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Ref.: SSO/SSO/NF102/Clients/OXOID Thermo Fisher Scientific/
Avis BT_Listeria Precis Enumeration_2018-09-11 (P1).docx

Subject: NF VALIDATION mark

OXOID Ltd, Part of Thermo Fisher Scientific
Mrs Ana-Maria LEONTE
Wade Road
RG24 8PW Basingstoke
Hampshire
United Kingdom

La Plaine Saint-Denis, September 11th, 2018

Dear Madam,

The NF VALIDATION certificate of the following alternative method:

Listeria Precis™ Enumeration	**Ref. UNI 03/05-09/06**

will expire on September 15th, 2018, before that complete results of the renewal study may be examined by the Technical Board of the NF VALIDATION mark (NF102), in its application to the food industry.

Consequently, following the positive agreement of the dedicated Technical Board, I declare that you can continue to refer to this certificate till March 31st, 2019.

Yours Sincerely.

Managing Director
Franck LEBEUGLE

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Ref.: SSO/AYV/NF102/Clients/OXOID Ltd
Avis BT_Listeria Precis_2014-07-03_(R2)

Subject: NF VALIDATION mark

OXOID Ltd
Monsieur Jonathan CLOKE
Wade Road
RG24 8PW Basingstoke
Hampshire
UK

La Plaine Saint-Denis, July 4th, 2014

Dear Sir,

Following the positive agreement expressed on July 3rd, 2014, 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:

Listeria Precis™

Validated for the enumeration of *Listeria monocytogenes* in all human food and production environment samples

Certificate reference n# UNI 03/05-09/06, with end of validity 15th-Sept.-2018

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
Florence MÉAUX

AFNOR Validation

Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: UNI 03/05 - 09/06

Validation date :	**09.15.2006**
Extension date :	**03.29.2007**
Renewal :	**07.02.2010**
End of validity :	**09.15.2014**

The company (Head office and Production site)	**OXOID Ltd** Wade Road Basingstoke, Hampshire RG24 8 PW, England, UK	**Distributeur**	**OXOID Thermo Fisher Scientifics** 6 route de Paisy 69571 Dardilly Cedex France

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

Listeria Precis™
(Enumeration method)

Protocol reference: **OCLA-D3 07-2010**

SCOPE

All human food products and environmental samples

RESTRICTIONS FOR USE

None

REFERENCE METHOD

EN ISO 11290-2 (1998) including **amendment A1** (2004) : Food Microbiology – horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2 : Enumeration method

Deputy General Manager
Jacques BESLIN

PRINCIPLE OF THE METHOD

The Listeria Precis™ method is a medium for isolation and presumptive identification of *Listeria monocytogenes*. The method consists of an incubation of a specific selective pre-enrichment broth, followed by an isolation of colonies onto the chromogenic medium Brilliance™ Listeria Agar.

In the context of AFNOR Validation, all samples identified as positive by Listeria Precis™ 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) from colonies isolated on a chromogenic media ;
- Implementing the OBIS MONO test from characteristic colonies isolated before onto Brilliance™ Listeria agar.

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

Note 1 : History of validation

The **extension study done in 2007** allowed to validate a new method of confirmation: OBIS MONO test.
Assays were performed on strains grown in a nutritious broth and isolated in parallel onto Brilliance™ Listeria and TSA-YE agars :

- 150 strains of *Listeria monocytogenes* from different sertoypes and origins were tested
- 100 strains not belonging to the genus *Listeria monocytogenes* wer tested

The results were in accordance with those expected.

AFNOR Validation **certification was renewed in 2010**. Since the previous validation, the alternative method was not modified and the reference method remains unchanged. Reanalysis of data of interlaboratory study realized in 2006 was done in accordance with the draft amendment 1 to EN ISO 16140 standard (version prA1 :2009). New results are in accordance with previous ones and are detailed in this certificate.

Note 2 : Modification of the trademark reference (February, 2008)

The alternative method formerly OCLA (Oxoid Chromogenic Listeria Agar) is named since 2008 **Listeria Precis™**. The name of the chromogenic media OCLA was replaced by **Brilliance™ Listeria**.

LINEARITY AND relative ACCURACY

Comparison of performances of the alternative method and the reference method

Linearity study:

Tests were performed in 2006 on the 6 food product/strain combinations and for the food categories given in the table below.

The samples were analyzed **in duplicate** with each of the **two methods**, at the five following artificial contamination levels:

- 10 à 50 CFU/g
- 50 à 100 CFU/g
- 100 à 500 CFU/g
- 500 à 1 000 CFU/g
- 1 000 à 10 000 CFU/g

The following results were obtained:

Food category	Food product/strain pair	Regression line
Meat products	Potted meat / *Listeria monocytogenes* 4e	y = 0.989 x+ 0.114
Dairy products	Raw milk / *Listeria monocytogenes* 1/2a	y = 1.019 x – 0.074
Vegetables	Lettuce / *Listeria monocytogenes* 1/2a	y = 1.008 x – 0.026
Seafood products	Smoked salmon / *Listeria monocytogenes* 1/2b	y = 1.042 x – 0.145
Egg products	Raw egg product / *Listeria monocytogenes* 1/2a	y = 0.978 x – 0.016
Environmental samples	Water process / *Listeria monocytogenes* 1/2a	y = 0.968 x + 0.132

y = log(N alternative method)
x = log(N reference method)

Accuracy study:

Tests were performed in 2006. The statistical interpretation was conducted on 65 results, including 12 naturally contaminated samples and 53 artificially contaminated samples, belonging to the following major food categories:

Meat products, Dairy products, Vegetables, Seafood products, Egg products, Environmental samples.

The samples were analyzed **in duplicate** with each of the **two methods**.

As an indication, the contamination (concentration) ranges were as follows:

Food category	Contamination range* (in log CFU/g)
Meat products	1.0 – 4.0
Dairy products	1.5 – 4.1
Seafood products	1.0 – 3.8
Vegetables	1.7 – 5.1
Egg products	1.3 – 5.0
Environmental samples	1.7 – 5.1

The equation of the regression line between the alternative method and the reference method, for all categories combined, is as follows:

y = 1.040 x – 0,182

y = log(N alternative method)
x = log(N reference method)

The repeatability for both methods and the bias between the two methods were determined according to the method of calculation used for the interlaboratory study (see sections 6.3.5 and 6.3.6 of the standard EN ISO 16140). These results provide additional information for the accuracy criterion.

The limits of repeatability (in log) obtained for the alternative method and the reference method are as follows:

Alternative method	Reference method
r = **0,311**	r = **0,264**

The bias (in log) between the two methods (alternative method - reference method) is as follows:

p = - 0,03 log CFU/g

Conclusion for linearity and relative accuracy:
Linearity and accuracy studies show that results obtained with alternative method are comparable to the ones obtained with reference method.

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)
Use of alternative method only

Study done in 2004 during validation of Listeria Precis™ detection method of *Listeria monocytogenes*

- 48 strains of *Listeria monocytogenes* were detected out of 50 tested, after 24 hours of incubation. The 2 strains which did not grow were collection strains of serotypes 3a and 4e.
- The study of 30 strains not belonging to the genus *Listeria monocytogenes* did not detect the presence of cross-reactions.

PRACTICABILITY
Use of alternative method only

- **Time of response :**
  - **Positive** results are obtained with the alternative method in 4 to 7 days as for the reference method.
  - **Negative** results are obtained in 2 days with the alternative method as for 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 4 to 7 days.

INTERLABORATORY STUDY

The interlaboratory study was conducted in 2006 with 13 participating laboratories. The analyses were carried out on samples of milk (mixture of 50 % of pasteurized whole milk and 50 % of pasteurized semi-skimmed milk), artificially contaminated with a *Listeria monocytogenes* 1/2a strain at the 4 following levels:

- level 0: < 10 CFU/ml
- level 1: 50 CFU/ml
- level 2: 500 CFU/ml
- level 3: 5 000 CFU/ml

The laboratories tested, using each of the **two methods, two replicates per contamination level.**

The results calculated in accordance with the draft amendment 1 to EN ISO 16140 standard (version prA1 :2009) were the following :

Contamination level	Number of samples taken into acccount*	Reference method		Alternative method		Bias
		Repeatability standard deviation S_r	Reproducibility standard deviation S_R	Repeatability standard deviation S_r	Reproducibility standard deviation S_R	
Level 1	10	0,2415	0,1982	0,2045	0,2433	1,14
Level 2 (a)	10	0,0543	0,0659	0,0914	0,1325	0,82
Level 2 (b)	10	0,2607	0,2082	0,1900	0,1953	0,35
Level 3	10	0,0103	0,0094	0,0130	0,0206	0,22

** Results of 3 laboratories were not taken into account (one did not receive the samples in time and 2 others did not respect the protocol)*

(a) : Calculation for Level 2 taking into account results obtained on 3 plates inoculated with mother suspension
(b) : Calculation for Level 2 taking into account results obtained on 1 or 3 plates inoculated with 0,1ml of mother suspension

NB: Limit of repeatability $r = 2.8\ S_r$, with S_r: repeatability standard deviation
Limit of reproducibility $R = 2.8\ S_R$, with S_R: reproducibility standard deviation

Conclusion

The interlaboratory study shows that the results obtained with the alternative method are similar to those obtained with 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