



**Alternative methods for agribusiness
Analytical performances certified**

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

Certificate No.: CHR- 21/2 – 12/06

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

**CHROMagar™ Listeria numeration
for enumeration of *Listeria monocytogenes***

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

SCOPE

All human food 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

CHROMagar™ *Listeria* numeration for enumeration of *Listeria monocytogenes* is a method including a suspension broth and the chromogenic culture media CHROMagar™ *Listeria*, allowing the specific detection of *Listeria monocytogenes*.

In case of positive result with the enumeration method, confirmation is not necessary when the presence of *Listeria monocytogenes* has already been confirmed by the detection method.

In the other cases, samples identified as positive by the alternative method must be confirmed by one of the following means:

- According to classical tests described in methods standardised by CEN or ISO (including a purification step), starting from CHROMagar™ *Listeria*.
- Directly from a typical colony well isolated on 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 results obtained.

NOTE (validation history)

In September 2009, the certification under AFNOR Validation mark was renewed for CHROMagar™ *Listeria* numeration test without complementary assays. Since the previous validation in 2006, the formula of CHROMagar™ *Listeria* numeration test has not changed, as well as reference method and the protocol described in standard EN ISO 16140.

LINEARITY AND relative ACCURACY

Comparison of performances of the alternative method and the reference method

Linearity study:

Tests were performed in 2006 on the 5 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 to 50, 50 to 100, 100 to 500, 500 to 1 000 and 1 000 to 10 000 CFU/g.

The following results were obtained:

Food category	Food product/strain pair	Regression line
Meat products	Pâté / <i>L. monocytogenes</i> 4e	$Y = 0,932 X + 0,305$
Dairy products	Raw milk / <i>L. monocytogenes</i> 1/2a	$Y = 1,011 X - 0,004$
Vegetal products	Salad / <i>L. monocytogenes</i> 1/2a	$Y = 1,024 X - 0,051$
Seafood products	Smoked salmon / <i>L. monocytogenes</i> 1/2b	$Y = 1,006 X - 0,031$
Environmental samples	Industrial water / <i>L. monocytogenes</i> 1/2a	$Y = 0,967 X + 0,122$

$Y = \log(N \text{ alternative method})$

$X = \log(N \text{ reference method})$

Accuracy study :

Tests were performed in 2006. The statistical interpretation was conducted on 54 results, including 10 naturally contaminated samples and 44 artificially contaminated samples, belonging to the following major food categories:

Meat products, dairy products, vegetal products, seafood products and 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	1,70 to 4,15
Dairy products	1,48 to 4,08
Vegetal products	1,60 to 4,00
Seafood products	1,84 to 5,11
Environmental samples	1,70 to 5,18

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

$$Y = 1,008 X - 0,012$$

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
r = 0,332

Reference method
r = 0,280

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

p = 0,02 if one consider the median value
or **D = 0,01** if one considers the average of individual bias.

Conclusion for linearity and relative accuracy:

Studies of linearity and relative accuracy show that results obtained with the alternative method are similar to those obtained with the reference method.

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)

Use of alternative method only

Specificity of the alternative method has been studied in 2001, during the Validation of the method for the detection of *Listeria monocytogenes*.

- 50 strains of were detected out of 50 tested.
- The study of 31 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 3 days (if confirmation with CHROMagar™ Identification Listeria test), or 4 to 7 days (if confirmation with classical tests), as opposed to 4 to 7 days with the reference method.
- **Negative** results are obtained in 2 days with both alternative method and reference method.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2006 with 12 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 *Listeria monocytogenes* 1/2a strain at the 4 following levels:

- < 10 CFU/ml
- 50 - 500 CFU/ml
- 500 - 5 000 CFU/ml
- 5 000 - 50 000 CFU/ml

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

The following results were obtained (for the 3 levels superior to 50 CFU/ml):

Contamination level	Number of samples taken into account*	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
Level 1	10	0,128	0,215	0,165	0,175	0,047
Level 2	10	0,106	0,106	0,152	0,204	-0,022
Level 3	10	0,182	0,182	0,197	0,197	0,000

* Two laboratories have been excluded, one for not receiving the sample in time, the other for not following the protocol of analysis.

Conclusion

Inter-laboratory study shows that results obtained with alternative method are comparable to those obtained with 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