STUDY REPORT

SureTect Salmonella species PCR Assay Workflow NF VALIDATION ISO 16140 – Extension Study: Relative Level of Detection

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Summary

As part of the NF VALIDATION™ ISO 16140 extension study of the Thermo Scientific™ SureTect™ Salmonella species PCR Assay workflow (alternative method), a method comparison study was conducted by ADRIA Développement, Quimper, France. The extension study was designed to validate the use of the SureTect Salmonella species PCR Assay with two real-time PCR systems: the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument with SureTect Software v1.2 for dairy samples and the Applied Biosystems™ 7500 Fast Real-Time PCR System and Applied Biosystems™ RapidFinder™ Express version 2.0 Software with SureTect Salmonella Kit File for meat products, dairy products, infant formula and production environment samples, along with a short enrichment protocol specifically for the analysis of raw seasoned and unseasoned raw beef meat. This report presents the results from the relative level of detection study.

Methodology

Choice of Strains and Matrices

Five *Salmonella* isolates were selected from the culture collection at ADRIA Développement and were spiked into representative matrices from the food categories analyzed during the NF VALIDATION ISO 16140 method comparison part of this validation study.

Protocol

Samples were prepared to give three batches of the matrices which consisted of five samples at 0 CFU/25 g, 20 samples at 0.5-1 CFU/25 g (to achieve fractional positive results) and 5 samples at 2 CFU/25 g. The samples were analyzed using the reference method detailed in ISO 6579:2002 prior to spiking in order to verify the absence of *Salmonella* spp. After inoculation, samples were tested using the ISO reference method and the alternative method.

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Alternative Method

Twenty-five gram samples were prepared following the protocol below:

- Powdered infant formula and environment samples were homogenised with 225 ml of room temperature Buffered Peptone Water (BPW) (ISO) and enriched by incubating for 16 to 20 hours at 37±1 °C.
- Raw beef meat samples were homogenised with 225 ml of pre-warmed BPW (ISO) and enriched by incubating for 9 to 24 hours at 41.5±1 °C.
- Meat samples (other than raw beef) and dairy product samples were homogenised with 225 ml of BPW (ISO) supplemented with 12 mg/l novobiocin and enriched by incubating for 20 to 24 hours at 37±1 °C.
- As an alternative to the protocol above dairy product samples were also homogenised with Thermo Scientific™ Oxoid™ ONE Broth™ Salmonella Base supplemented with 12 mg/l novobiocin and enriched by incubating for 20 to 24 hours at 37±1 °C.

Ten microlitres of SureTect Proteinase K Reagent were added to each of the required SureTect Lysis Tubes (supplied prefilled with Lysis Reagent 1). 10 µl of the enriched samples were added to the Lysis Tubes, which were then heated at 37±1 °C for 10 minutes, followed by 95±1 °C for 5 minutes. When performing PCR using the Applied Biosystems 7500 Fast Instrument, a negative control sample was also prepared by adding 10 µl sterile water to a SureTect Lysis Tube.

The Lysis Tubes were cooled at room temperature prior to transferring 20 µl aliquots of the lysates to PCR Tubes containing SureTect Salmonella species PCR tablets.

The PCR Tubes were then immediately sealed and transferred to the SureTect PikoReal Instrument or the Applied Biosystems 7500 Fast System for processing.

ISO Method

Twenty-five gram samples were analyzed according to ISO 6579:2002. Each sample was enriched by incubating at 37±1 °C for 16 to 20 hours in 225 ml of BPW (ISO). After primary enrichment, 0.1 ml of the BPW enrichment was inoculated in 10 ml of Rapapport-Vassiliadis Soya Peptone (RVS) Broth and incubated for 21 to 27 hours at 41.5±1 °C. After incubation, plates of Xylose-Lysine-Deoxycholate (XLD) Agar and another chromogenic agar were streaked and incubated for 21 to 27 hours at 37±1 °C. A further 1 ml of the BPW enrichment was inoculated in 10 ml of Muller-Kauffman Tetrathionate Broth supplemented with novobiocin (MKTTn) and incubated at 37±1 °C for 21 to 27 hours. After incubation plates of XLD Agar and another chromogenic agar were streaked and incubated for 21 to 27 hours at 37±1 °C. Up to five characteristic colonies were streaked onto Nutrient Agar to produce pure cultures and growth was confirmed by biochemical and serological identification tests, as detailed in the reference method.

Results

The relative level of detection was determined according to the ISO 16140-2:2016 standard. Table 1 shows the relative levels of detection obtained when using the alternative method with the SureTect PikoReal Instrument with Software and Table 2 shows the relative levels of detection obtained when using the alternative method with the Applied Biosystems 7500 Fast Instrument and Software. The aim was to determine the relative level of detection for all matrices analyzed during the AFNOR Certification validation study.

Table 1: Relative detection level results for the ISO reference method and alternative method according to ISO 16140-2:2016, when using the SureTect PikoReal Instrument with SureTect Software v1.2

Granted Certification	Matrix / Strain Pairs	Relative Level of Detection	Study Design / Acceptability Limit (AL)
March 2014	Poultry meat / *Salmonella* Braenderup Ad915	1.263 [0.403-3.955]	Paired; AL = 1.5
March 2014	Liquid egg / *Salmonella* Enteritidis 10	1.000 [0.522-1.917]	Paired; AL = 1.5
March 2014	Fresh vegetables / *Salmonella* Virchow Ad1721	1.000 [0.528-1.895]	Paired; AL = 1.5
March 2014	Raw milk / *Salmonella* Montevideo 606	BPW + novobiocin: 0.871 [0.369-2.061]	Unpaired; AL = 2.5
		ONE Broth Salmonella + novobiocin: 1.336 [0.534-3.342]	Unpaired; AL = 2.5
March 2014	Dried dog food / *Salmonella* Derby 630	2.076 [0.982-4.391]	Unpaired; AL = 2.5
November 2013	Ground beef / *Salmonella* Typhimurium A00C060	8 h: 0.527 [0.223-1.244]	Unpaired; AL = 2.5
		24 h: 0.527 [0.223-1.244]	Unpaired; AL = 2.5

Table 2: Relative detection level results for the ISO reference method and alternative method according to ISO 16140-2:2016, when using the Applied Biosystems 7500 Fast Instrument with RapidFinder Express v2.0 Software

Granted Certification	Matrix / Strain Pairs	Relative Level of Detection	Study Design / Acceptability Limit (AL)
June 2016	Raw chicken meat / *Salmonella* Bredeney 975	1.629 [0.696-3.814]	Unpaired; AL = 2.5
June 2016	Raw milk / *Salmonella* Ohio Ad1482	BPW + novobiocin: 0.871 [0.369-2.061]	Unpaired; AL = 2.5
		ONE Broth Salmonella + novobiocin: 1.000 [0.420-2.383]	Unpaired; AL = 2.5
June 2016	Infant formula with probiotics / *Salmonella* Anatum Ad298	1.000 [0.473-2.113]	Paired; AL = 1.5
June 2016	Ground beef / *Salmonella* Typhimurium A00C060	9 h: 0.554 [0.239-1.285]	Unpaired; AL = 2.5
		24 h: 0.554 [0.239-1.285	Unpaired; AL = 2.5
June 2016	Process water / *Salmonella* Livingstone A00L058	1.170 [0.437-3.132]	Paired; AL = 1.5
March 2017	Frozen spinach / *Salmonella* Virchow Ad1721	1 [0.385-2.599]	Unpaired; AL = 2.5

Conclusion

The relative level of detection study conducted as part of the NF VALIDATION extension study demonstrated that the alternative method showed a similar relative detection level for the matrices analyzed as the ISO reference method, detailed in ISO 6579:2002. When using the SureTect PikoReal Instrument and Software and the Applied Biosystems 7500 Fast Instrument with RapidFinder Express v2.0 Software, the relative levels of detection for all matrices were below the acceptability limits for both a paired and an unpaired study design. The NF VALIDATION certificate and validation report summary for this study are available from **http://nf-validation.afnor.org/en/.**

www.thermofisher.com/SureTect

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