



**Alternative methods for agribusiness  
Analytical performances certified**

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

**Certificate No.: RBP-31/01-06/08**

**Validation date: 30.06.2008  
End of validity: 30.06.2012**

**Manufacturer  
and distributor**      **R-BIOPHARM AG**  
Landwehrstr. 54  
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is hereby authorized to refer to this **AFNOR Validation certificate** for the following alternative **qualitative** analysis method:

**RIDASCREEN® Salmonella**

Protocol reference: RIDASCREEN® Salmonella 08-09-30

**SCOPE**

All human food products, animal feed and environmental samples (except samples of primary production).

**RESTRICTIONS OF USE**

None.

**REFERENCE METHOD**

**EN ISO 6579 (2002)** – Horizontal method for detection of *Salmonella* spp.

A handwritten signature in black ink, appearing to be "Jacques Beslin", written over a horizontal line.

**Deputy General Manager  
Jacques BESLIN**

**AFNOR Certification**

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## PRINCIPLE OF THE METHOD

The RIDASCREEN® *Salmonella* test is based on an ELISA-type immunoenzymatic reaction. The results can be read visually or by a micro plate reader.

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

- From the pre-enrichment broth according to the classic tests described in the methods standardized by the CEN or ISO (including the stage of purification).
- From the *Salmonella* broth according to the classic tests described in the methods standardized by the CEN or ISO (including the stage of purification). The selective broths can be used when the *Salmonella* broth does not give a *Salmonella* result.
- From the pre-enrichment broth according to all other certified AFNOR VALIDATION methods, based on a principle different from that of the RIDASCREEN® *Salmonella* method, following the conditions specified in the technical specification of the manufacturer.

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.

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

In **2007 and 2008**, tests were carried out on 380 product samples, of which 46 were naturally contaminated, 143 artificially contaminated, and 191 non-contaminated, belonging to the following principal food product categories :

Milk products, meat products, seafood and vegetables, miscellaneous, animal feed 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 = 184 <sup>(1)</sup>	Positive deviation A+/R- PD = 1 <sup>(1)</sup>
Alternative method negative (A-)	Negative deviation A-/R+ ND = 4 <sup>(2)</sup>	Negative agreement A-/R- NA = 191 <sup>(3)</sup>

(1) Confirmed positives

(2) Of which no sample presumed positive by the alternative method was negative after confirmation

(3) Of which 30 samples presumed positive by the alternative method were negative after confirmation

Percentages obtained compared to the reference method are as follows:

- Relative accuracy (AC): **99%**
- Relative specificity (SP): **99%**

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

- Relative sensitivity (SE): **98%**

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

$$\text{Alternative method :} \\ (PA + PD) / (PA + PD + ND) = 98\%$$

$$\text{Reference method :} \\ (PA + ND) / (PA + PD + ND) = 99\%$$

## Relative DETECTION LEVEL

### Comparison of performances of the alternative method and the reference method

Tests were carried out in 2007 and 2008, on 6 combinations of food products/strains.

These products represent the following food categories: milk products, meat products, seafood and vegetables, miscellaneous, animal feed 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 <sub>50</sub>	
Matrix	Strain	Alternative method	Reference method
Ground meat	<i>S. typhimurium</i>	0.8 [ 0.5 – 1.3 ]	0.6 [ 0.4 – 0.8 ]
Raw milk	<i>S. dublin</i>	0.7 [ 0.4 – 1.3 ]	0.7 [ 0.4 – 1.3 ]
Filet of coalfish	<i>S. enteritidis</i>	0.7 [ 0.4 – 1.0 ]	0.7 [ 0.5 – 1.1 ]
Whole eggs	<i>S. enteritidis</i>	0.7 [ 0.5 – 1.0 ]	0.7 [ 0.5 – 1.0 ]
Cat food	<i>S. infantis</i>	0.9 [ 0.5 – 1.4 ]	0.8 [ 0.5 – 1.3 ]
Process water	<i>S. typhimurium</i>	0.5 [ 0.3 – 0.6 ]	0.5 [ 0.4 – 0.7 ]

(3) **LOD<sub>50</sub>**: estimation of level of contamination enabling positive detection by alternative method in 50% of cases.

"Hitchins A. Proposed Use of a 50% Limit of detection Value in Defining Uncertainty Limits in the Validation of Presence-Absence Microbial detection Methods. Draft 10<sup>th</sup> December, 2003"

### Conclusion

The detection level of the alternative method is between 0.3 and 1.4 CFU/25 g.  
The detection level of the reference method is between 0.4 and 1.3 CFU/25 g.

## INCLUSIVITY / EXCLUSIVITY

### Implementation of alternative method only

- 75 strains of *Salmonella* were detected out of 75 tested.
- The study of 33 non-*Salmonella* strains showed cross reactions with two strains of *Citrobacter freundii*.

## PRACTICABILITY

### Implementation of alternative method only

- **Response time :**
- **Positive** results are obtained in 3 to 7 days using the alternative method against 5 to 7 days using the reference method.
- **Negative** results are obtained in 1day 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 after confirmation, these negative results are obtained in 3 to 7 days.

## INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2008 with 50 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a *Salmonella enteritidis* strain at the 3 following levels of contamination:

- 0 CFU / 25 ml
- 3 CFU / 25 ml
- 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	120	112	96	95	96	1	0
1	120	112	96	2	5	94	91
2	120	112	96	0	0	96	96

\*One laboratory did not receive samples on time.

\*\*Two laboratories encountered manipulation problems.

### Calculations

- Relative accuracy = **99%**
- Specificity = **100%**
- Sensitivity = **98%**

### Interpretation

The results of the interlaboratory study are comparable to those obtained in the preliminary study.

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

Alternative method :  

$$(PA + PD) / (PA + PD + ND) = 98\%$$

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**:

Contamination level	Accordance	Concordance	COR
L0	100%	100%	1.0
L1	91%	84%	1.9
L2	100%	100%	1.0

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

Contamination level	Accordance	Concordance	COR
L0	98%	98%	1.0
L1	95%	90%	1.1
L2	100%	100%	1.0

**Conclusion**

The variability of the alternative method (accordance, 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](http://www.afnor-validation.com)