afnor CERTIFICATION

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Ref.: SSO/JFY/NF102/Clients/bioMérieux/
Avis BT_AccuProbe Listeria monocytogenes_2015-02-06_R5

Subject: NF VALIDATION mark

La Plaine Saint-Denis, February 10th, 2015

Dear Sir,

Following the positive agreement expressed on February 6th, 2015, by the Technical Board "Food microbiology" of the NF VALIDATION mark (NF102), I beg to inform you that the **NF VALIDATION certification is renewed** for the following analysis method:

AccuProbe *Listeria monocytogenes*

Certificate reference n° BIO 12/04 - 02/95, with end of validity 7th-Feb.-2019

The alternative method is validated for the detection of *Listeria monocytogenes* in all human food products and industrial environment samples, by comparison to the reference method EN ISO 11290-1 :1997 and its amendment A1 :2005 and following the validation protocol EN ISO 16140 :2003.

A further letter will mention full conclusions and possible reservations made by the Technical Board. If reservations are mentioned, I ask you to take them into account without any delay.

Yours Sincerely.

Managing Director
Franck LEBEUGLE

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NF VALIDATION EN ISO 16140 BY AFNOR CERTIFICATION

Alternative methods for agribusiness
Analytical performances certified

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

Certificate No. : BIO 12/4 – 02/95

Validation date :	**23.03.1999**
Renewal dates :	**18.01.1999**
	06.02.2003
	02.02.2007*
	02.12.2010
End of validity :	**07.02.2015**

****Protocol EN ISO 16140 was used in 2006 for the preliminary study and the inter-laboratory study***

The company

BioMérieux SA
69280 Marcy l'Etoile (France)

Production site

GEN-PROBE Inc.
San Diego, CA 92121 (USA)

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

AccuProbe *Listeria monocytogenes*

Protocol reference: bioMérieux ref. 39500/Gen-Probe Cat. No. 2920 reference 103051F-01 Rev C

SCOPE

All human food products and environmental samples of production

RESTRICTIONS OF USE

None

REFERENCE METHOD

EN ISO 11290-1 (1997) including **amendment A1** (2004): Microbiology of food - horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Method of Detection

Deputy General Manager
Jacques BESLIN

cofrac CERTIFICATION DE PRODUITS INDUSTRIELS ACCRÉDITATION N° 5-0030 PORTÉE DISPONIBLE SUR WWW.COFRAC.FR

PRINCIPLE OF THE METHOD

After an enrichment phase in half Fraser broth followed by isolation on Palcam or Oxford agar, the AccuProbe *Listeria monocytogenes* test permits detection of *Listeria monocytogenes* by a nucleic acid hybridization test. The labelled RNA-DNA complexes formed are detected by photon emission with a luminometer.

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

- From the isolation medium (Palcam or Oxford agar), by implementation of conventional tests described in the standardized methods by CEN, ISO or AFNOR (including the purification stage),
- By isolation on an OAA chromogenic agar or on a chromogenic agar from a method with NF VALIDATION certification, according to instructions on the manufacturer's package insert (ALOA®, Compass®, CHROMagar, OAA and Rapid L'mono chromogenic media were used for the validation study)
- By using any other method certified NF VALIDATION with a different principle than the AccuProbe method. The whole of the validated protocol of the second method must be respected.

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

NOTE (history of validation)

For the renewal study of December 2010, no additional test according to EN ISO 16140 protocol was made because the AccuProbe *Listeria monocytogenes* method was not modified since the previous validation, and because the protocol of validation and the reference method remain unchanged.

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2006 on 346 product samples including 113 naturally contaminated, 56 artificially contaminated and 177 non-contaminated products, belonging to the following main food categories:

Dairy products, meat products, vegetable and miscellaneous products, seafoods and environmental samples.

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

Table of results obtained with Palcam agar (Cf. table 1 of standard EN ISO 16140):

Answer	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement A+ / R+ PA = **145** (1)	Positive deviation A+ / R- PD = **8** (1)
Alternative method negative (A-)	Negative deviation A- / R+ ND = **3** (2)	Negative agreement A- / R- NA = **178** (3)

(1) Confirmed positives
(2) Including no sample presumed positive by the alternate method was negative after confirmation
(3) Including 3 samples presumed positive by the alternate method that were non-confirmed

Percentages obtained, compared to the reference method are as follows:

- Relative accuracy: **AC = 96.8** %
- Relative specificity: **SP = 95.7** %
- Relative sensitivity: **SE = 98.1** %

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 the alternative method):

Alternative method:	Reference method:
(PA + PD) / (PA + PD + ND) = **98.2%**	(PA + ND) / (PA + PD + ND) = **95.2%**

Table of results obtained with Oxford agar for dairy products and with Palcam agar for the other products (Cf. table 1 of standard EN ISO 16140):

Answer	**Reference method positive (R+)**	**Reference method negative (R-)**
Alternative method positive (A+)	Positive agreement A+ / R+ PA = **156** (1)	Positive deviation A+ / R- PD = **8** (1)
Alternative method negative (A-)	Negative deviation A- / R+ ND = **4** 2)	Negative agreement A- / R- NA = **178** (3)

(1) Confirmed positives
(2) Including no sample presumed positive by the alternate method was negative after confirmation
(3) Including 3 samples presumed positive by the alternate method which were non-confirmed

Percentages obtained compared to the reference method are as follows:

- Relative accuracy: **AC = 96.5** %
- Relative specificity: **SP = 95.7** %
- Relative sensitivity: **SE = 97.5** %

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):

Alternative method:	Reference method:
(PA + PD) / (PA + PD + ND) = **97.6 %**	(PA + ND) / (PA + PD + ND) = **95.2 %**

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

For the results obtained with Palcam agar:
PD = 8, ND = 3 therefore Y = PD + ND = 11; 6 ≤ Y ≤ 22 m = 3, M= 1, therefore m > M

For the results obtained with Oxford agar for dairy products and with Palcam agar for the other products:
PD = 8, ND = 4 therefore Y = PD + ND = 12; 6 ≤ Y ≤ 22 m = 4, M= 2, therefore m > M

Conclusion

The two methods are not statistically different.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were performed in 2006 on the 5 food products/strain combinations described in the table below.

These products represent the following categories of food: dairy products, meat products, vegetable products and miscellaneous, seafoods and environmental samples

Products were analyzed **6 times**, by the **two methods**, at **4 levels** of contamination

The following results were obtained:

Matrix	Strain	Relative detection level LOD_{50} (3) With confidence interval (CFU/25g or 25 ml)	
		Alternative method	**Reference method**
Rillettes (Potted minced meat)	*Listeria monocytogenes* 1/2	0.5 [0.3 - 0.7]	0.5 [0.3 - 0.7]
Smoked salmon	*Listeria monocytogenes* 1/2a	0.2 [0.1 - 0.5]	0.2 [0.1 - 0.5]
Raw vegetables	*Listeria monocytogenes* 1/2	0.3 [0.1 - 0.6]	0.3 [0.1 - 0.6]
Raw milk	*Listeria monocytogenes* 4b	1.2 [0.3 - 5.2]	1.2 [0.3 - 5.2]
Processed water	*Listeria monocytogenes*	0.5 [0.2 - 1.2]	0.5 [0.2 - 1.2]

(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 level of detection of the alternative method and the reference method is between 0.1 and 5.2 CFU/25 g.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 50 strains of *Listeria monocytogenes* were detected out of the 50 tested.
- The study of 30 stains not belonging to the *Listeria monocytogenes* species did not detect the presence of cross-reactions.

PRACTICABILITY

Implementation of alternative method only

- **Response time:**

- **Positive** results are obtained in 3 to 6 days using the alternative method versus 11 to 12 days with the reference method.

- **Negative** results are obtained in 2 to 4 days with the alternative method versus 2 to 5 days with 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 to 6 days.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2006 with 13 participating laboratories. The analyses were carried out on samples of partially skimmed pasteurized milk, artificially contaminated with a strain of *Listeria monocytogenes* 4b at the 3 following levels of contamination:

- 0 CFU / 25ml
- 1 - 10 CFU / 25ml
- 5 - 50 CFU / 25 ml

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 exploited **	Number of negative results		Number of positive results	
				REF	ALT	REF	ALT
0	104	104	79**	79**	79**	0	0
1	104	104	80	0	0	80	80
2	104	104	80	0	0	80	80

* The results of three laboratories were not retained in the interpretation:
- One laboratory did not respect the protocol of the alternative method.
- Two laboratories obtained positive results by the alternative method after 48h of enrichment. One inter-contamination was suspected.

** One laboratory analyzed only 7 samples at this level (a bag was pierced on receipt).

Calculations

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

Interpretation

Results of the collaborative 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 samples 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%	98.8%	1.01
L1	100%	100%	1.00
L2	100%	100%	1.00

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

Contamination level	Accordance	Concordance	COR
L0	100%	98.8%	1.01
L1	100%	100%	1.00
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