



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
012401

The AOAC Research Institute hereby certifies the method known as

dd-Check STEC Solution

Corporate Location

**Bio-Rad Laboratories
2000 Alfred Nobel Drive
Hercules, CA 94547 USA**

Manufacturing Location

**Bio-Rad Laboratories
925 Alfred Nobel Drive
Hercules, CA 94547 USA**

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 19, 2025

Expiration Date

December 31, 2026

METHOD NAME
dd-Check Solution

CATALOG NUMBER
17004826

ORIGINAL CERTIFICATION DATE
January 5, 2024

PRINCIPLE OF THE METHOD

The dd-Check STEC Kit uses Droplet Digital PCR (ddPCR), Bio-Rad's unique digital PCR methodology, to distinguish double-positive linked virulence factors in samples from single-positive or unlinked virulence factors, and to differentiate samples that are true-positive STEC (*stx1* or 2 and *eae* together) from false-positive STEC (*stx1* or 2 or *eae* alone). This detection and linkage verification of targets in a single bacterium enhance the accuracy of food safety testing by reducing the number of false positive reactions.

ddPCR employs advanced microfluidics technology to generate thousands of highly uniform nanoliter-sized droplets in each sample, achieving sample partitioning on a massive scale. Once generated, the droplets can be amplified, read, and analyzed. In traditional PCR, a single measurement is performed on a single sample, while in ddPCR, thousands of measurements can be performed on each sample.

CERTIFIED CLAIM STATEMENT: The dd-Check STEC method is certified for the detection of Shiga toxin-producing *Escherichia coli* (STEC, *stx1* or 2 and *eae* together) within the scope of Tables 1-3.

Certified method includes:

1. Real-Time PCR Systems: CFX96 Touch Deepwell with CFX Maestro Software, IDE v4.0; and CFX Opus Deepwell with CFX Maestro Software, IDE v4.0
2. QX200 Droplet Reader
3. QX200 Droplet Generator
4. Use as secondary screening with fresh and frozen enrichments after initial screening with Bio-Rad iQ-Check STEC method

Table 1. Method Performance Claims – Detection

Matrix	Test Portion	Enrichment Conditions				Reference Method ^b	Claim ^c
		Broth ^a	Volume	Temperature	Time		
Raw beef trim	375 g	BPW+STEC supp	1125 mL	37 ± 1°C	16-24 h	MLG 5C.03	NSDD
	325 g	mTSB	975 mL	37 ± 1°C	15-24 h	MLG 5C.03	Eq
Raw ground beef	375 g	pw BPW+STEC supp	1125 mL	37 ± 1°C	16-24 h	MLG 5C.03	NSDD
	325 g	mTSB	975 mL	42 ± 1°C	15-24	MLG 5C.03	Eq
Carcass sampling cloth	1 cloth	BPW	200 mL	37 ± 1°C	8-16 h	MLG 5C.03	NSDD
	1 cloth	mTSB	200 mL	37 ± 1°C	15 h	MLG 5C.03	Eq
Fresh spinach	375 g	BPW+STEC supp	1125 mL	37 ± 1°C	10-16 h	BAM 4A	NSDD
	200 g	mBPWp+ACV	450 mL	37 ± 1°C ^d	5 h ^d	BAM 4A	Eq

^a pw = prewarmed; BPW+STEC supp = Buffered Peptone Water + Selective STEC supplement; mTSB = Modified Tryptone Soy Broth; mBPWp+ACV = Modified Buffered Peptone Water with pyruvate + Acriflavine, Cefsulodin, and Vancomycin.

^b MLG = Microbiology Laboratory Guidebook; BAM = Bacteriological Analytical Manual.

^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. Eq = Equivalence of candidate and reference methods demonstrated by 90% CI on dPOD_c meeting the criteria according to TR364 (Least Cost Formulations, Virginia Beach, VA).

^d After 5 h, move enrichment from 37°C to 42 ± 1°C and continue incubating for 8-24 h.

Table 2. Method Performance Claims – Secondary Screening^a

Matrix	Test Portion	Enrichment Conditions				Reference Method ^d	Claim ^e
		Broth ^b	Volume	Temperature	Time ^c		
Raw beef trim	375 g	BPW+STEC supp	1125 mL	37 ± 1°C	16-24 h	MLG 5C.03	Eq
	325 g	mTSB	975 mL	37 ± 1°C	15-24 h	MLG 5C.03	Eq
Raw ground beef	375 g	pw BPW+STEC supp	1125 mL	37 ± 1°C	16-24 h	MLG 5C.03	Eq
	325 g	mTSB	975 mL	42 ± 1°C	15-24	MLG 5C.03	Eq
Carcass sampling cloth	1 cloth	BPW	200 mL	37 ± 1°C	8-16 h	MLG 5C.03	Eq
	1 cloth	mTSB	200 mL	37 ± 1°C	15 h	MLG 5C.03	Eq
Fresh spinach	375 g	BPW+STEC supp	1125 mL	37 ± 1°C	10-16 h	BAM 4A	Eq
	200 g	mBPWp+ACV	450 mL	37 ± 1°C ^f	5 h ^f	BAM 4A	Eq

^a Initial screening performed with Bio-Rad iQ-Check STEC (PTM 121203) followed by dd-Check STEC as a secondary screening tool.

^b pw = prewarmed; BPW+STEC supp = Buffered Peptone Water + Selective STEC supplement; mTSB = Modified Tryptone Soy Broth; mBPWp+ACV = Modified Buffered Peptone Water with pyruvate + Acriflavine, Cefsulodin, and Vancomycin.

^c Enrichments tested fresh after certified enrichment times and after freezing at -20°C for 16-24 h for spinach and 7 days for all other matrixes.

^d MLG = Microbiology Laboratory Guidebook; BAM = Bacteriological Analytical Manual.

^e Eq = Equivalence of candidate and reference methods demonstrated by 90% CI on dPOD_c meeting the criteria according to TR364 (Least Cost Formulations, Virginia Beach, VA).

^f After 5 h, move enrichment from 37°C to 42 ± 1°C and continue incubating for 8-24 h.

Table 3. Method Selectivity

Broth ^a	Temperature	Inclusivity Strains		Exclusivity Strains	
		No. Tested	No. Positive	No. Tested	No. Positive
mBPWp+ACV	37 ± 1°C	100 ^b	100	100 ^c	0
mTSB	37 ± 1°C	100 ^b	100		
BPW	37 ± 1°C	100 ^b	100		
BPW+S	37 ± 1°C	100 ^b	100		

^a mBPWp = modified Buffered Peptone Water with pyruvate plus Acriflavin-Cefsulodin-Vancomycin supplement; mTSB = modified Tryptone Soy Broth; BPW = Buffered Peptone Water; BPW+S = Buffered Peptone Water + STEC Supplement.

^b Comprised of 17 strains O26, 6 strains O45, 15 strains O103, 21 strains O111, 5 strains O121, 12 strains O145 and 24 strains O157:H7.

^c Comprised of 37 species.

Table 4. Method History

No.	Date	Summary	Supporting Data
1	January 2024	Original certification	Certification Report
2	May 2025	Level 2 Modification: Addition of raw ground beef (325 g and 375 g).	Modification Report 1