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STUDY REPORT

SureTect Listeria Species PCR Assay Workflow NF VALIDATION ISO 16140-2 Validation Studies

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Summary

The Thermo Scientific™ SureTect™ Listeria species PCR Assay workflow (PT0200A) (alternative method) has been certified by NF VALIDATION (certification reference UNI 03/09-11/13) for the detection of *Listeria* spp. from meat, dairy, seafood, vegetable and production environment samples. The following report gives a summary of the validation studies performed to gain NF VALIDATION.

Methodology

For the initial validation studies, the performance of the alternative method was assessed as an unpaired study in comparison to the ISO reference method detailed in ISO 11290-1:1996, including Amendment 1:2004 'Microbiology of the food chain—Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp.—Part 1: Detection method'. PCR analysis was conducted using the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument.

The performance of the alternative method for the extension studies that were conducted to incorporate the Applied Biosystems™ 7500 Fast Food Safety System (7500 Fast Real-Time PCR Instrument and Applied Biosystems™ RapidFinder™ Express Software (version 2.0 or higher) (A30299/A30304) was assessed in comparison to ISO reference method detailed in ISO 11290-1:1996, including Amendment 1:2004.

The extension studies conducted to incorporate the Applied Biosystems™ QuantStudio™ 5 Food System (Applied Biosystems QuantStudio 5 Real-Time PCR Instrument and RapidFinder™ Analysis Software version 1.0, (A36320/A36328) assessed the alternative method performance in comparison to the updated ISO 11290-1:2017 in accordance with ISO 16140-2:2016.

The initial certification study and all subsequent extension studies were conducted by ADRIA Développement, Quimper, France.

The protocols for the alternative method and the reference method are summarized in Appendix 1 and 2, respectively.

Inclusivity and Exclusivity Study

Fifty-two inclusivity isolates were inoculated at 1-100 CFU/mL into 225 mL of Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) with 24 LEB Selective, and incubated for 18-24 hours at 37±1°C, prior to analysis with the alternative method.

Thirty exclusivity isolates were inoculated at approximately $1x10^5$ CFU into 225 mL Buffered Peptone Water (BPW) (ISO) and incubated for 24 hours at 37±1°C, prior to analysis with the alternative method.

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Inclusivity and Exclusivity Study

Fifty-one of the 52 inclusivity isolates tested returned a positive PCR result. One isolate of *Listeria roucourtiae* gave a negative PCR result and atypical colonies were observed during confirmation testing. This *L. roucourtiae* isolate was also tested with the reference method which returned a negative result using Half Fraser Broth and grew atypical colonies when cultured from Fraser Broth. All 30 exclusivity isolates gave a negative result with the alternative method.

The inclusivity and exclusivity study demonstrated that the alternative method is a sensitive and specific method.

Inter-laboratory Study

An inter-laboratory study was performed as part of the initial validation. A cheese matrix was prepared and spiked with a *Listeria monocytogenes* isolate and sent to all participating laboratories. Samples were analyzed following both the alternative method and ISO reference method. Of all samples tested, a third were unspiked, another third were 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).

Inter-laboratory Study Results

The relative sensitivity, specificity and accuracy of the alternative method are listed in Table 1.

The results displayed in Table 1 demonstrate that the alternative method is a reliable method to detect *Listeria* spp. from food and production environment samples.

Method Comparison Study

As part of the previous validation studies, a total of 370 samples were analyzed (including meat products, milk and dairy products, seafood and fishery product, vegetables and production environmental samples categories) using the alternative method with the Applied Biosystems 7500 Fast Food Safety System.

The aim of this extension study was to extend the AFNOR NF VALIDATION to add the category composite food to the certified claim as well as to incorporate the Applied Biosystems QuantStudio 5 Food Safety System.

A total of 378 samples (including meat products, milk and dairy products, seafood and fishery products, vegetables, composite foods and production environmental samples categories) were analyzed using the alternative method with the Applied Biosystems QuantStudio 5 Food System.

A total of 73 samples from the composite food category were analyzed using the alternative method with the Applied Biosystems 7500 Fast Food Safety System.

Method Comparison Study Results

The results for the method comparison study using the Applied Biosystems QuantStudio 5 Food Safety System are listed in Table 2.

The results for the method comparison study using the Applied Biosystems 7500 Fast Food Safety System, including the newly analysed composite food category are listed in Table 3.

Forty-six negative deviations (ND+PPND) were recorded when using the Applied Biosystems 7500 Fast Food Safety System, and the presence of *Listeria* spp. was detected in seven of these samples. Forty-one negative deviations were recorded when using the Applied Biosystems QuantStudio 5 Food Safety System.

The negative discordant results are 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, it is likely that no target cells were present in the portion of matrix used for the alternative method.

Forty-eight positive deviations were recorded when using the Applied Biosystems 7500 Fast Food Safety System and 42 when using the Applied Biosystems QuantStudio 5 Food Safety System. These results were detected as positive using the alternative method but failed to be detected with the reference method. All positive deviations were confirmed as true positives using the alternative method confirmation tests.

The analysis of discordant results according to the EN ISO 16140-2:2016 is given in Table 2 for the Applied Biosystems QuantStudio 5 Food Safety System and Table 3 for the Applied Biosystems 7500 Fast Food Safety System.

As agreed with the AFNOR technical committee for the Applied Biosystems QuantStudio 5 Food Safety System, no interpretation was made for a category if fewer than 30 positive samples were available.

The observed values ((ND + PPND) - PD) for the individual categories analyzed and for all the combined categories meet the Acceptability Limits (observed values ≤ AL) when using the Applied Biosystems 7500 Fast Food Safety System and the Applied Biosystems QuantStudio 5 Food Safety System.

The method comparison study shows that the alternative method is a reliable method for the detection of *Listeria* spp. from the milk and dairy, seafood, vegetables, composite food and production environmental samples categories.

Relative Level of Detection Study

For the relative level of detection (RLOD) study six individual *Listeria* spp. isolates were spiked into six matrices (tabbouleh, rillettes, raw milk, ready to cook vegetables, smoked salmon and process water) and analyzed using the Applied Biosystems 7500 Fast Food Safety System.

With agreement from the AFNOR technical committee, for the RLOD study, only four matrix/strain pairs were tested using the Applied Biosystems QuantStudio 5 Food Safety System as opposed to the six matrix/strain pairs that were previously tested for the Applied Biosystems 7500 Fast Food Safety System. This was due to there being insufficient lysates remaining for two of the matrices. This has been highlighted in Table 4.

The samples were analyzed using the reference method detailed in ISO 11290-1:1996, including Amendment 1:2004 prior to inoculation to verify the absence of *Listeria* spp. After inoculation, samples were tested using the ISO reference method and the alternative method.

Relative Level of Detection Study

The RLOD study results for each Food Safety System were calculated using the using the Excel™ spreadsheet available at http://standards.iso.org/iso/16140 (RLOD: clause 5-1-4-2. Calculation and interpretation of RLOD, version 06.07.2015). The results are displayed in Table 5.

As shown in Table 5, the relative level of detection study demonstrated that the alternative method gave an RLOD below the acceptability limit when used with either the Applied Biosystems QuantStudio 5 Food Safety System or the Applied Biosystems 7500 Fast Food Safety System.

Conclusion

The NF VALIDATION studies demonstrate that the alternative method is equivalent in performance to the reference method detailed in ISO 11290-1:1996, when using either the Applied Biosystems 7500 Fast Food Safety System or the Applied Biosystems QuantStudio 5 Food Safety System. The NF VALIDATION certificate is available from www.thermofisher.com/foodsafety.

Table 1. Inter-laboratory study results summary

	Alternative method result
Relative Accuracy	96.3%
Relative Sensitivity	95.6%
Relative Specificity	100.0%

Table 2: Method comparison study results for the alternative method on the Applied Biosystems QuantStudio 5 Food Safety System

Category	PA	NA	PD	ND	PPND	PPNA	(ND+PPND) -PD	AL
Composite foods (multi-component foods)	20	30	14	9	0	0	-5	3
Meat products	21	34	6	9	0	1	3	3
Milk & dairy products	17	21	8	5	1	5	-2	3
Seafood and fishery products	11	22	4	5	0	0	/	/
Vegetables	15	29	4	7	0	1	/	/
Production environment samples	23	45	6	3	2	0	-1	3
All categories	**107**	**181**	**42**	**38**	**3**	**7**	**-1**	**6**

Table 3: Method comparison study results for the alternative method on the Applied Biosystems 7500 Fast Food Safety System

Category	PA	NA	PD	ND	PPND	PPNA	(ND+PPND) -PD	AL
Composite foods	20	31	13	8	1	0	-4	3
Meat products	24	40	6	8	0	0	2	3
Milk & dairy products	23	29	9	7	2	6	0	3
Seafood and fishery products	23	30	6	4	0	0	-2	3
Vegetables	22	39	7	7	1	0	1	3
Production environment samples	16	45	7	8	0	1	1	3
All categories	**128**	**214**	**48**	**42**	**4**	**7**	**-2**	**6**

Key

PA: Positive Agreement, NA: Negative Agreement, PD: Positive Deviation, ND: Negative Deviation,

PP: Positive Presumptive Non Confirmed Samples, AL: Acceptability Limit (as defined by ISO 16140-2:2016)

PPND: Presumptive Positive Negative Deviation

PPNA: Presumptive Positive Negative Agreement

Table 4: Defined matrix/strain pairs for the RLOD determination

Category	Matrix	Inoculated strain	Storage conditions before analysis	Applied Biosystems Food Safety System
Composite food[a]	Tabbouleh	*Listeria seeligeri* Ad1293	5°C±3°C for 48 h	7500 Fast QuantStudio 5
Meat products	Rillettes	*Listeria innocua* Ad671	5°C±3°C for 48 h	7500 Fast[b]
Milk & dairy products	Raw milk	*Listeria ivanovii* Ad991	5°C±3°C for 48 h	7500 Fast[b]
Seafood and fishery products	Smoked salmon	*Listeria welshimeri* Ad1669	5°C±3°C for 48 h	7500 Fast QuantStudio 5
Vegetables	Ready-to-cook vegetables	*Listeria monocytogenes* Ad279	5°C±3°C for 48 h	7500 Fast QuantStudio 5
Production environmental samples[a]	Process water	*Listeria monocytogenes* Ad551	5°C±3°C for 48 h	7500 Fast QuantStudio 5

Key

[a] Categories analysed in current extension study

[b] Lysates from previous studies were unavailable for analysis with the Applied Biosystems QuantStudio 5 Food Safety System

Table 5: RLOD results summary

Matrix	Strain	QuantStudio 5 RLOD	7500 Fast RLOD	Acceptability level (≤)
Tabbouleh	*Listeria seeligeri* Ad1293	0.873	0.873	2.5
Rillettes	*Listeria innocua* Ad671	–	0.351	
Raw milk	*Listeria ivanovii* Ad991	–	1.000	
Smoked salmon	*Listeria welshimeri* Ad1669	0.986	0.986	
Ready-to-cook vegetables	*Listeria monocytogenes* Ad279	1.190	0.968	
Process water	*Listeria monocytogenes* Ad551	0.874	0.731	
	Combined RLOD	**0.994**	**0.828**	

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Appendix 1. Protocol for alternative method using the Applied Biosystems QuantStudio 5 Food Safety System

Day 0

For food samples (except meat), add 25 g of sample to 225 ml of 24 LEB with selective supplement, plus 10 mL buffer supplement.

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 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 in the Applied Biosystems™ SimpliAmp™ Thermal Cycler at 37±1°C for 10 minutes followed by 95±1°C for 5 minutes.

Transfer 20 µL of sample to SureTect PCR Tubes.

Load SureTect PCR Tubes to the Applied Biosystems 7500 Fast or the Applied Biosystems QuantStudio 5 PCR 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.

Appendix 2: Protocol for the reference method: ISO 11290-1:2017