



CERTIFICATION

AOAC Research Institute
***Performance Tested Methods*SM**

Certificate No.
051303

The AOAC Research Institute hereby certifies the method known as:

Thermo Scientific™ SureTect™ Salmonella species PCR Assay

manufactured by

Oxoid Ltd. part of Thermo Fisher Scientific
Wade Road
Basingstoke
Hampshire, RG248PW

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, Senior Director
Signature for AOAC Research Institute

Issue Date
Expiration Date

October 04, 2024
December 31, 2025

METHOD NAME

Thermo Scientific™ SureTect™ Salmonella species PCR Assay

CATALOG NUMBER

A56841

ORIGINAL CERTIFICATION DATE

May 20, 2013

PRINCIPLE OF THE METHOD

The SureTect™ Salmonella species PCR Assay is a real-time PCR test intended to be used with the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument with associated RapidFinder™ Analysis Software (version 3.0 or greater), and the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument with associated RapidFinder™ Express Software (version 2.0 or greater) for the detection of *Salmonella* spp. in human foods, pet food and select environmental surfaces.

This assay is based upon use of Solaris™ reagents for performing PCR. Dye-labeled probes target unique DNA sequences specific to *Salmonella* spp. and an internal positive control (IPC). Target DNA, if present, is detected by real-time PCR. Analysis software provides interpretation of results. The IPC template, primers, and probe provide an internal control with each reaction to show that the PCR process has occurred. It is unnecessary to incorporate positive control organisms with routine testing of samples. Enriched food or environmental samples are combined directly with ready-to-use Lysis Reagent 1 and Proteinase K, to lyse bacterial cells present in the sample and release their DNA into solution.

Lysates are transferred to the SureTect Salmonella species PCR Tubes to rehydrate the lyophilized PCR pellets. The pellets contain lyophilized target specific primers, dye-labelled probes, and PCR master mix components. The PCR tubes are sealed, loaded into the real-time PCR instrument, then the run is started using the RapidFinder software. After the run is complete, the software displays the interpreted results as simple positive or negative symbols. The results can be reported, stored, printed, and downloaded as required. Results are achieved approximately 80 minutes after loading the prepared sample into the instrument.

CLAIM STATEMENT: The Thermo Scientific™ SureTect™ Salmonella species PCR Assay method is certified for the detection of *Salmonella* within the scope of Tables 1 and 2.

Certification includes:

1. Applied Biosystems™ QuantStudio™ 5 Real-Time PCR with Thermo Scientific™ RapidFinder™ Analysis software version 3.0 or greater.
2. Applied Biosystems™ 7500 Fast Real-Time PCR with Applied Biosystems™ RapidFinder™ Express software version 2.0 or greater.
3. Alternative confirmation procedure: Direct streak from the primary enrichment onto Thermo Scientific *Brilliance* Salmonella Agar (BSA), or XLD Agar. Secondary enrichment and selective plating can be performed if high background present. Confirm colonies using the Thermo Scientific Oxoid Salmonella Test Kit (latex test) or Thermo Scientific Microbact GNB 24E Kit (biochemical gallery).

Table 1. Method Performance Claims

Matrix	Test portion	Enrichment Conditions				Reference method ^b	Claim ^c
		Broth ^a	Volume	Time	Temperature		
Pasteurized 2% milk	25 mL	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002 ^d	Eq
Non-Fat Dried Milk Powder	25 g	BPW	225 mL	18–26 h	34–38°C	ISO 6579:2002	Eq
Ice cream (vanilla)	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
Grated cheddar cheese	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579-1:2017 ^e	Eq
						BAM Ch. 5 (2019)	NSDD

Single cream (8% fat)	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579-1:2017 BAM Ch. 5 (2019)	Eq NSDD
Feta cheese	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579-1:2017 BAM Ch. 5 (2019)	Eq NSDD
Raw ground beef (80% lean)	25 g	pre- w BPW	225 mL	8–24 h	41.5 ± 1°C	ISO 6579:2002	NSDD
	375 g	pre-w mTSB	1500 mL	9–24 h	41.5 ± 1°C	ISO 6579:2002	NSDD
	375 g	pre-w BPW	1500 mL	8–24 h	42 ± 1°C	ISO 6579-1:2017	NSDD
Raw beef trim	375 g	pre-w mTSB	1500 mL	8–24 h	41.5 ± 1°C	MLG 4.10	NSDD
Raw ground pork	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
Pork frankfurters	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
Raw chicken breast	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
	375 g	BPW + 12 mg/L novo	1875 mL	20–28 h	42 ± 1°C	MLG 4.14	NSDD
	325 g	BPW	1875 mL	20–24 h	35 ± 1°C	MLG 4.14	NSDD
Chicken carcass rinse	30 mL	pre-w BPW + 12 mg/L novo	30 mL	16–24 h	42 ± 1°C	MLG 4.14	NSDD
	30 mL	pre-w BPW + 20 mg/L novo	30 mL	20–28 h	42 ± 1°C	MLG 4.14	NSDD
	30 mL	BPW	30 mL	20–24 h	35 ± 1°C	MLG 4.14	NSDD
	30 mL	BPW	30 mL	20–24 h	35 ± 1°C	MLG 4.14	NSDD
Pasteurized liquid whole egg	25 g	BPW	225 mL	18–26 h	34–38°C	ISO 6579:2002	Eq
Cooked shrimp (heads off)	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
Bagged lettuce	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
Mung bean sprouts	25 g	BPW+20 mg/L novo	225 mL	20–28 h	41.5 ± 1°C	ISO 6579:2002	NSDD
Cut cantaloupe	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
Cut mango	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579-1:2017	Eq
Cut mango						BAM Ch. 5 (2019)	Eq
Cut cabbage	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579-1:2017 BAM Ch. 5 (2019)	Eq NSDD
Raw spinach	375 g	pre-w BPW	3375 mL	10–24 h	41.5 ± 1°C	ISO 6579-1:2017	NSDD
Fresh bagged spinach	375 g	pre-w BPW	3375 mL	8–24 h	41.5 ± 1°C	BAM Ch. 5 (2019)	NSDD
Whole black peppercorns	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
Peanut butter	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
PIF with probiotics	375 g	pre-w BPW + 6 mg/L novo	1875 mL	18–26 h	34–38°C	ISO 6579-1:2017	NSDD
PIF without probiotics	375 g	pre-w BPW	1875 mL	18–26 h	34–38°C	ISO 6579-1:2017	NSDD
Dark chocolate (85% cocoa solids)	25 g	CSR	225 mL	20–28 h	34–38°C	ISO 6579:2002	NSDD
Cocoa powder	375 g	pre-w BPW	3375 mL	22–30 h	34–38°C	ISO 6579-1:2017	NSDD
		pre-w BPW				BAM Ch. 5 (2019)	Eq
		pre-w NFDM				ISO 6579-1:2017	Eq
		pre-w NFDM				BAM Ch. 5 (2019)	NSDD
Cocoa liquor	375 g	pre-w BPW	3375 mL	22–30 h	34–38°C	ISO 6579-1:2017	NSDD
		pre-w BPW				BAM Ch. 5 (2019)	NSDD
		pre-w NFDM				ISO 6579-1:2017	Eq
		pre-w NFDM				BAM Ch. 5 (2019)	NSDD
Cocoa butter	375 g	pre-w BPW	3375 mL	22–30 h	34–38°C	ISO 6579-1:2017	NSDD
		pre-w BPW				BAM Ch. 5 (2019)	NSDD

		pre-w NFD				ISO 6579-1:2017	Eq
		pre-w NFD				BAM Ch. 5 (2019)	NSDD
Dark chocolate (>70% cocoa solids)	375 g	pre-w BPW	3375 mL	22–30 h	34–38°C	ISO 6579-1:2017	NSDD
		pre-w BPW				BAM Ch. 5 (2019)	NSDD
		pre-w NFD				ISO 6579-1:2017	Eq
		pre-w NFD				BAM Ch. 5 (2019)	NSDD
Chilled ready-to-eat meal	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
Wet cat food	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
Dry dog food	25 g	BPW	225 mL	20–28 h	34–38°C	ISO 6579:2002	Eq
Cat liver pâté	375 g	pre-w BPW	1875 mL	20–28 h	34–38°C	ISO 6579-1:2017	NSDD
Cat kibble	150 g	BPW + 12 mg/L novo	1350 mL	20–28 h	34–38°C	ISO 6579-1:2017	NSDD
Stainless steel	4" x 4", sponge	BPW	100 mL	18–26 h	34–38°C	ISO 6579:2002	Eq
Plastic surface	1" x 1", swab	BPW	10 mL	18–26 h	34–38°C	ISO 6579:2002	Eq
Plastic surface	4" x 4", sponge	BPW	100 mL	18–26 h	34–38°C	ISO 6579:2002	Eq

^a BPW: Buffered peptone water (ISO formulation); pre-w mTSB = pre-warmed modified tryptone soya broth; pre-w BPW = pre-warmed BPW (ISO); BPW + 12 mg/L novo = BPW (ISO) with 12 mg/L novobiocin; pre-w BPW + 12 mg/L novo = pre-warmed BPW (ISO) with 12 mg/L novobiocin; pre-w BPW + 20 mg/L novo = pre-warmed BPW (ISO) with 20 mg/L novobiocin; BPW+20 mg/L novo = BPW (ISO) with 20 mg/L novobiocin; BPW + 6 mg/L novo = BPW (ISO) with 6 mg/L novobiocin; CSR = Cocoa sample recovery broth; NFD = Non-fat dried milk supplemented with 0.018% brilliant green dye solution.

^b ISO = International Organization for Standardization – Microbiology of the food chain; BAM = US Food and Drug Administration Bacteriological Analytical Manual; MLG = U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook.

^c 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 for unpaired studies. Expert opinion is that the method is appropriate for its intended use. For paired studies, Eq = Equivalence of candidate and reference method results demonstrated by 90% confidence interval on dPOD_c meeting the criteria according to TR364.

^d ISO 6579:2002 = ISO 6579:2002 including Technical Corrigendum 1:2004.

^e ISO 6579-1:2017 = ISO 6579-1:2017/Amd 1:2020.

^f Cocoa powder was also evaluated in an AOAC *Official Methods of Analysis*SM collaborative study comparing the BPW (ISO) enrichment to the BAM method.

Table 2. Method Selectivity

Enrichment		Inclusivity Strains		Exclusivity Species	
Broth ^a	Temp., °C	No. Tested	No. Positive	No. Tested	No. Positive
BPW	34–38°C	117 ^b	117	37 ^c	0
BPW+20 mg/L novo	41.5 ± 1°C	111 ^d	111	37 ^c	0

^a BPW = Buffered peptone water (ISO formulation); BPW+20 mg/L novo = BPW (ISO formulation) with 20 mg/L novobiocin

^b Comprised of 4 strains *S. bongori*, 3 strains *S. enterica* subsp. *arizonae*, 4 strains *S. enterica* subsp. *diarizonae*, 4 strains *S. enterica* subsp. *houtenae*, 3 strains *S. enterica* subsp. *indica*, 5 strains *S. enterica* subsp. *salamae*, and the remaining strains represent 94 serovars of *S. enterica* subsp. *enterica*.

^c 37 strains comprising 37 species. Exclusivity organisms were cultured under optimal conditions for growth.

^d Comprised of 4 strains *S. bongori*, 3 strains *S. enterica* subsp. *arizonae*, 3 strains *S. enterica* subsp. *diarizonae*, 3 strains *S. enterica* subsp. *houtenae*, 3 strains *S. enterica* subsp. *indica*, 3 strains *S. enterica* subsp. *salamae*, and the remaining strains represent 92 serovars of *S. enterica* subsp. *enterica*.

Table 3. Method History

No.	Date	Summary	Supporting Data
1	May 2013	Original certification.	Certification Report
2	August 2015	Level 3 Modification: Evaluation of a matrix extension.	Modification Report 1
3	February 2016	Level 2 Modification: Additional Applied Biosystems 750 Fast PCR instrument and change in target probe concentration.	Modification Report 2
4	April 2018	Level 2 Modification: Evaluation of workflow and lyophilization steps.	Modification Report 3
5	November 2018	Level 2 Modification: Evaluation of Applied Biosystems QuantStudio Real-Time PCR instrument with Applied Biosystems RapidFinder Analysis Software version 2.0.	Modification Report 4
6	October 2020	Level 2 Modification: Evaluation to upgrade software for Thermo Scientific RapidFinder Analysis PCR software to version 1.1 designed for use with the Applied Biosystems QuantStudio 5 Real-Time PCR.	Modification Report 5
7	October 2020	Level 2 Modification: Evaluation to upgrade the software for the Applied Biosystems RapidFinder Express to version 2.0 designed for use with the Applied Biosystems 7500 Fast Real-Time PCR.	Modification Report 6
8	January 2021	Level 2 Modification: Evaluation to use manual heat block or automated SimliAmp with Applied Biosystems 7500 Fast Real-Time PCR or Applied Biosystems QuantStudio 5 Real-Time PCR.	Modification Report 7
9	July 2022	Level 2 Modification: Evaluation of changes to improve handling steps and visual indicators.	Modification Report 8
10	January 2024	Level 2 Modification: Evaluation to include automated lysis procedure and PCR setup procedures.	Modification Report 9
11	July 2024	Level 2 Modification: Validation of a matrix extension.	Modification Report 10