STUDY REPORT

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

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Summary

The Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay (PT0300A) (alternative method) has been certified by NF VALIDATION™ (UNI 03/08-11/13) for the detection of *Listeria monocytogenes* from meat, dairy, seafood, vegetable and production environment samples. The following report gives a summary of the validation studies performed as part of the NF VALIDATION.

Methodology

Study	Reference method
Initial validation PCR analysis was conducted using the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument	**ISO 11290-1:1996, 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'
Extension study To incorporate the Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR System (7500 Fast Real-Time PCR Instrument and Applied Biosystems™ RapidFinder™ Express Software (version 2.0 or higher))	**ISO 11290-1:1996, 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'
Extension study • To incorporate the Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System (Applied Biosystems QuantStudio 5 Real-Time PCR Instrument and RapidFinder™ Analysis Software version 1.0) • To add the composite food category	**ISO 11290-1:2017** 'Microbiology of the food chain— Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp.—Part 1: Detection method'

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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 & exclusivity study

Fifty inclusivity isolates of *Listeria monocytogenes* and 30 exclusivity isolates were analyzed as part of the inclusivity and exclusivity study.

Inclusivity isolates were inoculated into 225 mL of Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) with selective supplement at 10 CFU/ 225 mL, before incubating for 18-24 hours at 37±1°C, prior to analysis with the alternative method.

Thirty exclusivity isolates were inoculated at approximately 10^5 CFU/mL into 225 mL Buffered Peptone Water (BPW) (ISO) and incubated for 24 hours at 37±1°C, prior to analysis with the alternative method.

Inclusivity & exclusivity results

The alternative method successfully identified all 50 inclusivity isolates for the presence of *Listeria monocytogenes* and correctly gave negative results for the 30 exclusivity isolates. 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 an isolate of *Listeria monocytogenes* and sent to all participating laboratories. Samples were analyzed following both the alternative method and the ISO reference method. Of all the samples tested, one third were unspiked, one third were spiked with a low-level inoculum (2 CFU/25 g) and the remaining third were spiked with a high level inoculum (24 CFU/25 g).

The relative sensitivity, specificity and accuracy of the interlaboratory study are listed in Table 1, which show the alternative method has high accuracy, sensitivity and specificity.

Table 1: Inter-laboratory study results.

Relative accuracy	96.7%
Relative sensitivity	96.9%
Relative specificity	100.0%

Method comparison study

As part of the previous validation studies a total of 393 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 the recently completed extension study was to extend the 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 387 samples (including meat products, milk and dairy products, seafood and fishery product, vegetables, composite food and production environmental samples categories) were analyzed using the alternative method with the Applied Biosystems QuantStudio 5 Food System.

A total of 67 samples from the composite food category were analyzed using the alternative method with the Applied Biosystems 7500 Fast Food Safety System.

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 analyzed composite food category are listed in Table 3.

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
1	Composite foods (multi-component foods)	13	36	11	6	1	0	-4	3
2	Meat products	17	41	7	6	1	0	0	3
3	Milk & dairy products	17	33	4	5	0	1	/	/
4	Seafood and fishery products	12	26	3	3	0	0	/	/
5	Vegetables	15	27	7	5	0	2	/	/
6	Production environmental samples	23	54	5	5	1	0	1	3
All catagories		**109**	**264**	**43**	**33**	**7**	**4**	**-4**	**5**

PA = Positive agreement PD = Positive deviation PPNA = Positive presumptive negative agreement AL = Acceptability limit (as defined by ISO 16140-2:2016) NA = Negative agreement
ND = Negative deviation PPND = Positive presumptive negative deviation

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
1	Composite foods (multi-component foods)	13	36	11	6	1	0	-4	3
2	Meat products	17	45	7	7	1	1	1	3
3	Milk & dairy products	22	45	4	5	1	1	2	3
4	Seafood and fishery products	22	33	5	3	1	1	-1	3
5	Vegetables	20	39	9	5	1	1	-3	3
6	Production environmental samples	15	66	7	7	2	0	2	3
All catagories		**109**	**264**	**43**	**33**	**7**	**4**	**-3**	**6**

PA = Positive agreement PD = Positive deviation PPNA = Positive presumptive negative agreement AL = Acceptability limit (as defined by ISO 16140-2:2016) NA = Negative agreement
ND = Negative deviation PPND = Positive presumptive negative deviation

Method comparison negative discordant results

Forty negative deviations (ND+PPND) were recorded when using the Applied Biosystems 7500 Fast Food Safety System. The presence of *Listeria monocytogenes* was detected in three of these samples after applying a subculture of the 24 LEB into Fraser Broth prior to spiking. Thirty-three negative deviations were recorded when using the Applied Biosystems QuantStudio 5 Food Safety System. For one of these samples the confirmatory tests concluded the presence of *Listeria monocytogenes* in the enrichment broth. Two of the negative deviations later detected *Listeria monocytogenes* after a subculture of the enriched 24 LEB into Fraser Broth prior to streaking onto selective agar plates. The remaining negative discordant results are likely due to the unpaired study design and the related sampling heterogeneity. As *Listeria monocytogenes* 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.

Method comparison positive discordant results

Forty-three positive deviations were recorded when using the Applied Biosystems 7500 Fast Food Safety System and thirty-seven 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 were

confirmed as true positives using the confirmation method. The analysis of discordant results according to the EN ISO 16140-2:2016 is given in Table 3 for the Applied Biosystems QuantStudio 5 Food Safety System and Table 4 for the Applied Biosystems 7500 Fast Food Safety System.

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

Method comparison conclusion

As shown in Table 3 and 4, 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 and the ISO reference method show equivalent performance and that the alternative method was shown to be a reliable alternative to the ISO reference method for the detection of *Listeria monocytogenes* from the milk and dairy, seafood, vegetables, composite food and production environmental samples categories.

Relative level of detection (RLOD) study

For the relative level of detection (RLOD) study, six *Listeria monocytogenes* isolates were spiked into six matrices (deli salad, rillettes, raw milk, smoked salmon, ready-to-cook vegetables 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 insufficient lysates for the remaining two 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 monocytogenes*. After inoculation, samples were tested using the ISO reference method and the alternative method. As shown in Table 5 the relative level of detection study demonstrated that the alternative method gave an RLOD below the acceptability limit of ≤2.5 for an unpaired study when used with the Applied Biosystems QuantStudio 5 Food Safety System and the Applied Biosystems 7500 Fast Food Safety System.

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

	Category	Matrix	Inoculated strain	Storage conditions before analysis	Applied Biosystems Food Safety System
1	Composite food[a]	Deli salad	*Listeria monocytogenes* Ad494	3±2°C for 48 h	7500 Fast QuantStudio 5
2	Meat products	Rillettes	*Listeria monocytogenes* Ad669	5±3°C for 48 h	7500 Fast[b]
3	Milk & dairy products	Raw milk	*Listeria monocytogenes* Ad153	5±3°C for 48 h	7500 Fast[b]
4	Seafood and fishery products	Smoked salmon	*Listeria monocytogenes* Ad670	5±3°C for 48 h	7500 Fast QuantStudio 5
5	Vegetables	Ready-to-cook vegetables	*Listeria monocytogenes* Ad279	5±3°C for 48 h	7500 Fast QuantStudio 5
6	Production environmental samples[a]	Process water	*Listeria monocytogenes* Ad551	3±2°C for 48 h	7500 Fast QuantStudio 5

[a]Categories analyzed in current extension study

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

The RLOD for each Food Safety System were calculated 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.

Table 5: RLOD results.

Matrix	Strain	QuantStudio 5 RLOD	7500 Fast RLOD	Acceptability limit (≤)
Deli salad	*Listeria monocytogenes* Ad494	2.000	2.00	2.5
Rillettes	*Listeria monocytogenes* Ad669	–	1.075	
Raw Milk	*Listeria monocytogenes* Ad153	–	0.224	
Smoked salmon	*Listeria monocytogenes* Ad670	1.072	0.761	
Ready-to-cook vegtables	*Listeria monocytogenes* Ad279	1.530	0.968	
Process water	*Listeria monocytogenes* Ad551	0.874	0.731	
	Combined RLOD	**1.295**	**0.851**	

Conclusion

The NF VALIDATION studies demonstrate that the SureTect Listeria monocytogenes PCR Assay workflow is equivalent in performance to the ISO reference method detailed in ISO 22964:2017 for the detection of *Listeria monocytogenes* from meat, dairy, seafood, vegetable and production environment samples when using the Applied Biosystems 7500 Fast or the Applied Biosystems QuantStudio 5 Food Safety Systems. The NF VALIDATION certificate and a summary of the validation report for this study are available from www.thermofisher.com/foodsafety.

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Appendix 1: Workflow for alternative method—SureTect Listeria monocytogenes PCR Assay for use with food, meat and environmental samples on the Applied Biosystems QuantStudio 5 Food Safety System and the Applied Biosystems 7500 Fast Food Safety System.

Day 0

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

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

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

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, 95±1°C for 5 minutes, and room temperature for 2 minutes before transferring 20 µL 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 Oxoid™ Microbact™ Listeria 12L Kit.

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

Day 0

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

Incubate at 30±1°C for 25±1 hours

Day 1

Add 100 µL of primary enrichment to 10 mL of Fraser Broth.

Incubate at 37±1°C for 24±2 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 2

Plate 10 µL of enrichment broth onto *Brilliance* Listeria Agar and Palcam Agar.

Incubate at 37±1°C for 24±3 hours. If small colonies or negative, reincubate a further 24 hours

Day 2, 3 or 4

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