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

Study Report

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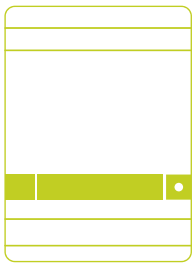
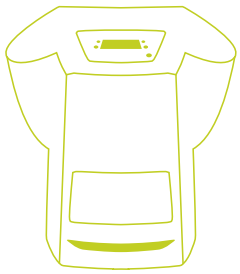


Applied Biosystems™ 7500 Fast Real-Time PCR Instrument



SureTect™ PikoReal™ Real-Time PCR Instrument

Summary

As part of the NF VALIDATION™ ISO 16140 extension study of the Thermo Scientific™ SureTect™ Listeria monocytogenes 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 monocytogenes PCR Assay with the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument with SureTect Software v1.2 for seafood, vegetables and production environment samples. Also part of this extension study was the validation of the use of the SureTect Listeria monocytogenes 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 monocytogenes* 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) and analyzed during the NF VALIDATION extension 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 11290-1:1996, including Amendment 1:2004 prior to inoculation in order to verify the absence of *L. monocytogenes.* 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 homogenized with 225 ml of fully supplemented Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB). The samples were enriched by incubating for 22 to 26 hours at 37±1 °C when analyzed with the SureTect PikoReal Instrument and for 24 to 28 hours at 37±1 °C when analyzed with the Applied Biosystems 7500 Fast System. 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). Ten microlitres of the enriched samples were added to each of the required number of Lysis Tubes, which were then heated at 37±1 °C for 10 minutes, followed by 95±1 °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 monocytogenes PCR Tubes containing PCR tablets.

When performing PCR using the 7500 Fast Instrument, a negative control sample was prepared by adding 10 µl sterile nuclease free water to a SureTect Lysis Tube.

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 to 27 hours in 225 ml of 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 to 27 hours. A further 100 µl of the Half Fraser Broth enrichment was inoculated into 10 ml Fraser Broth and incubated for 45 to 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 to 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 according to the Spearman-Kärber test, 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 [0.5-0.9]	0.8 [0.6-1.0]
Raw milk / *Listeria monocytogenes* 153	0.6 [0.5-0.7]	0.4 [0.3-0.5]
Ready to cook vegetables / *Listeria monocytogenes* Ad279	0.6 [0.4-0.9]	0.5 [0.3-0.7]
Smoked salmon / *Listeria welshimeri* Ad1669	0.6 [0.4-0.9]	0.5 [0.4-0.8]
Process water / *Listeria monocytogenes* Ad551	0.3 [0.3-0.5]	0.3 [0.2-0.4]

Table 2: Relative detection level results for the alternative method according to ISO 16140-2:2016

Matrix / Strain pairs	Relative level of detection (CFU/25 g)
Rillettes / *Listeria monocytogenes* Ad669	1.1 [0.4-2.9]
Raw milk / *Listeria monocytogenes* 153	0.2 [0.1-0.7]
Ready to cook vegetables / *Listeria monocytogenes* Ad279	0.7 [0.3-1.6]
Smoked salmon / *Listeria welshimeri* Ad1669	0.8 [0.3-1.7]
Process water / *Listeria monocytogenes* Ad551	0.7 [0.3-1.6]

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 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 according to the ISO 16140:2003 standard, the level of detection range was 0.3-0.9 CFU/25 g compared to a range of 0.2 – 1.0 CFU/25 g for the alternative method, demonstrating that the SureTect Listeria monocytogenes PCR Assay is an accurate alternative method for the detection of *L. monocytogenes* from the food categories analysed. When following the ISO 16140-2:2016 standard, it was demonstrated that the relative level of the alternative method met the acceptability limits for an unpaired study. The NF VALIDATION certificate and validation report summary for this study are available from **nf-validation.afnor.org/en/.**

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