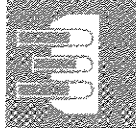




CERTIFICATE OF COMPLIANCE



MICROVAL

HEREBY DECLARES THAT THE CERTIFICATION ASSESSMENT BY
LLOYD'S REGISTER QUALITY ASSURANCE
HAS DEMONSTRATED THAT

RIDASCREEN® Salmonella

Manufactured and supplied by:
R-Biopharm AG
An der neuen Bergstraße 17
64297 Darmstadt
Germany

COMPLIES WITH

The MicroVal Rules and Certification Scheme version 5
The validation has been performed in accordance with EN ISO 16140: 2003
as demonstrated by Report: RIDASCREEN Salmonella Test Summary Report,
August 2008

Certificate no.: 2009-LR21

Validation date: 30-11-2009
Surveillance date: 30-11-2009
Expiry date: 29-11-2013

ISSUED BY: Lloyd's Register Nederland B.V.
Rotterdam, The Netherlands



USE OF EXTERNAL DATA

This certificate has been issued based upon Annex A of ISO16140. Specific rules for the acceptance of external results already obtained in a prior validation scheme. The MicroVal Certificate of The RIDASCREEN® Salmonella is based upon a study performed by Institut Scientifique d'Hygiène & d'Analyse (ISHA) in 2008.

PRINCIPLE OF THE METHOD

The RIDASCREEN® Salmonella test is based on an ELISA-type immuno-enzymatic reaction. The results can be read visually or by a micro plate reader. After an pre-enrichment in BPW at 37°C for 16 to 20 hours the broth is transferred to the microplate. The microplate with broth is incubated for 5.5 to 6 hours and then conjugate is added. The presence of *Salmonella* is indicated when the bound conjugate converts the substrate to a blue colour.

It is a screening method. All samples identified as positive by this test must be confirmed by one of the following means:

- from the pre-enrichment broth (BPW) according to the classic tests described in EN-ISO 6579:2002.
- from the selective enrichment broth (RVS) according the classic tests described in EN-ISO 6579:2002. The selective broth can be used when the pre-enrichment broth does not give a *Salmonella*-positive result.
- from the pre-enrichment broth according to any other *Salmonella* detection method validated according to EN-ISO 16140.

SCOPE

All human and animal food products, and environmental samples (except samples from the primary production stage).

RESTRICTION OF USE

None

REFERENCE METHOD

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

RELATIVE ACCURACY, RELATIVE SENSITIVITY and RELATIVE SPECIFICITY

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

Tests were done in 2007 and 2008 on 380 samples of products where 46 were naturally contaminated, 143 artificially contaminated and 191 not contaminated, belonging to the following principal food categories:

Meat products, milk products, seafood and vegetables, miscellaneous, animal feed and environmental samples.

All samples were analyzed in single using the two methods.



Table of results (Cf table 1 standard EN-ISO 16140:2003)

| Responses | Reference method positive (R+) | Reference method negative (R-) |
|----------------------------------|--|--|
| Alternative method positive (A+) | Positive agreement (A+R+) PA = 184 ¹ | Positive deviation (R-A+) PD = 1 ¹ |
| Alternative method negative (A-) | Negative deviation (A-R+) ND = 4 | Negative agreement (A-R-) NA = 191 ² |

- 1) Confirmed positives.
- 2) Of which 30 samples presumptive-positive by the alternative method were negative after confirmation.

Calculation of the relative sensitivity and relative specificity (Cf. table 2 EN-ISO 16140)

| Matrices | PA | NA | ND | PD | N | AC % | N+ | SE% | N- | SP % |
|------------------------|-----|-----|----|----|-----|------|-----|-----|-----|------|
| Meat products | 33 | 36 | 0 | 0 | 69 | 100 | 33 | 100 | 36 | 100 |
| Milk products | 31 | 32 | 1 | 0 | 64 | 98 | 32 | 97 | 32 | 100 |
| Seafood and vegetables | 31 | 30 | 1 | 0 | 62 | 98 | 32 | 97 | 32 | 100 |
| Miscellaneous | 28 | 30 | 2 | 0 | 60 | 97 | 30 | 93 | 30 | 100 |
| Animal feed | 31 | 31 | 0 | 1 | 63 | 98 | 31 | 100 | 32 | 97 |
| Environmental samples | 30 | 32 | 0 | 0 | 62 | 100 | 30 | 100 | 32 | 97 |
| Total | 184 | 191 | 4 | 1 | 380 | 99 | 188 | 98 | 192 | 99 |

Results before and after confirmation of presumptive-positive samples

| Matrices | Screening results (presumptive positive samples) | Confirmed positive samples | False-positive samples |
|------------------------|--|----------------------------|------------------------|
| Meat products | 44 | 33 | 11 |
| Milk products | 34 | 31 | 3 |
| Seafood and vegetables | 36 | 31 | 5 |
| miscellaneous | 31 | 28 | 3 |
| Animal feed | 33 | 31 | 2 |
| Environmental samples | 36 | 30 | 6 |
| Total | 214 | 184 | 30 |

CONCLUSION

The performance of the alternative method is equivalent to that of the reference method. Confirmation of suspect samples is required.

RELATIVE DETECTION LEVEL

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

Tests were done in 2007 and 2008 on the six combinations of food products/strains described in the table below.



These products represent the following food categories: Meat products, milk products, seafood and vegetables, miscellaneous, animal feed and environmental samples. The products were analyzed 6 times using the two methods at 4 levels of contamination.

The results obtained are the following:

| Relative level of detection LOD ₅₀ (1) With confidence interval (CFU/25 g or 25 ml) | | | |
|---|-----------------------|--------------------|------------------|
| 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] |

1) LOD₅₀: estimation of the contamination level enabling positive detection using the alternative method in 50% of cases. Hitchens A., 2003. Proposed Use of a 50% Limit of Detection Value in Defining Uncertainty Limits in the Validation of the Presence-Absence of Microbial Detection Methods (<http://www.cfsan.fda.gov/~acrobotat/bpmm-k.pdf>).

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.

All 75 *Salmonella* strains gave a positive result (= *Salmonella* detected) with the alternative method. The study of 33 non-*Salmonella* strains has shown cross-reactions with two strains of *Citrobacter freundii*.

INTERLABORATORY STUDY

The interlaboratory study was conducted in 2008 involving 15 collaborative laboratories. Analyses were carried out on samples of pasteurized milk, artificially contaminated with a *Salmonella* Enteritidis strain at 3 levels of contamination:

- L0: 0 cfu/25 ml
- L1: approximately 3 cfu/25 ml
- L2: approximately 30 cfu/25 ml

Each laboratory received 8 replicate samples for each level of contamination, which were tested by both methods.



Results interlaboratory study:

| Contamination level | Total number of samples | Number of samples analysed ¹ | Number of results evaluated ² | Number of negative results | | Number of positive results | |
|---------------------|-------------------------|---|--|----------------------------|-----|----------------------------|-----|
| | | | | Ref | Alt | Ref | Alt |
| L0 | 120 | 112 | 96 | 95 | 96 | 1 | 0 |
| L1 | 120 | 112 | 96 | 3 | 6 | 93 | 90 |
| L2 | 120 | 112 | 96 | 0 | 0 | 96 | 96 |

- 1) One laboratory did not receive samples on time
- 2) Two laboratories encountered manipulation problems

Alternative method results from the Method Comparison Study and Interlaboratory Study.

| | Method Comparison Study | Interlaboratory Study |
|-------------------------|-------------------------|-----------------------|
| Relative accuracy AC | 99% | 99% |
| Relative sensitivity SE | 98% | 98% |
| Relative specificity SP | 99% | 100% |

CONCLUSION

The results of the Interlaboratory Study are comparable to those obtained in the Method Comparison Study.

ACCORDANCE, CONCORDANCE AND CONCORDANCE ODDS RATIO

Accordance: percentage change 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. The concordance 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 (conditions of reproducibility). 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 the 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 the 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, concordance odds ratio) is equivalent to that of the reference method.

FINAL CONCLUSION

The results from the Method Comparison Study and the Interlaboratory Study revealed that there was no significant difference between the RIDASCREEN@Salmonella method, including a confirmation step and the reference method ISO 6579:2002 for the detection of *Salmonella*.

Please send any queries concerning the performance of the validated method to Lloyd's Register Quality Assurance.