Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: TEC 24/03 – 12/03

Validation date:	**12.12.2003**
Renewal dates:	**04.12.2007***
	10.05.2012
End of validity:	**12.12.2015**

** EN ISO 16140 was used in 2007 for the 1st renewal study.*

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

3M™ Tecra™ Unique Salmonella Test

Protocol reference: AV014410391

SCOPE

All human food products and animal feeding stuffs.

RESTRICTIONS

None.

REFERENCE METHOD

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

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PRINCIPLE OF THE METHOD

The 3M™ TECRA™ Unique Salmonella test is an Enzyme linked immunosorbent assay (ELISA) for the detection of motile and non-motile *Salmonella*. After a preenrichment in buffered peptone water, *Salmonella* in the sample are captured by highly specific antibodies distributed on a stick. After washing and enrichment, an ELISA reaction is applied. In the presence of Salmonella, a grey to violet colour appears on the last three-quarters of the stick, the first quarter serving as negative control.

In the context of NF VALIDATION, all samples identified as positive by the alternative method must be confirmed from the enrichment broth:

- According to classical tests described in methods standardized by CEN or ISO from colonies (including a purification step),
- Or using nucleic probes onto isolated colonies as described in EN ISO 7218 standard (including or not the purification step).

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

NOTE (History of validation)

The first validation study was done in 2003.

The EN ISO 16140 validation protocol was used in 2007 for the first renewal study. The 3M™ TECRA™ Unique Salmonella test remained the same. The validation study was totally repeated, taking into account some results obtained in 2003 for the accuracy study completed with new data in order to meet EN ISO 16140 requirements.

The 3M™ TECRA™ Unique Salmonella test includes two protocols which were tested during the renewal study of 2007:

1. General protocol corresponding to a pre-enrichment in modified Buffered Peptone Water at 37±1°C for 16 to 20 hours
2. Specific protocol for raw meats, animal feeding stuffs and egg products, corresponding to a pre-enrichment in modified Buffered Peptone Water supplemented with 1/20 of 3M™ TECRA™ Salmonella supplement + 2,25 ml of 3M™ Imbentin supplement, and heated at 42±1°C for 16 à 20 hours

In May 2012, the validation of 3M™ TECRA™ Unique Salmonella was renewed. This alternative method was not modified since the previous validation. The reference method and the validation protocol remained unchanged. Inclusivity study was completed according to NF VALIDATION specific requirements. The results are presented in this certificate.

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY

Comparison of performances of the alternative method and the reference method

Tests were carried in 203 and in 2007 out of 329 product samples, of which 44 were naturally contaminated, 124 artificially contaminated and 161 non-contaminated, belonging to the following principal food product categories:

- dairy products
- meat products vegetables
- seafood products and vegetables
- 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 = **161** (1)	Positive deviation A+ / R- PD = **1** (1)
Alternative method negative (A-)	Negative deviation A- / R+ ND = **6** (2)	Negative agreement A- / R- NA = **161** (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)**: **98%**
- Relative specificity **(SP)**: **99%**
- Relative sensitivity **(SE)**: **96%**

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

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

Alternative method :

(PA + PD) / (PA + PD + ND) = 96%

Reference method :

(PA + ND) / (PA + PD + ND) = 99%

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

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

Conclusion

The two methods are not statistically different.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2007, on 5 combinations of "food product/strain" described in the table below.

Products samples were belonging to the following food categories:

- dairy products
- meat products vegetables
- seafood products and vegetables
- egg products
- animal feeding stuffs

Products were analysed **6 times** by the **2 methods** at **4 levels** of contamination.

Results obtained are as follows:

		Relative detection level (CFU/25g or 25 ml) With confidence interval (3) LOD_{50}	
Matrix	**Strain**	**Alternative method**	**Reference method**
Minced meat	*Salmonella* Typhimurium	0.7 [0.4 – 1.3]	0.4 [0.2 – 0.8]
Smoked salmon	Salmonella Enteritidis	1.1 [0.7 – 1.8]	0.7 [0.5 – 1.0]
Raw milk	*Salmonella* Dublin	1.2 [0.6 – 2.4]	2.1 [1.0 – 4.3]
Egg	*Salmonella* Enteritidis	1.3 [0.7 – 2.4]	1.0 [0.6 – 1.5]
Feed for cat	*Salmonella* Typhimurium	1.1 [0.6 – 2.1]	0.9 [0.5 – 1.4]

(3) **LOD_{50}**: estimation of level of contamination enabling positive detection by alternative method in 50% of cases. *FDA. 2006. Final Report and Executive Summaries from the AOAC International Presidential Task Force on Best Practices in Microbiological Methodology. Appendix K. Statistics Working Group Tholen, D. W., D. S. Paulson, B. Jarvis, D. M. Mettler, B. Lombard, K. Newton, M. A. Mozola, and A. D. Hitchins.) Report Part 4a - LOD50.*

Conclusion

The relative detection level of the alternative method is between 0.4 and 2.4 CFU/25 g.
The relative detection level of the reference method is between 0.2 and 4.3 CFU/25 g.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

Inclusivity:

- Among 51 strains of *Salmonella* tested, one *S.* Paratyphi C was not detected whatever the protocol of the alternative method used and seven collection strains (*S.* Gallinarum, *S.* Infantis, *S.* Paratyphi A, *S.* Senftenberg, *S.* Typhi and two *S.* Paratyphi B) gave a negative result with the specific protocol and a positive result with the general protocol.

- Additional assays were conducted on serotypes not detected, at lower level of contamination, using the specific protocol supplemented with milk, and the general protocol:
  - One ***S.* Senftenberg** and two ***S.* Paratyphi B** gave a positive result with the specific protocol.
  - One ***S.* Paratyphi A** and one ***S.* Typhi** were not detected with the specific protocol, but were detected with the general protocol at lower inoculation levels.
  - One ***S.* Paratyphi C** was not detected whatever the protocol used.

- Moreover, four wild strains of ***S.* Infantis** gave a positive result with the general protocol, but a negative result with the specific protocol (however, additional accuracy study showed that these four strains reacted with the specific protocol in stress condition on raw meat matrix)

- Four wild strains of ***S.* Gallinarum** were not detected whatever the protocol of the alternative method used, but a positive result was obtained at inoculation level of about 10^7 CFU/ml. These 4 strains also gave a negative result with the reference method in stress condition on raw poultry matrix.

- Two ***S.* Arizonae** were also detected whatever the protocol of the alternative method used.

- For the renewal study of May 2012, 18 *Salmonella* strains were tested and one was not detected by the alternative method. It was a non-motile variant of *Salmonella* Typhimurium S.I 1,4,[5],12:-:-.

  Complementary tests were done in order to explain this result:

  - Others Salmonella strains with the same antigenic formula were tested and detected. The non-motile *S.* Typhimurium S.I 1,4,[5],12:-:- strains were not detected in normal conditions of use of the alternative method probably because of their particular antigenic formula (need of a very high level of inoculation to be detected).
  - Some S. Gallinarum / Pullorum strains tested were not detected probably due to their very low growth (there were not detected by the reference method in the same inoculation conditions).

Exclusivity:

- The study of 35 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 (including confirmation step) are obtained in 4 to 6 days using the 3M™ TECRA™ Unique Salmonella test against 5 to 7 days using the reference method.
- **Negative** results are obtained in 1 day using the 3M™ TECRA™ Unique Salmonella test 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 4 to 6 days.

INTERLABORATORY STUDY

The interlaboratory study was conducted in 2007 with 14 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a *Salmonella* Enteritidis strain at the following levels of contamination:

- Level 0: 0 CFU/25 mL
- Level 1: 3 CFU/25 mL
- Level 2: 30 CFU/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 processed**	Number of negative results		Number of positive results	
				REF	ALT	REF	ALT
0	112	104	80	78	79	2	1
1	112	104	80	4	2	76	78
2	112	104	80	0	0	80	80

** A laboratory received the samples out of delay and did not do the assays.*
*** Two laboratories found 8 negative results by the reference method on contaminated samples. A laboratory obtained 7 confirmed positive results with the reference method on non-contaminated samples. The results of these 3 laboratories were not used for the statistic interpretation.*

Calculations

- Relative accuracy = **96%**
- % specificity = **94%**
- % sensitivity = **97%**

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

Interpretation

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

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

Alternative method:	Reference method:
(PA + PD) / (PA + PD + ND) = 97.5%	(PA + ND) / (PA + PD + ND) = 97.0%

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= accordance x (100 - concordance) / concordance x (100 – accordance)

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

Contamination level	Accordance	Concordance	COR
L0	98%	97%	1.50
L1	96%	95%	1.30
L2	100%	100%	1.00

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

Contamination level	Accordance	Concordance	COR
L0	96%	95%	1.30
L1	95%	90%	2.10
L2	100%	100%	1.00

Conclusion

Variability of the alternative method (accordance, concordance, concordance odds ratio) is comparable to the one 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