



**Alternative methods for agribusiness
Analytical performances certified**

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

N° attestation : UNI 03/06 – 12/07

Validation date: 12/04/2007
Renewal date: 10/06/2011
End of validity: 12/04/2015

The company
(Head office and
Production site)

OXOID Ltd
Wade Road
Basingstoke, Hampshire
RG24 8 PW, England, UK

Distributor

OXOID Thermo Fisher Scientific
6 route de Paisy
69571 Dardilly cedex
France

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

Salmonella PreciTM

Protocol reference: INH 12/2011

SCOPE

All human and animal food products and environmental samples (excluding breeding samples)

RESTRICTIONS

None

REFERENCE METHOD

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

**Managing Director
Florence MÉAUX**



AFNOR Certification

11, rue Francis de Pressensé – 93571 La Plaine Saint-Denis Cedex – France
Phone +33 (0)1 41 62 80 00 – Fax +33 (0)1 49 17 90 00
www.afnor.org – www.afnor-validation.com

PRINCIPLE OF THE METHOD

The Salmonella Precis™ method includes an enrichment step in ONE Broth-Salmonella medium, followed by isolation on Brilliance™ Salmonella Agar chromogenic media. The technology improves recovery of *Salmonella* by reducing background flora and allows an easy identification and differentiation by producing brightly coloured colonies.

In the context of NF VALIDATION, all samples identified as positive by Salmonella Precis™ method must be confirmed by one of the following means:

- According to classical tests described in methods standardized by CEN or ISO (including a purification step) from colonies isolated on a Brilliance™ Salmonella chromogenic media.
- By implementing Oxoid Salmonella Latex Test. Re-isolation shall be done in parallel to verify the purity of the micro-organism.

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 validation): In October 2011, the renewal of validation was pronounced for Salmonella Precis™ method. This alternative method was not modified since the previous validation, and the reference method and the EN ISO 16140 standard remain unchanged. Inclusivity study was completed according to NF VALIDATION specific 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 2007 tests were carried out on 424 product samples, of which 72 were naturally contaminated, 144 artificially contaminated, and 208 non-contaminated, belonging to the following principal food product categories:

- Dairy products
- Meat products
- Vegetables, seafood products and miscellaneous
- Egg products
- Animal feeding stuffs
- Environmental samples

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 = 178 ⁽¹⁾	Positive deviation A+ / R- PD = 18 ⁽¹⁾
Alternative method negative (A-)	Negative deviation A- / R+ ND = 20 ⁽²⁾	Negative agreement A- / R- NA = 208 ⁽³⁾

(1) Confirmed positives

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

(3) Of which 5 samples presumed positive by the alternative method, negative after confirmation

Percentages obtained compared to the reference method are as follows:

- Relative accuracy (AC): 91.0%
- Relative specificity (SP): 92.0%
- Relative sensitivity (SE): 89.9%

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

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

Alternative method :	Reference method :
$(PA + PD) / (PA + PD + ND) = 90.7\%$	$(PA + ND) / (PA + PD + ND) = 91.7\%$

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

PD = 18, ND = 20 ; Y = PD + ND = 38 : Y > 18

Mc Nemar test : $d = |PD - ND| = 2$; $\chi^2 = d^2/Y = 0,105$ et $\chi^2 < 3.841$

Conclusion

The two methods are not statistically different.

Storage of One Broth Salmonella during 72 hours at 4°C

Confirmed positive results obtained for 226 samples just after incubation of One broth Salmonella were compared to those obtained after storage of One broth *Salmonella* during 72 hours at 2 to 8°C. The storage does not modify the results immediately obtained after incubation.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2007, on 6 combinations of food products/strains.

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
Escalope of raw turkey	<i>Salmonella</i> Typhimurium	0.3 [0.5 - 0.9]	0.2 [0.3 - 0.7]
Raw milk	<i>Salmonella</i> Anatum	0.3 [0.5 - 0.8]	0.3 [0.4 - 0.7]
Lettuce	<i>Salmonella</i> Enteritidis	0.1 [0.2 - 0.6]	0.4 [0.1 - 0.5]
Liquid egg	<i>Salmonella</i> Enteritidis	0.4 [0.2 - 1.0]	0.4 [0.2 - 1.1]
Biscuits for dog	<i>Salmonella</i> Anatum	0.1 [0.2 - 0.4]	0.2 [0.3 - 0.7]
Water process	<i>Salmonella</i> Give	0.2 [0.4 - 1.4]	0.3 [0.7 - 1.8]

(3) LOD₅₀: 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 detection level of the alternative method is between 0.1 and 1.8 CFU/25 g. It is identical to the relative detection level of the reference method.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 52 strains of *Salmonella* were detected out of 53 tested. The strain which did not grow in One broth *Salmonella* was *Salmonella* Dublin.
- The study of 40 strains not belonging to the genus *Salmonella* showed cross reactions on OSCMII Agar with 2 strains (*Citrobacter diversus* and *Enterobacter sakazakii*) but these strains gave negative results after confirmation by LATEX test.

PRACTICABILITY

Implementation of alternative method only

- **Response time :**
 - **Positive** results are obtained in 2 days (confirmation by LATEX test) or 4 days (confirmation by classical tests) using the alternative method against 5 days using the reference method.
 - **Negative** results are obtained in 2 days using the alternative method against 3 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 2 to 4 days.

INTERLABORATORY STUDY

The interlaboratory study was conducted in 2007 with 13 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a *Salmonella* Typhimurium strain at the 4 following 3 levels of contamination:

- Level 0: 0 CFU/25 ml
- Level 1: 5 CFU/25 ml
- Level 2: 25 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:

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	104	104	96	96	95	0	1
1	104	104	96 (REF) / 95 (ALT)	0	0	96	95 ^(a)
2	104	104	96	0	0	96	96

* One laboratory rendered positive results by the reference method on 4 samples not contaminated: their results were not taken into account.

(a) One laboratory did not correctly realize the dilution of one sample for alternative method: the sample was excluded of the interpretation.

Calculations

- Relative accuracy = **99.7%**
- % specificity = **100%**
- % sensitivity = **100%**

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 supplementary positives with alternative method):

$$\begin{aligned} &\text{Alternative method :} \\ &(PA + PD) / (PA + PD + ND) = 100\% \end{aligned}$$

$$\begin{aligned} &\text{Reference method :} \\ &(PA + ND) / (PA + PD + ND) = 99.5\% \end{aligned}$$

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	99	1.0
L1	100	100	1.0
L2	100	100	1.0

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

Contamination level	Accordance	Concordance	COR
L0	100	100	1.0
L1	100	100	1.0
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 AFNOR Certification.

You may download a summary document on the preliminary and inter-laboratory studies on www.afnor-validation.com