



**Alternative methods for agribusiness
Analytical performances certified**

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

Certificate No.: AES 10/05 - 09/06

Validation date : 2006.09.15

End of validity : 2010.09.15

The company
(head office,
distribution
and production site)

AES Laboratoire
Rue Maryse Bastié - Ker Lann
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is hereby authorized to refer to this **AFNOR Validation certificate** for the following alternative **qualitative** analysis method:

ALOA COUNT™

Protocol reference: **520080 30/06/08-S**

SCOPE

All foodstuffs for human consumption

RESTRICTIONS OF USE

None

REFERENCE METHOD

NF EN ISO 11290-2 including **amendment A1 (2004)**: Food microbiology - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Enumeration method

A handwritten signature in black ink, appearing to read "J. Beslin", written over a horizontal line.

**Deputy General Manager
Jacques BESLIN**

AFNOR Certification

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PRINCIPLE OF THE METHOD

The method includes a chromogenic agar medium (ALOA™) which is used to detect all *Listeria* by indicating the presence of β -glucosidase and distinguishing *Listeria monocytogenes* by the formation of a distinct halo of precipitation of phospholipids cleaved by its specific phospholipase.

The ALOA COUNT™ method is based on the use of an ALOA™ chromogenic medium inoculated (surface or poured plate technique) by revivified suspension, followed by incubation at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The final result of the count is obtained after 48 hrs. \pm 3hrs.

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, ISO or AFNOR (including a purification step), starting from colonies isolated from ALOA
- With ALOA Confirmation™ technique (according to the instructions in the ALOA Confirmation™ technical data sheet)
- By implementing any other AFNOR VALIDATION certified method based on a principle different from the alternative method, respecting specifications in the test instructions.

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.

NOTES

The two inoculation protocols proposed have been tested.

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 UFC/ gram
- 50 to 100 UFC/ gram
- 100 to 500 UFC/ gram
- 500 to 1,000 UFC/ gram
- 1,000 to 10,000 UFC/ gram

The following results were obtained:

With the ALOA COUNT™ poured plate protocol:

Food Category	Food product/strain pair	Regression line
Meat Products	Chopped Beef / <i>Listeria monocytogenes</i> 4b	$Y = 0.987 X - 0.005$
Seafood Products	Smoked Salmon / <i>Listeria monocytogenes</i> 4b	$Y = 1.006 X - 0.034$
Vegetable Products	Salad / <i>Listeria monocytogenes</i> 1/2 a	$Y = 0.980 X + 0.053$
Milk Products	Raw Milk / <i>Listeria monocytogenes</i> 1/2 a	$Y = 0.986 X + 0.080$
Eggs and Derivatives	Raw Egg / <i>Listeria monocytogenes</i> 1/2 a	$Y = 1.062 X - 0.242$

$Y = \log$ (N alternative method)

$X = \log$ (N reference method)

With the ALOA COUNT™ surface protocol:

Food Category	Food product/strain pair	Regression line
Meat Products	Chopped Beef / <i>Listeria monocytogenes</i> 4b	$Y = 0.935 X - 0.164$
Seafood Products	Smoked Salmon / <i>Listeria monocytogenes</i> 4b	$Y = 1.053 X - 0.111$
Vegetable Products	Salad / <i>Listeria monocytogenes</i> 1/2 a	$Y = 1.004 X + 0.031$
Milk Products	Raw Milk / <i>Listeria monocytogenes</i> 1/2 a	$Y = 1.069 X + 0.269$
Eggs and Derivatives	Raw Egg / <i>Listeria monocytogenes</i> 1/2 a	$Y = 1.111 X - 0.441$

Y = log(N alternative method)

X = log(N reference method)

Accuracy study:**With the ALOA COUNT™ poured plate protocol:**

Tests were performed in 2006. The statistical interpretation was conducted on 106 interpretable results including 15 naturally contaminated samples and 91 artificially contaminated samples, belonging to the following major food categories:

Meat products, seafood products, vegetable products, dairy products, and eggs& egg derivatives.

The samples were analyzed **twice** by each of the **two methods**.

As an indication, the areas of contamination (concentration) were the following:

Food Categories	Contamination range (in log CFU/g)
Meat Products	1 – 4.1
Seafood Products	1 – 5.2
Vegetable Products	1 – 2.8
Milk Products	1 – 3.7
Eggs & Derivatives	1 – 3.2

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

$$Y = 0.998 X - 0.010$$

Y = log(N alternative method)

X = log(N reference method)

With the ALOA COUNT™ surface protocol:

Tests were performed in 2006. The statistical interpretation was conducted on 102 interpretable results including 12 naturally contaminated samples and 90 artificially contaminated samples, belonging to the following major food categories:

Meat products, seafood products, vegetable products, dairy products, eggs and egg derivatives.

The samples were analyzed **twice** by each of the **two methods**.

As an indication, the areas of contamination (concentration) were the following:

Food Categories	Contamination range (in log CFU/g)
Meat Products	1 – 4.1
Seafood Products	1 – 5.2
Vegetable Products	1 – 2.8
Milk Products	1 – 3.7
Eggs & Egg Derivatives	1 – 3.2

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

$$Y = 1.003 X - 0.031$$

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:

With the ALOA COUNT™ poured plate protocol:

The limit of repeatability (in log) obtained for the alternative method is 0.300

The limit of repeatability (in log) obtained for the reference method is 0.224

The bias (in log) between the two methods (alternative – reference) is the following:
 $p = 0$ if we take the median or $D = 0$ if we take the average of the individual biases.

With the ALOA COUNT™ surface protocol:

The limit of repeatability (in log) obtained for the alternative method is 0.384

The limit of repeatability (in log) obtained for the reference method is 0.233

The bias (in log) between the two methods (alternative – reference) is the following:
 $p = 0$ if we take the median or $D = 0.01$ if we take the average of individual biases.

Conclusion for linearity and relative accuracy:

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

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)

Use of alternative method only

This study was done in 2004 during the approval of the ALOA ONE DAY™ method.

- 50 strains of *Listeria monocytogenes* were detected out of 50 tested.
- The study of 30 non-*Listeria monocytogenes* strains did not indicate the presence of cross-reactions. It should be noted that certain colonies of *Listeria ivanovii* show a typical aspect with a thin halo after the first 24 hours of incubation.

PRACTICABILITY

Use of alternative method only

- **Positive** results are obtained in 3 days (if confirmation by ALOA Confirmation™) or 8 days (if confirmation by usual tests) with the alternative method versus 4 to 8 days with the reference method.
- **Negative** results are obtained in 48 hours with the alternative method as with the reference method.

INTER-LABORATORY STUDY

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

- level 0: < 10 CFU/ml
- level 1: 10 to 100 CFU/ml
- level 2: 100 to 1,000 CFU/ml
- level 3: 1,000 to 10,000 CFU/ml
- level 4: 10,000 to 100,000 CFU/ml

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

The following results were obtained:

Contamination level	Number of laboratories giving results taken into account*	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
Level 1	8	0.439	0.591	0.294	0.469	0.12
Level 2	8	0.118	0.131	0.187	0.203	0.00
Level 3	8	0.131	0.167	0.266	0.282	-0.10
Level 4	8	0.160	0.244	0.105	0.169	-0.05

* 5 laboratories were excluded from statistical interpretation for non-compliance with the protocol

Conclusion

The inter-laboratory study shows that the results obtained with the alternative method are comparable 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