Study Report

SureTect Listeria monocytogenes PCR Assay Workflow NF VALIDATION ISO 16140: Inclusivity and Exclusivity

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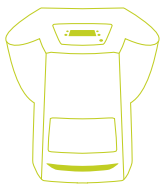

SureTect™ PikoReal™ Real-Time PCR Instrument

Summary

As part of the NF VALIDATION™ ISO 16140 of the Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay workflow (alternative method), an inclusivity and exclusivity study was conducted by ADRIA Développement, Quimper, France. The following is a summary of that study.

Methodology

Choice of strains: Fifty inclusivity isolates of *Listeria monocytogenes* and thirty exclusivity (non-*L. monocytogenes*) isolates were analyzed as part of the NF VALIDATION certification from AFNOR Certification ISO 16140 inclusivity and exclusivity study.

Culture enrichment: Inclusivity isolates were inoculated into 225 ml of Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) at between 1 and 28 CFU, before incubating for 18 to 24 hours at 37±1 °C, prior to analyzing with the alternative method.

Each exclusivity isolate was 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 analyzing with the SureTect Assay protocol.

Method: 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.

Results: Inclusivity and exclusivity results are summarized in tables one and two respectively. All fifty inclusivity isolates tested returned positive results and all of the thirty exclusivity isolates gave negative results with the alternative method.

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Table 1: Inclusivity results for the alternative method

Isolate	ID	Result	Isolate	ID	Result
L. monocytogenes	1011/1410	Positive	*L. monocytogenes*	Ad494	Positive
L. monocytogenes	153	Positive	*L. monocytogenes*	Ad534	Positive
L. monocytogenes	1972/2399	Positive	*L. monocytogenes*	Ad544	Positive
L. monocytogenes	1973/2400	Positive	*L. monocytogenes*	Ad546	Positive
L. monocytogenes	2760/3145	Positive	*L. monocytogenes*	Ad548	Positive
L. monocytogenes	32.183	Positive	*L. monocytogenes*	Ad551	Positive
L. monocytogenes	38/181	Positive	*L. monocytogenes*	Ad618	Positive
L. monocytogenes	5721/6179	Positive	*L. monocytogenes*	Ad623	Positive
L. monocytogenes	7111/7516	Positive	*L. monocytogenes*	Ad625	Positive
L. monocytogenes	850/109	Positive	*L. monocytogenes*	Ad626	Positive
L. monocytogenes	877/133	Positive	*L. monocytogenes*	Ad630	Positive
L. monocytogenes	913/048	Positive	*L. monocytogenes*	Ad665	Positive
L. monocytogenes	A00C022	Positive	*L. monocytogenes*	A00M009	Positive
L. monocytogenes	A00C036	Positive	*L. monocytogenes*	A00M032	Positive
L. monocytogenes	A00C039	Positive	*L. monocytogenes*	Ad235	Positive
L. monocytogenes	A00C040	Positive	*L. monocytogenes*	Ad249	Positive
L. monocytogenes	A00C041	Positive	*L. monocytogenes*	Ad253	Positive
L. monocytogenes	A00C042	Positive	*L. monocytogenes*	Ad260	Positive
L. monocytogenes	A00C043	Positive	*L. monocytogenes*	Ad265	Positive
L. monocytogenes	A00C044	Positive	*L. monocytogenes*	Ad266	Positive
L. monocytogenes	A00C052	Positive	*L. monocytogenes*	Ad267	Positive
L. monocytogenes	A00C053	Positive	*L. monocytogenes*	Ad268	Positive
L. monocytogenes	A00E082	Positive	*L. monocytogenes*	Ad270	Positive
L. monocytogenes	A00L097	Positive	*L. monocytogenes*	Ad273	Positive
L. monocytogenes	A00M009	Positive	*L. monocytogenes*	Ad274	Positive
L. monocytogenes	A00M032	Positive	*L. monocytogenes*	Ad285	Positive

Table 2: Exclusivity results for the alternative method

Isolate	ID	Result	Isolate	ID	Result
Bacillus cereus	Ad465	Negative	*Lactococcus lactis cremoris*	Ad136	Negative
Bacillus coagulans	Ad731	Negative	*Leuconostoc camosum*	Ad411	Negative
Bacillus licheniformis	Ad978	Negative	*Listeria grayi*	CIP76124	Negative
Bacillus mycoides	Ad762	Negative	*Listeria grayi*	ATCC® 19120™	Negative
Bacillus pseudomycoides	Ad765	Negative	*Listeria innocua*	Ad658	Negative
Bacillus pumilus	Ad284	Negative	*Listeria innocua*	Ad655	Negative
Bacillus weihenstephanensis	Ad726	Negative	*Listeria innocua*	Ad660	Negative
Brochothrix thermosphacta	EN15129	Negative	*Listeria ivanovii*	Ad466	Negative
Brochotrix compessis	CIP1029205	Negative	*Listeria ivanovii*	L2-11	Negative
Carnobacterium piscicola	Ad369	Negative	*Listeria ivanovii*	L2-9	Negative
Enterococcus durans	Ad149	Negative	*Listeria seeligeri*	BR18	Negative
Enterococcus faecalis	89L326	Negative	*Listeria seeligeri*	Ad674	Negative
Lactobacillus curvatus	Ad380	Negative	*Listeria welshimeri*	191424	Negative
Lactobacillus fermentum	Ad482	Negative	*Listeria welshimeri*	Ad650	Negative
Lactobacillus sakei	Ad473	Negative	*Staphylococcus aureus*	Ad165	Negative

Conclusions

The inclusivity and exclusivity study conducted as part of this NF VALIDATION study demonstrated that the SureTect Listeria monocytogenes PCR Assay workflow showed 100% inclusivity and 100% exclusivity with all of the isolates analyzed. 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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