Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: UNI 03/01 - 05/91

Validation date:	**05.30.1991**
Renewal dates:	09.08.1995
	09.07.1999
	12.11.2003
	12.04.2007*
	07.01.2011
End of validity:	**09.07.2015**

**** EN ISO 16140 protocol was used in 2007 for the 4th renewal of validation***

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

OXOID *SALMONELLA* RAPID TEST (OSRT)

Protocol reference (temporary): **12/2007**

SCOPE: All human and animal food products and environmental samples (excluding primary production stage samples).

RESTRICTIONS FOR USE: The method is adapted to the detection of motile *Salmonella* and is not suitable for the detection of non motile *Salmonella* (non motile strains or strains having lost motility).

REFERENCE METHOD: EN ISO 6579 (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 Oxoid *Salmonella* Rapid Test (OSRT) is designed for the presumptive detection of motile *Salmonella*. It consists in a pre-enrichment of a homogenised sample in a suitable medium, followed by inoculation of the culture vessel containing a *Salmonella* selective medium with pre-enriched culture.

The culture vessel is equipped with two tubes both containing a selective medium and an upper indicator medium. *Salmonella* migrate actively through the lower selective medium to the upper indicator media where their presence is indicated by a colour change.

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

- According to classical tests described in methods standardized by CEN or ISO from colonies (including a purification step)
- Implementing Oxoid *Salmonella* LATEX Test starting from one tube indicator positive result. Agglutination of latex particles confirms the presence of motile *Salmonella*
- Using any other NF VALIDATION certified method, the principle of which is different from the OSRT method. The protocol of the second validated method shall be followed entirely. All steps that are before the step from which the confirmation is done shall be common to both methods.

In the event of discordant results (positive with alternative method, non-confirmed by means of options described above, in particular by the Latex test), the laboratory must follow the necessary steps to ensure validity of the result obtained.

Note (History of validation)

For NF VALIDATION renewal of 2007, the comparative and interlaboratory validation studies were entirely repeated following the new validation protocol described in EN ISO 16140 standard. Some results obtained in 2003 were re-investigated for relative accuracy/specificity/sensibility study.

At the same time, the OSRT method was modified as follow: (i) the scope of validation was extended to environmental samples; (ii) a new protocol of confirmation was added (Oxoid *Salmonella* LATEX test); (iii) the possibility to store pre-enrichment buffered peptone water broth (to be used prior to selective enrichment) during 72 hours at 2 to 8°C was validated.

In July 2011, the renewal of validation was pronounced without performing additional tests, because the OSRT method was not modified since the previous validation, and because the reference method and the protocol of validation remain unchanged. 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 2003 and 2007 tests were carried out on 447 product samples, of which 123 were naturally contaminated, 96 artificially contaminated and 228 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 = **207** [1]	Positive deviation A+ / R- PD = **9** [1]
Alternative method negative (A-)	Negative deviation A- / R+ ND = **3** [2]	Negative agreement A- / R- NA = **228** [3]

(1) Confirmed positives

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

(3) Of which 8 samples presumed positive by the alternative method, negative after confirmation

Percentages obtained compared to the reference method are as follows:

- Relative accuracy **(AC)**: **97.3%**
- Relative specificity **(SP)**: **96.2%**
- Relative sensitivity **(SE)**: **98.6%**

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 :

(PA + PD) / (PA + PD + ND) = 98.6%

Reference method :

(PA + ND) / (PA + PD + ND) = 95.9%

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

PD = 9, ND = 3 Y = PD + ND = 12 So $6 \leq Y \leq 22$ m = 3, M = 2 So $m > M$

Conclusion

The two methods are not statistically different.

Storage of buffered peptone water broths at 4°C during 72 hours

In 2007, all confirmed positive results obtained just after incubation of buffered peptone water broth were compared to those obtained after storage of buffered peptone water broth during 72 hours at 4°C.

Among 107 confirmed positives results obtained just after incubation:

- 3 samples gave negative results after storage of the buffered peptone water broth at 4°C during 72 hours (whereas they were supplementary positives results with OSRT just after incubation).
- 2 samples negative before storage were positive after storage.

The storage does not modify noticeably 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 samples were belonging to the following food categories:

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

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 beef meat	*Salmonella* Infantis	0.3 [0.1 – 0.8]	0.3 [0.1 – 0.8]
Raw milk	*Salmonella* Typhimurium	0.3 [0.1 – 1.0]	0.3 [0.1 – 0.9]
Cod filet	*Salmonella* Saintpaul	0.8 [0.5 – 1.3]	0.8 [0.5 – 1.3]
Liquid egg	*Salmonella* Enteritidis	0.2 [0.1 – 0.8]	0.3 [0.1 – 1.0]
Meatball for cat	*Salmonella* Agona	0.6 [0.3 – 1.8]	0.6 [0.3 – 1.8]
Water process	*Salmonella* Derby	0.3 [0.1 – 1.1]	0,3 [0.1 – 1.1]

(3) **LOD_{50}**: estimation of level of contamination enabling positive detection by alternative method in 50% of cases.
"*Hitchins A. Proposed Used 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 relative detection level of the alternative method is identical to the relative detection level of the reference method and ranges between 0.1 and 1.8 CFU/25g.

INCLUSIVITY / EXCLUSIVITY
Implementation of alternative method only

- 52 strains of *Salmonella* were detected out of 55 tested. The three strains which did not grow on OSRT test were *Salmonella* Paratyphi A strains. These strains were positive by LATEX test, gave characteristical colonies on OSCM II Agar, but were non characteristical on XLD Agar.
- 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 2 days (confirmation by LATEX test) or 5 days (confirmation by classical tests) using OSRT 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 5 days.

INTERLABORATORY STUDY

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

- 0 CFU/25 ml
- 1-10 CFU/25 ml
- 5-50 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	104	104	88	88	88	0	0
1	104	104	88	0	0	88	88
2	104	104	88	0	0	88	88

Calculations

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

Interpretation

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 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** and 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 identical 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