NordVal International / NMKL
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NordVal International Certificate

Issued for:	Salmonella Velox
NordVal No:	046
First approval date:	7 June 2016
Valid until:	7 June 2018

Salmonella Velox

Manufactured and supplied by:
DNA Diagnostic A/S
Voldbjergvej 14
8240 Risskov
Denmark

fulfils the requirements for NordVal Certification. The reference method was EN ISO 6579:2002: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

NordVal International has studied the enclosures to the application and evaluated the results obtained in the validations conducted by the expert laboratory AnalyTech Miljølaboratorium AS, Denmark. The comparison study is conducted according to ISO/DIS 16140-2. The interlaboratory study is carried out according to NordVal Protocol of 2009. The interlaboratory study was carried out on enriched samples instead of food samples. Salmonella Velox is a rapid test that can be carried out within 5.5 hours. NordVal International has concluded that it has been satisfactorily demonstrated that Salmonella Velox performs satisfactorily for raw meat and ready-to-cook meat products including poultry, and raw and ready-to-cook fish and seafood.

The results document no statistical difference in the performances between the Salmonella Velox method and the reference method. The Salmonella Velox can be used without further confirmation.

Date: 15 February 2017

Yours sincerely

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary General

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, 25 – 100 g for minced meat and 25 - 125 g raw meat. Then 100 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.

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 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 target. Probes with FAM fluorophore and probes with ROX fluorophore are present for detection of the IAC and Salmonella 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 28-37. A ROX signal below Ct = 38 show the sample is positive for *Salmonella* bacteria. A ROX signal with Ct > 38 may be a positive sample. Retest to confirm presence of Salmonella. No Ct value shows that the sample is negative (not detected) for *Salmonella*.

FIELD OF APPLICATION:

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

HISTORY

It has been added, that the Salmonella Velox can be used without further confirmation. In addition, it has been specified, that this method applies to raw meat and ready-to-cook meat products including poultry, and raw and ready-to-cook fish and seafood.

COMPARISON STUDY

The comparison study was carried out by AnalyTech Miljølaboratorium AS in February 2016. The study was conducted according to the ISO/DIS 16140-2.

Selectivity study

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 selecticity is 100% for both the alternative method and the reference method.

Sensitivity study

The study was conducted on artificially contaminated samples, spiked with three different cold-stressed Salmonella serovars; *Salmonella enteritidis*, *Salmonella typhimurium* and *Salmonella newport*. The matrixes tested are given 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	*Salmonella typhimurium* or *Salmonella enteritidis* or *Salmonella Newport*
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	

For each of the three matrix categories, three matrix types, each with minimum 20 samples were tested with both the Salmonella Velox method and the reference method. The total number of samples was: 2 methods x 3 categories x 3 types x 20 samples = 2 x 180 samples (here: 2 x 186 samples tested).

For each of the nine 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_3: 2x5 samples were spiked with 10-100 cfu.

The results are given in Table 2.

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

Matrix type	PA	NA	ND (FN)	PD (TP)	FP	Sum	AC%	SE%	SP%
1.1	17	5	0	0	0	22	100.0	100.0	100.0
1.2	16	5	0	1 (1)	0	22	95.5	106.3	100.0
1.3	17	5	0	0	0	22	100.0	100.0	100.0
sum 1	**50**	**15**	**0**	**1 (1)**	**0**	**66**	**98.5**	**102.0**	**100.0**
2.1	14	5	0	1 (1)	0	20	95.0	107.1	100.0
2.2	15	5	0	0	0	20	100.0	100.0	100.0
2.3	12	5	0	3 (3)	0	20	85.0	125.0	100.0
sum 2	**41**	**15**	**0**	**4 (4)**	**0**	**60**	**93.3**	**109.8**	**100.0**
3.1	15	5	0	0	0	20	100.0	100.0	100.0
3.2	15	5	0	0	0	20	100.0	100.0	100.0
3.3	15	5	0	0	0	20	100.0	100.0	100.0
sum 3	**45**	**15**	**0**	**0**	**0**	**60**	**100.0**	**100.0**	**100.0**

PA = positive agreement, NA = negative agreement, ND = negative deviation, FN = false negative, PD = positive deviation, TP = true positive, FP = false positive, AC=relative accuracy, SE= relative sensitivity, SP=relative specificity

According to ISO/DIS 16140-2, the results are considered satisfactory if ND+PD ≤ 10 (for paired studies, no limit for unpaired). From the results in Table 2, ND = 0 and PD = 5, ND+PD=5, and hence the sensitivity of the Salmonella Velox is considered satisfactory.

For some of the results in Table 2, the relative sensitivity is above 100% meaning that the results were positive, and confirmed positive, with the alternative method but was found negative with the reference method. The sensitivity is therefore somewhat better with the alternative method than the reference method.

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 matrix categories, one matrix item were used; raw pork cutlet (1.1.), raw chicken breast (2.2.) and raw salmon fillet (3.1.), respectively. The results from the sensitivity study for L_0 and L_1 were used in addition to another low level (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 analyzed for each matrix type: 5 negative level replicates, 20 low level replicates, 5 high level replicates (= 30 replicates). The RLOD for each matrix and the RLOD combined for all matrixes are given in Table 3.

Table 3: RLOD of Salmonella Velox / Reference Method

	Pork Cutlet		**Chicken breast**		**Salmon fillet**		**Combined**	
Matrix Level	$L_{50\%}$ (cfu)	L_1 (cfu)	$L_{50\%}$ (cfu)	L_1 (cfu)	$L_{50\%}$ (cfu)	L_1 (cfu)	$L_{50\%}$ (cfu)	L_1 (cfu)
	0.50	6.8	0.83	3.7	0.67	6.6		
Number of samples	20	12	20	10	20	10	60	32
Number of positives with ISO 6579	9	12	13	10	13	10	35	32
Number of positives with Salmonella Velox	9	12	14	10	11	10	34	32
Alternative / Reference (RLOD)	1		0.872		1.315		1.047	

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.

INTERLABORATORY STUDY

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

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 4h30min 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 deepwell 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 4.

Table 4: 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 analyzing 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 Samlonella Velox method. The sensitivity and the specificity for this study are 100%.