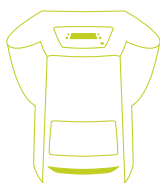


# SureTect *Listeria monocytogenes* PCR Assay Workflow

## 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<sup>5</sup> 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.

Table 1: Inclusivity results for the alternative method

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

Table 2: Exclusivity results for the alternative method

Isolate	ID	Result	Isolate	ID	Result
<i>Bacillus cereus</i>	Ad465	Negative	<i>Lactococcus lactis cremoris</i>	Ad136	Negative
<i>Bacillus coagulans</i>	Ad731	Negative	<i>Leuconostoc carnosum</i>	Ad411	Negative
<i>Bacillus licheniformis</i>	Ad978	Negative	<i>Listeria grayi</i>	CIP76124	Negative
<i>Bacillus mycoides</i>	Ad762	Negative	<i>Listeria grayi</i>	ATCC® 19120™	Negative
<i>Bacillus pseudomycooides</i>	Ad765	Negative	<i>Listeria innocua</i>	Ad658	Negative
<i>Bacillus pumilus</i>	Ad284	Negative	<i>Listeria innocua</i>	Ad655	Negative
<i>Bacillus weihenstephanensis</i>	Ad726	Negative	<i>Listeria innocua</i>	Ad660	Negative
<i>Brochothrix thermosphacta</i>	EN15129	Negative	<i>Listeria ivanovii</i>	Ad466	Negative
<i>Brochotrix compessis</i>	CIP1029205	Negative	<i>Listeria ivanovii</i>	L2-11	Negative
<i>Camobacterium piscicola</i>	Ad369	Negative	<i>Listeria ivanovii</i>	L2-9	Negative
<i>Enterococcus durans</i>	Ad149	Negative	<i>Listeria seeligeri</i>	BR18	Negative
<i>Enterococcus faecalis</i>	89L326	Negative	<i>Listeria seeligeri</i>	Ad674	Negative
<i>Lactobacillus curvatus</i>	Ad380	Negative	<i>Listeria welshimeri</i>	191424	Negative
<i>Lactobacillus fermentum</i>	Ad482	Negative	<i>Listeria welshimeri</i>	Ad650	Negative
<i>Lactobacillus sakei</i>	Ad473	Negative	<i>Staphylococcus aureus</i>	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](http://www.thermofisher.com/SureTect)

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993-195  
LT2219A  
January 2016

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