



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.

011103

The AOAC Research Institute hereby certifies the method known as

Reveal[®] 2.0 for *E. coli* O157:H7

manufactured by

**Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912 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

January 19, 2026

Expiration Date

December 31, 2026

METHOD NAMEReveal® 2.0 for *E. coli* O157:H7**CATALOG NUMBERS**9734 (SKU 700002805), 9714 (SKU 700002798),
9727 (SKU 700002802)**ORIGINAL CERTIFICATION DATE**

January 01, 2011

PRINCIPLE OF THE METHOD

The Reveal 2.0 for *E. coli* O157:H7 method is a lateral-flow format, immunodiagnostic test that facilitates rapid and accurate detection of *Escherichia coli* O157:H7 in raw beef samples. A portion (200 µL) of the final enrichment culture is treated with promoter reagent to enhance antigen availability and introduced to the Reveal 2.0 for *E. coli* O157:H7 device. The sample is wicked through a reagent zone, which contains specific antibodies conjugated to colloidal gold particles. If *E. coli* O157:H7-specific antigens are present in the sample, they will bind to the colloidal gold conjugated antibodies. This antigen-antibody complex then leaves the reagent zone and travels through the nitrocellulose membrane, which contains a zone of anti-*E. coli* O157:H7 antibodies. The immune complex with colloidal gold conjugate is captured and aggregates in this zone, displaying a visible line. The remainder of the sample continues to migrate to the end of the membrane, where it will eventually be deposited into a waste pad. The reagent zone also contains a colloidal gold conjugate of a second antigen, which is also eluted by the sample. The colloidal gold-conjugated control indicator migrates through the membrane to the negative control capture zone (containing antibody to the second antigen), where it is captured and aggregated to form a visible line. Regardless of the presence of *E. coli* O157:H7 antigen, the control line will form in the control zone, ensuring that the test is working properly. Positive assay results must be confirmed by standard culture methods.

CERTIFIED CLAIM STATEMENT: The Reveal 2.0 for *E. coli* O157:H7 method is certified for the detection of *Escherichia coli* O157:H7 within the scope of Tables 1 and 2.

Certified method includes:

1. Visual results read only
2. Alternative confirmation protocol of direct streak from primary Reveal® 2.0 for *E. coli* O157:H7 Medium to CHROMagar

Table 1. Method Performance Claims

Matrix	Test Portion	Enrichment Conditions				Reference Method ^b	Claim ^c
		Broth ^a	Volume	Temperature	Time		
Raw beef trim	65 g	Reveal	325 mL	42 ± 1°C	12-20 h	MLG 5.04	NSDD
	375 g	Reveal	1875 mL	42 ± 1°C	12-20 h	MLG 5.04	NSDD
Raw ground beef	65 g	Reveal	325 mL	42 ± 1°C	12-20 h	MLG 5.04	NSDD
	375 g	Reveal	1875 mL	42 ± 1°C	12-20 h	MLG 5.04	SDD-C

^a Reveal = Reveal 2.0 for *E. coli* O157:H7 Medium

^b MLG = USDA 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. 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.

Table 2. Method Selectivity

Broth	Temperature	Inclusivity Strains		Exclusivity Strains	
		No. Tested	No. Positive	No. Tested	No. Positive
Reveal 2.0 for <i>E. coli</i> O157:H7 Medium	42 ± 1°C	51 ^a	51 ^b	41 ^c	3 ^d

^a Comprising *E. coli* O157:H7, *E. coli* O157:NM and *E. coli* O157:H-

^b 51 positive at 10⁶ colony forming units/mL and 50 positive at 10⁵ colony forming units/mL

^c Comprising 16 non-*E. coli* species and 25 non-target *E. coli* serogroups

^d *E. coli* O157:H38 and 1 strain *E. coli* O157:H43 produced positive results.

Table 3. Method History

No.	Date	Summary	Supporting Data
1	January 2011	Original certification	Certification Report