



**Alternative methods for agribusiness
Analytical performances certified**

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

Certificate No. : BIO 12/16 – 09/05

Validation date:	20.09.2005
Extension on :	04.12.2007
Renewal date :	03.07.2009
End of validity :	20.09.2013

The company
(head office, distribution, production, site)

**BIOMERIEUX
69280 MARCY L'ETOILE
FRANCE**

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

VIDAS Easy Salmonella – Ref. 30 702

Protocol reference: 06984 version (Q)

SCOPE

All human and animal food products and environment samples (except stock farming environment)

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 be "Jacques Beslin", written over a horizontal line.

PRINCIPLE OF THE METHOD

The VIDAS *Salmonella* test is an enzyme immunoassay test which detects *Salmonella* antigens using the ELFA (Enzyme Linked Fluorescent Assay) method on the VIDAS or mini VIDAS analyzers. The VIDAS Easy *Salmonella* method consists of an enrichment in BPW, after which 0.1 ml of this suspension is transferred into SX2 broth. The VIDAS *Salmonella* test is then performed with a heated aliquot SX2 broth.

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 test: washing solution, alkaline phosphatase-labeled anti *Salmonella* antibodies and substrate.

In the context of AFNOR VALIDATION mark, all samples identified as positive by the VIDAS Easy *Salmonella* must be confirmed from the SX2 broth according to classical tests (including a purification step) described in methods standardized by CEN or ISO. The confirmation can be performed after until 72 hours of storage of the SX2 broth at 2-8°C.

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

NOTE (History of validation)

1/ The method is validated since September 2005 with SX broth.

An **extension study** was realized in 2007. This new study allowed to validate the use of the SX2 broth and reduction of the incubation time. Complementary assays were done with SX2 broth, for the following parameters: relative accuracy/specificity/sensitivity, relative detection level and inclusivity. The results are included in this certificate. The Interlaboratory study done in 2005 with SX broth was not tested with SX2 broth in accordance with EN ISO 16140 standard.

In June 2010, a new version of this certificate was edited to take into account the **definitive replacement of SX broth by SX2 broth**.

2/ For the **renewal study of 2009**, as the VIDAS *Salmonella* reagents were not modified since previous validation, as well as the reference method and the validation protocol, no additional test was performed.

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

Tests were carried out in 2007 **using SX2 broth** on 414 product samples, of which 54 were naturally contaminated, 142 artificially contaminated, and 218 non-contaminated, belonging to the following principal food product categories:

Meat products, dairy products, seafood products and vegetables, miscellaneous (pastries, eggs and egg products), environment samples (surfaces samples, varied waters, residues) and animal feed.

All samples were analysed in single by both methods.

Table of results with SX2 broth (Cf. Table 1 of the EN ISO 16140 standard):

	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement A+ / R+ PA = 192 ⁽¹⁾	Positive deviation A+ / R- PD = 2 ⁽¹⁾
Alternative method negative (A-)	Negative deviation A- / R+ ND = 2 ⁽²⁾	Negative agreement A- / R- NA = 218 ⁽³⁾

(1) Confirmed positives

(2) and (3) Of which none sample presumed positive by the alternative method was negative after confirmation

The obtained percentages compared to the reference method are as follows:

- Relative accuracy (AC): 99.0%
- Relative specificity (SP): 99.1%

Note: a relative specificity below 100 % is due to additional confirmed positive results of the alternative method, considered as positive deviation (false positive) by the EN ISO 16140 standard.

- Relative sensitivity (SE): 99.0%

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

Alternative method :	Reference method :
$(PA + PD) / (PA + PD + ND) = 99.0 \%$	$(PA + ND) / (PA + PD + ND) = 99.0 \%$

Conclusion

Both methods are not different in statistical term.

Conservation of SX2 broth during 72 hours at 2-8°C

During the 2007 extension study, SX2 broths were kept 72 hours at 2-8°C and then they were retested with the VIDAS SLM. Results were identical to those obtained directly after incubation except for one negative result which became positive (with a value very near threshold and with *Salmonella* colonies only found on SMID2 agar after sub-culturing from SX2 to RVS broth).

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2005 and 2007, on the combinations of food products/strains described in the table below.

These products represent the following kinds of food products: meat products, dairy products, seafood products and vegetables, miscellaneous, environment samples and animal feed.

Products were analysed 6 times by both 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	
		VIDAS Easy Salmonella with SX2 broth	Reference method
Ground poultry	S. Hadar	0.4 [0.2 - 0.6]	0.4 [0.2 - 0.6]
Raw milk	S. Typhimurium	0.5 [0.3 - 0.8]	0.5 [0.3 - 0.8]
Fish fillet	S. Virchow	0.5 [0.3 - 0.9]	0.5 [0.3 - 0.9]
Raw eggs	S. Enteritidis	0.5 [0.3 - 0.8]	0.5 [0.3 - 0.8]
Processed water	S. Newport	0.4 [0.2 - 0.7]	0.4 [0.2 - 0.7]
Animal feed	S. Senftenberg	0.5 [0.3 - 1.0]	0.5 [0.3 - 1.0]

(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 detection level of the alternative method with **SX2 broth** is identical to the one of the reference method: it is assessed between 0.2 and 1.0 CFU/25g.

INCLUSIVITY / EXCLUSIVITY (studies in 1994, 2002 and 2007)

Implementation of alternative method only

- In 2007, 51 strains of *Salmonella* were detected out of 51 tested with VIDAS Easy *Salmonella* using SX2 broth.
- 30 non *Salmonella* strains were tested as a whole. Crossed reactions were obtained using VIDAS SLM with the following strains and from culture in non selective broth (BPW): *Citrobacter diversus* (2 strains) and *Citrobacter freundii*.

These strains were re-tested with the whole protocol of the alternative method and results remain positive for *Citrobacter diversus*. Nevertheless colonies isolated from SX2 broth on selective agars are not typical of *Salmonella*.

PRACTICABILITY

Implementation of alternative method only

- **Response time :**
 - **Positive** results are obtained in 5 to 7 days using the alternative method (*including confirmation according to classical tests of the reference method, with purification step included*) like using the reference method.
 - **Negative** results are obtained in 2 days using the alternative method against 3 to 7 days using the reference method.
 - In the case of results presumed positive using the alternative method, but rendered negative after confirmation, these negative results are obtained in 3 to 7 days.

INTERLABORATORY STUDY

The interlaboratory study was conducted in 2005 with 15 participating laboratories. The analyses were carried out on samples of pasteurized milk artificially contaminated with a *Salmonella* Typhimurium strain at 3 levels of contamination:

- 0
- 3 cells/ml
- 30 cells/ml

The laboratories tested, using **both methods**, **8 replicate samples for each level** of contamination. The assays for VIDAS Easy *Salmonella* method were realized with **SX** broth.

The following results were obtained:

Contamination 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	120	112	104	101	97 ⁽¹⁾	3	7
1	120	112	104	4	4	100	100
2	120	112	104	0	0	104	104

* One laboratory did not realize the analysis since the samples were received after the deadline

**** One laboratory's results were not exploited because all samples even non contaminated ones were positive when using both methods. A cross contamination probably occurred at the step at the pre-enrichment preparation step.**

(1) One sample positive when using VIDAS *Salmonella* but not confirmed.

Comments: several laboratories obtained positive results with non contaminated samples with the reference method (3 samples) and the alternative method (7 samples of which one in common with the reference method). In all cases, the strains were isolated and identified as the same one as the strain used in the artificial contamination. The hypothesis of cross contamination is confirmed.

Calculations

- Relative accuracy = **97.4 %**
- Specificity = **93.3 %**

Note: a relative specificity below 100 % is due to additional confirmed positive results of the alternative method, considered as positive deviation (false positive) by the EN ISO 16140 standard.

- Sensitivity = **98.1 %**

Interpretation

Results of the collaborative study are comparable to those obtained during the preliminary study for the relative accuracy and the sensitivity. They are different for the specificity because of positive results issued from non contaminated samples.

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

Alternative method :

$$(PA + PD) / (PA + PD + ND) = 99.1 \%$$

Reference method :

$$(PA + ND) / (PA + PD + ND) = 97.2 \%$$

Accordance, concordance and concordance odds ratio:

Accordance: percentage chance of finding the same result (i.e. both negative or both positive) from two identical test portions 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. The concordance is the percentage of all pairings of duplicates giving the same result.

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

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

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

Contamination level	Accordance	Concordance	COR
L0	90 %	87 %	1.03
L1	94 %	92 %	1.01
L2	100 %	100 %	1.00

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

Contamination level	Accordance	Concordance	COR
L0	95 %	94 %	1.01
L1	94 %	92 %	1.01
L2	100 %	100 %	1.00

Conclusion

Variability of the alternative method (accordance, concordance, concordance, odds ratio) and variability of the reference method are equivalent.

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