STUDY REPORT

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

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Summary

As part of the NF VALIDATION™ ISO 16140 extension study of the Thermo Scientific™ SureTect™ Listeria species 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 species PCR Assay with the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument with SureTect Software v1.2 for meat samples (Initial validation was obtained on the dairy, seafood, vegetables and production environment samples) . Also part of this extension study was the validation of the use of the SureTect Listeria species 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 expert laboratory study conducted to validate the alternative method against the ISO reference method detailed in ISO 11290-1:1996, including Amendment 1:2004.

Methodology

Expert laboratory study

A total of 325 samples covering the meat, milk and dairy, seafood, vegetables and production environment categories, were analyzed as part of the expert laboratory study, which was designed to validate the performance of the SureTect Listeria species PCR Assay on the SureTect PikoReal Instrument and 370 samples were analysed for the use of the SureTect Listeria species PCR Assay with the Applied Biosystems 7500 Fast System and RapidFinder Express v2.0 Software with SureTect Salmonella Kit File (for meat, dairy, seafood, vegetables and production environment categories).

To validate the use of the alternative method on the SureTect PikoReal Instrument, 69 samples were artificially contaminated with 23 different *Listeria* spp. To validate the use of the alternative method on the Applied Biosystems 7500 Fast Instrument, 107 samples were artificially contaminated with 45 different *Listeria* spp. isolates.

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Protocol

Alternative method

Twenty-five gram samples were homogenized with 225 ml Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) fully supplemented with Thermo Scientific™ Oxoid™ 24 Listeria Selective Supplement and Thermo Scientific™ Oxoid™ 24 Listeria Buffer Supplement.

When analyzed with the SureTect PikoReal Instrument and SureTect Software v1.2, food (except the meat samples) and production environment samples were enriched by incubating for 22-26 hours at 37±1 °C. Meat samples were enriched for 24-28 hours at 37±1 °C.

When analysed with the Applied Biosystems 7500 Fast Instrument and RapidFinder Express V 2.0 Software, all food and production environment samples were enriched by incubating for 24-28 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). 10 µl 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 species PCR Tubes containing SureTect Listeria species PCR tablets.

When performing PCR using the Applied Biosystems 7500 Fast Instrument, a negative control sample was prepared by adding 10 µl sterile nuclease free water or sterile media to a SureTect Lysis Tube in place of the enriched sample.

The PCR Tubes were then immediately sealed and transferred to the SureTect PikoReal Instrument or the Applied Biosystems 7500 Fast System 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 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 21-27 hours in 225 ml Half Fraser Broth. 10 µl 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 21-27 hours. A further 100 µl of the Half Fraser Broth enrichment was inoculated into 10 ml Fraser Broth and incubated for 45-51 hours at 37±1 °C. Following this secondary enrichment, 10 µl was streaked onto OCLA (ISO) and PALCAM Agar plates which were then incubated for 21-27 hours at 37±1 °C. Up to five presumptive positive colonies were confirmed by Gram stain, haemolysis, catalase, CAMP test and biochemical identification.

Alternative method: Protocol used for all food and production environment samples using the SureTect PikoReal Instrument

Day: 0

For food samples (except meat) add 25 g of sample to 225 ml of 24 LEB fully supplemented

For production environment samples add

- 1 swab to 10 ml of supplemented 24 LEB
- 1 sponge to 100 ml of supplemented 24 LEB
- 1 wipe to 225 ml of supplemented 24 LEB

Incubate at 37±1 °C for 22-26 hours

For meat samples add 25 g of sample to 225 ml of 24 LEB fully supplemented

Incubate at 37±1 °C for 24-28 hours

Day: 1

Add 10 µl of SureTect Proteinase K, followed by adding 10 µl of SureTect Lysis Reagent 2 to each required SureTect Lysis Tube.

Add 10 µl enriched sample to the SureTect Lysis Tube.

Incubate SureTect Lysis Tubes at 37±1 °C for 10 minutes followed by 95±1 °C for 5 minutes.

Allow lysates to cool at room temperature for at least 2 minutes, then transfer 20 µl to SureTect PCR Tubes.

Load SureTect PCR Tubes to the SureTect PikoReal Instrument. Start PCR and review results at end of run

Report negative results

Day: 2 or 3

Confirm positive results by plating 10 µl of enrichment onto *Brilliance* Listeria Agar and confirming presumptive positive colonies with the Microbact Listeria 12L Kit.

Alternative method: Protocol used for all food and production environment samples using the Applied Biosystems 7500 Fast Instrument

Day: 0

For all food samples add 25 g of sample to 225 ml of 24 LEB fully supplemented

For production environment samples add

- 1 swab to 10 ml of supplemented 24 LEB
- 1 sponge to 100 ml of supplemented 24 LEB
- 1 wipe to 225 ml of supplemented 24 LEB

Incubate at 37±1 °C for 24-28 hours

Day: 1

Add 10 µl of SureTect Proteinase K, followed by adding 10 µl of SureTect Lysis Reagent 2 to each required SureTect Lysis Tube.

Add 10 µl enriched sample to the SureTect Lysis Tube.

Incubate SureTect Lysis Tubes at 37±1 °C for 10 minutes followed by 95±1 °C for 5 minutes.

Allow lysates to cool at room temperature for at least 2 minutes, then transfer 20 µl to SureTect PCR Tubes.

Load SureTect PCR Tubes to the Applied Biosystems 7500 Fast Instrument. Start PCR and review results at end of run

Report negative results

Day: 2 or 3

Confirm positive results by plating 10 µl of enrichment onto *Brilliance* Listeria Agar and confirming presumptive positive colonies with the Microbact Listeria 12L Kit.

ISO reference method: Protocol used for all food and production environment samples

Day: 0

Add 25 g of sample to 225 ml of Half Fraser Broth.

Incubate at 30±1 °C for 21-27 hours

Day: 1

Add 0.1 ml of Half Fraser Broth enrichment to 10 ml of Fraser Broth.

Incubate at 37±1 °C for 45-51 hours

Streak 10 µl of Half Fraser Broth enrichment onto PALCAM Agar and OCLA (ISO).

Incubate at 37±1 °C for 24 hours. If small colonies or negative, reincubate a further 24 hours

Day: 3

Streak 10 µl of Fraser Broth enrichment onto PALCAM Agar and OCLA (ISO)

Incubate at 37±1 °C for 24 hours. If small colonies or negative, reincubate a further 24 hours

DAY: 2-3 Half Fraser DAY: 4-5 Fraser

Confirm up to 5 presumptive positive colonies by biochemical identification and serological tests

Results

Expert Laboratory

The alternative method was shown to be a reliable alternative to the ISO reference method for the detection of *Listeria* spp. from the food and production environment categories analyzed during the expert laboratory study.

Twenty-eight negative deviation results were recorded during the expert laboratory study, when using the SureTect PikoReal Instrument. The presence of *Listeria* spp. was detected in 3 of these samples. Thirty-seven negative deviation results were recorded, when using the Applied Biosystems 7500 Fast Instrument. The presence of *Listeria* spp. was detected in 7 of these samples.

The remaining negative discordant results were reported by the expert laboratory as being most likely due to the unpaired study design and the related sampling heterogeneity, as *Listeria* spp. 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.

Twenty-four positive deviation results were recorded when using the SureTect PikoReal Instrument and 35 when using the Applied Biosystems 7500 Fast Instrument.

The relative sensitivity, specificity and accuracy of the alternative method are listed in Table 2.

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

Table 1: NF VALIDATION ISO 16140 extension study confirmed results for the alternative and reference methods

		ISO reference method positive results	ISO reference method negative results
SureTect PikoReal Instrument (initial validation)	Alternative method positive results	114	24
	Alternative method negative results	28	159
Applied Biosystems 7500 Fast System (extension study)	Alternative method positive results	108	35
	Alternative method negative results	37	190

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

	SureTect PikoReal Instrument	Applied Biosystems 7500 Fast Instrument
Relative Accuracy	84.0%	80.5%
Relative Specificity	86.9%	84.4%
Relative Sensitivity	80.3%	74.5%

Conclusion

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 analysed to 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 System and RapidFinder Express v2.0 Software. The NF VALIDATION certificate and a summary of the expert laboratory report for this study are available from **http://nf-validation.afnor.org/en/.**

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