



**Alternative methods for agribusiness
Analytical performances certified**

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

Certificate No.: BIO 12/26 – 07/09

Validation date : 03.07.2009

End of validity : 03.07.2013

The company
(head office, distribution
and production site)

**BIOMERIEUX
Chemin de l'Orme
69280 MARCY L'ETOILE
FRANCE**

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

**VIDAS® Salmonella Xpress (SLMX)
Réf. 30 709**

Protocol reference: **14563 version C**

SCOPE

Raw beef and veal meats (including frozen, not flavoured) and pasteurized egg products.

RESTRICTIONS OF USE

None.

REFERENCE METHOD

EN ISO 6579 (2002): Microbiology of food and animal feedings stuffs. Horizontal method for the detection of *Salmonella* spp.

**Deputy General Manager
Jacques BESLIN**

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

AFNOR Certification

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PRINCIPLE OF THE METHOD

VIDAS® *Salmonella* Xpress (SLMX) is an enzyme immunoassay which detects *Salmonella* antigens using the ELFA (Enzyme Linked Fluorescent Assay) method on the VIDAS® automated system. The VIDAS SLMX method consists in an enrichment in buffered peptone water (incubated for 16-24 hours at 41,5°C±1°C) followed by the VIDAS® SLMX test.

Each test is composed of two parts:

- The disposable SPR®, which serves both as the solid phase and the pipetting device for the test. The SPR® is coated with anti *Salmonella* antibodies adsorbed on its surface.
- The strip, which contains all ready-to-use reagents necessary for the immunological test.

In the context of AFNOR Validation, all samples identified as presumptive positives by the VIDAS® *Salmonella* Xpress method must be confirmed within 72 hours after incubation, from the non-heated enrichment broth stored at 2-8°C, by one of the following means:

- From 1 to 5 isolated colonies on 2 selective medias by classical tests described in standardized methods by CEN or ISO (including a purification step) ;
- Using any other AFNOR Validation certified method, the principle of which is different from the VIDAS® *Salmonella* Xpress method. The protocol of the other validated method shall be followed entirely. All steps that are before the step from which the confirmation is done shall be common to both methods.

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

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY Comparison of performances of the alternative method and the reference method

Tests were performed in 2009 on 124 product samples, of which none was naturally contaminated, 63 artificially contaminated, and 61 non-contaminated, belonging to the following principal food product categories:

Raw beef and veal meats (including frozen, not flavoured) and pasteurized egg products.

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

Table of results (Cf. Table 1 of the EN ISO 16140 standard):

Answer	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement A+ / R+ PA = 63 ^{(1)**}	Positive deviation A+ / R- PD = 0 ⁽¹⁾
Alternative method negative (A-)	Negative deviation A- / R+ ND = 0 ⁽²⁾	Negative agreement A- / R- NA = 61 ⁽³⁾

(1) Confirmed positives

(2) (3) Of which no sample presumed positive by the alternative method was negative after confirmation

** Nota :

- "Pasteurized egg products" category: of which 1 positive VIDAS test result (1,6%) only confirmed on XLD Agar ;
- "Beef and veal raw meats" category: from which 6 positive VIDAS test results (9.5 %) not confirmed after direct plating on Chrom ID *Salmonella* Agar and 7 positive VIDAS test results (11 %) not confirmed on XLD Agar, from which 2 negative results on both agar media.

Percentages obtained compared to the reference method are as follows:

- Relative Accuracy (AC) = 100%
- Relative Specificity (SP) = 100%
- Relative Sensitivity (SE) = 100%

Sensitivity was also recalculated taking into account all confirmed positives (including supplementary positives by alternative method):

$$\text{Alternative method:} \\ (PA + PD) / (PA + PD + ND) = 100\%$$

$$\text{Reference method:} \\ (PA + ND) / (PA + PD + ND) = 100\%$$

Conclusion

Performances of VIDAS® *Salmonella* Xpress method appear similar to those of the reference method.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were performed in 2009, on 6 combinations of food products/strains described in the table below.

These products represent the following categories of food: raw beef and veal meats (including frozen, not flavoured) and pasteurized egg products.

Products were analysed **6 times** by the **two methods** at **4 levels** of contamination.

Results obtained are as follows:

Matrix	Strain	Relative detection level (CFU/25g or 25 ml) With confidence interval (3) LOD ₅₀	
		Alternative method	Reference method
Minced beef meat	<i>Salmonella</i> Montevideo	0.5 [0.3 – 0.8]	0.8 [0.5 – 1.2]
Liquid egg	<i>Salmonella</i> Enteritidis	0.5 [0.3 – 0.7]	0.4 [0.3 – 0.6]

(3) LOD₅₀: estimation of level of contamination enabling positive detection by alternative method in 50% of cases.

"Hitchins A. Proposed Use 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 limit of detection of the alternative method ranges between 0.3 and 0.8 CFU/25 grams.
The limit of detection of the reference method ranges between 0.3 and 1.2 CFU/25 grams.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 52 strains of *Salmonella* spp were detected out of 52 tested.
- The study of 30 strains not belonging to the genus *Salmonella* showed cross reactions with 2 strains of *Citrobacter diversus*. These strains were not positive by the reference method.

PRACTICABILITY

Implementation of alternative method only

- **Positive** results are obtained in three to six days using the alternative method (according to the confirmation level used) compared to five to seven days using the reference method.
- **Negative** results are obtained in one day using the alternative method compared to three to seven days using the reference method.
- In case of presumptive positive results using the alternative method, but found negative after confirmation, these negative results are obtained in two to six days.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2009 with 14 participating laboratories. Analyses were carried out on samples of minced beef meat, artificially contaminated with a *Salmonella* Typhimurium strain at the 3 following levels of contamination:

- 0 CFU / 25ml
- 3 CFU / 25ml
- 30 CFU / 25ml

The laboratories tested, using **both methods, 8 replicate samples** for **each level** of contamination, giving a total of 24 analyses for each participating laboratory, corresponding to a total of 336 analyses.

The following results were obtained:

Contamin- ation 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	112	112	80	80	80	0	0
1	112	112	80	0	0	80	80
2	112	112	80	0	0	80	80

* The results of 4 laboratories were excluded from interpretation: 2 laboratories because they did not received the samples in time, another laboratory because of cross-contamination of samples.

Calculations

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

Interpretation

Results of the collaborative study are comparable to those obtained during the preliminary study.

Sensitivity was also recalculated taking into account all confirmed positive results (this includes supplementary positives with alternative method):

$$\begin{array}{l} \text{Alternative method:} \\ (PA + PD) / (PA + PD + ND) = \mathbf{100\%} \end{array}$$

$$\begin{array}{l} \text{Reference method:} \\ (PA + ND) / (PA + PD + ND) = \mathbf{100\%} \end{array}$$

Accordance, concordance and concordance odds ratio:

Accordance: percentage chance of finding the same result (i.e. both negative and 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 (reproducibility conditions). The concordance is the percentage of all pairings of duplicates giving the same result

Concordance odds ratio (COR): defined by the following formula:

$$\text{COR} = \frac{\text{accordance} \times (100 - \text{concordance})}{\text{concordance} \times (100 - \text{accordance})}$$

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

Contamination level	Accordance	Concordance	COR
L0	100%	100%	1.00
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%	100%	1.00
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