CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

012102

The AOAC Research Institute hereby certifies the method known as:

Thermo Scientific™ SureTect™ Escherichia coli O157:H7 and STEC Screening PCR Assay
Thermo Scientific™ SureTect™ Escherichia coli STEC Identification 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.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date	November 21, 2024
Expiration Date	December 31, 2025

METHOD NAME
Thermo Scientific™ SureTect™ Escherichia coli O157:H7 and STEC Screening PCR Assay
Thermo Scientific™ SureTect™ Escherichia coli STEC Identification PCR Assay

CATALOG NUMBER
A56838, A56840

ORIGINAL CERTIFICATION DATE
January 11, 2021

PRINCIPLE OF THE METHOD

Thermo Scientific™ SureTect™ Escherichia coli O157:H7 and STEC Screening PCR Assay and Thermo Scientific™ SureTect™ Escherichia coli STEC Identification PCR Assay are real-time PCR assays designed as a complete workflow for the rapid detection of *Escherichia coli* O157:H7 and other stipulated STEC serotypes (O26, O45, O103, O111, O121, O145). The assay is designed for use with the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument and associated Applied Biosystems™ RapidFinder Analysis software (version 3.0 or higher).

The assay is supplied as a kit containing all necessary reagents to conduct the sample lysis, including prefilled lysis tubes and lyophilized PCR pellets, containing all necessary PCR reagents (target-specific primers, dye-labelled probes, and PCR master mix components) to easily conduct the PCR analysis of the sample. PCR probes are short oligonucleotides with a quencher molecule at one end that, when not bound to target DNA, greatly reduces fluorescence from the dye label at the opposite end of the probe molecule. The SureTect Escherichia coli O157:H7 and STEC Screening PCR Assay contains four different channels (*stx*, *eae*, O157:H7 target 1 and O157:H7 target 2) that utilize different fluorophores to identify the presence of a STEC isolate. Both the *stx* and *eae* channel must have a positive result for the overall well call to be presumptive positive for the presence of STEC. The O157:H7 target 1 and target 2 channels must be positive for an *E. coli* O157:H7 positive result, *stx* and *eae* channels may be positive or negative. The SureTect Escherichia coli STEC Identification PCR Assay should be run alongside the SureTect Escherichia coli O157:H7 and STEC Screening PCR Assay. The identification kit differentiates between the non-*E. coli* O157:H7 STEC serotypes via unique fluorophores contained in each of the five channels allowing for the presence of O26, O145, O103, O111, or O121/O45 (this is a single channel) to be characterized. Within the identification kit, the dye-labeled probes target unique DNA sequences specific to the *E. coli* STEC serotypes. If relevant *E. coli* STEC serotypes are present, the target DNA sequences will be amplified and the increasing fluorescent signal generated will be detected by the QuantStudio 5 Real-Time PCR instrument and interpreted by the RapidFinder Analysis software.

In addition to detection of any target DNA, the PCR pellets contain probes, primers, and DNA templates for an internal positive control (IPC). During PCR cycling, the IPC template is amplified regardless of if any target DNA is present or not. The probe used for the IPC is labelled with a different colored fluorescent dye to the probes used within the assay to detect target DNA, and so can be detected by the QuantStudio 5 Real-Time PCR instrument through a separate dye channel. If there is no presence of target DNA, the presence of the IPC amplification curve indicates that the PCR process has occurred successfully. A warning call will occur if the IPC is not amplified, which alerts the end user about a potential error which could have occurred during the lysis or PCR setup procedure.

The PCR probes used in the SureTect Escherichia coli O157:H7 and STEC Screening and SureTect Escherichia coli STEC Identification PCR Kits are based on TaqMan™ PCR technology. Results are achieved approximately 80 minutes after loading the prepared sample into the PCR instrument and are displayed via the RapidFinder Analysis software on the attached computer screen as simple positive or negative symbols with an attached PCR amplification plot that is easily accessible for review. All results interpreted by the software can be reported, stored, printed, and downloaded as required, by the user.

CERTIFIED CLAIM STATEMENT: The Thermo Scientific™ SureTect™ Escherichia coli O157:H7 and STEC Screening PCR Assay and Thermo Scientific™ SureTect™ Escherichia coli STEC Identification PCR Assay is certified for the detection of Shiga-toxin producing *Escherichia coli* (STEC): *E. coli* O157:H7, *E. coli* O26, *E. coli* O45, *E. coli* O103, *E. coli* O111, *E. coli* O121, and *E. coli* O145 within the scope of Tables 1 and 2.

***Certification includes**:*

1. Applied Biosystems™ SimpliAmp™
2. Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument, with Thermo Scientific™ RapidFinder™ Analysis Software v3.0 or later
3. E. coli-STEC-ID-ST-A56840-QS5-1.2 or later.
4. Alternative confirmation procedures include direct streak to CT-SMAC agar (Oxoid™) for *E. coli* O157:H7 positive results, and either Chromogenic Coliform Agar (Oxoid™) or CHROMagar™ STEC agar (Oxoid™) for non-O157:H7 positive results. IMS (Dynabeads™), followed by plating as described, can be used if presumptive colonies cannot be isolated by direct streak. Confirm isolated colonies by PCR using the SureTect™ Escherichia coli O157:H7 and STEC Screening PCR Assay (together with/and) the SureTect™ Escherichia coli STEC Identification PCR Assay. Remel™ RIM E. coli O157:H7 Latex Test can also be used to confirm presumptive *E. coli* O157:H7 colonies.

Table 1. Method Performance Claims

Matrix	Test Portion	Enrichment Conditions				Reference Method[b]	Claim[c]
		Broth[a]	Volume	Temperature	Time		
Fresh raw spinach	25 g	Pre-warmed BPW	225 mL	41.5 ± 1°C	8–24 h	BAM Ch 4A	NSDD
Fresh raw spinach	375 g	Pre-warmed BPW	3,375 mL	41.5 ± 1°C	8–24 h	BAM Ch 4A	NSDD
Fresh baby leaves	25 g	Pre-warmed BPW	225 mL	41.5 ± 1°C	8–24 h	ISO/TS 13136	NSDD
Fresh cut tomatoes	25 g	Pre-warmed BPW	225 mL	41.5 ± 1°C	8–24 h	ISO/TS 13136	NSDD
Frozen raw ground beef	25 g	Pre-warmed BPW	225 mL	41.5 ± 1°C	8–24 h	ISO/TS 13136	NSDD
Frozen raw ground beef	375 g	Pre-warmed BPW	1,500 mL	41.5 ± 1°C	8-24 h	ISO/TS 13136	NSDD
Raw beef trim	25 g	Pre-warmed BPW	225 mL	41.5 ± 1°C	8–24 h	MLG 5C.00	NSDD
Raw beef trim	375 g	Pre-warmed mTSB	1,500 mL	41.5 ± 1°C	8–24 h	MLG 5C.00	NSDD
Beef carcass sponge	Sponge	Pre-warmed mTSB	200 mL	41.5 ± 1°C	8–24 h	MLG 5C.00	NSDD
Beef trim sampling cloth without nBPW	Cloth	Pre-warmed BPW	225 mL	41.5 ± 1°C	8–24 h	MLG 5C.03	NSDD
Beef trim sampling cloth with nBPW	Cloth	Pre-warmed BPW	200 mL	41.5 ± 1°C	8–24 h	MLG 5C.03	NSDD
Beef trim sampling cloth with nBPW	Cloth	mTSB	200 mL	42 ± 1°C	15–23 h	MLG 5C.03	Eq

[a] BPW = Buffered peptone water; TSBn = tryptic soy broth with novobiocin.

[b] MLG = BAM = US FDA Bacteriological Analysis Manual; ISO/TS = International Organization for Standardization/Technical Specification; US FSIS 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 in unpaired studies. Expert opinion is that the method is appropriate for its intended use. Eq = Equivalence of candidate and reference method results demonstrated by 90% confidence interval on $dPOD_C$ meeting the criteria according to TR364.

Table 2. Method Selectivity – Detection

Enrichment		Inclusivity Strains		Exclusivity Strains	
Broth[a]	Temp., °C	No. Tested	No. Positive	No. Tested	No. Positive
BPW	41.5 ± 1°C	61[b]	61[c]	35[d]	0

[a] BPW = Buffered peptone water (ISO formulation).

[b] Comprised of 8 strains O26, 8 strains O103, 8 strains O111, 8 strains O145, 8 strains O121, 8 strains O45, and 13 strains O157:H7.

[c] O157:H7 strains tested negative with the SureTect STEC Identification PCR Assay as expected.

[d] 35 isolates were tested, comprised of 9 strains *stx* and/or *eae* negative *E. coli,* and the remaining species representing other organisms. Exclusivity organisms were cultured under optimal conditions for growth.

Table 3. Method Selectivity – Confirmation

Enrichment		Inclusivity Strains		Exclusivity Strains	
Culture media[a]	Temp., °C	No. Tested	No. Positive	No. Tested	No. Positive
TSA/Blood agar[b], CCA, CHROMagar STEC, CT-SMAC	37 ± 1°C	162[c]	162[d]	105[e]	0

[a] Culture media used in the ISO 16140-6:2019 study; CCA = Chromogenic Coliform Agar; CT-SMAC = Sorbitol MacConkey Agar with Cefixime-Tellurite Supplement.

[b] TSA/Blood agar used to culture exclusivity strains as well as inclusivity strains.

[c] Comprised of 21 strains O103, 25 strains O111, 24 strains O121, 17 strains O145, 25 strains O26, 25 strains O45, and 25 strains O157:H7.

[d] All O157:H7 strains tested negative with the SureTect STEC Identification PCR Assay as expected.

[e] 105 isolates were tested, comprised of 25 closely related non-*E. coli* strains and 85 non-target *E. coli* strains, as required by ISO 16140-6:2019.

Table 4. Method History

No.	Date	Summary	Supporting Data
1	January 2021	Original certification.	Certification Report
2	July 2022	Level 2 Modification: Changes made to improve handling steps and visual indicators.	Modification Report 1
3	January 2024	Level 2 Modification: Addition of automated lysis procedure and PCR setup procedure.	Modification Report 2
4	November 2024	Level 2 Modification: Matrix extension to include beef trim sampling clothes (with and without neutralizing Buffered Peptone Water), and up to 375 g frozen raw ground beef.	Modification Report 3