



Alternative methods for agribusiness
Analytical performances certified

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

Certificate No. : BIO 12/1 – 04/94

Validation date :	06.04.1994
Renewal:	06.09.1998
	18.09.2002
	15.09.2006*
	20.05.2010
End of validity :	09.06.2014

* EN ISO 16140 protocol was used during the 3rd renewal in 2006

The company
(Head office, distributor
and production site)

bioMérieux
Chemin de l'Orme
69280 MARCY L'ETOILE
FRANCE

is hereby authorized to refer to this NF VALIDATION certificate for the following alternative qualitative analysis method :

VIDAS Salmonella (VIDAS SLM)
Dual selective enrichment for the rapid detection of *Salmonella*

Protocol reference: Ref. 30 702 - 06984 version S

SCOPE

All human food products and Pet Food.

RESTRICTIONS

None.

REFERENCE METHOD

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

A handwritten signature in blue ink, appearing to read 'FM', with a long horizontal line extending to the right.

Managing Director
Florence MÉAUX



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 SLM method consists in a pre-enrichment in buffered peptone water followed by enrichment in selective broths (RVS and MKTTn broths during 6 to 8 hours) each followed by a post-enrichment in M broth during 16 to 20 hours, after which the VIDAS test is conducted.

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 NF VALIDATION, all samples identified as positive by the VIDAS *Salmonella* must be confirmed from the RVS and MKTTn broths of which the incubation was continued until 16 – 20 hours, according to classical tests (including a purification step) described in methods standardized by CEN or ISO.

In the event of discrepant 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

Some results obtained in 2002 during the comparative study were maintained for the renewal in 2006. The comparative study was complemented according to standard EN ISO 16140 for:

- The relative accuracy/sensitivity/specificity part for the pet food category, and the other categories for the new artificial contaminations,
- The inclusivity/exclusivity part.

The inter laboratory study was completely repeated according to the new protocol in standard EN ISO 16140.

The validation of VIDAS SLM dual selective enrichment protocol was renewed in May 2010 without additional assays, as the alternative method was not modified, and neither the reference method nor the protocol of validation did change since the previous validation. Inclusivity study was completed according to specific NF VALIDATION requirements. The results were in conformity with those expected (not available in this certificate).

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY

Comparison of performances of the alternative method and the reference method

In 2002 and 2006 tests were carried out on 328 product samples, of which 65 were naturally contaminated, 87 artificially contaminated, and 176 non-contaminated, belonging to the following principal food product categories: meat products, dairy products, seafood products, vegetables, miscellaneous, pet food products.

All samples were analysed in single by both methods.

Table of results (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 = 151 ⁽¹⁾	Positive deviation A+ / R- PD = 0 ⁽¹⁾
Alternative method negative (A-)	Negative deviation A- / R+ ND = 1 ⁽²⁾	Negative agreement A- / R- NA = 176 ⁽³⁾

(1) Confirmed positives

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

Percentages obtained compared to the reference method are as follows:

- Relative accuracy : AC: **99.7 %**
- Relative specificity : SP: **100 %**
- Relative Sensitivity : SE: **99.3 %**

Conclusion

Both methods are not different in statistical terms.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2006, on the following 5 combinations of food products/strains described in the table below.

These products represent the following food products categories: meat products, dairy products, seafood products, vegetables, miscellaneous, pet food products.

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	
		Alternative method	Reference method
Ground poultry	<i>Salmonella</i> Hadar	0.5 [0.3 – 0.9]	0.5 [0.3 – 0.9]
Raw milk	<i>Salmonella</i> Typhimurium	0.7 [0.4 – 1.1]	0.7 [0.4 – 1.1]
Fish filet	<i>Salmonella</i> Enteritidis	0.7 [0.3 – 1.4]	0.7 [0.3 – 1.4]
Raw eggs	<i>Salmonella</i> Virchow	0.4 [0.3 – 0.5]	0.4 [0.3 – 0.5]
Pet food (mash)	<i>Salmonella</i> Senftenberg	0.7 [0.5 – 1.1]	0.7 [0.5 – 1.1]

(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 is identical to the reference method's one. It is assessed between 0.3 and 1.4 cells/25g.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 51 strains of *Salmonella* were detected out of 51 tested.
- The study of 30 non-*Salmonella* strains showed the presence of cross-reactions with the following strains, cultured in non selective broth (BPW): *Citrobacter diversus* (2 strains) and *Citrobacter freundii*.

Only both strains of *Citrobacter diversus* showed a positive result when using the complete protocol of the alternative method.

PRACTICABILITY

Implementation of alternative method only

- **Response time :**
- **Positive** results are obtained in 5 to 7 days using the VIDAS SLM kit (including confirmation according to classical tests of the reference method, with purification step included) or the reference method.
- **Negative** results are obtained in 2 day(s) 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 following confirmation, 3 to 7 days are required.

INTERLABORATORY STUDY

The interlaboratory study was conducted in 2006 with 15 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a Salmonella Typhimurium strain at the following 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, giving a total of 24 analysis for each participating laboratory.

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	95	79	79***	79	0	0
1	120	96	80	0	0	80	80
2	120	96	80	0	0	80	80

* 3 laboratories did not realize the analysis.

** 2 laboratories did not perform the analysis properly. Their results were not exploited.

*** One laboratory supplied only 7 results out of 8 expected for the LO level, following to a problem during the analysis.

Calculations

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

Interpretation

The results of the interlaboratory study are comparable to those obtained during the preliminary study.

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 accordancy 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:

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

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

Contamination level	Accordance	Concordance	COR
L0	100	98,8*	1.01
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	98,8*	1.01
L1	100	100	1.00
L2	100	100	1.00

* The concordance percentage for both reference method and alternative method is lower than 100 % at the L0 level because one laboratory supplied only 7 results out of 8 expected, following to a problem during the analysis.

Conclusion

Variability of the alternative method (accordance, concordance, concordance odds ratio) is equivalent to the reference method's one.

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