



**Alternative methods for agribusiness
Analytical performances certified**

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

Certificate No.: AES-10/04-05/04

Validation date :	07.05.2004
Extension:	02.07.2007
Renewal date:	27.03.2008
End of validity :	07.05.2012

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

SIMPLE METHOD FOR SALMONELLA (SMS™)

Protocol reference: Technical data sheet SMS™: 520069 : 27/03/08 - Q

SCOPE

All products for human and animal consumption and samples of environment (except breeding samples)

RESTRICTIONS OF USE

The method is intended for the detection of mobile *Salmonella* and is not adapted to the detection of non-motile *Salmonella* (non-motile strains or having lost their mobility)

REFERENCE METHOD

NF EN ISO 6579: Horizontal method for the detection of *Salmonella* spp.

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

**Deputy General Manager
Jacques BESLIN**

AFNOR Certification

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PRINCIPLE OF THE METHOD

The principle of the SMS™ lies on the detection of *Salmonella*'s motility and their ability to decarboxylate L-lysine. After pre-enrichment, incubated sample in buffered peptone water is deposited on the edge of the plate containing SMS™ agar medium. After incubation at 41°C +/- 1°C during 24hrs +/- 1hr, *Salmonella* produce migration zones superior to 2 cm, associated a colour change from green to red of the agar.

In the context of AFNOR Validation, all samples identified as positive by the alternative method must be confirmed by one of the following means :

1. According to classical tests described in methods standardized by CEN, ISO or AFNOR (including a purification step), starting by a subculture from a SMS plate onto a selective medium for *Salmonella* .
2. by transplanting or insulation starting from a box of SMS presumed positive, and implemented by SMS Confirmation test (2 options) according to the instruction described in the note for this test
3. By implementing any other AFNOR VALIDATION validated 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.

NOTE : History of the Validation

The first validation was pronounced in May 2004 according to the protocol of the standard EN ISO 16140.

In February 2007, an extension study was undertaken to add an option of additional confirmation (cf 2nd option presented above, with the use of the SMS Confirmation test). Tests were carried out on pure strains :

- 150 strains of *Samonella spp* of varied serotypes and origins were tested
- 105 non-target strains (the choice being directed by the genetic proximity to *Salmonella spp*) were tested.

The results obtained were in conformity with those expected.

In March 2007, the validation was renewed without the carrying out of complementary tests, since neither the SMS method, nor neither the method taken in reference, nor protocol of validation were modified.

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

In (2004) tests were carried out on 378 product samples, of which 47 were naturally contaminated, 138 artificially contaminated, and 193 non-contaminated, belonging to the following principal food product categories: meat products, seafood, egg products, dairy products, environment samples, animal feeding stuffs.

All samples were analysed in **single** by the **two 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 = 181 ⁽¹⁾	Positive deviation A+ / R- PD = 3 ⁽¹⁾
Alternative method negative (A-)	Negative deviation A- / R+ ND = 1 ⁽²⁾	Negative agreement A- / R- NA = 193 ⁽³⁾

1. Confirmed positives
2. Of which no sample presumed positive by the alternative method was negative after confirmation
3. Of which no samples presumed positive by the alternative method were negative after confirmation

Percentages obtained compared to the reference method are as follows :

- Relative accuracy : **99 %**
- Relative specificity :**99 %**

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

- Relative sensitivity : 98 %

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

Alternative method :	Reference method :
$(PA + PD) / (PA + PD + ND) = 98.4\%$	$(PA + ND) / (PA + PD + ND) = 99.5\%$

Conclusion

The performance of the SMS method appears equal to that of the reference method.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in (2004), on combinations of food products/strains in the following categories meat products, seafood, egg products, dairy products, environment samples, animal feeding stuffs.

Products were analysed **6 times** by the **2 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	<i>S.typhimurium</i>	0.46 [0.315 , 0.673]	0.46 [0.315 , 0.673]
Fresh milk	<i>S.dublin</i>	0.50 [0.362 , 0.703]	0.50 [0.362 , 0.703]
Egg	<i>S.enteritidis</i>	0.36(0.261, 0.506]	0.36 [0.261, 0.506]
Fillet of coalfish	<i>S. virchow</i>	0.25 [0.151 , 0.428]	0.25 [0.151, 0.428]
Process water	<i>S. typhimurium</i>	0.46 [0.315 , 0.673]	0.46 [0.315, 0.673]

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

Conclusion

The limit of detection of the alternative method ranges between 0.151 and 0.703 CFU/25 g

The relative levels of detection obtained for the alternative method are identical to those obtained for the reference method, whatever the matrix/strain combination used.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 57 strains of *Salmonella* were detected out of 64 tested. The strains which were not detected are *gallinarum* (not motile) and *paratyphi* A. One strain of *S.infantis* out of 5 tested and one strain of *S.paratyphi* C were not detected.
- The study of 30 strains not belonging to the genus *Salmonella* did not detect the presence of any cross-reaction.

PRACTICABILITY

Implementation of alternative method only

Response time :

- Positive** results are obtained in 5 to 6 days using the alternative method (*including confirmation according to classical tests of the reference method, with purification step included*) against 5 to 7 days using the reference method.
- Negative** results are obtained in 2 day(s) using the alternative method against 5 to 7 days using 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 5 days.

Personnel training : *if applicable*

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2004 with 14 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a *Salmonella enteritidis* strain at the following 3 levels of contamination:

- Level 0
- Level slightly superior to relative detection level
- Level 10 times superior to previous level

The laboratories tested, using **both methods**, **8 replicate samples** for each level of contamination, giving a total of 672 analyses for each participating laboratory.

The following results were obtained:

Contamin- ation level	Total number of samples	Number of samples analysed	Number of results processed	Number of negative results		Number of positive results	
				REF	ALT	REF	ALT
0	112	112	104	104	104	0	0
1	112	112	104	5	5	99	99
1	112	112	104	5	5	104	104

The results of one laboratory were not used because, at the time of first sending, two unmatched results were observed (samples achieving positive with confirmation by the reference method and negative by the alternative method). The laboratory invoked a sample error in the suspension-mother sacs. The counter-analysis, requested by the laboratory expert starting from the peptoned swab water conserved at 4°C gave results in conformity (the 2 samples gave a negative result for the 2 methods). However this counter-analysis was carried out one week after the date of the performance of the tests by the participating laboratories, reason for which this laboratory has been excluded from the interpretation

Calculations

- Relative accuracy = **100%**
- % specificity = **100%**
- % sensitivity = **98%**

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) :

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

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

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

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

Contamination level	Accordance	Concordance	COR
L0	100%	100%	1
L1	92%	91%	1.14
L2	100%	100%	1

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.

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