human food products and environmental samples.

RESTRICTIONS OF USE

None.

REFERENCE METHOD

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

Deputy General Manager
Jacques BESLIN

PRINCIPLE OF THE METHOD

CHROMagar™ Listeria method includes a single enrichment step in half Fraser broth followed by a isolation of 100µl onto a single CHROMagar™ Listeria plate which is a chromogenic medium allowing the specific detection of *Listeria monocytogenes* after 24 hours.
In case of absence of typical colonies after 24 hours of incubation on CHROMagar™ Listeria, the method indicates absence of *L.monocytogenes,* thus after a total of 48 hours.

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

- According to classical tests described in methods standardized by CEN or ISO (including a purification step), starting from CHROMagar™ Listeria.
- Directly from a typical colony from CHROMagar™ Listeria by spotting the colony onto CHROMagar™ Identification Listeria: *Listeria monocytogenes* displays a mauve colour surrounded by a white opaque halo.

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)

1/ 2005 renewal study: Since its first validation granted on 2001, the CHROMagar™ Listeria method has not been modified. A new mode of confirmation for the positive samples by CHROMagar™ Identification Listeria test has been added.

The reference method has been changed (addition of amendment A1) and the protocol described in standard EN ISO 16140 has been applied. The study has been entirely done again, except for the practicability study from 2001 completed to test the use of the new confirmation option.

2/ 2009 renewal study: Since the previous validation in 2006, the formula of CHROMagar™ Listeria numeration test has not changed, as well as the reference method and the protocol described in standard EN ISO 16140. No complementary assays of validation were done for this validation renewal.

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 439 product samples, of which 88 were naturally contaminated, 76 artificially contaminated, and 275 non-contaminated, belonging to the following principal food product categories: meat products, dairy products, seafood, vegetables 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 = **150** (1)	Positive deviation A+ / R- PD = **3** (1)
Alternative method negative (A-)	Negative deviation A- / R+ ND = **11** (2)	Negative agreement A- / R- ND = **275** (3)

(1) Confirmed positives

(2) Of which 0 sample presumed positive by the alternative method was negative after confirmation

(3) Of which 0 samples presumed positive by the alternative method were negative after confirmation

Percentages obtained compared to the reference method are as follows:

- Relative accuracy : **AC = 96.8%**
- Relative specificity : **SP = 98.9%**

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

- Relative sensitivity : **SE = 93.2%**

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) = 93,3%	(PA + ND) / (PA + PD + ND) = 98,2%

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

PD = 3, ND = 11 therefore Y = PD + ND = 14 ; $6 \leq Y \leq 22$ m = 3, M = 2 therefore m > M

Conclusion

Both methods are not statistically different.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2005, on 5 combinations of food products/strains.

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**
Pâté	*L. monocytogenes* 4e	0.4 [0.2 - 1.0]	0,4 [0.2 – 1.0]
Smoked salmon	*L. monocytogenes* 1/2b	0.3 [0.1 - 1.0]	0.3 [0.1 - 1.0]
Salad	*L. monocytogenes* 1/2a	0.9 [0.5 - 1.6]	0.9 [0.5 - 1.6]
Raw milk	*L. monocytogenes* 1/2a	0.5 [0.2 - 1.3]	0.5 [0.2 - 1.3]
Industrial water	*L. monocytogenes* 1/2a	0.9 [0.3 - 2.2]	0.7 [0.3 - 1.9]

(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 detection limit of the alternative method is between 0.1 and 2.2 CFU/25g.
The detection limit of the reference method is between 0.1 and 1.9 CFU/25g.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 50 strains of *L.monocytogenes* were detected out of 50 tested.
- The study of 31 strains not belonging to the species *L.monocytogenes* did not detect the presence of any cross-reaction.

PRACTICABILITY

Implementation of alternative method only

- **Response time**:
  - **Positive** results are obtained in 3 days using the alternative method *(when confirmation is done with the CHROMagar™ Identification Listeria* test) or 5 to 8 days (*including confirmation according to classical tests of the reference method, with purification step included*) against 7 to 11 days using the reference method.
  - **Negative** results are obtained in 2 days using the alternative method against 5 days (if absence of typical colonies) to 11 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 3 days*(when confirmation is done with the CHROMagar™ Identification Listeria test*) and up to 5 to 8 days (if confirmation is done with classical tests).

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2006 with 14 participating laboratories. The analyses were carried out on samples of milk (obtained by mixing 50% of pasteurized milk with 50% of half skimmed milk) artificially contaminated with a strain of *L.monocytogenes* serotype 1/2a the 3 following levels of contamination:

- 0
- slightly superior to relative detection level
- 10 times superior to previous level

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

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	112	112	112	112	112	0	0
1	112	112	112	0	0	112	112
2	112	112	112	0	0	112	112

REF: reference method
ALT: alternative method

Calculations

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

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
L1	100	100	1
L2	100	100	1

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

Contamination level	Accordance	Concordance	COR
L0	100	100	1
L1	100	100	1
L2	100	100	1

Conclusion

Variability of the alternative method (accordance, 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