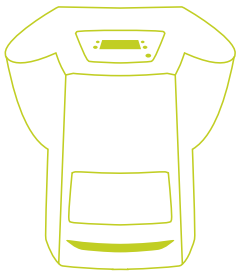
SureTect Listeria species PCR Assay Workflow NF VALIDATION ISO 16140: Method Comparison

Study Report

Ana-Maria Leonte and Jonathan Cloke, Thermo Fisher Scientific, Wade Road, Basingstoke, Hampshire, RG24 8PW, UK



SureTect™ PikoReal™ Real-Time PCR Instrument

Summary

As part of the NF VALIDATION™ ISO 16140 validation of the Thermo Scientific™ SureTect™ Listeria species PCR Assay workflow, 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 the SureTect assay workflow (alternative method) against the ISO reference method detailed in ISO 11290-1:1996, including Amendment 1:2004. The collaborative study was conducted using a spiked cheese matrix which was analyzed by 12 independent laboratories across six European countries. The following is a summary of the method comparison study.

Methodology

Expert laboratory study

A total of 325 samples were analyzed as part of the expert laboratory study, which was designed to validate the performance of the alternative method with meat, dairy, seafood, vegetable and production environment samples.

A total of 111 samples were naturally contaminated with *Listeria* spp., 69 samples were artificially contaminated (by spiking with 23 different *Listeria* spp. isolates) and 14 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 *Listeria monocytogenes* isolate and sent to all participating collaborative laboratories. Samples were analyzed following both the alternative method and the ISO reference method. Of all the samples tested, a third was unspiked, another third was spiked with a low level inoculum (2 CFU/25 g) and the remaining samples were 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). Samples were then enriched by incubating 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 number of 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 then cooled for at least 2 minutes at room temperature prior to transferring 20 µl aliquots of the lysates to SureTect PCR Tubes containing SureTect Listeria species PCR tablets. The PCR Tubes were then immediately sealed and transferred to the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument for processing.

The presence of *Listeria* spp. was confirmed by direct streaking of the 24 LEB enrichment onto Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria Agar. Additional confirmation of presumptive positive colonies was performed using the Thermo Scientific™ Oxoid™ Microbact™ Listeria 12L Kit.

ISO reference Method:

Twenty-five gram samples were analyzed according to the reference 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. After primary enrichment, 10 µl of the Half Fraser Broth enrichment was inoculated on both Thermo Scientific™ Oxoid™ Chromogenic Listeria Agar (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 for 46 to 50 hours at 37±1 °C, from which OCLA (ISO) and PALCAM Agar plates were streaked and incubated for 22 to 26 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 species PCR Assay workflow was shown to be a reliable alternative to the ISO reference method for the detection of *Listeria* spp. from the food and production environment samples analyzed during the expert laboratory study, as shown in Table 1.

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

	ISO reference method positive results	ISO reference method negative results
Alternative method positive results	114	24
Alternative method negative results	28	159

Twenty-eight negative deviation results were recorded during the expert laboratory study. The presence of *Listeria* spp. was detected by culture in three of these samples.

The remaining 25 negative discordant results were reported by the expert laboratory as being most likely due to a lack of homogeneity during the sample preparation, as *Listeria* spp. could not be isolated from the samples by following the confirmation steps.

Collaborative laboratory study

The collaborative laboratory study demonstrated the SureTect Listeria species PCR Assay workflow is a reliable method to detect *Listeria* spp. from food and production environment samples. The relative sensitivity, specificity and accuracy of the alternative method (for both the expert and collaborative laboratory studies) 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	84.0%	96.3%
Relative Sensitivity	86.6%	95.6%
Relative Specificity	80.3%	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 show equivalent performance (accordance, concordance, odds ratio).

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

	Spike level	Alternative method	ISO reference method
Accordance	Unspiked	100%	100%
	Low level	84.7%	95.6%
	High level	100%	100%
Concordance	Unspiked	100%	100%
	Low level	84.0%	95.1%
	High level	100%	100%
Odds ratio	Unspiked	1.00	1.00
	Low level	1.06	1.13
	High level	1.00	1.00

Conclusions

The method comparison study conducted as part of this NF VALIDATION study demonstrates that the SureTect Listeria species 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

Contact Information:
microbiology@thermofisher.com
USA +1 800 255 6730
International +44 (0) 1256 841144

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