



Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: BIO 12/22 – 05/07

Validation date: 24.05.2007
End of validity: 24.05.2011

The company
(head office, distributor and 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 Immuno-Concentration Salmonella II (VIDAS ICS2) + SLM
Reference 30708

Protocol reference: 13614 version G

SCOPE

All human food products (except raw milk) and pet foods

RESTRICTIONS OF USE

None

REFERENCE METHOD

EN ISO 6579:2002 - Food Microbiology - horizontal method for detection of *Salmonella* spp

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

Deputy General Manager
Jacques BESLIN

AFAQ AFNOR Certification

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PRINCIPLE OF THE METHOD

The VIDAS ICS2 + SLM assay involves an automated immunological capture and a specific release process for the concentration of *Salmonella* from a pre-enrichment broth. The released bacteria are then detected by VIDAS SLM immunoassay after culture in a specific ICS broth.

In the context of AFNOR Validation, all samples identified as positive by the VIDAS SLM test must be confirmed from unheated ICS broth (kept at 2-8 °C) on selective agar. The confirmation must be initiated within 48 hours of the end of the incubation of ICS broth at 41.5°C.

VIDAS SLM assays may be performed from ICS broth just after incubation or storage of ICS broths at 2 - 8°C for up to 48h.

Typical colonies on selective media must be identified in one of the following ways

- Identification of 1 to 5 colonies by the conventional tests described in the standardized methods by CEN, ISO or AFNOR (including the purification stage),
- Preparation of an API® test strip from an isolated colony directly seeded on selective agar, by checking in parallel the purity of the strain on nutrient agar.

In the event of discordant results (positive by the alternative method, non-confirmed by the tests described in the standardised methods by CEN, ISO or AFNOR) the laboratory must follow the necessary steps to ensure validity of the result obtained.

NOTE

Six pre-enrichment protocols are proposed according to the following categories of food product:

- Chocolate products except cocoa
- Cocoa
- Egg products
- Raw poultry meat
- Powder milk
- Other products (general protocol)

All the positive samples (artificially and naturally contaminated), were retested after 48h of storage of ICS broth at 2 - 8°C, to validate the possibility of carrying out the tests after storage of the broths for 48h.

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

Tests were carried out in 2007 on 308 product samples including 115 naturally contaminated, 40 artificially contaminated and 153 non-contaminated products, belonging to the following main food categories:

Dairy products, meat products, seafood and vegetable products, miscellaneous and animal feeds.

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

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

	Reference method (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement A+ / R+ PA = 147 ⁽¹⁾	Positive deviation A+ / R- PD=1 ⁽¹⁾
Alternative method negative (A-)	Negative deviation A- / R+ ND = 7 ⁽²⁾	Negative agreement A- / R- NA = 153 ⁽³⁾

(1) Confirmed positives

(2) Of which one sample presumed positive by the alternative method, was negative after confirmation

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

Percentages obtained compared to the reference method were as follows:

- Relative accuracy: **AC = 97.4%**
- Relative specificity: **SP = 99.4%**

NB: **relative specificity** below 100% results from a number of confirmed supplementary positives and not from false positives

- Relative sensitivity: **SE = 95.5%**

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

Alternative method	Reference method:
$(PA + PD) / (PA + PD + ND) = 95.5\%$	$(PA + ND) / (PA + PD + ND) = 99.4\%$

Analysis of discrepant results (according to appendix F of standard EN ISO 16140)

PD= 1, ND= 7 therefore Y = PD + ND = 8; $6 \leq Y \leq 22$ m=1, M=0 therefore m > M

Conclusion

The two methods are not statistically different.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were performed in 2007 on the 5 food product/strain combinations described in the table below.

These products represent the following categories of food: dairy products, meat products, seafood and vegetable products, miscellaneous and animal feeds.

Products were analyzed **6 times**, by the **two methods**, at **4 levels** of contamination

The following results were obtained:

Matrix	Strain	Relative detection level LOD ₅₀ (3) With confidence interval (CFU/25g)	
		Alternative method	Reference method
Raw poultry meat	<i>Salmonella</i> Hadar	0.4 [0.2-0.7]	0.6 [0.4-1.1]
Powder milk	<i>Salmonella</i> Agona	0.4 [0.2-0.6]	0.3 [0.2-0.6]
Egg product	<i>Salmonella</i> Enteritidis	0.4 [0.3-0.7]	0.3 [0.2-0.5]
Cocoa	<i>Salmonella</i> Anatum	0.5 [0.3-0.8]	0.6 [0.3-1.0]
Dog food	<i>Salmonella</i> Senftenberg	0.4 [0.3-0.5]	0.4 [0.3-0.5]

(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 level of detection of the alternative method is between 0.2 and 0.8 CFU/25 g .

The level of detection of the reference method is between 0.2 and 1.1 CFU/25 g.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 51 *Salmonella* strains were detected out of the 51 tested.
- For 34 strains not belonging to the *Salmonella* genus, 2 strains of *Citrobacter diversus* were scored positive by VIDAS SLM.

PRACTICABILITY

Implementation of alternative method only

- **Response time:**
 - **Positive** results are obtained in 3 to 4 days using the alternative method versus 5 to 6 days with the reference method.
 - **Negative** results are obtained in 1 day with the alternative method versus 3 to 7 days with the reference method.
 - In the case of results presumed positive using the alternative method, but rendered negative following confirmation, these negative results are obtained in 3 to 4 days.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2007 with 18 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a strain of *Salmonella typhimurium* serotype at the 3 following levels of contamination:

- Level 0
- Level slightly higher than the relative detection level (3 cells/25 ml)
- Level 10 times higher than previous level (30 cells/25 ml)

The laboratories tested, using **both methods, 8 replicate samples** for each level of contamination, giving a total of 24 analyses 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	144	144	144	135	139	9*	5*
1	144	144	144	2	2	142	142
2	144	144	144	0	0	144	144

* Comments: Several laboratories obtained positive results for uncontaminated samples, with the reference method (9 samples) and with the alternative method (5 samples). In all these cases, the strain was detected and was the same as that used to contaminate the positive samples, validating the cross-contamination hypothesis.

Calculations

- Relative accuracy = 98.2%
- % specificity = 96.5 %
- % sensitivity = 99.3 %

NB: a **relative specificity** less than 100% is due to additional confirmed positives and not to false positives

Interpretation:

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

Sensitivity was also recalculated by taking into account all the confirmed positive (including the additional positives of the alternative method):

Alternative method:

$$(PA + PD) / (PA + PD + ND) = 98.0\%$$

Reference method:

$$(PA + ND) / (PA + PD + ND) = 99.3\%$$

This difference is due to the fact that additional positives of the reference method result from cross-contamination of negative samples by positive samples.

Accordance, concordance and concordance odds ratio:

Accordance: percentage chance of finding the same result from two identical samples analysed in the same laboratory, under repeatability conditions. 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:

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

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

Contamination level	Accordance	Concordance	COR
L0	95.0	93.2	1.4
L1	97.6	97.2	1.1
L2	100	100	1.0

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

Contamination level	Accordance	Concordance	COR
L0	93.6	88.0	2.0
L1	97.6	97.2	1.1
L2	100	100	1.0

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 AFAQ AFNOR Certification.

On request, AFAQ AFNOR Certification will send you a summary document (in French) on the preliminary and collaborative studies.