Thermo Scientific SureTect Listeria monocytogenes Assay: NF Validation Using The Applied Biosystems 7500 Fast PCR Instrument

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ABSTRACT

Introduction
The Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay is a real-time PCR assay intended for the detection of *Listeria monocytogenes* from food products and environmental samples, which has previously gained NF VALIDATION™ by AFNOR Certification using the Thermo Scientific™ SureTect™ PikoReal™ PCR instrument and Thermo Scientific SureTect Software version 1.2.

Purpose
The purpose of this study was to conduct an NF VALIDATION by AFNOR Certification extension study to validate use of the SureTect Listeria monocytogenes PCR Assay on the Applied Biosystems™ 7500 Fast PCR Instrument with Applied Biosystems™ RapidFinder™ Express version 2.0 Software (the alternative method) with meat products, milk and dairy products, seafood and fishery products, vegetables plus environmental samples.

Methods
A method comparison study and relative limit of detection (RLOD) study was conducted. For the alternative method, all samples underwent an enrichment step followed by direct lysis. Following direct lysis, PCR was run and results were automatically interpreted by the software. The reference method was conducted according to EN ISO 11290-1/A1:2004.

Results
A total of 393 food and environmental samples were tested using the alternative and reference methods. For the RLOD study, five different *L. monocytogenes* isolates were spiked into representative matrices and tested as per the alternative method protocol and the reference method. The alternative method demonstrated equivalent performance for all human food and environment samples analyzed to the reference method. The alternative method showed a similar RLOD (0.2-1.0 CFU/25 g) to the EN reference method (0.3-0.9 CFU/25 g).

Significance
The alternative method proved to be a suitable substitute to reference method for *L. monocytogenes* detection.

INTRODUCTION

The SureTect Listeria monocytogenes PCR Assay is a real-time PCR kit for the detection of *Listeria monocytogenes* from food and production environmental samples. The kit combines pre-dispensed lysis reagents and lyophilised and tableted PCR reagents to simplify and improve assay handling, along with software to automatically interpret and display results. This NF VALIDATION by AFNOR Certification ISO 16140 extension study was conducted to extend the use of the SureTect Listeria monocytogenes PCR Assay to the Applied Biosystems 7500 Fast 96-well PCR instrument with RapidFinder Express 2.0 Software for meat, dairy, seafood, vegetable and environmental samples.

MATERIALS AND METHODS

Method comparison study
A total of 393 food and environmental samples (78 meat products, 78 milk and dairy products, 65 seafood and fishery products, 75 vegetables plus 97 environmental samples) were analyzed as part of the expert laboratory study,

A total of 137 samples were artificially contaminated: 126 samples by a seeding protocol, six samples by a spiking protocol and five samples were contaminated by cross-contamination. A total of 42 different *Listeria monocytogenes* isolates were used for the artificial contamination of the samples; all 42 isolates were stressed by exposure to low temperatures (-20°C or 2 to 8 °C).

Relative limit of detection (RLOD) study
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. 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.

SureTect Listeria monocytogenes PCR Assay method
Twenty-five gram samples were homogenized with 225 ml of fully supplemented Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) then enriched by incubating for 24 to 28 hours at 37±1 °C. Ten microliters 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 microliters of the enriched samples were added to each of the required number of Lysis Tubes. A negative control sample was also prepared by adding 10 µl sterile nuclease free water (or sterile media) to a SureTect Lysis Tube. The lysis tubes 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 PCR Tubes containing SureTect Listeria monocytogenes PCR tablets. The PCR Tubes were then immediately sealed and transferred to the Applied Biosystems 7500 Fast System for processing. Regardless of the PCR result, all samples were confirmed 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. Refer to figure 1 for a summary of this method.

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 22 to 26 hours in 225 ml of Half Fraser Broth. Ten microlitrers 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 22 to 26 hours. A further 100 µl of the Half Fraser Broth enrichment was inoculated into 10 ml Fraser Broth and incubated at 37±1 °C for 46 to 50 hours. Following this secondary enrichment,10 µl was streaked onto OCLA (ISO) and PALCAM Agar plates which were then incubated at 37±1 °C for 22 to 26 hours. Up to five presumptive positive colonies were confirmed by Gram stain, haemolysis, catalase, CAMP test and biochemical identification

RESULTS

Method comparison study
SureTect Listeria monocytogenes PCR Assay was shown to be a reliable alternative to the ISO reference method for the detection of *L. monocytogenes* from the food and production environment categories analyzed during the method comparison study. See table 1 for a summary of the SureTect Listeria monocytogenes PCR Assay results versus the ISO reference method results.

Thirty-three negative deviation results were recorded during the method comparison study. The presence of *L. monocytogenes* was detected in two of these samples (by subculture of the 24 LEB Broth into Fraser Broth before streaking onto selective agar plates).

The remaining 31 negative discordant results were reported during the method comparison study were determined to be most likely due to the unpaired study design and the related sampling heterogeneity, as *L. monocytogenes* could not be isolated from the samples by the culture confirmation method meaning that it is likely that no target cells were present in the portion of matrix used for the alternative method.

Thirty-two positive deviation results were recorded during the method comparison study.

The relative sensitivity, specificity and accuracy of the SureTect Listeria monocytogenes PCR Assay method are listed in Table 2.

Relative limit of detection (RLOD) study
The level of detection for the alternative method and the ISO reference method were determined according to the ISO 16140-2:2016 standard (Table 3). The aim was to determine the relative level of detection for both matrices analyzed during the AFNOR Certification validation study.

Figure 1. SureTect Listeria monocytogenes PCR Assay protocol used for all food and production environment samples using the Applied Biosystems 7500 Fast Instrument

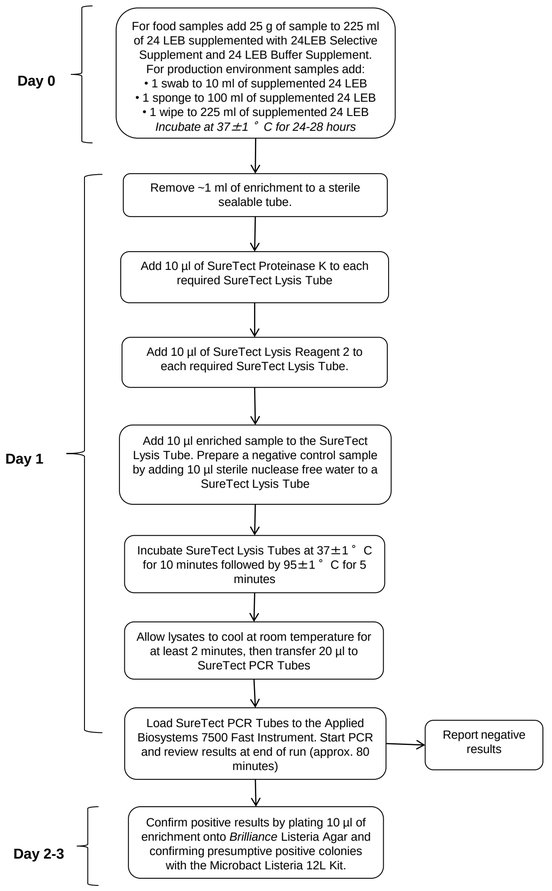

Table 1. SureTect Listeria monocytogenes PCR Assay versus the ISO reference method

SureTect Listeria monocytogenes PCR Assay Method	ISO 11290-1:1996, including Amendment 1:2004 reference method		Total
	positive	negative	
positive	96	32	128
negative	33	232	265
Total	129	264	393

Table 2. Relative sensitivity, specificity and accuracy of the SureTect Listeria monocytogenes PCR Assay

	SureTect Assay methods comparative study
Relative Accuracy	83.5%
Relative Sensitivity	74.4%
Relative Specificity	87.9%

Table 3: Relative detection level results for the SureTect Listeria monocytogenes PCR Assay 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 method comparison study conducted as part of this NF VALIDATION extension study demonstrated that the alternative method is equivalent in performance for the food and production environment samples analyzed to the ISO reference method detailed in ISO 11290-1:1996, including Amendment 1:2004, when using the Applied Biosystems 7500 Fast System and RapidFinder Express v2.0 Software.

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 when using the Applied Biosystems 7500 Fast Instrument with RapidFinder Express v2.0 Software. When the results were analyzed according to the ISO 16140-2:2016 standard, the level of detection ranged from of 0.2 to 1.1 CFU/25 g demonstrating that the SureTect Listeria monocytogenes PCR Assay is an accurate alternative method for the detection of *L. monocytogenes* from the food categories analyzed.

REFERENCES

1. ISO 11290-1:1996. Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 1: Detection method
2. ISO 11290-1:1996/Amd 1:2004. Modification of the isolation media and the haemolysis test, and inclusion of precision data.
3. ISO 16140-2:2016. Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

TRADEMARKS/LICENSING

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