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NordVal International Certificate

Issued for:	**VETPOD *Sal-LAMP* kit for detection of *Salmonella* spp. in meat**
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VETPOD Sal-LAMP kit for detection of Salmonella spp. In meat

Manufactured and supplied by:

DNA Diagnostic A/S
Voldbjergvej 14
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Denmark

fulfils the requirements for NordVal Certification. The reference method was EN ISO 6579-1:2017/ ISO 6579-1 2017/Amd 1 Edition 2020 Microbiology of food and animal feeding stuffs – Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp.

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

Date: 30 May 2025

Yours sincerely,

Hrólfur Sigurðsson
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

The VETPOD system detects *Salmonella* spp. in liquid samples after pre-enrichment in e.g. Buffered Peptone Water (ISO 6579 procedure) or equivalent pre-enrichment procedures. The detection method is based on detection of nucleic acids (bacterial DNA) in the sample in question.

LAMP (Loop Mediated Isothermal Amplification) is used for multiplication of nucleic acids to a detectable level. The detection principle is based on the detection of the turbidity in the reaction wells, formed by precipitates during the LAMP reaction.

The proprietary method consists of a detection instrument ready-to-use chips to be loaded with the sample after a short treatment to release bacterial DNA. Ten samples can be analysed at the same time and the detection time is less than 60 min. The VETPOD system is portable and thus well suited for on-site use at e.g. slaughterhouses and food producing establishments.

FIELD OF APPLICATION

The method is applicable for the detection of *Salmonella* spp. in raw meat and ready-to-cook meat products and raw poultry and ready-to-cook poultry products.

HISTORY

30.05.2025: The reference method used in the original validation study, ISO 6579-1 2017, has been replaced by ISO 6579-1 2017/Amd 1 Edition 2020. The changes in the new edition of the reference method have been evaluated by NordVal not to affect the results of validation, and the reference method in the certificate has been updated to the current edition.

COMPARISON STUDY

The Microbiological Laboratory of the Danish Food and Veterinary Authority in Ringsted analysed the samples using the reference method, and DTU Bioengineering at the Technical University of Denmark analysed the pre-enrichment broth using the VETPOD system.

Sensitivity study

The study was conducted using artificially contaminated samples spiked with known concentrations of *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis in separate samples. The study was done as a paired study.

Both the reference method and the VETPOD protocol were used for the detection of *Salmonella* spp. analysing the three following categories:

Category 1: Raw poultry and ready-to-cook poultry products. (Sample size: 25 g).

- 20 samples of chicken neck skin (matrix type: Fresh meat /unprocessed),
- 20 samples of fresh chicken meat (matrix type: Fresh meat /unprocessed),
- 20 samples of frozen seasoned chicken breasts (matrix type: Ready-to-cook products (processed)).

Category 2: Raw meat and ready-to-cook meat products (except poultry).

- 20 samples of swabs from pig carcasses (matrix type: Fresh meat (unprocessed)),
- 20 samples of minced beef meat (matrix type: Fresh meat (unprocessed)),
- 20 samples of frozen burger patties (matrix type: Ready-to-cook products (processed)).

Each matrix type from each category was divided into two groups containing 10 samples in each group. The group 1 from each matrix type was artificially contaminated with cold stressed *Salmonella* Typhimurium and group 2 from each matrix type was artificially contaminated with *Salmonella* Enteritidis at a level near the expected LOD50 for the matrix (2 to 3 CFU/25 g) for both groups.

The results obtained by the reference and the VETPOD method for all the sample types were analysed and compared as shown in the **Table 1**.

Table 1. Results of the sensitivity study

Category	PA	NA	PD	ND	FP	Sum	RT(%)	SE(%) VETPOD	SE(%) ISO	FPR (%)
1	39	18	1	2	1	60	95.0	95.2	97.6	5.6
2	44	15	0	1	0	60	98.3	97.8	100	0.0
Total	83	33	1	3	1	120	96.7	96.5	98.8	3.0

PA = positive agreement, NA = negative agreement, ND = negative deviation, PD = positive deviation, FP = false positive, FPR = false positive ratio, RT = relative trueness, SE = sensitivity

According to the NordVal requirements, for paired studies and 2 categories, the acceptability limit (AL) is ND+PD ≤ 8 and ND-PD ≤ 5. In the sensitivity study, there ND+PD = 4 and ND-PD = 2. The results for the two categories are satisfactory.

Level of detection (LOD) and relative level of detection (RLOD) study

A single sample type within each of the three categories (raw meat and ready-to-cook meat products and raw poultry and ready to-cook poultry products) were tested at three different levels of contamination artificially contaminated as specified in **Table 2**.

Table 2: Details of the samples included in the study

Category	Matrix types and organisms	No. of samples	Spiking levels (CFU/25 g)
1	Frozen chicken breast meat Spiked with *S.* Enteritidis	5	Nil
		20	≈1
		5	2-3
2	Minced beef meat Spiked with *S.* Typhimurium	5	Nil
		20	≈1
		5	2-3

- For category 1, poultry: there where only 1 positive sample for L1 and 1 positive sample for L2 (for both methods), - there should be fractional recovery of 25 – 75%, and hence the LOD and the RLOD for the interlaboratory study is used.
- For category 2, the meat (not poultry): all samples were positive with the reference method for L1 and for L2. For the alternative method, the results had a fractional recovery of 85%. The LOD was calculated to 0.09 cfu, which is about the results obtained in the ILS study, see **Table 6**.

Selectivity (inclusivity/exclusivity) study

Inclusivity: All the 100 target strains tested (40 strains of *S*. Typhimurium, 30 strains of *S*. Enteritidis, 15 strains of *S*. Infantis, 10 strains of *S*. Derby and 5 strains of *S*. Dublin) were found to be positive.

Exclusivity: Neither of the 30 non-target strains sharing some properties with *Salmonella* or species common in chicken intestines (such as *Campylobacter spp*., *E. coli*, *Citrobacter spp*., *Enterobacter spp*., *Lactobacillus spp*.) were detected.

The selectivity was satisfactory for the VETPOD system for detection of *Salmonella spp*.


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INTERLABORATORY STUDY

The interlaboratory study (ILS) was organised by the Department of Bioengineering at the Technical University of Denmark (DTU) according to the NordVal Protocol No. 1 / ISO 16140-2. 14 collaborators participated (from 10 laboratories) producing 14 data sets of results using the VETPOD system and the reference method ISO 6579-1:2017.

The ILS was conducted using artificially contaminated samples, spiked with known concentrations of *Salmonella enterica* serovar Typhimurium at two spiking levels along with a negative control level (**Table 3**).

Table 3. Artificial contamination of samples

Levels of inoculation		Concentration of culture
1	L0 (negative)	Nil
2	L1 (around LOD50)	About 2-3 cfu/25 g
3	L2 (higher concentration than LOD50)	About 68-70 cfu/25 g

Each collaborator analysed 8 blind replicates of each contamination level (L1 and L2) along with 8 blind replicates of negative level (L0) using both the alternative method and the reference method. The results are given in **Table 4**.

Table 4. The results by the reference method

	Reference method			Alternative method					
Level ->	**L_0**	**L_1**	**L_2**	**L_0**		**L_1**		**L_2**	
Collaborators				**Scr**	**Conf**	**Scr**	**Conf**	**Scr**	**Conf**
1	0/8[a]	7/8[b]	8/8[c]	0/8	0/8	8/8	7/8	8/8	8/8
2	0/8	5/8	8/8	1/8	1/8	5/8	5/8	8/8	8/8
3	0/8	4/8	8/8	0/8	0/8	4/8	4/8	8/8	8/8
4	1/8	5/8	8/8	1/8	1/8	5/8	5/8	8/8	8/8
5	0/8	5/8	8/8	1/8	0/8	5/8	5/8	8/8	8/8
6	0/8	4/8	8/8	2/8	0/8	4/8	4/8	8/8	8/8
7	0/8	5/8	8/8	0/8	0/8	5/8	5/8	8/8	8/8
8	0/8	6/8	8/8	1/8	0/8	6/8	6/8	8/8	8/8
9	0/8	5/8	8/8	0/8	0/8	5/8	5/8	8/8	8/8
10	2/8	5/8	8/8	0/8	0/8	4/8	4/8	8/8	8/8
11	0/8	5/8	8/8	1/8	0/8	5/8	5/8	8/8	8/8
12	0/8	5/8	7/8	0/8	0/8	6/8	5/8	8/8	7/8
13	0/8	6/8	8/8	0/8	0/8	6/8	6/8	8/8	8/8
14	0/8	6/8	8/8	0/8	0/8	6/8	6/8	8/8	8/8
Total	P_0	P_1	P_2	P0	CP_0	P1	CP1	P2	CP2
	3/112	**73/112**	**111/112**	**7/112**	**2/112**	**74/112**	**72/112**	**112/112**	**111/112**

L_0 = negative control
L_1 = 2-3 cells per 25 g
L_2 = 68-70 cells per 25 g
[a] Number of positive reference method results at level 0
[b] Number of positive reference method results at level 1
[c] Number of positive reference method results at level 2

P0 = Number of positive results at level 0
CP0 = Number of confirmed alternative method results at level 0
P1 = Number of positive results at level 1
CP1 = Number of confirmed alternative method results at level 1
P2 = Number of positive results at level 2
CP2 = Number of confirmed alternative method results at level 2

The specificity (%), SP = $\left[1 - \frac{P_0}{N_-}\right] \cdot 100\%$:
For the reference method the specificity was 97.3%.
For the VETPOD before confirmation the specificity was found to be 93.8% and after confirmation it was found to be 98.2%. There is no confirmation step in the VETPOD.

The results of the 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 rate (FPR) is provided in **Table 5**.

Table 5. Results of the interlaboratory study (sensitivity, relative trueness, false positive rate)

Level	PA	NA	PD	ND	FP	N	SE_{alt} (%)	SE_{ref} (%)	RT (%)	FPR (%)
L0		105			6	112				5.7
L1	72	38	1	1	2	112	98.6	98.6	98.2	5.3
L2	111	0	0	1	1	112	99.1	100.0	99.1	-
Total L1+ L2	184	38	1	2		224	99.5	98.9	98.7	
Total		143			9					6.3

The results of the sensitivity study illustrates that the method has a good sensitivity (close to 100%). There are some false positives with the alternative method, and hence confirmation of positive samples should be confirmed.

The results by the VETPOD method are in line with the acceptability limit (AL). For paired studies, involving 14 to 16 data sets, ND+PD ≤ 6 and ND-PD ≤ 4. In the study, the ND+PD = 2+1=3 and the ND-PD = 2-1=-1 and hence the acceptability level is met.

Level of detection (LOD) and relative level of detection (RLOD) study

Artificially contaminated samples were used in the study of LOD and RLOD. The matrix used in the ILS was frozen chicken meat (raw poultry and ready-to-cook poultry products). The inoculation levels, the number of samples and the obtained results are given in Table 6.

Table 6. The LOD of the methods and RLOD of the VETPOD compared to ISO 6579

				ISO 6579		VETPOD		
	cfu/g	No. of samples	No. of positive samples	LOD: cfu/g	LOD: cfu/25g	LOD: cfu/g	LOD: cfu/25g	**RLOD**
Inoculation level	0	88	0	0.10	2.5	0.10	2.5	**1.0**
	0.1	88	58					
	2.76	88	87					

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 results from the comparison study and the interlaboratory study show that the alternative method performs equivalent to the reference method ISO 6579-1 2017/Amd 1 Edition 2020 for the matrices tested.