



## CERTIFICATE OF COMPLIANCE

# MICROVAL



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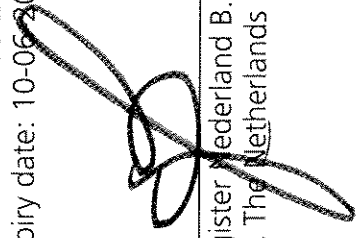
**Nestec Laboratory Instruction LI-00.713-5 (dd  
5-5-2009) for the detection of *Salmonella***

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 number 2009.502

Certificate no.: 2007-LR06

Validation date: 11-06-2009  
Surveillance date: 11-06-2009  
Expiry date: 10-06-2013

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

Certificate no.: 2007-LR06

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This certificate includes 5 pages



## PRINCIPLE OF THE METHOD

The main validated principle for all the Categories under study was the use of only RVS (alternative method) instead of both RVS and MKTtn (ISO reference method) as selective enrichment broth.

Additionally, an alternative pre-enrichment broth was used for two types of products, both within the Category Dairy products containing Probiotics:

-Dehydrated products (eg infant formula) with probiotic bacteria at a level  $< 10^8$  cfu/g: BPW with 10 mg/l vancomycin.

-Dehydrated products (eg infant formula) with probiotic bacteria at a level  $\square 10^8$  cfu/g: double strength BPW with 10 mg/l vancomycin, 100 mg/l malachite green and 100 g/l non-fat dry milk powder.

Four different isolation media were used in parallel and all are part of the validation study (both the method comparison study and the interlaboratory study, including confirmation of isolates on all 4 plates). Each combination of 2 isolation media was evaluated on its performance.

## SCOPE

All human and animal food products, and environmental samples.

## 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.

The tests were performed in 2008/2009 on 6 food categories. A total of 28 samples were naturally contaminated, 156 samples were artificially contaminated, and 180 were non-contaminated. The principle food product categories tested were dairy products, dairy products containing probiotics, chocolate products, other products, animal feed, and environmental samples.

All samples were analysed in single by 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 = 172	Positive deviation (R-A+) PD = 1
Alternative method negative (A-)	Negative deviation (A-R+) ND = 3	Negative agreement (A-R-) NA = 188



**Calculation of the relative accuracy, the relative sensitivity and the relative specificity**  
(Cf table 2 standard EN/ISO 16140:2003)

Matrices	PA	NA	ND	PD	Sum	Relative Accuracy AC (%)	N+	Relative sensitivity SE (%)	N-	Relative specificity SP (%)
					N	100 x (PA+NA)/N	PA+ND	100 x PAVN+	NA+PD	100 x NAVN-
Dairy products	25	35	0	0	60	100.0%	25	100.0%	35	100.0%
Dairy products plus Probiotics	28	30	1	1	60	96.7%	29	96.6%	31	96.8%
Chocolates	31	33	0	0	64	100.0%	31	100.0%	33	100.0%
Other products	27	33	0	0	60	100.0%	27	100.0%	33	100.0%
Animal Feed	28	30	2	0	60	96.7%	30	93.3%	30	100.0%
Environmental samples	33	27	0	0	60	100.0%	33	100.0%	27	100.0%
<b>TOTAL</b>	<b>172</b>	<b>188</b>	<b>3</b>	<b>1</b>	<b>364</b>	<b>98.9%</b>	<b>175</b>	<b>98.3%</b>	<b>189</b>	<b>99.5%</b>

### CONCLUSION

The performance of the alternative method appears equivalent to that of the reference method.

### RELATIVE DETECTION LEVEL

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

Tests were carried out in 2008, on 6 combinations of products/strains as described in the list below. These products represent the following (food) categories: dairy products, dairy products containing probiotics, chocolate products, other products, animal feed, and environmental samples. Products were analysed 6 times by the 2 methods at 5 levels of contamination.

The results obtained are as follows:

Type of product	Strain	Relative detection level with confidence interval LOD <sub>50</sub> <sup>(1)</sup> (cfu/25 g)	
		Reference method	Alternative method
Infant formula	<i>S. Typhimurium</i>	0,4 [0,3-0,7]	0,4 [0,3-0,7]
Infant formula containing Probiotics	<i>S. Panama</i>	0,6 [0,3-1,2]	0,4 [0,3-0,8]
Cocoa powder	<i>S. Senftenberg</i>	0,4 [0,2-0,6]	0,4 [0,2-0,6]
Dried chicken soup	<i>S. Derby</i>	0,3 [0,3-0,4]	0,3 [0,3-0,4]
Dog food (dry)	<i>S. Virchow</i>	0,4 [0,3-0,6]	0,4 [0,3-0,6]
Vacuum cleaner residues	<i>S. Enteritidis</i>	0,3 [0,2-0,5]	0,3 [0,2-0,6]

(1) LOD<sub>50</sub> : estimation of level of contamination enabling positive detection in 50% of cases. Hitchins, A., 2003. Proposed Use of a 50 % Limit of Detection Value in Defining Uncertainty Limits in the Validation of Presence-Absence Microbial Detection Methods. <http://www.cfsan.fda.gov/~acrobat/bprm-k.pdf> and Revealed calculations and formula LOD 50%.xls



## CONCLUSION

The detection limit for the alternative method is between 0,2 and 0,8 cfu/25 g.  
The detection limit for the reference method is between 0,2 and 1,2 cfu/25 g.

## INCLUSIVITY/EXCLUSIVITY

Implementation of alternative method only

All 50 *Salmonella* strains gave a positive result (= *Salmonella* detected) with the alternative method.  
All 30 non-*Salmonella* strains gave a negative result (= *Salmonella* not detected) with the alternative method.

## INTERLABORATORY STUDY

The interlaboratory study was conducted in 2009 involving 15 collaborative laboratories from 7 European countries. Analyses were carried out on samples of pasteurized milk, artificially contaminated with a *Salmonella* Infantis strain at 3 levels of contamination:

- L0: 0 cfu/25 ml
- L1: around 3 cfu/25 ml
- L2: around 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	Number of results evaluated*	Number of negative results		Number of positive results	
				Ref	Alt	Ref	Alt
L0	120	120	96	96	0	0	
L1	120	120	96	0	96	96	
L2	120	120	96	0	96	96	

\*The data provided by 3 laboratories was omitted from the statistical analysis because they reported one or more false-positive results. This probably could have been due to leakage of sample material during transport, which may have had caused cross-contamination.

Alternative method results from the MCS and ILS.

	Method Comparison Study	Interlaboratory Study
Relative accuracy AC	98,9%	100%
Relative sensitivity SE	98,3%	100%
Relative specificity SP	99,5%	100%

## CONCLUSION

The results of the interlaboratory study are comparable to those obtained during 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 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 (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 both the reference and the alternative method:

Contamination level	Accordance	Concordance	COR
L0	100%	100%	1,0
L1	100%	100%	1,0
L2	100%	100%	1,0

## CONCLUSION

The variability of the alternative method (accordance, concordance, concordance odds ratio) is identical 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 Nestec Laboratory Instruction LI-00.713-5 method and the reference method ISO 6579:2003 for the detection of *Salmonella*.

This is valid for each of the combinations of plating media used for the Alternative method (XLD or BGA, XLD or MLCB, XLD or SMID2, BGA or MLCB, BGA or SMID2, MLCB or SMID2).

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