



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.

**071304**

The AOAC Research Institute hereby certifies the method known as:

### **Thermo Scientific™ SureTect™ Listeria species PCR Assay**

manufactured by

**Oxoid Ltd., part of Thermo Fisher Scientific**

**Wade Road**

**Basingstoke, RG24 8PW, Hampshire, UK**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*<sup>SM</sup> Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date

August 06, 2025

Expiration Date

December 31, 2025

**METHOD NAME**Thermo Scientific™ SureTect™ *Listeria* species PCR Assay**CATALOG NUMBER**

A56842

**ORIGINAL CERTIFICATION DATE**

July 25, 2013

**PRINCIPLE OF THE METHOD**

The SureTect™ *Listeria* species PCR Assay enables real-time PCR detection of *Listeria* species from food and environmental samples. These PCR kits are for use in laboratories undertaking microbiological analysis. The SureTect *Listeria* species PCR Assay is based upon Solaris™ reagents for performing PCR. Dye-labeled probes target unique DNA sequences specific to *Listeria* species, and an internal positive control (IPC). Target DNA, if present, is detected by real-time PCR. Analysis software provides interpretation of results. The assay includes an IPC for each reaction to confirm that the PCR process has occurred. It is unnecessary to incorporate positive control organisms with routine testing of samples.

**CERTIFIED CLAIM STATEMENT:** The SureTect™ *Listeria* species PCR method is certified for the detection of *Listeria* species within the scope of Tables 1 and 2 and with the modifications indicated in Table 3.

**Certified method includes:**

1. CyBio™ FeliX Instrument for SureTect™ PCR Automation Workflow (for lysis and PCR setup)
2. Applied Biosystems™ SimpliAmp™ Thermal Cycler (for lysis)
3. Applied Biosystems™ QuantStudio™ 5 Thermal Cycler, 0.1 mL block, with Thermo Scientific™ RapidFinder™ Analysis Software v3.0
4. Applied Biosystems™ 7500 Fast Thermal Cycler with Applied Biosystems™ RapidFinder™ Express Software v2.0
5. Alternative confirmation protocol of plating of the enrichment broth onto Thermo Scientific™ Oxoid™ *Brilliance*™ *Listeria* Agar (ISO) and confirming presumptive positive colonies with the Thermo Scientific™ Oxoid™ Microbact™ *Listeria* 12L Kit.

**Table 1. Method Performance Claims**

Matrix	Test Portion	Enrichment Conditions					Reference Method <sup>c</sup>	Claim <sup>d</sup>
		Broth <sup>a</sup>	24 LEB Volume	24 LEB Buffer <sup>b</sup> Volume	Temperature	Time		
Raw ground beef (80% lean)	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Raw pork frankfurters	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Raw ground pork	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Pork rillettes	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	20–28 h	ISO 11290-1:2017	SDD-C
Raw pork sausage	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Raw ground turkey	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Salami	25 g	24 LEB	475 mL	20 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	SDD-C
Cooked sliced ham	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Cooked sliced turkey	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD

Ready-to-eat poultry and pork-based meat pie	125 g	pw 24 LEB	1,125 mL	50 mL	37 ± 1°C	20–28 h	MLG 8.15	NSDD
Deli salad	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	20–28 h	ISO 11290-1:2017	NSDD
Fresh bagged spinach	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Bagged lettuce	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	SDD-C
Ready-to-cook vegetables	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	20–28 h	ISO 11290-1:2017	NSDD
Cut cantaloupe	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Processed cheese	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Pasteurized brie cheese	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	SDD-C
Cottage cheese (4% fat)	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	BAM Ch. 10 (2019)	NSDD
Blue cheese	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	BAM Ch. 10 (2019)	NSDD
Pasteurized 2% fat milk	25 mL	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Raw milk	25 mL	24 LEB	225 mL	10 mL	37 ± 1°C	20–28 h	ISO 11290-1:2017	NSDD
	125 mL	pw 24 LEB	1,125 mL	50 mL	37 ± 1°C	20–28 h	BAM Ch. 10 (2022)	NSDD
Vanilla ice cream	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Greek yogurt	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	BAM Ch. 10 (2019)	NSDD
	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:2017	NSDD
Smoked salmon	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	20–28 h	ISO 11290-1:2017	NSDD
Cooked prawns (heads off)	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Raw cod	25 g	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Stainless steel	4" x 4", sponge <sup>e</sup>	24 LEB	100 mL	4.4 mL	37 ± 1°C	22–30 h	BAM Ch. 10 (2019)	NSDD
	1" x 1", swab <sup>e</sup>	24 LEB	10 mL	0.44 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
Plastic	4" x 4", sponge <sup>e</sup>	24 LEB	225 mL	10 mL	37 ± 1°C	22–30 h	ISO 11290-1:1996	NSDD
	1" x 1", swab <sup>e</sup>	24 LEB	10 mL	0.44 mL	37 ± 1°C	22–30 h	BAM Ch. 10 (2019)	NSDD
Ceramic tile	4" x 4", sponge <sup>e</sup>	24 LEB	100 mL	4.4 mL	37 ± 1°C	22–30 h	BAM Ch. 10 (2019)	SDD-C
Concrete	4" x 4", sponge <sup>e</sup>	24 LEB	100 mL	4.4 mL	37 ± 1°C	22–30 h	BAM Ch. 10 (2019)	NSDD
Process water	25 mL	24 LEB	225 mL	10 mL	37 ± 1°C	20–28 h	ISO 11290-1:2017	NSDD

<sup>a</sup> pw = prewarmed to 37 ± 1°C, all other matrixes the media should be at 23 ± 5°C; 24 LEB = Oxoid 24 Listeria Enrichment Broth + Oxoid 24 Listeria Enrichment Broth Selective Supplement.

<sup>b</sup> 24 LEB Buffer = Oxoid 24 Listeria Enrichment Broth Buffer Supplement.

<sup>c</sup> BAM = Bacteriological Analysis Manual; ISO = International Organization for Standardization.

<sup>d</sup> NSDD = No statistical difference detected using SLV study design from OMA Appendix J (2012). The SLV qualitative method comparison study design from OMA Appendix J (2012) is not intended to demonstrate statistical equivalence in unpaired studies. Expert opinion is that the method is appropriate for its intended use. SDD-C = Statistical difference detected with a positive bias for the Candidate method.

<sup>e</sup> Swabs and sponges premoistened in Dey-Engley broth.

**Table 2. Method Selectivity**

Broth <sup>a</sup>	Temperature	Inclusivity Strains		Exclusivity Strains	
		No. Tested	No. Positive	No. Tested	No. Positive
24 LEB + LEB Buffer	37 ± 1°C	68 <sup>b</sup>	68 <sup>c</sup>	33 <sup>d</sup>	0 <sup>c</sup>

<sup>a</sup> 24 LEB = Oxoid 24 Listeria Enrichment Broth + Oxoid 24 Listeria Enrichment Broth Selective Supplement.

<sup>b</sup> Comprised of *L. monocytogenes*, *L. grayi*, *L. innocua*, *L. ivanovii*, *L. welshimeri*, and *L. seeligeri*.

<sup>c</sup> Results were identical when evaluating the QuantStudio 5 and the 7500 Fast.

<sup>d</sup> Comprised of 33 non-*Listeria* species.

**Table 3. Method History**

No.	Date	Summary	Supporting Data
1	June 2013	Original certification: Included smoked salmon, processed cheese, fresh bagged spinach, cut cantaloupe, cooked prawns (head off), cooked sliced turkey meat, vanilla ice cream, pork frankfurters, salami, ground raw beef meat (12% fat), plastic and stainless steel.	Certification Report
2	September 2015	Level 3 Modification: Addition of raw ground turkey, raw ground pork, bagged lettuce, raw pork sausages, pasteurized 2% fat milk, raw cod, pasteurized brie cheese and ice cream.	Modification 1 Report
3	November 2015	Level 2 Modification: Validation of Applied Biosystems 7500 Fast instrument.	Modification 2 Report
4	April 2018	Level 2 Modification: Evaluation of workflow and lyophilization.	Modification 3 Report
5	October 2018	Level 2 Modification: Validation of Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument and addition of stainless steel (100 mL, sponge) and stainless steel (10 mL, swab).	
6	October 2020	Level 2 Modification: Thermo Scientific™ RapidFinder™ Analysis software v1.1 software upgrade for use with the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument.	Modification 4 Report
7	October 2020	Level 2 Modification: Applied Biosystems™ RapidFinder™ Express software v2.0 software upgrade for use with the Applied Biosystems™ 7500 Fast™ Real-Time PCR Instrument.	Modification 5 Report
8	January 2021	Level 2 Modification: Replacement of incubators with SimpliAmp in SureTect workflow.	Modification 6 Report
9	February 2022	Level 2 Modification: Addition of cottage cheese (4% fat), blue cheese, Greek yogurt, plastic (1"x1", swab), stainless steel (4"x4", sponge), ceramic (4"x4", sponge) and concrete (4"x4", sponge).	Modification 7 Report
10	July 2022	Level 2 Modification: Workflow improvements including new handling tools, blue dye relocation, pierceable lysis seal and improved plastics.	Modification 8 Report
11	June 2023	Level 2 Modification: Addition of deli salad, pork rillettes, raw milk, ready-to-cook vegetables, and process water and evaluation of New Brilliance Listeria Agar (ISO) for SureTect confirmation protocol.	Modification 9 Report
12	January 2024	Level 2 Modification: Extend the scope of the methods to include an automated lysis and PCR setup procedure.	Modification 10 Report
13	June 2025	Level 2 Modification: Addition of Ready-to-eat poultry and pork-based meat pie (125 g) and raw milk (125 g).	Modification 11 Report