



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.

062501

The AOAC Research Institute hereby certifies the method known as

SureFast® Listeria 3plex ONE

manufactured by

CONGEN Biotechnologie GmbH

Robert Roessle Str. 10

D-13125 Berlin, Germany

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM 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*SM 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

November 28, 2025

Expiration Date

December 31, 2026

METHOD NAME

SureFast® *Listeria* 3plex ONE

CATALOG NUMBER

F5217

ORIGINAL CERTIFICATION DATE

June 05, 2025

PRINCIPLE OF THE METHOD

The SureFast® *Listeria* 3plex ONE Kit detects and differentiates *Listeria* spp. and *L. monocytogenes* in a variety of food and one environmental surface, stainless steel. The method consists of four steps: cultural enrichment, DNA-extraction, specific real-time PCR detection, and interpretation of results. A second DNA extraction kit, SureFast® PREP Bacteria Kit, can also be used with SureFast *Listeria* 3plex ONE Kit to provide an alternative option for DNA extraction.

The SureFast *Listeria* 3plex ONE Kit real-time PCR assay can be performed with commonly used real-time PCR instruments, equipped for detection of four fluorescence emissions in the channels FAM, VIC/HEX, ROX and Cy5 at the same time. Each PCR reaction contains an internal amplification control (IAC). If the DNA extract contains PCR-inhibiting substances, the signal of the amplification control can be affected, or the amplification will be suppressed. Examples for PCR-inhibiting substances are alcohols (e.g., ethanol, isopropanol), surfactants (e.g., cetrimonium bromide, SDS, Triton X-100) and salts (e.g., sodium chloride). In addition, spices, herbs, algae, cocoa and other sample matrices can have PCR-inhibiting effects.

CERTIFIED CLAIM STATEMENT: The SureFast® *Listeria* 3plex ONE method is certified for the detection of *L. grayi*, *L. fleischmannii* subsp. *fleischmannii*, *L. innocua*, *L. ivanovii*, *L. marthii*, *L. rocourtiae*, *L. seeligeri*, *L. welshimeri*, and *L. weihenstephanensis* and *L. monocytogenes* within the scope of Tables 1 and 2.

Certified method includes:

1. Bio-Rad CFX96 Deep Well DX, Bio-Rad Opus Deepwell and R-Biopharm RIDA®CYCLER.
2. SureFast® *Listeria* 3plex ONE Lysis and SureFast® PREP Bacteria Lysis.

Table 1. Method Performance Claims: *Listeria* species

Matrix	Test Portion	Enrichment Conditions				Reference Method ^d	Claim ^e
		Broth ^a	Volume	Temperature	Time		
Frankfurter sausage	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Deli ham	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Smoked deli turkey	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Smoked salmon	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Frozen cooked shrimp	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Liquid whole egg	25 mL	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Raw milk ^b	25 mL	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Pasteurized milk ^b	25 mL	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Vanilla ice cream ^b	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Gorgonzola cheese ^b	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD

Gouda cheese ^b	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Brie cheese ^b	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Bagged salad	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Canned Enoki mushrooms	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Ground soy vegan meat substitute	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Guacamole	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Stainless steel	1" x 1", swab ^c	pw Half Fraser	9 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Stainless steel	4" x 4", sponge ^c	pw Half Fraser	90 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Process water	25 mL	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD

^a Half Fraser prewarmed.

^b Dairy products require a secondary enrichment (0.1 mL Enrichment add into 10 mL of Fraser broth, incubate at 37 °C for 24 ± 2 h).

^c Swabs and sponges premoistened in 1 mL and 10 mL Hi-Cap Neutralizing Broth, respectively.

^d ISO = International Organization for Standardization.

^e 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. Expert opinion is that the method is appropriate for its intended use.

Table 2. Method Performance Claims: *Listeria monocytogenes*

Matrix	Test Portion	Enrichment Conditions				Reference Method ^d	Claim ^e
		Broth ^a	Volume	Temperature	Time		
Frankfurter sausage	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Deli ham	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Smoked deli turkey	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Frozen cooked shrimp	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Liquid whole egg	25 mL	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Raw milk ^b	25 mL	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Pasteurized milk ^b	25 mL	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Gouda cheese ^b	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Brie cheese ^b	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Bagged salad	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Canned Enoki mushrooms	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Ground soy vegan meat substitute	25 g	pw Half Fraser	225 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Stainless steel	1" x 1", swab ^c	pw Half Fraser	9 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD
Stainless steel	4" x 4", sponge ^c	pw Half Fraser	90 mL	37 ± 1°C	18-20 h	ISO 11290-1:2017	NSDD

^a Half Fraser prewarmed.

^b Dairy products require a secondary enrichment (0.1 mL Enrichment add into 10 mL of Fraser broth, incubate at 37 °C for 24 ± 2 h).

^c Swabs and sponges premoistened in 1 mL and 10 mL Hi-Cap Neutralizing Broth, respectively.

^d ISO = International Organization for Standardization.

^e 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. Expert opinion is that the method is appropriate for its intended use.

Table 3. Method Selectivity

Target	Lysis Method	Broth	Temperature	Inclusivity Strains		Exclusivity Strains	
				No. Tested	No. Positive	No. Tested	No. Positive
<i>Listeria</i> spp.	SureFast® Listeria 3plex ONE	Half Fraser	37 ± 1°C	106 ^a	100 ^b	30 ^c	0
<i>Listeria</i> spp.	SureFast® PREP Bacteria	Half Fraser	37 ± 1°C	106	101 ^d	30	0
<i>L. monocytogenes</i>	SureFast® Listeria 3plex ONE	Half Fraser	37 ± 1°C	51 ^e	51	30	0
<i>L. monocytogenes</i>	SureFast® PREP Bacteria	Half Fraser	37 ± 1°C	51	51	30	0

^a Comprising 10 strains *L. innocua*, 6 strains *L. grayi*, 8 strains *L. ivanovii*, 9 strains *L. seeligeri*, 5 strains *L. welshimeri*, 51 strains *L. monocytogenes* and 17 additional *Listeria* spp.

^b Strains not detected: *L. grandensis*, *L. floridensis*, *L. rocourtiae*, *L. cornellensis*, *L. aquatica*, and *L. portnoyi*.

^c Comprising 28 species.

^d Strains not detected: *L. grandensis*, *L. floridensis*, *L. cornellensis*, *L. aquatica*, and *L. portnoyi*.

^e Comprising 51 strains of *Listeria monocytogenes* including 2 strains ser. 1, 7 strains ser. 1/2a, 5 strains ser. 1/2b, 3 strains ser. 1/2c, 1 strain ser. 2, 2 strains ser. 3, 1 strains ser. 3a, 2 strains ser. 3b, 1 strain ser. 3c, 2 strain ser. 4a, 10 strains ser. 4b, 2 strains ser. 4c, 1 strain ser. 4d, 1 strain ser. 4e, 1 strain ser. 7.

Table 4. Method History

No.	Date	Summary	Supporting Data
1	May 2025	Original Certification	Certification Report