NMKL - NordVal International
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NordVal International Certificate

Issued for:	**Salmonella Velox spp. and Salmonella Velox SE + ST**
NordVal No:	046
First approval date:	07 June 2016
Renewal date:	01 October 2025
Valid until:	02 October 2027

Salmonella Velox spp. and Salmonella Velox SE + ST

Manufactured and supplied by:

DNA Diagnostic A/S
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fulfils the requirements for NordVal Certification. The reference method was EN ISO 6579:2017: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. AnalyTech Miljølaboratorium A/S, Denmark and ISI Food Protection ApS, Denmark have conducted validation studies according to ISO 16140-2/NordVal Protocol No. 1.

Salmonella Velox spp. and S. Velox SE + ST are rapid qPCR tests that can be carried out within 5.5 hours for a broad range of foods. The method was validated using the matrices raw meat, poultry and fishery products as well as ready-to-cook, ready-to-reheat and ready-to-eat meat, on swabs performed on raw food samples and in environmental and primary production samples.

The production of the kits is fulfilling the requirements given in ISO 13485.

Date: 01. October 2025
(updated 30 Apr 2026)

Yours sincerely,

Hrólfur Sigurðsson

Hrólfur Sigurðsson
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

The method includes description of an enrichment step, a DNA extraction step, a qPCR step and how to interpret the results.

Enrichment
The enrichment step uses a Salmonella Velox Bag into which the cold sample (2-10°C) is inserted.
The amount of the sample is maximum 25 g ± 5 g chicken, fish and seafood, ready-to-eat or ready-to-reheat; 25 – 100 g for minced meat; 25 - 125 g raw meat or 25 g ± 5 g food swabs; maximum 25 g ± 5 g environmental samples; maximum 25 g ± 5 g primary production samples.
Following, 100 mL to 225 mL preheated (42.5°C ±0.5°C for sample sizes 75 – 125 g, or 41°C ± 0.5°C for sample sizes 25 – 75 g) Salmonella Velox Broth is added to the bag, and the bag is inserted in a rack between Salmonella Velox Heating Packs, also preheated to 42.5°C ± 0.5°C or 41°C ± 0.5°C. The rack with bags is incubated at 42.5°C ± 0.5°C or 41°C ± 0.5°C for 4.5 – 24 hours. For ready-to-eat and ready-to-reheat meat products, and primary production samples, the minimum required time is 5 hours and 7 hours, respectively.

DNA extraction
The DNA extraction step uses the Salmonella Velox DNA Extraction Kit for DNA extraction from 1.5 -1.8 mL of the enrichment. Bacteria are pelleted by centrifugation. The supernatant is removed by a plate wash instrument and cells are lysed by addition of Lysis Buffer 1 and heating at 95°C ± 5°C. Finally, Lysis Buffer 2 is added and cell debris is pelleted by centrifugation. Now the DNA is in the supernatant.

PCR
The qPCR step uses the Salmonella Velox spp. qPCR Kit or the Salmonella SE+ST qPCR Kit and 5 uL DNA extract. The DNA sample is added to a well in a 96 well qPCR plate containing qPCR reaction mix. The qPCR reaction runs for approximately 40 minutes. A single stranded DNA template is present in the qPCR reaction mix as an internal amplification control (IAC) template. The qPCR reaction mix contains primers for amplification of the IAC and primers for the amplification of the Salmonella specific targets. For Salmonella Velox S. spp, probes with FAM fluorophore and probes with ROX fluorophore are present for detection of the IAC and Salmonella amplicons respectively.
For Salmonella Velox SE + ST, probes with FAM fluorophore and probes with ROX and Cy5 fluorophores are present for detection of the IAC and the Salmonella Enteritidis and Typhimurium amplicons respectively.

Interpretation
The functionality of the qPCR reaction is controlled by the presence of IAC amplicons resulting in a FAM signal with a Ct value at 28-35. Note that the FAM Ct can be > 35 or missing if the Ct for ROX/CY5 is low. Reactions with no ROX/CY5 signal and FAM signal absent or outside the interval 28-35 must be retested.

Ct-value	Salmonella Velox	Salmonella Velox SE+ST
ROX<38	The reaction is positive for a *Salmonella spp*.	The reaction is positive for a *Salmonella* Enteritidis
CY5<38	N/A	The reaction is positive for a *Salmonella* Typhimurium
ROX / CY5 38-40	The reaction may be interpreted as positive for *Salmonella spp.* if retesting	The reaction may be interpreted as positive for *Salmonella* Enteritidis/Typhimurium if

	(second qPCR using the same DNA extract as for the first qPCR) results in a similar or lower Ct value as the first test.	retesting (second qPCR using the same DNA extract as for the first qPCR) results in a similar or lower Ct value as the first test.
ROX / CY5 No Ct	The reaction is negative for *Salmonella spp.*	The reaction is negative for *Salmonella* Enteritidis/Typhimurium

FIELD OF APPLICATION

The method is applicable for the detection of *Salmonella* spp. and/or *Salmonella Enteritidis* + *Typhimurium* in a broad range of foods. The matrices used in the validation were: raw meat, poultry and fishery products as well as ready-to-cook, ready-to-reheat and ready-to-eat meat, on swabs from raw food and in environmental and primary production samples.

HISTORY

In 2017, the performance on ready-to-reheat and ready-to-eat products has been validated in sensitivity and detection level study.

In 2019, LOD was included and results were recalculated according to the new NordVal International protocol.

In 2021, the alternative method “Salmonella Velox spp.” was extended by environmental and primary production samples. Sensitivity study and selectivity studies were also carried out for the Salmonella Velox SE+ST qPCR compared to the reference method. For the detection study, the Salmonella Velox SE +ST were compared to the qPCR CT curves of Salmonella Velox spp. The studies were performed by the expert laboratory ISI Food Protection.

In 2023 a new ILS was made according to the EN ISO 16140-2:2016 and the NordVal Protocol No. 1 from January 2022.

30 Apr 2026, it was included in the certificate that the method validations fulfil the requirements for applicability to a broad range of matrices.

COMPARISON STUDY

The comparison studies have been carried out by AnalyTech Miljølaboratorium A/S according to the ISO 16140-2:2016. The study on raw meat and ready to cook meat products, poultry and fish and seafood was carried out in February 2016 and the analysis on swabs were performed in April 2017. The studies on environmental samples and production samples of Salmonella Velox spp and the studies on Salmonella Velox SE+ST qPCR were carried out in 2021.

Selectivity study

Salmonella Velox spp

Inclusivity study: 110 Salmonella strains were tested with Salmonella Velox and ISO 6579. Two samples were excluded due to failure of spiking and failure of qPCR reaction, respectively. The remaining 108 Salmonella strains, representing 101 different Salmonella serovars, all tested positive with both Salmonella Velox and the reference method.

Exclusivity study: 30 non-salmonella bacteria were tested with Salmonella Velox and ISO 6579. All tested negative with both methods.

It can be concluded that the selectivity is 100% for both the alternative method and the reference method.

Salmonella Velox SE + ST qPCR

Inclusivity study: 151 strains of Salmonella were tested of which 25 strains were *S.* Typhimurium, 32 strains were *S.* Enteritidis and 94 strains were Salmonella spp. that are not the target serotypes *S.* Typhimurium or *S.* Enteritidis. Three Salmonella spp. gave false positive results: *S*. Rissen, *S*. Goldcoast and *S*. Moscow.
S. Derby, *S*. California, *S*. Infantis, *S*. Anatum, *S*. Muenchen and *S*. Bovismorbificans all gave true negative results.

Exclusivity study: 45 non-Salmonella bacteria were tested and all gave true negative result.

Sensitivity study

The study was conducted on artificially contaminated samples, spiked with different cold-stressed Salmonella serovars: *S*. Enteritidis, *S*. Typhimurium, *S*. Newport and *S*. Infantis. The matrices tested are given in **Table 1**.

Table 1. The matrix category, matrix type and the bacteria inoculated used in the samples.

Matrix Category	Matrix type	Bacteria
1. Raw meat and ready-to-cook meat products (except poultry) (125 g of sample)	1.1. Raw pork cutlet * 1.2. Raw bovine minced meat* 1.3. Raw sheep meat cuts*	*S*. Typhimurium or *S*. Enteritidis or *S*. Newport or *S*. Infantis
2. Raw poultry and ready-to-cook poultry products (25 g of sample)	2.1. Raw chicken carcass 2.2. Raw chicken breast 2.3. Raw seasoned chicken breast	
3. Raw and ready-to-cook fish and seafood (unprocessed) (25 g of sample)	3.1. Raw salmon fillet 3.2. Raw tiger shrimps 3.3. Raw blue mussels	
4. Swabs (25 g of sample)	4.1. Raw pork meat*	
5. Ready-to-eat and ready-to-reheat (25 g of sample)	5.1 Cooked chicken breast (poultry, cooked) 5.2 Salami (meat/pork, fermented) 5.3 Smoked salmon (fishery, raw cured) 5.4 Sausage (meat/pork, cooked)	
Extension study 2021 both Salmonella Velox spp and Salmonella Velox SE+ST qPCR		
6. Raw poultry and ready-to-cook poultry	6.1 Raw chicken breast (unprocessed)	*S*. Enteritidis
	6.2 Raw barbeque seasoned chicken fillets (unprocessed)	*S*. Typhimurium
7. Ready-to-eat and ready-to-reheat meat products and poultry products	7.1 Chicken nuggets (poultry. processed. breaded)	*S*. Typhimurium

	7.2 Chicken sausages (poultry. processed. cooked)	*S*. Enteritidis
	7.3 Salami (red meat/pork. processed. fermented)	*S*. Enteritidis
8. Environmental samples (food or feed production)	8.1 Environmental swabs (surface swabs from meat processing areas)	*S*. Typhimurium
9. Primary production samples (PPS)	9.1 Swine Feces (from pigsty)	*S*. Enteritidis
	9.2 Boot swabs from broiler house	*S*. Enteritidis
	9.3 Litter from cow enclosure	*S*. Typhimurium

** lysates from the matrix items are also used for analysis of Salmonella Velox SE+ST qPCR*

The matrix categories 1-5 were used in the studies conducted in 2016 and 2017, and the matrix categories 6-9 were used in the validation of 2021. In addition, lysates from matrix categories 1 and 4 were used in the validation conducted in 2021.
For each matrix type minimum 20 samples were tested with both the Salmonella Velox method and the reference method.

For each of the matrix types, the 20 x 2 samples were spiked to give the following levels:
L_0: 2x5 samples no spiking (negative),
L_1: 2x10 minimum samples were spiked with low level (1-10 cfu) and
L_2: 2x5 samples were spiked with 10-100 cfu.

The results are given in **Table 2** and **Table 3**.

Table 2. The results obtained by the Salmonella Velox spp compared against ISO 6579

Matrix type	PA	NA	PD	ND	FP	Sum N	Relative trueness, RT% (PA+NA) ·100 / N	Sensitivity alternative method SE_{alt} (%) (PA+PD) ·100 / PA+PD+ND	Sensitivity reference method SE_{ref} (%) (PA+ND) · 100 / PA+PD+ND	FPR(%) FP ·100 / N
1.1	17	5	0	0	0	22	100.0	100.0	100.0	0
1.2	16	5	1	0	0	22	95.5	100.0	94.1	0
1.3	17	5	0	0	0	22	100.0	100.0	100.0	0
Sum 1	**50**	**15**	**1**	**0**	**0**	**66**	**98.5**	**100.0**	**98.0**	**0**
2.1	14	5	1	0	0	20	95.0	100.0	93.3	0
2.2	15	5	0	0	0	20	100.0	100.0	100.0	0
2.3	12	5	3	0	0	20	85.0	100.0	80.0	0
Sum 2	**41**	**15**	**4**	**0**	**0**	**60**	**93.3**	**100.0**	**91.1**	**0**
3.1	15	5	0	0	0	20	100.0	100.0	100.0	**0**
3.2	15	5	0	0	0	20	100.0	100.0	100.0	0
3.3	15	5	0	0	0	20	100.0	100.0	100.0	0
Sum 3	**45**	**15**	**0**	**0**	**0**	**60**	**100.0**	**100.0**	**100.0**	**0**
4.1	**13**	**5**	**2**	**0**	**0**	**20**	**90.0**	**100.0**	**86.7**	**0**
5.1	15	5	0	0	0	20	100.0	100.0	100.0	**0**
5.2	15	5	0	0	0	20	100.0	100.0	100.0	0
5.3	15	5	0	0	0	20	100.0	100.0	100.0	0
5.4	15	5	0	0	0	20	100.0	100.0	100.0	0
Sum 5	**60**	**20**	**0**	**0**	**0**	**80**	**100.0**	**100.0**	**100.0**	**0**
6.1	15	5	0	0	0	20	100.0	100.0	100.0	0
6.2	15	4	0	1	0	20	95.0	93.8	100.0	0
Sum 6	**30**	**9**	**0**	**1**	**0**	**40**	**98.5**	**96.8**	**98.0**	**0**
7.1	8	11	1	0	0	20	95.0	100.0	88.9	0
7.2	15	5	0	0	0	20	100.0	100.0	100.0	0
7.3	15	5	0	0	0	20	100.0	100.0	100.0	0
Sum 7	**38**	**21**	**1**	**0**	**0**	**60**	**98.5**	**100.0**	**98.0**	**0**
8.1	15	5	0	0	0	20	100.0	100.0	100.0	0
9.1	15	9	1	0	0	25	96.0	100.0	93.8	0
9.2	18	7	0	0	0	25	100.0	100.0	100.0	0
9.3	15	8	2	0	0	25	92.0	100.0	88.2	0
Sum 9	**48**	**24**	**3**	**0**	**0**	**75**	**98.5**	**100.0**	**98.0**	**0**

PA = positive agreement, NA = negative agreement, ND = negative deviation, PD = positive deviation,

FP = false positive, FPR = false positive ratio

Table 3. The results obtained by the Salmonella Velox spp SE+ST compared against ISO 6579

Matrix type	PA	NA	PD	ND	FP	Sum N	Relative trueness, RT% (PA+NA) ·100 / N	Sensitivity alternative method SE_{alt} (%) (PA+PD) ·100 / PA+PD+ND	Sensitivity reference method SE_{ref} (%) (PA+ND) · 100 / PA+PD+ND	FPR(%) FP ·100 / N
1.1	17	5	0	0	0	22	100.0	100.0	100.0	0
1.2	16	5	1	0	0	22	95.5	100.0	94.1	0
1.3	17	5	0	0	0	22	100.0	100.0	100.0	0
Sum 1	**50**	**15**	**1**	**0**	**0**	**66**	**98.5**	**100.0**	**98.0**	**0**
4.1	**13**	**5**	**2**	**0**	**0**	**20**	**90.0**	**100.0**	**86.7**	**0**
6.1	15	5	0	0	0	20	100.0	100.0	100.0	0
6.2	15	4	0	1	0	20	95.0	93.8	100.0	0
Sum 6	**30**	**9**	**0**	**1**	**0**	**40**	**98.5**	**96.8**	**98.0**	**0**
7.1	8	11	1	0	0	20	95.0	100.0	88.9	0
7.2	15	5	0	0	0	20	100.0	100.0	100.0	0
7.3	15	5	0	0	0	20	100.0	100.0	100.0	0
Sum 7	**38**	**21**	**1**	**0**	**0**	**60**	**98.5**	**100.0**	**98.0**	**0**
8.1	15	5	0	0	0	20	100.0	100.0	100.0	0
9.1	15	9	1	0	0	25	96.0	100.0	93.8	0
9.2	18	7	0	0	0	25	100.0	100.0	100.0	0
9.3	15	7	3	0	0	25	88.0	100.0	100.0	0
Sum 9	**48**	**23**	**4**	**0**	**0**	**75**	**98.5**	**100.0**	**98.0**	**0**

PA = positive agreement, NA = negative agreement, ND = negative deviation, PD = positive deviation, FP = false positive, FPR = false positive ratio

According to ISO/DIS 16140-2, the results are considered satisfactory if ND-PD ≤ 5 for 5 categories. As there are more positive deviations than negative deviation, ND-PD will be below zero, hence the sensitivity of the Salmonella Velox spp. and Salmonella Velox SE+ST are considered satisfactory.

Level of Detection (LOD) and the Relative Level of Detection (RLOD)

The level of detection (LOD) of the Salmonella Velox method was compared against the LOD of the reference method. For each of the three food matrix categories, one matrix item was used; raw pork cutlet (1.1.), raw chicken breast (2.2.) and raw salmon fillet (3.1.), respectively. For the swab matrix category, swabs from raw pork meat were used. For the ready-to-eat (R-t-E) and ready-to-reheat (R-t-R) category cooked chicken breast was used. The results from the sensitivity study for L_0 and L_1 were used in addition to another low level ($L_{50\%}$)(around 0.7 cfu per sample on average aiming at approximately 50% positive

samples). For both the Salmonella Velox method and the reference method, the following numbers of replicates were analysed for each matrix type: 5 negative level replicates, 20 low level replicates, 5 high level replicates (= 30 replicates). For swabs and ready-to-eat and ready-to-reheat similar samples were analysed except for 10 high level replicates (= 35 replicates). The LOD and RLOD for each matrix and the LOD respectively the RLOD combined for all matrixes are given in **Table 4**.

Table 4. The LOD of the Salmonella Velox method and the RLOD of Salmonella Velox / Reference Method

	Pork Cutlet (125 g)		**Chicken breast** (25 g)		**Salmon fillet** (25 g)		**Swabs** (1 swab)		**R-t-E/R-t-R** (25 g)		**Combined**	
Matrix Level	$L_{50\%}$	L_1	$L_{50\%}$	L_1	$L_{50\%}$	L_1	$L_{50\%}$	L_1	$L_{50\%}$	L_1	$L_{50\%}$	L_1
(CFU/ sample)	0.50	6.8	0.83	3.7	0.67	6.6	0.50	3.1	0.25	5.1		
Number of samples	20	12	20	10	20	10	20	10	20	10	100	52
Number of positives with ISO 6579	9	12	13	10	13	10	6	8	7	10	48	50
Number of positives with Salmonella Velox	9	12	14	10	11	10	9	10	8	10	51	52
$LOD_{50\%}$* Salmonella Velox (CFU/g)	0.005		0.019		0.023		0.021		0.014		0.016	
Alternative / Reference (RLOD)	1.00		0.87		1.32		0.47		0.84		0.82	

The Acceptability Limit (AL) for the RLOD for unpaired study data is 2.5, i.e. the LOD for the alternative method may not be higher than 2.5 times the LOD of the reference method. Table 3 shows that all the RLODs are below 2.5, i.e. the LOD for Salmonella Velox is satisfactory.
* $LOD_{50\%}$ calculations according to Wilrich & Wilrich, Journal of AOAC International 92(6), 1763-1772 (2009)

In the extension study, a Ct comparison study was carried out between Salmonella Velox spp qPCR and Salmonella Velox SE+ST qPCR. The level tested was at LOD_{50}. Concentrations of DNA copy number at the detection limit showed a minimum of difference for Salmonella Velox SE+ST qPCR compared against Salmonella Velox spp. qPCR.

INTERLABORATORY STUDY FROM 2016

The interlaboratory study was organised by AnalyTech Miljølaboratorium A/S in April/May 2016 according to the NordVal Protocol of 2009. Eight laboratories participated, using three different qPCR (Real time) instruments.

The interlaboratory study was carried out on enriched samples instead of food samples. The selected matrix was raw unprocessed pork samples; 125 g for the Salmonella Velox and 25 g for the reference method. The pork samples were inoculated with cold stressed

***Salmonella* Typhimurium** by the expert laboratory, before being enriched for 4 h 30 min in Salmonella Velox broth.

Three different levels of contamination were used:
L_0: Negative control, no Salmonella
L_1: Low level, 8.0 cfu
L_2: High level, 80 cfu

The expert laboratory analysed the samples with both the Salmonella Velox method and the reference method. The participating laboratories analysed the pre-enriched samples (10-15 deep-well plates or 2 mL tubes containing the pelleted samples). Two blind replicates of each contamination level were tested by each collaborator using the Salmonella Velox method. The results are given in **Table 5**.

Table 5. Results obtained at the expert laboratory using both methods

	Contamination level		
By the expert laboratory	L_0	L_1	L_2
No. of positives Salmonella Velox	0/2	2/2	2/2
No. of positives ISO 6579	0/2	2/2	2/2
8 Collaborators analysing 2 replicates using Salmonella Velox	0/16	16/16	16/16

All the samples expected to be positive are positive, and the samples expected to be negative are negative with the Salmonella Velox method.

Sensitivity of the alternative method: $SE_{alt} = 100\%$

Sensitivity of the reference method: $SE_{ref} = 100\%$

Relative trueness: RT = 100%

INTERLABORATORY STUDY FROM 2023

The interlaboratory study was organised by Adria in May/June 2023 according to the NordVal Protocol 1 of 2022 and the EN ISO 16140-2:2016. Eight laboratories participated, involving 15 collaborators, and 12 sets of data were generated. The study was an unpaired study as the reference method and alternative method use different enrichment step.

The interlaboratory study was carried out on 25 g of cooked ham. The pork samples were inoculated with Salmonella Enteritidis 2532 isolated from meat. For alternative method the sample was put into Velox bag and enriched for 5 hours in Salmonella Velox broth.
For reference method (ISO 6579-1(2017/A1(2020)) the sample was put into stomacher bag and enriched for 18 +/- 2 hours.

Three different levels of contamination were used:
L_0: Negative control, no Salmonella
L_1: Low level, 1 cfu
L_2: High level, 8 cfu

The expert laboratory analysed the samples with both the Salmonella Velox method and the reference method. The results are given in **Table 6**, **Table 7**, and **Table 8**.

Table 6. Results obtained at the expert laboratory using both methods.

Level	Reference method	Alternative method
L0	0/8	0/8
L1	5/8	4/8
L2	8/8	8/8

Table 7. Positive results by the reference method Salmonella spp. and Salmonella SEST

Collaborators	Contamination level		
	L0	L1	L2
A1	0	6	8
A2	0	6	8
A3	0	5	8
B	0	6	8
C2	0	5	8
C3	0	3	8
E1	0	6	8
E2	0	7	8
G	0	7	8
H	0	7	8
TOTAL	**$P_0 = 0$**	**$P_1 = 58$**	**$P_2 = 80$**

Table 8. Positive results (before and after confirmation) by the alternative method Salmonella spp. and Salmonella SEST

Collabo-rators	Contamination level								
	L0			L1			L2		
	PCR results	Confirm-ation results	Final results	PCR results	Confirm-ation results	Final results	PCR results	Confirm-ation results	Final results
A1	0	0	0	4	5	4	8	8	8
A2	0	0	0	5	5	5	8	8	8
A3	0	0	0	7	7	7	8	8	8
B	0	0	0	4	4	4	8	8	8
C2	0	0	0	4	4	4	8	8	8
C3	0	0	0	3	3	3	8	8	8
E1	0	0	0	5	5	5	8	8	8
E2	0	0	0	6	6	6	8	8	8
G	0	0	0	8	8	8	8	8	8
H	0	0	0	4	4	4	8	8	8
TOTAL	**$P_0 = 0$**	**$C_0 = 0$**	**$CP_0 = 0$**	**$P_1 = 50$**	**$C_1 = 51$**	**$CP_1 = 50$**	**$P_2 = 80$**	**$C_2 = 80$**	**$CP_2 = 80$**

The Specificity percentage (SP) of the reference method and of the alternative method, using the data after confirmation, based on the results of level L0 are presented in **Table 9**.

Table 9. Specificity percentage Salmonella spp. and Salmonella SEST

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) x\ 100\ \% =$	100 %
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-}\right)\right) x\ 100\ \% =$	100 %

Calculation of the sensitivity of the alternative method (SE_{alt}), the sensitivity for the reference method (SE_{ref}), the relative trueness (RT) and the false positive ratio for the alternative method (FPR). Fractional positive results were obtained for the low inoculation level (L1). This inoculation level was thus retained for calculation.

A summary of the results of the collaborators retained for interpretation, obtained with the reference and the alternative methods for Level 1 is provided in **Table 10**.

Table 10. Summary of the obtained results with the reference method and the alternative method for Level 1 Salmonella spp. and Salmonella SEST

Level	Response	Reference method positive (R+)	Reference method negative (R-)
1	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 38**	Positive deviation (R-/A+) **PD = 12**
	Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 20 (PPND= 0)**	Negative agreement (A-/R-) **NA = 10 (PPNA= 0)**

Based on the data summarized in Table 10, the calculated values of the sensitivity of the alternative and reference methods, as well as the relative trueness and false positive ratio for the alternative method (taking account the confirmations) are presented in **Table 11**.

Table 11. Sensitivity, relative trueness and false positive ratio percentages

Salmonella spp. and Salmonella SEST

		Level 1	Level 2
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \; x \; 100\% =$	71,4 %	100%
Sensitivity for the reference method	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \; x \; 100\% =$	82,9 %	100%
Relative trueness	$RT = \frac{(PA+NA)}{N} \; x \; 100\% =$	60,0 %	100%
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} \; x \; 100\% =$	0 %	0%

For an unpaired study design, the difference between (ND – PD) is calculated for the level(s) where fractional recovery is obtained (so L_1 and possibly L_2). The observed value found for (ND – PD) shall not be higher than the Acceptability Limit, AL. The AL is defined as [$(ND - PD)_{max}$] and calculated per level where fractional recovery is obtained as described below using the following three parameters:

$$(p+)_{\text{ref}} = \frac{P_x}{N_x}$$

where

P_x = number of samples with a positive result obtained with the reference method at level x (L_1 *or* L_2) for all the collaborators

N_x = number of samples tested at level *x* (L_1 *or* L_2) with the reference method by all the collaborators

$$(p+)_{\text{alt}} = \frac{CP_x}{N_x}$$

where

CP_x = number of samples with a confirmed positive result obtained with the alternative method at level x (L_1 *or* L_2) for all the collaborators.

N_x = number of samples tested at level x (L_1 *or* L_2) with the alternative method by all the collaborators.

$$(\text{ND-PD})_{\max} = \sqrt{3N_x \times \left((p+)_{\text{ref}} + (p+)_{\text{alt}} - 2\left((p+)_{\text{ref}} \times (p+)_{\text{alt}}\right)\right)}$$

where

N_x = number of samples tested for level *x* (L_1 *or* L_2) with the reference method by all the collaborators.

The AL is not met when the observed value (L_1 *or* L_2) is higher than the AL. Thus, investigations were made in order to provide an explanation of the observed results. In this study, fractional recovery was observed at Level 1. No fractional recovery was observed in Level 2. The calculations are the following, according to the EN ISO 16140-2:2016 (**Table 12**).

Table 12. Calculations Salmonella spp. and Salmonella SEST

	Level 1
N_X	80
$(p+)_{ref}$	0,73
$(p+)_{alt}$	0,61
AL = (ND - PD) max	10,39
ND - PD	8
Conclusion	**ND – PD ≤ AL**

The ISO 16140-2 (2016) requirements are fulfilled as (ND - PD) meet the AL (ND-PD ≤ AL).

CONCLUSION

The results from the comparison study and both interlaboratory studies show that the alternative method performs equivalent to, or better, than the reference method for the matrices tested.