Study Report

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

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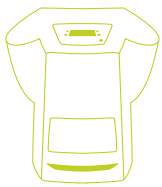

SureTect™ PikoReal™ Real-Time PCR Instrument

Summary

As part of the NF VALIDATION™ ISO 16140 of the Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay workflow (alternative method), a relative level of detection (RLOD) study was conducted by ADRIA Développement, Quimper, France. The following is a summary of that study.

Methodology

Choice of strains and matrices: Five individual *Listeria monocytogenes* isolates from the culture collection at ADRIA Développement were spiked into each of the five matrices (rillettes, smoked salmon, raw milk, raw vegetables and process water) analyzed during the NF VALIDATION™ certification from AFNOR Certification study according to ISO 16140.

Protocol: For each matrix/strain combination, four levels of contamination were prepared and for each level of contamination, six replicates were analyzed. The target contamination levels were: 0 CFU/g or ml, the contamination level required to obtain 0% to 50% positive samples, the contamination level required to obtain 50% to 75% positive sample and the contamination level required to get 75% to 100% positive samples. 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 SureTect Listeria monocytogenes Assay method.

Alternative Method: Twenty-five gram samples were homogenized with 225 ml of supplemented Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB). The sample was then enriched by incubation for 22 to 26 hours at 37±1 °C. 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 each of the required number of 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. The tubes were cooled for 5 minutes at room temperature prior to transferring 20 µl aliquots of the lysates to SureTect L. monocytogenes PCR Tubes containing PCR tablets. The PCR Tubes were then immediately sealed and transferred to the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument for processing.

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ISO method: Twenty-five gram samples were analyzed according to ISO 11290-1:1996, including Amendment 1:2204. Each sample was enriched by incubating at 30±1 °C, for 22 to 26 hours in 225 ml of Half Fraser Broth. Following enrichment, 10 µl of the Half Fraser Broth was inoculated onto both Thermo Scientific™ Oxoid™ Chromogenic Listeria Agar (ISO) (OCLA (ISO)) and PALCAM Agar and incubated at 37±1 °C, for 22 to 26 hours. A further 100 µl of the Half Fraser Broth was inoculated into 10 ml Fraser Broth and incubated for 46 to 50 hours at 37±1 °C, from which OCLA (ISO) and PALCAM Agar plates were later streaked and incubated for 24 hours at 37±1 °C. Presumptive positive growth from up to five colonies was confirmed by Gram stain, haemolysis, catalase, CAMP test and biochemical identification.

Results: The levels of detection for the alternative method and the ISO method were determined according to the Spearman-Kärber (LOD_{50}) method (table 1). The aim was to obtain the relative levels of detection for each of "strain-matrix" combinations analyzed during the AFNOR Certification study.

Table 1: Relative detection level results for the alternative method and ISO reference method

Strain / matrix pairs	ISO reference method (CFU/25 g)	Alternative method (CFU/25 g)
Rillettes / *L. monocytogenes* Ad 669	0.308 [0.183-0.518]	0.494 [0.280-0.872]
Smoked salmon / *L. monocytogenes* BR32	0.398 [0.226-0.703]	0.316 [0.197-0.508]
Raw milk / *L. monocytogenes* 153	0.442 [0.239-0.818]	0.638 [0.345-1.18]
Raw vegetables / *L. monocytogenes* 1016/1413	0.603 [0.339-1.071]	0.603 [0.356-1.021]
Process water / *L. monocytogenes* 877/113	0.541 [0.387-0.756]	0.618 [0.464-0.824]

Conclusions

The relative limit of detection study conducted as part of the NF VALIDATION study demonstrated that the SureTect Listeria monocytogenes PCR Assay workflow showed a similar detection level for the matrices analyzed as the ISO reference method, detailed in ISO 11290-1:1996, including Amendment 1:2004. For the ISO reference method, the range in the level of detection for all food categories studied during the validation was 0.1-1.0 CFU/25 g, compared to a range of 0.1-1.1 CFU/25 g for the alternative method, demonstrating that the SureTect Listeria monocytogenes PCR Assay workflow is an accurate alternative method for the detection of *Listeria monocytogenes*. The NF VALIDATION certificate and the validation report summary for this study are available from **http://nf-validation.afnor.org/en/**.

www.thermofisher.com/SureTect

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