NMKL - NordVal International
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NordVal International Certificate

Issued for:	foodproof® Salmonella Genus plus Enteritidis and Typhimurium Detection LyoKit
NordVal No:	055
First approval date:	28 December 2021
Renewal date:	24 November 2025
Valid until:	31 December 2027

Manufactured and supplied by:

Hygiena Diagnostics GmbH
Hermannswerder 17,
D-14473 Potsdam, Germany.

NordVal International has reviewed the method validation documentation. The validation was conducted by Campden BRI (Chipping Campden) Limited according to ISO 16140-2. The reference methods were ISO 6579-1:2017 Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp., and ISO/TR 6579-3:2014 Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 3: Guidelines for serotyping of *Salmonella* spp.

NordVal International concludes that it has been satisfactorily demonstrated that the data and interpretations comply with the EN ISO 16140-2:2016 requirements and demonstrate comparable performance of the alternative method **food**proof® *Salmonella* Genus plus Enteritidis and Typhimurium Detection LyoKit, to the ISO reference method for the detection of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium and for the serotyping of *Salmonella* Enteritidis and S*almonella* Typhimurium in raw and ready to cook meat and poultry products and environmental samples.

The production of the kits fulfills the requirements outlined in ISO 9001.

Date: 24 November 2025

Yours sincerely,

Hrólfur Sigurðsson

Chair of NordVal International

Eystein Oveland

NMKL Executive Director

PRINCIPLE OF THE METHOD

The **food**proof® *Salmonella* Genus plus Enteritidis and Typhimurium Detection LyoKit is a polymerase chain reaction (PCR) based assay that targets DNA specific to *Salmonella* spp. and two specific serotypes - *Salmonella* Enteritidis and *Salmonella* Typhimurium. The method consists of three distinct stages:

- Sample enrichment in Buffered Peptone Water for 16-20h at 37°C ± 1°C
- DNA extraction, including a heat lysis step in the presence of beads to release the genomic DNA into solution, using either:
  - **food**proof® StarPrep Three Kit (Order No. KIT 230187), or
  - **food**proof® StarPrep Three 8-Strip Kit (Order No. KIT 230188)

  Both kits use the same volume of reagents and there is a declaration of equivalence for the two kits, with the only difference being the format of the tubes used for DNA extraction.
- Real-time PCR analysis of the DNA extracts using the **food**proof® *Salmonella* Genus plus Enteritidis and Typhimurium Detection LyoKit – 5' Nuclease (Order No. KIT 230134, KIT 230135, KIT 230136). All three targets are detected using genus and serotype specific primers and DNA detection is reported through an increase in fluorescence when the DNA is replicated during PCR.

FIELD OF APPLICATION

The **food**proofPP®PP *Salmonella* Genus plus Enteritidis and Typhimurium Detection LyoKit is applicable for the detection of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in raw and ready to cook meat and poultry products and environmental samples.

HISTORY

In 2025, an additional rebranding affecting layouts, images and order numbers was introduced.

METHOD COMPARISON STUDY

Selectivity study

Inclusivity: All 25 *S.* Enteritidis tested were positive and confirmed as *S.* Enteritidis, all 25 *S.* Typhimurium were positive and confirmed as *S.* Typhimurium. These isolates together with 75 other *Salmonella* strains were tested for *Salmonella* spp. All 75 out of the 75 *Salmonella* spp. strains tested showed the expected positive result.

Exclusivity: All the 30 non-*Salmonella* strains tested showed the expected negative result and none of the strains showed a positive result.

The selectivity study shows that the alternative **food**proof® *Salmonella* Genus plus Enteritidis and Typhimurium Detection LyoKit method is selective and specific for *S.* Enteritidis, *S.* Typhimurium and *Salmonella* spp.

Sensitivity study

To ensure that samples were contaminated with the two specific target *Salmonella s*erotypes (Enteritidis and Typhimurium), artificial contamination of samples were carried out using a seeding protocol. All samples were inoculated at a level of 1-5 cfu/sample, with a maximum of 7 cfu/sample. The categories and types tested are provided in **Table 1**.

Table 1. The catogories and types tested

Category		Type	
1	Raw meat and ready to cook meat products	a	Fresh meats (unprocessed)
		b	Ready to cook (processed)
		c	Ready to eat and ready to reheat products
2	Raw poultry and ready to cook poultry products	a	Fresh meats (unprocessed)
		b	Ready to cook (processed)
		c	Ready to eat and ready to reheat products
3	Environmental samples	a	Dust and residues
		b	Cleaning and process waters
		c	Surface samples

The sensitivity parameters calculated for each category and type are shown in **Tables 2a**, **2b,** and **2c**.

Table 2a. *S.* Enteritidis - Overview calculated sensitivity parameters per category and type

Category	Type	PA	NA	PD	ND	FP	SEalt(%)	SEref(%)	RT(%)	FPR(%)
Raw meat and ready to cook meat products	a	10	10	0	0	0	100	100	100	0.0
	b	10	10	0	0	0	100	100	100	0.0
	c	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Raw poultry and ready to cook poultry products	a	10	10	0	0	0	100	100	100	0.0
	b	10	10	0	0	0	100	100	100	0.0
	c	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Environmental samples	a	10	10	0	0	0	100	100	100	0.0
	b	10	10	0	0	0	100	100	100	0.0
	c	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Overall		90	90	0	0	0	100	100	100	0.0

Table 2b. *S.* Typhimurium - Overview calculated sensitivity parameters per category and type

Category	Type	PA	NA	PD	ND	FP	SEalt(%)	SEref(%)	RT(%)	FPR(%)
Raw meat and ready to cook meat products	a	10	10	0	0	0	100	100	100	0.0
	b	10	10	0	0	0	100	100	100	0.0
	c	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Raw poultry and ready to cook poultry products	a	10	10	0	0	0	100	100	100	0.0
	b	10	10	0	0	0	100	100	100	0.0
	c	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Environmental samples	a	10	10	0	0	0	100	100	100	0.0
	b	10	10	0	0	0	100	100	100	0.0
	c	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Overall		90	90	0	0	0	100	100	100	0.0

Table 2c. *Salmonella* spp. - Overview calculated sensitivity parameters per category and type

Category	Type	PA	NA	PD	ND	FP	SEalt(%)	SEref(%)	RT(%)	FPR(%)
Raw meat and ready to cook meat products	a	10	10	0	0	0	100	100	100	0.0
	b	10	10	0	0	0	100	100	100	0.0
	c	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Raw poultry and ready to cook poultry products	a	10	10	0	0	0	100	100	100	0.0
	b	10	10	0	0	0	100	100	100	0.0
	c	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Environmental samples	a	10	10	0	0	0	100	100	100	0.0
	b	10	10	0	0	0	100	100	100	0.0
	c	10	9	0	0	1	100	100	100	11.1
	Total	30	29	0	0	1	100	100	100	3.4
Overall		90	89	0	0	1	100	100	100	1.1

PA = number of obtained results that are positive with both the alternative and the reference method.
NA = number of obtained results that are negative with both the alternative and the reference method.
ND = number of obtained results that are negative with the alternative method and positive with the reference method.
PD = number of obtained results that are positive with the alternative method and negative with the reference method.
FP = number of false positives.
RT = relative trueness method.
SE = the sensitivity; the ability of the method to detect the analyte.
FPR = the false positive rate.

The results of the sensitivity study were satisfactory.

Level of Detection (LOD_{50}) and Relative Level of Detection (RLOD)

A single sample type within each of the three categories (raw meat and ready to cook meat products, raw poultry and ready to poultry products and environmental samples) were tested at three different levels of contamination: no contamination, low and high. The number of samples analyzed for each was:

- 5 blank samples with no contamination to act as the negative controls
- 20 samples at a low level of contamination (0.2-2 cfu per portion)
- At least 5 samples at a high level (2-5 cfu per portion)

Each sample type was inoculated with a different strain.

The $LODRR_{50}RR$ varied from 0.6 – 1.0 cfu/test portion.

The RLOD values (using the confirmed alternative method results) for all three targets of the **food**proof® *Salmonella* Genus plus Enteritidis and Typhimurium Detection LyoKit meet the acceptability limit, which is 1.5 for paired studies.

The LOD50 per Category is given in **Table 3a, 3b, and 3c**.

Table 3a. LOD50 after confirmation of the alternative method results *S.* Enteritidis

Type (Category)	LOD50 cfu per portion	Lower confidence limit cfu per portion	Upper confidence limit cfu per portion
Raw poultry and ready to cook poultry	0.792	0.451	1.390
Environmental	1.040	0.584	1.852
Combined	0.909	0.608	1.359

Table 3b. LOD50 after confirmation of the alternative method results *S.* Typhimurium

Type (Category)	LOD50 cfu per portion	Lower confidence limit cfu per portion	Upper confidence limit cfu per portion
Raw meat and ready to cook meat products	0.702	0.402	1.225

Table 3c. LOD50 after confirmation of the alternative method results *Salmonella* spp

Type (Category)	LOD50 cfu per portion	Lower confidence limit cfu per portion	Upper confidence limit cfu per portion
Raw meat and ready to cook meat products	0.728	0.419	1.267
Raw poultry and ready to cook poultry	0.792	0.451	1.390
Environmental	0.570	0.299	1.089
Combined	0.706	0.503	0.990

INTERLABORATORY STUDY

Samples were sent to 14 laboratories in six different countries. Single collaborators were involved in the study for ten laboratories and two collaborators were included from two laboratories.

Samples of cooked sliced chicken were inoculated with *Salmonella* Enteritidis strain Campden ref 3739.

All the samples were pre-weighed in stomacher bags in 25 g amounts and individually inoculated at the required level.

The target inoculation levels were:

- Level 0: 0 CFU/25 g,
- Level 1: 2.4 CFU/25 g,
- Level 2: 8 CFU/25 g.

Each laboratory received 24 samples of 25 g, i.e. eight samples per inoculation level.

In the calculations, results from four laboratories were removed: Two laboratories had insufficient data for the reference method and two laboratories had positive results for the blank samples due the potential for cross contamination.

The results before and after confirmation are provided in Table 4 and Table 5 for *Salmonella* spp., and for *S.* Enteritidis, respectively.

Table 4. *Salmonella* spp. - Valid results by the alternative method and the reference method

Collaborator	Contamination level								
	positive results at L 0			***positive results at L 1***			***positive results at L 2***		
	Before confirm.	*After confirm.*	*Ref. method*	*Before confirm.*	*After confirm.*	*Ref. method*	*Before confirm.*	*After confirm.*	*Ref. method*
3	8/8	0/8	0/8	8/8	3/8	3/8	8/8	8/8	8/8
6	1/8	0/8	0/8	2/8	2/8	2/8	8/8	8/8	8/8
7	1/8	0/8	0/8	4/8	4/8	4/8	8/8	8/8	8/8
8	3/8	0/8	0/8	4/8	4/8	4/8	8/8	8/8	8/8
9	0/8	0/8	0/8	3/8	3/8	3/8	8/8	8/8	8/8
10	0/8	0/8	0/8	3/8	3/8	3/8	8/8	8/8	8/8
11	0/8	0/8	0/8	4/8	4/8	4/8	8/8	8/8	8/8
12	1/8	0/8	0/8	3/8	3/8	3/8	8/8	8/8	8/8
13	0/8	0/8	0/8	6/8	5/8	5/8	8/8	8/8	8/8
14	0/8	0/8	0/8	2/8	2/8	2/8	8/8	8/8	8/8
TOTAL	**14/80**	**CP 0 =0/80**	**P 0 =0/80**	**39/80**	**33/80**	**33/80**	**80/80**	**80/80**	**80/80**

Table 5. *S.* Enteritidis - Valid results by the alternative method and the reference method

Collaborator	Contamination level								
	positive results at L_0			*positive results at L_1*			*positive results at L_2*		
	Before confirm.	*After confirm.*	*Ref. method*	*Before confirm.*	*After confirm.*	*Ref. method*	*Before confirm.*	*After confirm.*	*Ref. method*
3	8/8	0/8	0/8	8/8	2/8	2/8	8/8	8/8	8/8
6	0/8	0/8	0/8	2/8	2/8	2/8	8/8	8/8	8/8
7	1/8	0/8	0/8	4/8	4/8	4/8	8/8	8/8	8/8
8	1/8	0/8	0/8	4/8	4/8	4/8	7/8	7/8	8/8
9	0/8	0/8	0/8	3/8	3/8	3/8	8/8	8/8	8/8
10	0/8	0/8	0/8	3/8	3/8	3/8	8/8	8/8	8/8
11	0/8	0/8	0/8	4/8	4/8	4/8	8/8	8/8	8/8
12	0/8	0/8	0/8	3/8	3/8	3/8	8/8	8/8	8/8
13	0/8	0/8	0/8	5/8	5/8	5/8	8/8	8/8	8/8
14	0/8	0/8	0/8	2/8	2/8	2/8	8/8	8/8	8/8
TOTAL	**10/80**	**CP_0=0/80**	**P_0=0/80**	**38/80**	**32/80**	**32/80**	**79/80**	**79/80**	**80/80**

The percentage specificity (SP) of the reference method and of the alternative method based on the results of level L_0 are provided in **Table 6**.

Table 6. Percentage specificity

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

N = total number of all L_0 tests.
P_0 = total number of false-positive results obtained with the reference method.
CP_0 = total number of false-positive results obtained with the alternative method.

Calculation of the sensitivity for 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) is provided in **Table 7**.

Table 7. Results of the interlaboratory study (sensitivity, relative trueness and false positive rate)

Method	Level	PA	NA	PD	ND	FP	N	SEalt(%)	SEref(%)	RT(%)	FPR(%)
S. spp.	L1	33	47	0	0	0	80	100.0	100.0	100.0	0
	L2	80	0	0	0	0	80	100.0	100.0	100.0	-
S. Enteritidis	L1	33	47	0	0	0	80	100.0	100.0	100.0	0
	L2	79	0	0	1	0	80	98.8	100.0	98.8	-

See abbreviations under **Table 2c**.

The relative level of detection, RLOD was also calculated and found to be 1.0 for *Salmonella* spp. and 1.1 for *S.* Enteritidis, which meets the acceptability limit of the RLOD of 1.5.

CONCLUSION

The acceptability limits for the sensitivity and RLOD are met in both the method comparison study and in the interlaboratory study.

The data and interpretations comply with the EN ISO 16140-2:2016 requirements and demonstrate comparable performance of the alternative method **food**proof® *Salmonella* Genus plus Enteritidis and Typhimurium Detection LyoKit, to the ISO reference method, for the detection of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in raw and ready to cook meat and poultry products and environmental samples.