STUDY REPORT

Thermo Scientific RapidFinder Salmonella species, Typhimurium and Enteritidis PCR Kit NF VALIDATION ISO 16140: Relative Level of Detection

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Summary

As part of the NF VALIDATION™ ISO 16140 certification study of the Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis PCR Kit (alternative method) for use with the Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR Instrument and associated Applied Biosystems™ RapidFinder™ Express Software (version 2.0 or greater) and with the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument with Thermo Scientific™ RapidFinder™ Analysis Software v1.0, a relative level of detection study (RLOD) was conducted. The following is a summary of the RLOD study.

Methodology

Choice of strains

Three *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: raw poultry and pork meat, ready-to-eat poultry and pork and environmental samples. The environmental sample matrix was spiked with two different *Salmonella* strains.

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 five samples at 2 CFU/25 g. The samples were analyzed using the reference method detailed in ISO 6579-1:2017 and ISO/TR 6579-3:2014 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.

Alternative method

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

- Raw and ready-to-eat poultry and pork meats were homogenised with 225 ml of Buffered Peptone Water (BPW) (ISO) supplemented with 12 mg/l novobiocin and enriched by incubating for 14 to 18 hours at 41.5±1 °C

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- Environmental samples were homogenised with 225 ml of BPW (ISO) and enriched by incubating for 16 to 20 hours at 37±1 °C

Ten microliters of Proteinase K reagent were added to each of the required number of Lysis Tubes (supplied prefilled with Lysis Reagent 1) followed by 10 µl of the enriched sample. The Lysis Tubes were then transferred to the Applied Biosystems™ SimpliAmp™ Thermal Cycler Instrument which heated the tubes at 37±1 °C for 10 minutes, followed by 95±1 °C for 5 minutes, and cooled for at least 2 minutes at room temperature. Twenty microliter aliquots of the lysates were transferred to PCR Tubes containing RapidFinder Salmonella species, Enteritidis, and Typhimurium PCR pellets. The PCR Tubes were sealed and immediately transferred to the 7500 Fast PCR Instrument or to the QuantStudio 5 PCR Instrument for processing.

ISO Method

Twenty-five gram samples were analyzed according to ISO 6579-1:2017. 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. Serotyping was then performed according to ISO/TR 6579-3:2014.

Results

The relative level of detection was determined according to the ISO 16140-2:2016 standard. Table 1 shows the relative levels of detection. 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—7500 Fast and QuantStudio 5 PCR platforms

Target	Matrix / Strain pairs	Relative level of detection	Study design
Salmonella spp	Turkey ham/*S.* Enteritidis Ad2524	0.728	Unpaired 2.5
	Raw pork meat/ *S.* Typhimurium Ad1872	1.023	Unpaired 2.5
	Process water/*S.* Typhimurium Ad1070 and *S.* Derby A00E084	1.000	Paired 1.5
	Combined	0.931	/
Salmonella Enteritidis	Turkey ham/*S.* Enteritidis Ad2524	0.728	Unpaired 2.5
Salmonella Typhimurium	Raw pork meat/*S.* Typhimurium Ad1872	1.105	Unpaired 2.5
	Process water/*S.* Typhimurium Ad1070 and *S.* Derby A00E084	0.562	Paired 1.5
	Combined	0.831	/

Conclusions

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 6579-1:2017. The relative levels of detection for all matrices were below the acceptability limits for both the 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/.**

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