



Alternative methods for agribusiness  
Analytical performances certified

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

Certificate No : BRD 07/05 – 09/01

Validation date :	28.09.2001
Renewal date* :	09.12.2005
	<b>02.07.2009</b>
Extension dates :	28.09.2006
	25.09.2008
End of validity :	<b>28.09.2013</b>

\* EN ISO 16140 protocol was used on renewal in 2005

**The company** **BIO-RAD**  
(Head office) 3 Boulevard Raymond Poincaré  
92430 MARNES LA COQUETTE  
FRANCE

**Production** **BIO-RAD**  
**site** Route de Cassel  
59114 STEENVOORDE  
FRANCE

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

**RAPID'L. MONO**  
**Enumeration method**

Protocol reference : RAPID'L. Mono (356-3694 et 355-5294) – V11  
Test Rhamnose (355-3669) – V0

**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

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  
[certification@afnor.com](mailto:certification@afnor.com) - [www.afnor-validation.com](http://www.afnor-validation.com)

## PRINCIPLE OF THE METHOD

The method enables enumeration on a single medium (RAPID'L. *Mono*) after preparing samples (revivification) according to reference method ISO 11290-2.

The RAPID'L. *Mono* is an agar plate for specific detection of *Listeria monocytogenes* by chromogenic revelation of PIPLC activity (Phosphatidyl Inositol Phospholipase C) in *L. monocytogenes* and *L. ivanovii*, which form blue colonies. Xylose fermentation is used to differentiate *L. ivanovii* (xylose +ve: forms a yellow halo around the colony) from *Listeria monocytogenes* (xylose –ve: no halo around the colony).

If there is a positive result from the enumeration method, confirmation is not necessary insofar as the presence of *Listeria monocytogenes* has been confirmed during detection.

In other cases, confirmation of positive samples must be carried out for colonies isolated by RAPID'L. *Mono* in one of the following ways :

- Purification of colonies and identification according to classical tests described in methods standardized by CEN or ISO;
- Using nucleic probes as described in ISO 7218 standard, using isolated colonies (with or without purification step) ;
- By implementing a spot sub-culture of an isolated colony on an Ottaviani & Agosti *Listeria* plate, directly from RAPID'L. *Mono* ;
- By carrying out Rhamnose test ;
- By implementing any other AFNOR validated method based on a principle different from the alternative method, respecting specifications in the test instructions referenced above. The validated second method protocol should be respected in its entirety, i.e. all stages preceding the intermediary stage from which confirmation is sought must be common to the two methods.

## NOTE (VALIDATION HISTORY)

### 1/Renewal in 2005 with extension of scope

The renewal study carried out in 2005 has made it possible to extend the scope of the RAPID'L. *Mono* method to all food products and environmental samples.

In order to take into account the new validation protocol EN ISO 16140, the study has been almost entirely redone, except for the sections concerning inclusivity/exclusivity and practicability, for which previous results were used.

### 2/ Extension in 2006 for 24 hour reading

A growth activator was added to the formula for RAPID'L. *Mono* medium so that a final reading of *Listeria monocytogenes* could be made after 24 hours  $\pm$  2 hours of incubation (instead of 24 and 48 hours, as before).

The validation/renewal carried out in 2005 was completed for the relative accuracy part on 29 positive interpretable samples covering the whole scope of the procedure. The EN ISO 16140 protocol was used for analysing these samples

The results for all these categories tested showed equivalence of readings of plates incubated for 24 hours at 37°C or incubated for 48 hours at 37°C. These additional results are not included in this certificate.

### 3/ Extension in 2008 for a new test of confirmation

In September of 2008, a new test of confirmation was validated : Rhamnose test.

Tests were done with pure strains on :

- 150 strains 'cibles' of *Listeria monocytogenes* of different serotypes and origin
- 105 strains not 'cibles'

The results obtained were in accordance with those expected.

## 2/ Renewal in 2009

The RAPID'L. *Mono* method was renewed without additional test, the kit having not been modified since the last validation, and the reference method as well as EN ISO 16140 protocol having remained the same.

### LINEARITY AND relative ACCURACY

#### Comparison of performances of the alternative method and the reference method

##### Linearity study :

Tests were performed in 2005 on the 5 food product/strain combinations and for the food categories given in the table below.

The samples were analysed **in duplicate** by 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, 1 000 to 10,000 CFU/g.

The following results were obtained :

Food category	Food product/strain pair	Regression line
Meat products	Rillettes / <i>Listeria monocytogenes</i> 1/2b	$y = 0.950 x + 0.031$
Dairy products	Untreated milk / <i>Listeria monocytogenes</i> 1/2b	$y = 0.998 x - 0.030$
Vegetables	Cabbage / <i>Listeria monocytogenes</i> 4b	$y = 0.965 x + 0.078$
Fishery products	Smoked salmon / <i>Listeria monocytogenes</i> 1/2a	$y = 1.001 x - 0.047$
Environmental samples	Process water / <i>Listeria monocytogenes</i> 1/2c	$y = 1.014 x - 0.148$

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

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

##### Accuracy study :

Tests were performed in 2005. The statistical interpretation analysis was conducted on 54 results, including 30 naturally contaminated samples and 24 artificially contaminated samples, belonging to the following major food categories :

Meat products, dairy products, vegetables, fishery products, environmental samples.

The samples were analysed **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	1.1 – 6.5
Dairy products	1.0 – 5.1
Vegetables	2.1 – 5.5
Fishery products	2.9 – 6.6
Environmental samples	2.0 – 5.3

The regression line between the alternative method and the reference method, for all categories combined, has the following equation :

$$\text{Equation of the regression line : } y = 1.019 x - 0.170$$

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

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

The repeatability for both methods and the bias between the two methods were determined according to the method of calculation used for the inter-laboratory 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
<b>r = 0.21</b>	<b>r = 0.16</b>

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

$$D = -0.09 \log \text{CFU/g}$$

#### **Conclusion for linearity and relative accuracy :**

The linearity and accuracy studies show that the results obtained with the alternative method are comparable to the results obtained with the reference method.

## **SELECTIVITY (INCLUSIVITY/EXCLUSIVITY) 1998 et 2008**

### **Use of alternative method only**

Studies carried out by the Pasteur Institute in Paris (reference centre for *Listeria*) and by the AFNOR VALIDATION expert laboratory in charge of validation assays :

- 357 strains of *Listeria monocytogenes* were detected out of 358 tested. The unrecognised strain was a *Listeria monocytogenes* 3a which did not express PIPLC activity.
- The study of 109 strains not belonging to the genus *Listeria monocytogenes* and 100 non *Listeria* strains did not detect the presence of cross-reactions.

## **PRACTICABILITY**

### **Use of alternative method only**

- **Time to results :**
  - **Positive** results are obtained with the alternative method in 2 days (or 4 – 7 days with confirmation where confirmation was necessary) as opposed to 4 - 7 days with the reference method.
  - **Negative** results are obtained in 2 days by the alternative method as opposed to 2 days (with no confirmation) or 4 – 7 days (with confirmation) by the reference method.

## **INTER-LABORATORY STUDY**

The inter-laboratory study was conducted in 2005 with 15 participating laboratories. The analyses were carried out on samples of pasteurised milk, artificially contaminated with a *Listeria monocytogenes* 1/2b strain at the 4 following levels (in cells/ml) :

- level 0
- level 1: 50 - 500
- level 2: 500 – 5,000
- level 3: 5,000 – 50,000

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

The following results were obtained :

Contamination level	Number of laboratories whose results were taken into account*	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias (alt-ref)
Level 1	11	0.367	0.367	0.563	0.654	-0.054
Level 2	11	0.140	0.140	0.146	0.162	-0.030
Level 3	11	0.050	0.138	0.082	0.194	-0.005

\* Samples from four laboratories were excluded from the results analysis (one due to late receipt and three others due to receipt temperatures that did not comply with requirements).

### **Conclusion for the inter-laboratory study**

The inter-laboratory study shows that the results obtained using the alternative method are comparable with those obtained using the reference method:

- The repeatabilities are comparable for the three levels
- The reproducibility of level 1 is lower than for the reference method, whereas the repeatabilities of levels 2 and 3 are comparable.
- The hypothesis of zero bias between the two methods is statistically accepted for all levels.

### **General conclusion**

The RAPID<sup>L</sup>. Mono method was compared with the method NF EN ISO 11290-2: 2005.

The results obtained enable the conclusion that:

- The linearity of the alternative method is satisfactory,
- The relative accuracy of the alternative method compared to the reference method is satisfactory.

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](http://www.afnor-validation.com)