NordVal International / NMKL
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# NordVal International Certificate

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| Issued for: | Salmonella Velox |
| NordVal No: | 046 |
| First approval date: | 7 June 2016 |
| Renewal date: | 12 September 2017 |
| Valid until: | 12 September 2019 |

## 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 A/S, Denmark. The comparison study is conducted according to ISO/DIS 16140-2. The interlaboratory study is carried out according to NordVal Protocol of 2009. 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, poultry and fishery products as well as ready-to-cook, ready-to-reheat and ready-to-eat meat, poultry and fishery products as well as swabs performed on raw food samples.

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 according to ISO 20838:2006 as the method uses a fluorescent DNA probe specific for a target gene specific for Salmonella.

Date: 12 September 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, ready-to-eat or ready-to-reheat; 25 – 100 g for minced meat; 25 - 125 g raw meat or 25g ± 5g swabs. Following, 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. For ready-to-eat and ready-to-reheat meat products, the minimum required time is 5hours.

### 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, poultry and fishery products as well as ready-to-cook, ready-to-reheat and ready-to-eat meat, poultry and fishery products and on swabs on raw food.

## HISTORY

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

## 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.

### 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 selectivity is 100% for both the alternative method and the reference method.

### Sensitivity study

The study was conducted on artificially contaminated samples, spiked with different cold-stressed Salmonella serovars; *Salmonella enteritidis*, *Salmonella typhimurium, Salmonella Newport and Salmonella infantis*. 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)<br>(125 g of sample) | 1.1. Raw pork cutlet<br>1.2. Raw bovine minced meat<br>1.3. Raw sheep meat cuts | *Salmonella typhimurium*<br>or<br>*Salmonella enteritidis*<br>or<br>*Salmonella Newport*<br>or<br>*Salmonella infantis* |
| 2. Raw poultry and ready-to-cook poultry products<br>(25 g of sample) | 2.1. Raw chicken carcass<br>2.2. Raw chicken breast<br>2.3. Raw seasoned chicken breast | |
| 3. Raw and ready-to-cook fish and seafood (unprocessed)<br>(25 g of sample) | 3.1. Raw salmon fillet<br>3.2. Raw tiger shrimps<br>3.3. Raw blue mussels | |
| 4. Swabs<br>(25 g of sample) | 4.1. Raw pork meat | |
| 5. Ready-to-eat and ready-to-reheat | 5.1 Cooked chicken breast (poultry, cooked)<br>5.2 Salami (meat/pork, fermented)<br>5.3 Smoked salmon (fishery, raw cured)<br>5.4 Sausage (meat/pork, cooked) | |

For each of the three matrix categories 1, 2 and 3 given in table 1, three matrix types, each with minimum 20 samples were tested with both the Salmonella Velox method and the reference method. The total number of food samples was: 2 methods x 3 categories x 3 types x 20 samples = 2 x 180 samples (here: 2 x 186 samples tested). For swabs the total number of samples was 2 methods x 35 samples = 70 samples. For ready-to-eat and ready-to-reheat the number of samples was 2 methods x 3 types x 20 samples = 2 x 60 samples.

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
**$L_2$**: 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% | Kappa |
|---|---|---|---|---|---|---|---|---|---|---|
| 1.1 | 17 | 5 | 0 | 0 | 0 | 22 | 100.0 | 100.0 | 100.0 | 1.0 |
| 1.2 | 16 | 5 | 0 | 1 (1) | 0 | 22 | 95.5 | 106.3 | 100.0 | 1.0 |
| 1.3 | 17 | 5 | 0 | 0 | 0 | 22 | 100.0 | 100.0 | 100.0 | 1.0 |
| **sum 1** | **50** | **15** | **0** | **1 (1)** | **0** | **66** | **98.5** | **102.0** | **100.0** | **1.0** |
| | | | | | | | | | | |
| 2.1 | 14 | 5 | 0 | 1 (1) | 0 | 20 | 95.0 | 107.1 | 100.0 | 1.0 |
| 2.2 | 15 | 5 | 0 | 0 | 0 | 20 | 100.0 | 100.0 | 100.0 | 1.0 |
| 2.3 | 12 | 5 | 0 | 3 (3) | 0 | 20 | 85.0 | 125.0 | 100.0 | 1.0 |
| **sum 2** | **41** | **15** | **0** | **4 (4)** | **0** | **60** | **93.3** | **109.8** | **100.0** | **1.0** |
| | | | | | | | | | | |
| 3.1 | 15 | 5 | 0 | 0 | 0 | 20 | 100.0 | 100.0 | 100.0 | 1.0 |
| 3.2 | 15 | 5 | 0 | 0 | 0 | 20 | 100.0 | 100.0 | 100.0 | 1.0 |
| 3.3 | 15 | 5 | 0 | 0 | 0 | 20 | 100.0 | 100.0 | 100.0 | 1.0 |
| **sum 3** | **45** | **15** | **0** | **0** | **0** | **60** | **100.0** | **100.0** | **100.0** | **1.0** |
| | | | | | | | | | | |
| **4.1** | **13** | **5** | **0** | **2 (2)** | **0** | **20** | **100.0** | **115.4** | **100** | **1.0** |
| | | | | | | | | | | |
| 5.1 | 15 | 5 | 0 | 0 | 0 | 20 | 100.0 | 100.0 | 100.0 | 1.0 |
| 5.2 | 15 | 5 | 0 | 0 | 0 | 20 | 100.0 | 100.0 | 100.0 | 1.0 |
| 5.3 | 15 | 5 | 0 | 0 | 0 | 20 | 100.0 | 100.0 | 100.0 | 1.0 |
| 5.4 | 15 | 5 | 0 | 0 | 0 | 20 | 100.0 | 100.0 | 100.0 | 1.0 |
| **Sum 5** | **60** | **20** | **0** | **0** | **0** | **80** | **100.0** | **100.0** | **100.0** | **1.0** |

*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 = 7, ND+PD=7, and hence the sensitivity of the Salmonella Velox is considered satisfactory.

The statistical test, Cohen's kappa, κ, is used as an estimate for the agreement between the methods. For method validation, "very good agreement" is required, i.e. $\kappa > 0.80$. In this study, kappa was above 0.80 for all levels, indicating very good agreement between the reference method and the alternative method.

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 food matrix categories, one matrix item were 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 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** | | **Swabs** | | **R-t-E/R-t-R** | | **Combined** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Matrix Level | $L_{50\%}$ | $L_1$ | $L_{50\%}$ | $L_{50\%}$ | $L_{50\%}$ | $L_1$ | $L_{50\%}$ | $L_1$ | $L_{50\%}$ | $L_1$ | $L_{50\%}$ | $L_1$ |
| (CFU) | 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 |
| 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.

**INTERLABORATORY STUDY**
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 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 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. The sensitivity and the specificity for this study are 100%.