Study Report

SureTect Listeria monocytogenes PCR Assay Workflow NF VALIDATION ISO 16140: Method Comparison

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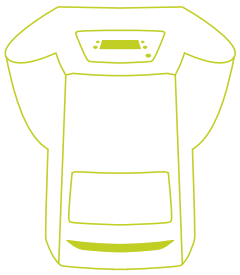

SureTect™ PikoReal™ Real-Time PCR Instrument

Summary

As part of the NF VALIDATION™ ISO 16140 of the Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay, a method comparison study was conducted by the AFNOR Certification Expert laboratory, ADRIA Développement, Quimper, France. This report presents the results from both the expert and collaborative laboratory studies conducted to validate the performance of this SureTect assay workflow (alternative method) against the ISO reference method detailed in the ISO 11290-1:1996, including Amendment 1:2004. The collaborative study was conducted using a matrix of spiked cheese which was analyzed by 12 independent laboratories across Europe. The following is a summary of the method comparison study.

Methodology

Expert Laboratory Study

A total of 339 samples were analyzed as part of the expert laboratory study, which was designed to validate the performance of the SureTect Listeria monocytogenes PCR Assay for meat, dairy, seafood, vegetable and production environment samples.

Eighty-four samples were naturally contaminated with *Listeria monocytogenes*, 96 samples were artificially contaminated (by spiking with 23 different *L. monocytogenes* isolates) and 16 samples were contaminated by cross contamination. All isolates used for the artificial contamination of the samples were stressed by exposure to heat, low temperatures (-20 °C) or acidic or alkaline levels of pH.

Collaborative Laboratory Study

A cheese matrix was prepared and spiked with a *L. monocytogenes* isolate and sent to all participating collaborative laboratories. Samples were analyzed following both the alternative method and the ISO reference method. A third of samples remained unspiked, another third was spiked with a low level inoculum (2 CFU/25 g) and the remaining third was spiked with a high level inoculum (24 CFU/25 g).

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Protocol

Alternative Method:

Twenty-five gram samples were homogenized with 225 ml of supplemented Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB). The enriched sample was then incubated 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.

Regardless of the PCR result, all 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 colony with the Thermo Scientific™ Oxoid™ Microbact™ Listeria 12 L 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 test 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 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 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

Expert Laboratory Study

The SureTect Listeria monocytogenes PCR Assay workflow was shown to be a reliable alternative to the ISO reference method for the detection of *L. monocytogenes* from the categories analyzed during the expert laboratory study.

Table 1: NF VALIDATION ISO 16140 confirmed results for the alternative method and the ISO reference method

	ISO method positive results	ISO method negative results
Alternative method positive results	97	34[1]
Alternative method negative results	26	182

[1]Samples positive by alternative method but negative by reference method. Alternative method positive results were all confirmed by culture.

Twenty-six negative deviation results were recorded during the expert laboratory study. The presence of *L. monocytogenes* was detected by culture in 6 of these samples.

The remaining 20 negative discordant results were reported by the expert laboratory as being most likely due to a lack of homogeneity during the sample preparation, as *L. monocytogenes* could not be isolated from the samples.

The supplementary positive results detected by the alternative method were not included in this table.

Collaborative Laboratory Study

The collaborative laboratory study demonstrated the SureTect Listeria monocytogenes PCR Assay workflow is a reliable method for the detection of *L. monocytogenes* from food and production environment samples. The relative sensitivity, specificity and accuracy of the SureTect method are listed in Table 2.

Table 2: NF VALIDATION ISO 16140 relative accuracy, sensitivity and specificity results

	Expert laboratory study	Collaborative laboratory study
Relative Accuracy	82.3%	96.7%
Relative Sensitivity	78.9%	96.9%
Relative Specificity	84.3%	100.0%

The Accordance, Concordance and Odds ratios of the alternative method and the ISO reference method were also determined. The results are shown in Table 3.

The observed data and results confirmed that the alternative method and the ISO reference method show equivalent performance.

Table 3: NF VALIDATION ISO 16140 accordance, concordance and odds ratio results

		Alternative method	ISO reference method
Accordance	Level 0	100%	100%
	Level 1	95.6%	89.1%
	Level 2	100%	100%
Concordance	Level 0	100%	100%
	Level 1	95.1%	88.2%
	Level 2	100%	100%
Odds ratio	Level 0	1.00	1.00
	Level 1	1.13	1.09
	Level 2	1.00	1.00

Conclusions

The method comparison study conducted as part of this NF VALIDATION study demonstrated that the SureTect Listeria monocytogenes PCR Assay workflow is equivalent in performance to the ISO reference method detailed in ISO 11290-1:1996, including Amendment 1:2004. The NF VALIDATION certificate and a summary of the validation report for this study are available from **http://nf-validation.afnor.org/en/**.

www.thermofisher.com/SureTect

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