STUDY REPORT

SureTect Listeria 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™ Listeria species PCR Assay workflow (alternative method), a method comparison study was conducted by ADRIA Développement, Quimper, France. The extension study aims to validate the use of the SureTect Listeria species PCR Assay with the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument with SureTect Software v1.2 for meat samples (Initial validation was obtained on the dairy, seafood, vegetables and production environment samples). Also part of this extension study was the validation of the use of the SureTect Listeria species PCR Assay with the Applied Biosystems™ 7500 Fast Real-Time PCR System and Applied Biosystems™ Rapid Finder™ Express version 2.0 Software for meat, dairy, seafood, vegetable and production environment samples. This report presents the results from the relative level of detection study.

Methodology

Choice of strains and matrices

Five individual *Listeria* species isolates from the culture collection at ADRIA Développement were spiked into each of five matrices (rillettes, raw milk, ready to cook vegetables, smoked salmon and process water) analyzed during the NF VALIDATION extension study.

Protocol

For the alternative method workflow using the SureTect PikoReal Instrument, for each matrix/strain combination 4 levels of contamination were prepared. For each level of contamination, 6 replicates were analyzed. The target contamination levels were 0 CFU/g or ml, the contamination level required to obtain up to 50% positive samples, the contamination level required to obtain 50% to 75% positive samples and the contamination level required to get 75% to 100% positive samples. The samples were analyzed

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using the reference method detailed in ISO 11290-1:1996, including Amendment 1:2004 prior to inoculation in order to verify the absence of *Listeria* spp.

For the alternative method workflow using the Applied Biosystems 7500 Fast Instrument extension, 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 11290-1:1996, including Amendment 1:2004 prior to inoculation in order to verify the absence of *Listeria* spp. After inoculation, samples were tested using the ISO reference method and the alternative method.

Alternative method

Twenty-five gram samples were homogenized with 225 ml Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) fully supplemented with Thermo Scientific™ Oxoid™ 24 Listeria Selective Supplement and Thermo Scientific™ Oxoid™ 24 Listeria Buffer Supplement. The samples were enriched by incubating for 22-26 hours at 37±1 °C when analyzed with the SureTect PikoReal Instrument and for 24-28 hours at 37±1 °C when analyzed with the Applied Biosystems 7500 Fast Instrument.

Ten microlitres of SureTect Proteinase K Reagent and 10 µl of SureTect Lysis Reagent 2 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 each of the required number of Lysis Tubes, which were then heated at 37±3 °C for 10 minutes, followed by 95±3 °C for 5 minutes. The tubes were cooled for 5 minutes at room temperature prior to transferring 20 µl aliquots of the lysates to SureTect Listeria species PCR Tubes containing PCR tablets. When performing PCR using the Applied Biosystems 7500 Fast Instrument, a negative control sample was prepared by adding 10 µl sterile nuclease free water to a SureTect Lysis Tube instead of enriched sample.

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

Positive samples were confirmed using the alternative method confirmation protocol by plating 10 µl of the enrichment onto Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria Agar and confirming at least one of any presumptive positive colonies with the Thermo Scientific™ Oxoid™ Microbact™ Listeria 12L Kit.

ISO reference method

Twenty-five gram samples were analyzed according to the method detailed in ISO 11290-1:1996, including Amendment 1:2004. Each sample was enriched by incubating at 30±1 °C for 21-27 hours in 225 ml Half Fraser Broth. Ten microlitres of the Half Fraser Broth enrichment was inoculated onto both Thermo Scientific™ Oxoid™ Chromogenic Listeria Agar (ISO) (OCLA (ISO)) and PALCAM Agar and incubated at 37±1 °C for 21-27 hours. A further 100 µl of the Half Fraser Broth enrichment was inoculated into 10 ml Fraser Broth and incubated for 45-51 hours at 37±1 °C. Following this secondary enrichment, 10 µl were streaked onto OCLA (ISO) and PALCAM Agar plates which were then incubated for 21-27 hours at 37±1 °C. Presumptive positive colonies were confirmed by Gram stain, haemolysis, catalase, CAMP test and biochemical identification.

Results

The level of detection for the alternative method and the ISO reference method were determined according to the ISO 16140:2003 standard (Table 1) and according to the ISO 16140-2:2016 standard (Table 2). The aim was to determine the relative level of detection for both matrices analyzed during the AFNOR Certification validation study.

Table 1: Relative detection level results for the ISO reference method and alternative method when using SureTect PikoReal Instrument, according to ISO 16140:2003

Matrix / Strain pairs	Relative level of detection	
	ISO reference method (CFU/25 g)	Alternative Method (CFU/25 g)
Rillettes / *Listeria monocytogenes* Ad669	0.7 [0.3-1.3]	0.4 [0.2-0.7]
Raw milk / *Listeria ivanovii* Ad991	0.7 [0.4-1.3]	0.8 [0.4-1.5]
Raw vegetables / *Listeria seeligeri* Ad 1293	0.6 [0.3-1.1]	0.6 [0.4-1.0]
Smoked salmon / *Listeria innocua* 1	0.8 [0.5-1.2]	0.6 [0.4-0.9]
Process water / *Listeria welshimeri* Ad1252	0.8 [0.5-1.3]	0.8 [0.5-1.3]

Table 2: Relative detection level results for the ISO reference method and alternative method, when using Applied Biosystems 7500 Fast Instrument, according to ISO 16140:2003

Matrix / Strain pairs	Relative level of detection	
	ISO reference method (CFU/25 g)	Alternative Method (CFU/25 g)
Rillettes / *Listeria innocua* Ad671	1.0 [0.8-1.2]	0.6 [0.4-0.8]
Raw milk / *Listeria ivanovii* Ad991	1.0 [0.6-1.6]	1.0 [0.6-1.6]
Ready to cook vegetables / *Listeria monocytogenes* Ad279	0.6 [0.4-0.9]	0.5 [0.3-0.7]
Smoked salmon / *Listeria welshimeri* Ad1669	0.4 [0.3-0.5]	0.4 [0.3-0.6]
Process water / *Listeria species* Ad551	0.3 [0.3-0.5]	0.3 [0.2-0.4]

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 11290-1:1996, including Amendment 1:2004, when using either the SureTect PikoReal Instrument with SureTect Software v1.2 or the Applied Biosystems 7500 Fast Instrument with RapidFinder Express v2.0 Software. When the results were analysed using the SureTect PikoReal Instrument, the level of detection range was 0.2-1.5 CFU/25 g compared to a range of 0.3 – 1.3 CFU/25 g for the reference method. When the results were analysed using the Applied Biosystems 7500 Fast Instrument, the level of detection range was 0.2-1.6 CFU/25 g compared to a range of 0.3 – 1.6 CFU/25 g for the reference method. Regardless of the PCR platform used during the study, the SureTect Listeria species PCR Assay workflow is an accurate alternative method for the detection of *Listeria* spp. from the food categories analysed. The NF VALIDATION certificate and validation report summary for this study are available from **http://nf-validation.afnor.org/en/.**

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