AFNOR Validation

Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: TRA 02/8 - 03/01

Validation date : 23.03.2001
Renewal date*: 03.02.2005
End of validity : 03.02.2009

**** EN ISO 16140 protocol was used in 2005***

The company (head office and production site)	**RAISIO DIAGNOSTICS SAS** 8, rue Saint Jean de Dieu **69007 LYON** France	**Distribution**	**RAISIO DIAGNOSTICS AB** FO Petersons Gata 32 **SE-421 31 Västra Frölunda** **Sweden**

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

TRANSIA® PLATE Salmonella GOLD

Art. Nr SA 0180 (1 microplate) and Art. Nr SA 0190 (10 microplates)

Protocol reference: **NOT COM 510J** 02/06 (SA 0180) and **NOT COM 710K** 02/06 (SA 0190)

SCOPE:

Food and animal feeding stuffs and environmental samples

RESTRICTIONS OF USE:

None

REFERENCE METHOD:

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

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PRINCIPLE OF THE METHOD

TRANSIA PLATE Salmonella Gold is an Enzyme Linked Immuno Sorbent Assay (ELISA) based on a sandwich-type reaction. The 2 step enrichment protocol includes a pre-enrichment in buffered peptone water followed by a selective enrichment in Rappaport Vassiliadis Soya broth (RVS). The detection step is then made in a microplate coated with antibodies specific to *Salmonella* spp. The reading is made with a microplate reader at 450 nm.

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

- According to classical tests described in methods standardized by CEN, ISO or AFNOR (including a purification step), starting from the RVS broth followed by isolation on 2 different selective media
- By implementing any other AFNOR validated method based on a principle different from the TRANSIA PLATE Salmonella Gold method, respecting specifications in the test instructions.

In the event of discrepant 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

The TRANSIA PLATE Salmonella Gold method has been validated in 2001 according to the previous validation protocol against the EN 12824 reference method for food and animal feeding stuffs. The renewal was performed in 2005 according to the EN ISO 16140 (2003) standard and against the new reference method EN ISO 6579 (2002). Some variations from the original protocol were also included (test performed on the RVS immediately after incubation and heat inactivation or after storage of RVS broth at 3°C ± 2°C up to 48 h prior to heat inactivation) and the validation was extended to environmental samples.
The preliminary study and the collaborative study were totally repeated according to the EN ISO 16140 protocol. In addition, all positive (spiked and naturally contaminated) and doubtful samples were tested again after 48 h storage at 3°C ± 2°C, in order to validate the possibility to do the detection test after storage of the broths in a fridge up to 48 hours.

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 429 product samples, of which 69 were naturally contaminated, 118 artificially contaminated, and 242 non-contaminated, belonging to the following main food product categories: meat products, dairy products, vegetables and seafood products, miscellaneous (egg products, ready meals, pastries), animal feeds and 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 = **186**[(1)]	Positive deviation A+ / R- PD = **0**
Alternative method negative (A-)	Negative deviation A- / R+ ND = **1**[(2)]	Negative agreement A- / R- NA = **242**[(3)]

- (1) Confirmed positives
- (2) (3) Of which no sample presumed positive by the alternative method was negative after confirmation

Percentages obtained compared to the reference method are as follows:

- Relative accuracy: **99.8** %
- Relative specificity: **100** %
- Relative sensitivity: **99.5** %

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) = **99.5** %	(PA + **ND**) / (PA + **PD** + ND) = **100** %

Conclusion
TRANSIA PLATE Salmonella Gold method performances are equivalent to those 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 6 combinations of food products/strains.

These products represent the following product categories: meat products, dairy products, vegetables and seafood products, miscellaneous (egg products, ready meals, pastries), animal feeds and 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**
Ground poultry	*Salmonella* Hadar	0.3 [0.2 - 0.5]	0.3 [0.2 - 0.5]
Raw milk	*Salmonella* Thyphimurium	0.8 [0.4 - 1.4]	0.8 [0.4 - 1.4]
Raw eggs	*Salmonella* Enteritidis	0.7 [0.4 - 1.3]	0.7 [0.4 - 1.3]
Fish filet	*Salmonella* Virchow	0.3 [0.2 - 0.5]	0.3 [0.2 - 0.5]
Animal feeds	*Salmonella* Senftenberg	0.5 [0.3 - 0.9]	0.5 [0.3 - 0.9]
Process water	*Salmonella* Infantis	0.7 [0.4 - 1.5]	0.7 [0.4 - 1.5]

(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 detection level is assessed between 0.2 and 1.5 CFU/25 g for both reference and alternative methods.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 50 strains of *Salmonella* were detected out of 50 tested.
- The study of 30 non-*Salmonella* strains did not detect the presence of any cross-reaction.

PRACTICABILITY

Implementation of alternative method only

Time to result:

- **Positive** results are obtained in 5 to 7 days using the alternative method *(including confirmation according to classical tests of the reference method, with purification step included*) or the reference method.
- **Negative** results are obtained in 2 days using the alternative method against 3 to 7 days using the reference method.
- In the case of results presumed positive using the alternative method, but rendered negative after confirmation, these negative results are obtained in 3 to 7 days.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2004 with 11 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:

- 0
- slightly superior to relative detection level (3 cells / 25 mL)
- 10 times superior to previous level (30 cells / 25 mL)

The laboratories tested, using **both methods**, **8 replicate samples** for **each level** of contamination, giving a total of 260 analyses for the participating laboratories as a whole.

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	88	80	80	80	80	0	0
1	88	80	80	4	5	76	75
2	88	80	80	0	0	80	80

** a laboratory did not receive the samples in time and did not perform the analyses*

Calculations

- Relative accuracy = **99.6** %
- % specificity = **100 %**
- % sensitivity = **96.9 %**

Interpretation

The 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) = **99.4 %**

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

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

Contamination level	Accordance	Concordance	COR
L0	100 %	100 %	1.00
L1	91 %	87.7 %	1.04
L2	100 %	100 %	1.00

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

Contamination level	Accordance	Concordance	COR
L0	100 %	100 %	1.00
L1	93 %	90.1 %	1.03
L2	100 %	100 %	1.00

Conclusion

The variability of the alternative method (accordance, concordance, concordance odds ratio) is equivalent to the reference method's one.

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.