



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.  
**102003**

The AOAC Research Institute hereby certifies the method known as:

### **BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact**

manufactured by

**Hygiena**

**2 Boulden Circle**

**New Castle, DE 19720**

**USA**

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated in the applicability of the method. 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*<sup>SM</sup> 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 that reads 'Scott Coates'.

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

Issue Date                      December 20, 2023

Expiration Date                December 31, 2024

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MODIFICATION APRIL 2023: Nisha Corrigan, Julie Weller, Deja Latney, Margaret Morris, and Stacy Stoltenberg

**SUBMITTING COMPANY**

Hygiena  
2 Boulden Circle  
New Castle, DE 19720 USA

**METHOD NAME**

BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact

**CATALOG NUMBER**

KIT2039

**INDEPENDENT LABORATORY**

Q Laboratories  
1930 Radcliff Drive  
Cincinnati, OH 45204 USA

**INDEPENDENT LABORATORY**

MODIFICATION JANUARY 2022  
TEQ Analytical Laboratories, Inc.  
12635 E. Montview Blvd.  
Suite 175  
Aurora, CO 80045 USA

**APPLICABILITY OF METHOD**

Target Organism – *E. coli* O157:H7.

Matrixes – Fresh raw ground beef (25 and 375 g, 73% lean), fresh raw beef trim (325 and 375 g), leafy greens (375 g), and raw fluid milk (25 g)  
MODIFICATION JANUARY 2022: (AOAC SMPR 2020.12; 10 g) dried cannabis flower [ $>0.3\%$  delta 9-tetrahydrocannabinol (THC)] and dried hemp flower ( $\leq 0.3\%$  THC).

MODIFICATION APRIL 2023 – Sampling cloths swabbed from 375 g beef trim portions.

Performance claims – The BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact method showed no differences in results compared to the reference culture methods in the U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (MLG), 5C.00, *Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponges* (2) for fresh raw ground beef and beef trim, the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (BAM), Chapter 4A (2018), *Diarrheagenic Escherichia coli* (3) for leafy greens, and ISO 16654:2001/AMD 1:2017, *Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Escherichia coli O157 – Amendment 1: Annex B: Results of interlaboratory studies* (4) for raw fluid milk. Furthermore, the BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact is an effective method for the colony confirmation of *E. coli* O157:H7 from appropriate selective agars.

MODIFICATION JANUARY 2022: BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact test kit is effective in screening for *E. coli* O157:H7 in dried cannabis flower ( $>0.3\%$  THC) and dried hemp flower ( $\leq 0.3\%$  THC) at a 10 g test portion size and met the requirements of *Standard Method Performance Requirements* (SMPRs®) for Detection of Shiga toxin-producing *Escherichia coli* in Cannabis and Cannabis Products (AOAC SMPR 2020.012; 6).

MODIFICATION APRIL 2023: The study data were unable to find a statistically detectable difference in results from zero between the BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact and the United States Department of Agriculture Food Safety and Inspection Service MLG 5C.03 *Detection, Isolation, and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products, Carcass, and Environmental Sponges* (8) from sampling cloths swabbed from 375 g beef trim test portions in 8–24 h using MP media or modified Tryptic Soy Broth with casamino acids (mTSB +caa).

**REFERENCE METHODS**

U.S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook*, 5C.00, *Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponges* (February 2019) (2)

U.S. Food and Drug Administration *Bacteriological Analytical Manual* (BAM), Chapter 4A (2018), *Diarrheagenic Escherichia coli* (October 2018) (3)

ISO 16654:2001/AMD 1:2017, *Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Escherichia coli O157 – Amendment 1: Annex B: Results of interlaboratory studies* (4)

*Standard Method Performance Requirements* (SMPRs®) for Detection of Shiga toxin-producing *Escherichia coli* in Cannabis and Cannabis Products (AOAC SMPR 2020.012) (6)

United States Department of Agriculture Food Safety and Inspection Service MLG 5C.03 *Detection, Isolation, and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products, Carcass, and Environmental Sponges*. (8)

<b>ORIGINAL CERTIFICATION DATE</b> October 16, 2020	<b>CERTIFICATION RENEWAL RECORD</b> Renewed annually through December 2024.
<b>METHOD MODIFICATION RECORD</b> 1. December 2021 Level 1 2. January 2022 Level 2 3. April 2023 Level 2 4. December 2023 Level 1	<b>SUMMARY OF MODIFICATION</b> 1. Editorial/clerical changes. 2. Dried cannabis flower (>0.3% THC) and dried hemp flower (≤0.3% THC). 3. Matrix extension to include sampling cloths for 375 g beef trim portions. 4. Editorial/clerical changes.
<b>Under this AOAC Performance Tested Methods<sup>SM</sup> License Number, 102003 this method is distributed by:</b> NONE	<b>Under this AOAC Performance Tested Methods<sup>SM</sup> License Number, 102003 this method is distributed as:</b> NONE

**PRINCIPLE OF THE METHOD (1)**

The BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact method is based on the use of target-specific dye-labeled probes for fluorescent detection of *E. coli* O157:H7 utilizing real-time polymerase chain reaction technology. Probes bind to the targeted DNA fragment while the fluorophore is separated, producing a fluorescent signal. The BAX System uses multiple filters to measure the fluorescent signal at the end of each cycle. These signals are then clearly displayed after approximately 55 minutes as positive or negative for easy interpretation.

**DISCUSSION OF THE VALIDATION STUDY (1)**

In the method comparison study, the BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact demonstrated no significant differences between presumptive and confirmed results or between candidate, alternative confirmation and reference method results, demonstrating comparable performance than the corresponding USDA MLG, FDA BAM or ISO reference methods. The BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact demonstrated no significant differences between presumptive and confirmed results or between candidate, alternative confirmation and reference method results after 8, 10 and 24 h of incubation when enriched with BAX MP media and mTSB for both test portion sizes (25 and 375 g) of fresh raw ground beef; after 8, 10, 12 and 24 h of incubation when enriched in MP media (375 g) and after 10 and 24 h of incubation when enriched with mTSB media (375 g) for fresh raw beef trim; after 6, 8 and 24 h of incubation when enriched in MP media (375 g) for leafy greens; and after 12, 20 and 24 h of incubation for raw fluid milk when enriched in double strength BPW.

In the inclusivity and exclusivity studies for the detection method completed by the method developer, the BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact was able to correctly detect all 50 *E. coli* O157:H7 inclusivity isolates and correctly did not detect any of the 30 exclusivity strains. For the inclusivity/exclusivity confirmation method studies completed by the Method developer, the BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact was able to correctly identify all 25 *E. coli* O157:H7 colony isolates from CT-SMAC, RBA and mRBA and not detect the 103 exclusivity strains. In the inclusivity/exclusivity confirmation method evaluation completed by the Independent Laboratory, the BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact was able to correctly detect all 16 *E. coli* O157:H7 isolates from isolated from CT-SMAC, RBA and mRBA.

**Table 1. Inclusivity Results for BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact for Detection (1)**

No.	Strain	Source	Origin	BAX Result		
				BAX MP Media	mTSB <sup>a</sup>	2X BPW <sup>b</sup>
1	<i>E. coli</i> O157:H7	PSU <sup>c</sup> 991762	Human	POS	POS	POS
2	<i>E. coli</i> O157:H7	PSU 991766	Human	POS	POS	POS
3	<i>E. coli</i> O157:H7	PSU 991774	Human	POS	POS	POS
4	<i>E. coli</i> O157:H7	PSU 991793	Human	POS	POS	POS
5	<i>E. coli</i> O157:H7	PSU 991797	Human	POS	POS	POS
6	<i>E. coli</i> O157:H7	PSU 991799	Human	POS	POS	POS
7	<i>E. coli</i> O157:H7	PSU 991800	Human	POS	POS	POS
8	<i>E. coli</i> O157:H7	PSU 991801	Human	POS	POS	POS
9	<i>E. coli</i> O157:H7	PSU 991802	Human	POS	POS	POS
10	<i>E. coli</i> O157:H7	PSU 991803	Human	POS	POS	POS
11	<i>E. coli</i> O157:H7	PSU 991805	Human	POS	POS	POS
12	<i>E. coli</i> O157:H7	PSU 991819	Human	POS	POS	POS
13	<i>E. coli</i> O157:H7	PSU 991821	Human	POS	POS	POS
14	<i>E. coli</i> O157:H7	PSU 991824	Human	POS	POS	POS
15	<i>E. coli</i> O157:H7	PSU 991825	Human	POS	POS	POS
16	<i>E. coli</i> O157:H7	PSU 991826	Human	POS	POS	POS
17	<i>E. coli</i> O157:H7	PSU 991827	Human	POS	POS	POS
18	<i>E. coli</i> O157:H7	PSU 992004	Bovine	POS	POS	POS
19	<i>E. coli</i> O157:H7	PSU 992005	Bovine	POS	POS	POS
20	<i>E. coli</i> O157:H7	PSU 992008	Bovine	POS	POS	POS
21	<i>E. coli</i> O157:H7	PSU 992011	Bovine	POS	POS	POS
22	<i>E. coli</i> O157:H7	PSU 992012	Bovine	POS	POS	POS
23	<i>E. coli</i> O157:H7	PSU 992013	Bovine	POS	POS	POS
24	<i>E. coli</i> O157:H7	PSU 992014	Bovine	POS	POS	POS
25	<i>E. coli</i> O157:H7	PSU 992016	Bovine	POS	POS	POS
26	<i>E. coli</i> O157:H7	PSU 992017	Bovine	POS	POS	POS
27	<i>E. coli</i> O157:H7	PSU 992019	Bovine	POS	POS	POS
28	<i>E. coli</i> O157:H7	PSU 992020	Bovine	POS	POS	POS
29	<i>E. coli</i> O157:H7	PSU 992022	Bovine	POS	POS	POS
30	<i>E. coli</i> O157:H7	PSU 992024	Bovine	POS	POS	POS
31	<i>E. coli</i> O157:H7	PSU 992025	Bovine	POS	POS	POS
32	<i>E. coli</i> O157:H7	PSU 992026	Bovine	POS	POS	POS
33	<i>E. coli</i> O157:H7	PSU 992027	Bovine	POS	POS	POS
34	<i>E. coli</i> O157:H7	PSU 992028	Bovine	POS	POS	POS

35	<i>E. coli</i> O157:H7	PSU 992030	Bovine	POS	POS	POS
36	<i>E. coli</i> O157:H7	PSU 992031	Bovine	POS	POS	POS
37	<i>E. coli</i> O157:H7	PSU 992032	Bovine	POS	POS	POS
38	<i>E. coli</i> O157:H7	PSU 992033	Bovine	POS	POS	POS
39	<i>E. coli</i> O157:H7	PSU 992034	Bovine	POS	POS	POS
40	<i>E. coli</i> O157:H7	PSU 992036	Bovine	POS	POS	POS
41	<i>E. coli</i> O157:H7	PSU 992037	Bovine	POS	POS	POS
42	<i>E. coli</i> O157:H7	PSU 992038	Bovine	POS	POS	POS
43	<i>E. coli</i> O157:H7	PSU 992040	Bovine	POS	POS	POS
44	<i>E. coli</i> O157:H7	PSU 992045	Bovine	POS	POS	POS
45	<i>E. coli</i> O157:H7	PSU 992046	Bovine	POS	POS	POS
46	<i>E. coli</i> O157:H7	PSU 992047	Bovine	POS	POS	POS
47	<i>E. coli</i> O157:H7	PSU 992048	Bovine	POS	POS	POS
48	<i>E. coli</i> O157:H7	PSU 992049	Bovine	POS	POS	POS
49	<i>E. coli</i> O157:H7	PSU 992051	Bovine	POS	POS	POS
50	<i>E. coli</i> O157:H7	PSU 992052	Bovine	POS	POS	POS

<sup>a</sup>Modified Tryptone Soya Broth.

<sup>b</sup>Double strength Buffered Peptone Water.

<sup>c</sup>The Pennsylvania State University Department of Veterinary Science *E. coli* Reference Center, University Park, PA.

**Table 2. Exclusivity Results for BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact – Non- *E. coli* O157:H7 organisms for Detection (1)**

No.	Strain	Qualicon Culture Collection No.	Source	Origin	BAX Result
1	<i>E. coli</i> O1:H7	DD2434	HCC <sup>a</sup>	Unknown	NEG
2	<i>E. coli</i> O113:H7	DD2520	HCC	Unknown	NEG
3	<i>E. coli</i> O114:H32	DD1721	HCC	Unknown	NEG
4	<i>E. coli</i> O119:H27	DD1723	HCC	Unknown	NEG
5	<i>E. coli</i> O125:H19	DD1725	HCC	Unknown	NEG
6	<i>E. coli</i> O127:H40	DD1811	HCC	Unknown	NEG
7	<i>E. coli</i> O128:H2	DD1718	HCC	Unknown	NEG
8	<i>E. coli</i> O136:H8	DD1818	HCC	Unknown	NEG
9	<i>E. coli</i> O157:H19	DD2443	HCC	Unknown	NEG
10	<i>E. coli</i> O158:H23	DD1716	HCC	Unknown	NEG
11	<i>E. coli</i> O18:H14	DD1724	HCC	Unknown	NEG
12	<i>E. coli</i> O25:H7	DD1908	HCC	Unknown	NEG
13	<i>E. coli</i> O26:HNM	DD1720	HCC	Unknown	NEG
14	<i>E. coli</i> O28:H16	DD1810	HCC	Unknown	NEG
15	<i>E. coli</i> O29:H51	DD1834	HCC	Unknown	NEG
16	<i>E. coli</i> O55:H10	DD5883	HCC	Unknown	NEG
17	<i>Escherichia hermannii</i>	DD6719	HCC	Sesame seeds	NEG
18	<i>Raoultella species</i>	DD849	ATCC <sup>b</sup> 21073	Soil	NEG
19	<i>Escherichia vulnaris</i>	DD850	ATCC 33821	Human wound	NEG
20	<i>Hafnia alvei</i>	DD5588	HCC	Ground beef	NEG
21	<i>Klebsiella oxytoca</i>	DD6523	HCC	Ground beef	NEG
22	<i>Listeria monocytogenes</i>	DD1152	HCC	Pate	NEG
23	<i>Morganella morganii</i>	DD3064	HCC	Environmental swab	NEG
24	<i>Proteus mirabilis</i>	DD6121	HCC	Gull, cloacal swab	NEG
25	<i>Pseudomonas aeruginosa</i>	DD3982	ATCC 27853	Blood culture	NEG
26	<i>Salmonella</i> Anatum	DD2274	HCC	Unknown	NEG
27	<i>Salmonella</i> Dublin	DD7005	HCC	Unknown	NEG
28	<i>Salmonella</i> Newport	DD1261	HCC	Duck	NEG
29	<i>Shigella boydii</i>	DD1081	ATCC 8700	Unknown	NEG
30	<i>Staphylococcus aureus</i>	DD610	ATCC 13565	Ham	NEG

<sup>a</sup>Hygiena Culture Collection, New Castle, DE.

<sup>b</sup>American Type Culture Collection, Manassas, VA.

**Table 3. Inclusivity List BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact– *E. coli* O157:H7 Strains for Confirmation (1)**

No.	Strain	Source	Origin	BAX Result		
				RBA <sup>a</sup>	mRBA <sup>b</sup>	CT-SMAC <sup>c</sup>
1	<i>E. coli</i> O157:H7	PSU <sup>d</sup> 991762	Human	POS	POS	POS
2	<i>E. coli</i> O157:H7	PSU 991766	Human	POS	POS	POS
3	<i>E. coli</i> O157:H7	PSU 991774	Human	POS	POS	POS
4	<i>E. coli</i> O157:H7	PSU 991793	Human	POS	POS	POS
5	<i>E. coli</i> O157:H7	PSU 991797	Human	POS	POS	POS
6	<i>E. coli</i> O157:H7	PSU 991799	Human	POS	POS	POS
7	<i>E. coli</i> O157:H7	PSU 991800	Human	POS	POS	POS
8	<i>E. coli</i> O157:H7	PSU 991801	Human	POS	POS	POS

9	<i>E. coli</i> O157:H7	PSU 991802	Human	POS	POS	POS
10	<i>E. coli</i> O157:H7	PSU 991803	Human	POS	POS	POS
11	<i>E. coli</i> O157:H7	PSU 991805	Human	POS	POS	POS
12	<i>E. coli</i> O157:H7	PSU 991819	Human	POS	POS	POS
13	<i>E. coli</i> O157:H7	PSU 991821	Human	POS	POS	POS
14	<i>E. coli</i> O157:H7	PSU 991824	Human	POS	POS	POS
15	<i>E. coli</i> O157:H7	PSU 991825	Human	POS	POS	POS
16	<i>E. coli</i> O157:H7	PSU 991826	Human	POS	POS	POS
17	<i>E. coli</i> O157:H7	PSU 991827	Human	POS	POS	POS
18	<i>E. coli</i> O157:H7	PSU 992004	Bovine	POS	POS	POS
19	<i>E. coli</i> O157:H7	PSU 992005	Bovine	POS	POS	POS
20	<i>E. coli</i> O157:H7	PSU 992008	Bovine	POS	POS	POS
21	<i>E. coli</i> O157:H7	PSU 992011	Bovine	POS	POS	POS
22	<i>E. coli</i> O157:H7	PSU 992012	Bovine	POS	POS	POS
23	<i>E. coli</i> O157:H7	PSU 992013	Bovine	POS	POS	POS
24	<i>E. coli</i> O157:H7	PSU 992014	Bovine	POS	POS	POS
25	<i>E. coli</i> O157:H7	PSU 992016	Bovine	POS	POS	POS

<sup>a</sup>RBA = Rainbow Agar.

<sup>b</sup>mRBA = Modified Rainbow Agar.

<sup>c</sup>CT-SMAC = MacConkey Agar with Sorbitol, Cefixime, and Tellurite Agar.

<sup>d</sup>The Pennsylvania State University Department of Veterinary Science *E. coli* Reference Center, University Park, PA.

**Table 4. Exclusivity List BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact for Confirmation (1)**

No.	Strain	Qualicon Culture			BAX Result		
		Collection No.	Source	Origin	RBA <sup>a</sup>	mRBA <sup>b</sup>	CT-SMAC <sup>c</sup>
1	<i>E. coli</i> O1:H7	2434	HCC <sup>d</sup>	Unknown	NEG	NEG	NEG
2	<i>E. coli</i> O101:K-:K99	655	ATCC <sup>e</sup> 31619	Calf Intestine	NEG	NEG	NEG
3	<i>E. coli</i> O103	R66	MSU <sup>f</sup>	Human Clinical	NEG	NEG	NEG
4	<i>E. coli</i> O104:H4	13493	ATCC- BAA-2326	Stool sample from German outbreak 2011	NEG	NEG	NEG
5	<i>E. coli</i> O111	R70	CDC <sup>g</sup>	Human Clinical	NEG	NEG	NEG
6	<i>E. coli</i> O111	R72	CDH <sup>h</sup>	Human Clinical	NEG	NEG	NEG
7	<i>E. coli</i> O112:HNM	2502	HCC	Unknown	NEG	NEG	NEG
8	<i>E. coli</i> O113:H7	2520	HCC	Unknown	NEG	NEG	NEG
9	<i>E. coli</i> O113:H21	2533	HCC	Unknown	NEG	NEG	NEG
10	<i>E. coli</i> O114:H32	1721	HCC	Unknown	NEG	NEG	NEG
11	<i>E. coli</i> O115:HNM	2037	HCC	Unknown	NEG	NEG	NEG
12	<i>E. coli</i> O117:H4	2441	HCC	Unknown	NEG	NEG	NEG
13	<i>E. coli</i> O118:HNM	2438	HCC	Unknown	NEG	NEG	NEG
14	<i>E. coli</i> O121	R76	MSU	Human Clinical	NEG	NEG	NEG
15	<i>E. coli</i> O124:HNM	2501	HCC	Unknown	NEG	NEG	NEG
16	<i>E. coli</i> O125:H19	1725	HCC	Unknown	NEG	NEG	NEG
17	<i>E. coli</i> O125:HNM	1824	HCC	Unknown	NEG	NEG	NEG
18	<i>E. coli</i> O127: HNM	1722	HCC	Unknown	NEG	NEG	NEG
19	<i>E. coli</i> O127:H-	1835	HCC	Unknown	NEG	NEG	NEG
20	<i>E. coli</i> O127:H10	1812	HCC	Unknown	NEG	NEG	NEG
21	<i>E. coli</i> O127:H40	1811	HCC	Unknown	NEG	NEG	NEG
22	<i>E. coli</i> O128:H2	1718	HCC	Unknown	NEG	NEG	NEG
23	<i>E. coli</i> O135:HNM	2451	HCC	Unknown	NEG	NEG	NEG
24	<i>E. coli</i> O136:H8	1818	HCC	Unknown	NEG	NEG	NEG
25	<i>E. coli</i> O136:HNM	2487	HCC	Unknown	NEG	NEG	NEG
26	<i>E. coli</i> O139	2028	HCC	Unknown	NEG	NEG	NEG
27	<i>E. coli</i> O143:HNM	2001	HCC	Unknown	NEG	NEG	NEG
28	<i>E. coli</i> O145	R79	MSU	Human Clinical	NEG	NEG	NEG
29	<i>E. coli</i> O146:H21	2463	HCC	Unknown	NEG	NEG	NEG
30	<i>E. coli</i> O148:H-	2019	HCC	Unknown	NEG	NEG	NEG
31	<i>E. coli</i> O153:H-	2026	HCC	Unknown	NEG	NEG	NEG
32	<i>E. coli</i> O153:H25	2462	HCC	Unknown	NEG	NEG	NEG
33	<i>E. coli</i> O153:HNM	2041	HCC	Unknown	NEG	NEG	NEG
34	<i>E. coli</i> O157:H19	2443	HCC	Unknown	NEG	NEG	NEG
35	<i>E. coli</i> O158:H23	1716	HCC	Unknown	NEG	NEG	NEG
36	<i>E. coli</i> O159:H27	2030	HCC	Unknown	NEG	NEG	NEG
37	<i>E. coli</i> O163:H19	2433	HCC	Unknown	NEG	NEG	NEG
38	<i>E. coli</i> O165:H-	2432	HCC	Unknown	NEG	NEG	NEG
39	<i>E. coli</i> O167:H5	2431	HCC	Unknown	NEG	NEG	NEG
40	<i>E. coli</i> O18:HNM	1819	HCC	Unknown	NEG	NEG	NEG
41	<i>E. coli</i> O2:H5	2517	HCC	Unknown	NEG	NEG	NEG
42	<i>E. coli</i> O2:H7	2491	HCC	Unknown	NEG	NEG	NEG
43	<i>E. coli</i> O20:HNM	1827	HCC	Unknown	NEG	NEG	NEG
44	<i>E. coli</i> O25:H7	1908	HCC	Unknown	NEG	NEG	NEG

45	<i>E. coli</i> O25:H8	1825	HCC	Unknown	NEG	NEG	NEG
46	<i>E. coli</i> O25:HNM	2484	HCC	Unknown	NEG	NEG	NEG
47	<i>E. coli</i> O26	R144	US MARC <sup>d</sup>	Ground beef	NEG	NEG	NEG
48	<i>E. coli</i> O26:HNM	1720	HCC	Unknown	NEG	NEG	NEG
49	<i>E. coli</i> O26:H11	1831	HCC	Unknown	NEG	NEG	NEG
50	<i>E. coli</i> O28:H16	1810	HCC	Unknown	NEG	NEG	NEG
51	<i>E. coli</i> O28:H8,4,3	1822	HCC	Unknown	NEG	NEG	NEG
52	<i>E. coli</i> O28:HNM	1719	HCC	Unknown	NEG	NEG	NEG
53	<i>E. coli</i> O29:H-	1817	HCC	Unknown	NEG	NEG	NEG
54	<i>E. coli</i> O29:H25	2000	HCC	Unknown	NEG	NEG	NEG
55	<i>E. coli</i> O29:H51	1834	HCC	Unknown	NEG	NEG	NEG
56	<i>E. coli</i> O38:H26	2447	HCC	Unknown	NEG	NEG	NEG
57	<i>E. coli</i> O4:H5	2452	HCC	Unknown	NEG	NEG	NEG
58	<i>E. coli</i> O4:HNM	2512	HCC	Unknown	NEG	NEG	NEG
59	<i>E. coli</i> O45	R63	MSU	Human Clinical	NEG	NEG	NEG
60	<i>E. coli</i> O50:H4	2437	HCC	Unknown	NEG	NEG	NEG
61	<i>E. coli</i> O55:H7	2477	HCC	Unknown	NEG	NEG	NEG
62	<i>E. coli</i> O55:H9	1833	HCC	Unknown	NEG	NEG	NEG
63	<i>E. coli</i> O55:H10	5883	HCC	Unknown	NEG	NEG	NEG
64	<i>E. coli</i> O55:H-	1821	HCC	Unknown	NEG	NEG	NEG
65	<i>E. coli</i> O6:H-	1814	HCC	Unknown	NEG	NEG	NEG
66	<i>E. coli</i> O84:H21	2444	HCC	Unknown	NEG	NEG	NEG
67	<i>E. coli</i> O86:H8	1820	HCC	Unknown	NEG	NEG	NEG
68	<i>E. coli</i> O91:H21	2525	HCC	Unknown	NEG	NEG	NEG
69	<i>Shimwellia blattae</i>	846	ATCC 29907	Cockroach	NEG	NEG	NEG
70	<i>Escherichia fergusonii</i>	847	ATCC 35469	Human feces	NEG	NEG	NEG
71	<i>Escherichia hermanii</i>	6719	HCC	Sesame seeds	NEG	NEG	NEG
72	<i>Raoultella species</i>	849	ATCC 21073	Soil	NEG	NEG	NEG
73	<i>Escherichia vulnaris</i>	850	ATCC 33821	Human wound	NEG	NEG	NEG
74	<i>Bacillus cereus</i>	2901	HCC	Cream cake	NEG	NEG	NEG
75	<i>Edwardsiella tarda</i>	2614	ATCC 15947	Human feces	NEG	NEG	NEG
76	<i>Cronobacter sakazakii</i>	11338	HCC	Food	NEG	NEG	NEG
77	<i>Enterococcus faecalis</i>	3981	HCC	urine	NEG	NEG	NEG
78	<i>Hafnia alvei</i>	5588	HCC	Ground beef	NEG	NEG	NEG
79	<i>Klebsiella oxytoca</i>	6523	HCC	Ground beef	NEG	NEG	NEG
80	<i>Lactobacillus acidophilus</i>	7344	ATCC 4356	Human	NEG	NEG	NEG
81	<i>Carnobacterium maltaromaticum</i>	687	ATCC 43225	Vacuum packed lamb	NEG	NEG	NEG
82	<i>Listeria innocua</i>	922	HCC	cured ham	NEG	NEG	NEG
83	<i>Listeria ivanovii</i>	649	ATCC 19119	sheep	NEG	NEG	NEG
84	<i>Listeria monocytogenes</i>	1152	HCC	Pate	NEG	NEG	NEG
85	<i>Morganella morganii</i>	3064	HCC	Environmental swab	NEG	NEG	NEG
86	<i>Proteus mirabilis</i>	6121	HCC	Gull, cloacal swab	NEG	NEG	NEG
87	<i>Pseudomonas aeruginosa</i>	3982	ATCC 27853	Blood culture	NEG	NEG	NEG
88	<i>Pseudomonas fluorescens</i>	661	ATCC 13525	pre-filter tanks	NEG	NEG	NEG
89	<i>Pseudomonas stutzeri</i>	577	ATCC 17588	Human	NEG	NEG	NEG
90	<i>Salmonella</i> Abaetetuba	2166	HCC	Unknown	NEG	NEG	NEG
91	<i>Salmonella</i> Dublin	3017	HCC	Unknown	NEG	NEG	NEG
92	<i>Salmonella</i> Infantis	733	HCC	Unknown	NEG	NEG	NEG
93	<i>Salmonella</i> Weltevreden	13629	Taxonomics <sup>e</sup>	Human Stool	NEG	NEG	NEG
94	<i>Salmonella</i> Anatum	2274	HCC	Unknown	NEG	NEG	NEG
95	<i>Salmonella</i> Javiana	13569	ATCC- BAA-1593	Human stool	NEG	NEG	NEG
96	<i>Salmonella</i> Lille	2992	HCC	Unknown	NEG	NEG	NEG
97	<i>Salmonella</i> Mbandaka	2341	HCC	Unknown	NEG	NEG	NEG
98	<i>Salmonella</i> Newport	1261	HCC	Duck	NEG	NEG	NEG
99	<i>Shigella boydii</i>	1081	ATCC 8700	Unknown	NEG	NEG	NEG
100	<i>Shigella sonnei</i>	6832	HCC	Unknown	NEG	NEG	NEG
101	<i>Staphylococcus aureus</i>	4160	HCC	Howler monkey	NEG	NEG	NEG
102	<i>Leclercia adecarboxylata</i>	13141	ATCC 23216	Dry Sausage	NEG	NEG	NEG
103	<i>E. coli</i> O126:H2	1726	HCC	Unknown	NEG	NEG	NEG
104	<i>E. coli</i> O119:H27	1723	HCC	Unknown	NEG	NEG	NEG

<sup>a</sup>RBA = Rainbow Agar.<sup>b</sup>mRBA = Modified Rainbow Agar.<sup>c</sup>CT-SMAC = MacConkey Agar with Sorbitol, Cefixime, and Tellurite Agar.<sup>d</sup>HCC=Hygiene Culture Collection, New Castle, DE.<sup>e</sup>ATCC = American Type Culture Collection, Manassas, VA.<sup>f</sup>MSU = Michigan State Culture Collection, East Lansing, MI.

<sup>9</sup>CDC = Centers for Disease Control, Atlanta, GA.

<sup>8</sup>CDH = Connecticut Department of Health, Hartford, CT.

<sup>4</sup>US MARC = United States Meat and Animal Research Center, Clay Center, NE.

<sup>7</sup>Taxonometrics= Taxonometrics, West Chester, PA.

Table 6. BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact presumptive vs. confirmed results (confirmed by Reference method) (1)

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	BAX Exact presumptive			BAX Exact confirmed by Reference method				
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI	dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
Ground beef (25 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 43895	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (25 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (25 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.11, 0.21
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (25 g, mTSB media, 8 h)	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (25 g, mTSB media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.21, 0.11
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (25 g, mTSB media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, mTSB media, 8 h)	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, mTSB media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, mTSB media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	7	0.40	0.22, 0.61	7	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 QL <sup>i</sup> 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.68 (0.38, 1.14)	20	0	0.00	0.00, 0.16	6	0.30	0.15, 0.52	-0.30	-0.54, -0.06
		6.53 (2.30, 18.54)	5	1	0.20	0.00, 0.62	5	1.00	0.57, 1.00	-0.80	-1.00, -0.16
Beef trim	<i>E. coli</i> O157:H7	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47



Hygiena BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact, AOAC Performance Tested Methods<sup>SM</sup> certification number 102003

(375 g, BAX MP media, 10 h)	QL 164673	0.68 (0.38, 1.14)	20	2	0.10	0.03, 0.30	6	0.30	0.15, 0.52	-0.20	-0.41, -0.01
		6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.68 (0.38, 1.14)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim <sup>k</sup> (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 DD13078 <sup>l</sup>	N/A	5	0	0.00	0.00, 1.00	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.83 (0.50, 1.4)	20	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	-0.11, 0.21
		1.5 (0.75, 2.75)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim <sup>k</sup> (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 DD13078	N/A	5	0	0.00	0.00, 1.00	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.83 (0.50, 1.4)	20	10	0.50	0.30, 0.70	10	0.50	0.34, 0.74	0.00	-0.13, 0.13
		1.5 (0.75, 2.75)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim <sup>k</sup> (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 DD13078	N/A	5	0	0.00	0.00, 1.00	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.83 (0.50, 1.4)	20	10	0.50	0.30, 0.70	10	0.50	0.34, 0.74	0.00	-0.13, 0.13
		1.5 (0.75, 2.75)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim <sup>m</sup> (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.62 (0.37, 0.94)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		2.10 (1.13, 4.99)	5	5	0.20	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim <sup>m</sup> (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.62 (0.37, 0.94)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		2.10 (1.13, 4.99)	5	5	0.20	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim <sup>m</sup> (375 g, BAX MP media, 12 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.62 (0.37, 0.94)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		2.10 (1.13, 4.99)	5	5	0.20	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim <sup>m</sup> (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.62 (0.37, 0.94)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		2.10 (1.13, 4.99)	5	5	0.20	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim (375 g, mTSB, 8 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.68 (0.38, 1.14)	20	4	0.20	0.08, 0.42	6	0.30	0.15, 0.52	-0.10	-0.28, 0.08
		6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim (375 g, mTSB, 10 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.68 (0.38, 1.14)	20	5	0.25	0.11, 0.47	6	0.30	0.15, 0.52	-0.05	-0.21, 0.11
		6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim (375 g, mTSB, 24 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.68 (0.38, 1.14)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Leafy greens (375 g, BAX® MP media, 6 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.45 (0.22, 0.78)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.11, 0.21
		3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Leafy greens (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.45 (0.22, 0.78)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Leafy greens (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.45 (0.22, 0.78)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw milk	<i>E. coli</i> O157:H7	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47

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(25 g 2X BPW, 12 h)	QL 14077.4	0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.11, 0.21
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw milk (25 g 2X BPW, 20 h)	<i>E. coli</i> O157:H7 QL 14077.4	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.11, 0.21
Raw milk (25 g 2X BPW, 24 h)	<i>E. coli</i> O157:H7 QL 14077.4	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.34 (0.14, 0.61)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>x = Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>CC</sub> = Candidate method presumptive positive outcomes confirmed by the reference method divided by the total number of trials.

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>ATCC = American Type Culture Collection, Manassas, VA.

<sup>i</sup>Not applicable.

<sup>j</sup>QL = Q Laboratories, Cincinnati, OH.

<sup>k</sup>Matrix conducted in the method developer laboratory, Hygiena, New Castle, DE. All other matrixes were conducted by the independent laboratory, Q Laboratories, Cincinnati, OH.

<sup>l</sup>DD = Qualicon Culture Collection Number, Hygiena Culture Collection, New Castle, DE.

<sup>m</sup>Repeat Beef trim study completed by Independent Laboratory.

**Table 7. BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact results (confirmed by Reference method) vs. Reference method results (1)**

Matrix <sup>a</sup>	Strain	MPN <sup>b</sup> /test portion	N <sup>c</sup>	BAX Exact results confirmed			Reference <sup>f</sup> method results			dPOD <sub>CP</sub> <sup>h</sup>	95% CI <sup>i</sup>
				X <sup>d</sup>	POD <sub>CC</sub> <sup>e</sup>	95% CI	x	POD <sub>R</sub> <sup>g</sup>	95% CI		
Ground beef (25 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A <sup>k</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (25 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (25 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef <sup>m</sup> (25 g, mTSB, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef <sup>m</sup> (25 g, mTSB, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.21, 0.11

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10 h)		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef <sup>a</sup> (25 g, mTSB, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A 0.39 (0.17, 0.68) 4.12 (1.58, 10.72)	5 20 5	0 8 5	0.00 0.40 1.00	0.00, 0.43 0.22, 0.61 0.57, 1.00	0 8 5	0.00 0.40 1.00	0.00, 0.43 0.22, 0.61 0.57, 1.00	0.00 0.00 0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Ground beef (375 g, mTSB, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A 0.39 (0.17, 0.68) 4.12 (1.58, 10.72)	5 20 5	0 6 5	0.00 0.30 1.00	0.00, 0.43 0.15, 0.52 0.57, 1.00	0 8 5	0.00 0.40 1.00	0.00, 0.43 0.22, 0.61 0.57, 1.00	0.00 -0.10 0.00	-0.43, 0.43 -0.36, 0.18 -0.43, 0.43
Ground beef (375 g, mTSB, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A 0.39 (0.17, 0.68) 4.12 (1.58, 10.72)	5 20 5	0 6 5	0.00 0.30 1.00	0.00, 0.43 0.15, 0.52 0.57, 1.00	0 8 5	0.00 0.40 1.00	0.00, 0.43 0.22, 0.61 0.57, 1.00	0.00 -0.10 0.00	-0.43, 0.43 -0.36, 0.18 -0.43, 0.43
Ground beef (375 g, mTSB, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A 0.39 (0.17, 0.68) 4.12 (1.58, 10.72)	5 20 5	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0 8 5	0.00 0.40 1.00	0.00, 0.43 0.22, 0.61 0.57, 1.00	0.00 -0.05 0.00	-0.43, 0.43 -0.32, 0.23 -0.43, 0.43
Beef trim (375 g, BAX® MP media, 8 h)	<i>E. coli</i> O157:H7 QL' 164673	N/A 0.68 (0.38, 1.14) 6.53 (2.30, 18.54)	5 20 5	0 0 1	0.00 0.00 0.20	0.00, 0.43 0.00, 0.16 0.00, 0.62	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0.00 -0.35 -0.80	-0.43, 0.43 -0.57, -0.12 -1.00, -0.19
Beef trim (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.68 (0.38, 1.14) 6.53 (2.30, 18.54)	5 20 5	0 0 5	0.00 0.00 1.00	0.00, 0.43 0.00, 0.16 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0.00 -0.35 0.00	-0.43, 0.43 -0.57, -0.12 -0.43, 0.43
Beef trim (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.68 (0.38, 1.14) 6.53 (2.30, 18.54)	5 20 5	0 6 5	0.00 0.30 1.00	0.00, 0.43 0.15, 0.52 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0.00 -0.05 0.00	-0.43, 0.43 -0.32, 0.23 -0.43, 0.43
Beef trim <sup>m</sup> (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 DD13078 <sup>n</sup>	N/A 0.83 (0.50, 1.4) 1.5 (0.75, 2.75)	5 20 5	0 10 5	0.00 0.50 1.00	0.00, 1.00 0.30, 0.70 0.57, 1.00	0 11 5	0.00 0.55 1.00	0.00, 0.43 0.34, 0.74 0.57, 1.00	0.00 -0.05 0.00	-0.43, 0.43 -0.33, 0.24 -0.43, 0.43
Beef trim <sup>m</sup> (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 DD13078	N/A 0.83 (0.50, 1.4) 1.5 (0.75, 2.75)	5 20 5	0 10 5	0.00 0.50 1.00	0.00, 1.00 0.30, 0.70 0.57, 1.00	0 11 5	0.00 0.55 1.00	0.00, 0.43 0.34, 0.74 0.57, 1.00	0.00 -0.05 0.00	-0.43, 0.43 -0.33, 0.24 -0.43, 0.43
Beef trim <sup>m</sup> (375 g, BAX® MP media, 24 h)	<i>E. coli</i> O157:H7 DD13078	N/A 0.83 (0.50, 1.4) 1.5 (0.75, 2.75)	5 20 5	0 10 5	0.00 0.50 1.00	0.00, 1.00 0.30, 0.70 0.57, 1.00	0 11 5	0.00 0.55 1.00	0.00, 0.43 0.34, 0.74 0.57, 1.00	0.00 -0.05 0.00	-0.43, 0.43 -0.33, 0.24 -0.43, 0.43
Beef trim <sup>o</sup> (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 QL164673	N/A 0.62 (0.37, 0.94) 2.10 (1.13, 4.99)	5 20 5	0 9 5	0.00 0.45 0.20	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0.00 0.10 0.00	-0.43, 0.43 -0.19, 0.37 -0.43, 0.43
Beef trim <sup>o</sup> (375 g, BAX MP media, 10 h) <sup>o</sup>	<i>E. coli</i> O157:H7 QL164673	N/A 0.62 (0.37, 0.94) 2.10 (1.13, 4.99)	5 20 5	0 9 5	0.00 0.45 0.20	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0.00 0.10 -0.80	-0.43, 0.43 -0.19, 0.37 -1.00, -0.19
Beef trim <sup>o</sup> (375 g, BAX MP media, 12 h) <sup>o</sup>	<i>E. coli</i> O157:H7 QL 164673	N/A 0.62 (0.37, 0.94) 2.10 (1.13, 4.99)	5 20 5	0 9 5	0.00 0.45 0.20	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0.00 0.10 0.00	-0.43, 0.43 -0.19, 0.37 -0.43, 0.43
Beef trim <sup>o</sup> (375 g, BAX MP media, 24 h) <sup>o</sup>	<i>E. coli</i> O157:H7 QL 164673	N/A 0.62 (0.37, 0.94) 2.10 (1.13, 4.99)	5 20 5	0 9 5	0.00 0.45 0.20	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0.00 0.10 0.00	-0.43, 0.43 -0.19, 0.37 -0.43, 0.43
Beef trim (375 g, mTSB, QL 164673	<i>E. coli</i> O157:H7 QL 164673	N/A 0.68 (0.38, 1.14)	5 20	0 3	0.00 0.15	0.00, 0.43 0.05, 0.36	0 7	0.00 0.35	0.00, 0.43 0.18, 0.57	0.00 -0.20	-0.43, 0.43 -0.44, 0.07

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8 h)		6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim (375 g, mTSB, 10 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.68 (0.38, 1.14)	5 20	0 5	0.00 0.25	0.00, 0.43 0.11, 0.47	0 7	0.00 0.35	0.00, 0.43 0.18, 0.57	0.00 -0.10	-0.43, 0.43 -0.36, 0.18
Beef trim (375 g, mTSB, 24 h)	<i>E. coli</i> O157:H7 QL 164673	6.53 (2.30, 18.54) N/A 0.68 (0.38, 1.14)	5 5 20	5 0 6	1.00 0.00 0.30	0.57, 1.00 0.00, 0.43 0.15, 0.52	5 0 7	1.00 0.00 0.35	0.57, 1.00 0.00, 0.43 0.18, 0.57	0.00 0.00 -0.05	-0.43, 0.43 -0.43, 0.43 -0.32, 0.23
Leafy greens (375 g, BAX MP media, 6 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A 0.45 (0.22, 0.78) 3.70 (1.52, 9.02)	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 8 5	0.00 0.40 1.00	0.00, 0.43 0.22, 0.61 0.57, 1.00	0.00 0.05 0.00	-0.43, 0.43 -0.24, 0.33 -0.43, 0.43
Leafy greens (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A 0.45 (0.22, 0.78) 3.70 (1.52, 9.02)	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 8 5	0.00 0.40 1.00	0.00, 0.43 0.22, 0.61 0.57, 1.00	0.00 0.05 0.00	-0.43, 0.43 -0.24, 0.33 -0.43, 0.43
Leafy greens (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A 0.45 (0.22, 0.78) 3.70 (1.52, 9.02)	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 8 5	0.00 0.40 1.00	0.00, 0.43 0.22, 0.61 0.57, 1.00	0.00 0.05 0.00	-0.43, 0.43 -0.24, 0.33 -0.43, 0.43
Raw milk (25 g 2X BPW, 12 h)	<i>E. coli</i> O157:H7 QL 14077.4	N/A 0.34 (0.14, 0.61) 4.12 (1.58, 10.72)	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0.00 0.10 0.00	-0.43, 0.43 -0.19, 0.37 -0.43, 0.43
Raw milk (25 g 2X BPW, 20 h)	<i>E. coli</i> O157:H7 QL 14077.4	N/A 0.34 (0.14, 0.61) 4.12 (1.58, 10.72)	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0.00 0.10 0.00	-0.43, 0.43 -0.19, 0.37 -0.43, 0.43
Raw milk (25 g 2X BPW, 24 h)	<i>E. coli</i> O157:H7 QL 14077.4	N/A 0.34 (0.14, 0.61) 4.12 (1.58, 10.72)	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00, 0.43 0.18, 0.57 0.57, 1.00	0.00 0.10 0.00	-0.43, 0.43 -0.19, 0.37 -0.43, 0.43

<sup>a</sup>Results of the matrix study were analyzed as an unpaired study, with the exception of 25 g ground beef in mTSB, which was analyzed as a paired study.

<sup>b</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

<sup>c</sup>N = Number of test portions.

<sup>d</sup>x = Number of positive test portions.

<sup>e</sup>POD<sub>c</sub> = Candidate method presumptive positive outcomes confirmed positive confirmed by the reference method divided by the total number of trials.

<sup>f</sup>Reference method = MLG 5C.00 for ground beef and beef trim, BAM 4A for leafy greens and ISO 16654 for raw milk.

<sup>g</sup>POD<sub>R</sub> = Confirmed reference method positive outcomes divided by the total number of trials.

<sup>h</sup>dPOD<sub>c</sub> = Difference between the candidate method and reference method POD values.

<sup>i</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>j</sup>ATCC = American Type Culture Collection, Manassas, VA.

<sup>k</sup>Not applicable.

<sup>l</sup>QL = Q Laboratories, Cincinnati, OH.

<sup>m</sup>Matrix conducted in the method developer laboratory, Hygiena, New Castle, DE. All other matrices were conducted by the independent laboratory, Q Laboratories, Cincinnati, OH.

<sup>n</sup>DD = Qualicon Culture Collection Number, <sup>o</sup>Hygiena Culture Collection, New Castle, DE.

<sup>p</sup>Repeat Beef trim study completed by Independent Laboratory.

Table 8. BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact presumptive vs. confirmed results (confirmed by BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact assay) (1)

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	BAX Exact presumptive			BAX Exact confirmed by BAX® Exact				
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI	dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
Ground beef (25 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 43895	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (25 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (25 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.11, 0.21
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (25 g, mTSB media, 8 h)	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (25 g, mTSB media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.21, 0.11
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (25 g, mTSB media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, mTSB media, 8 h)	<i>E. coli</i> O157:H7 ATCC <sup>h</sup> 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, mTSB media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, mTSB media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	7	0.40	0.22, 0.61	7	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 QL <sup>i</sup> 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.68 (0.38, 1.14)	20	0	0.00	0.00, 0.16	6	0.30	0.15, 0.52	-0.30	-0.54, -0.06
		6.53 (2.30, 18.54)	5	1	0.20	0.00, 0.62	5	1.00	0.57, 1.00	-0.80	-1.00, -0.16
Beef trim (375 g, BAX MP	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.68 (0.38, 1.14)	20	2	0.10	0.03, 0.30	6	0.30	0.15, 0.52	-0.20	-0.41, -0.01

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media, 10 h)		6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.68 (0.38, 1.14) 6.53 (2.30, 18.54)	5 20 5	0 6 5	0.00 0.30 1.00	0.00, 0.43 0.15, 0.52 0.57, 1.00	0 6 5	0.00 0.30 1.00	0.00, 0.43 0.15, 0.52 0.57, 1.00	0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Beef trim <sup>k</sup> (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 DD13078 <sup>l</sup>	N/A 0.83 (0.50, 1.4) 1.5 (0.75, 2.75)	5 20 5	0 11 5	0.00 0.55 1.00	0.00, 1.00 0.34, 0.74 0.57, 1.00	0 10 5	0.00 0.50 1.00	0.00, 0.43 0.30, 0.70 0.57, 1.00	0.00 0.05 0.00	-0.47, 0.47 -0.11, 0.21 -0.47, 0.47
Beef trim <sup>k</sup> (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 DD13078	N/A 0.83 (0.50, 1.4) 1.5 (0.75, 2.75)	5 20 5	0 10 5	0.00 0.50 1.00	0.00, 1.00 0.30, 0.70 0.57, 1.00	0 10 5	0.00 0.50 1.00	0.00, 0.43 0.34, 0.74 0.57, 1.00	0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Beef trim <sup>k</sup> (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 DD13078	N/A 0.83 (0.50, 1.4) 1.5 (0.75, 2.75)	5 20 5	0 10 5	0.00 0.50 1.00	0.00, 1.00 0.30, 0.70 0.57, 1.00	0 10 5	0.00 0.50 1.00	0.00, 0.43 0.34, 0.74 0.57, 1.00	0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Beef trim <sup>m</sup> (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.62 (0.37, 0.94) 2.10 (1.13, 4.99)	5 20 5	0 9 5	0.00 0.45 0.20	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Beef trim <sup>m</sup> (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.62 (0.37, 0.94) 2.10 (1.13, 4.99)	5 20 5	0 9 5	0.00 0.45 0.20	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Beef trim <sup>m</sup> (375 g, BAX MP media, 12 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.62 (0.37, 0.94) 2.10 (1.13, 4.99)	5 20 5	0 9 5	0.00 0.45 0.20	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Beef trim <sup>m</sup> (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.62 (0.37, 0.94) 2.10 (1.13, 4.99)	5 20 5	0 9 5	0.00 0.45 0.20	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Beef trim (375 g, mTSB, 8 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.68 (0.38, 1.14) 6.53 (2.30, 18.54)	5 20 5	0 4 5	0.00 0.20 1.00	0.00, 0.43 0.08, 0.42 0.57, 1.00	0 6 5	0.00 0.30 1.00	0.00, 0.43 0.15, 0.52 0.57, 1.00	0.00	-0.47, 0.47 -0.28, 0.08 -0.47, 0.47
Beef trim (375 g, mTSB, 10 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.68 (0.38, 1.14) 6.53 (2.30, 18.54)	5 20 5	0 5 5	0.00 0.25 1.00	0.00, 0.43 0.11, 0.47 0.57, 1.00	0 6 5	0.00 0.30 1.00	0.00, 0.43 0.15, 0.52 0.57, 1.00	0.00	-0.47, 0.47 -0.21, 0.11 -0.47, 0.47
Beef trim (375 g, mTSB, 24 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.68 (0.38, 1.14) 6.53 (2.30, 18.54)	5 20 5	0 6 5	0.00 0.30 1.00	0.00, 0.43 0.15, 0.52 0.57, 1.00	0 6 5	0.00 0.30 1.00	0.00, 0.43 0.15, 0.52 0.57, 1.00	0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Leafy greens (375 g, BAX MP media, 6 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A 0.45 (0.22, 0.78) 3.70 (1.52, 9.02)	5 20 5	0 10 5	0.00 0.50 1.00	0.00, 0.43 0.30, 0.70 0.57, 1.00	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0.00	-0.47, 0.47 -0.11, 0.21 -0.47, 0.47
Leafy greens (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A 0.45 (0.22, 0.78) 3.70 (1.52, 9.02)	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Leafy greens (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A 0.45 (0.22, 0.78) 3.70 (1.52, 9.02)	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0 9 5	0.00 0.45 1.00	0.00, 0.43 0.26, 0.66 0.57, 1.00	0.00	-0.47, 0.47 -0.13, 0.13 -0.47, 0.47
Raw milk (25 g 2X BPW, 12	<i>E. coli</i> O157:H7 QL 14077.4	N/A 0.34 (0.14, 0.61)	5 20	0 10	0.00 0.50	0.00, 0.43 0.30, 0.70	0 9	0.00 0.45	0.00, 0.43 0.26, 0.66	0.00 0.05	-0.47, 0.47 -0.11, 0.21

h)		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw milk (25 g 2X BPW, 20 h)	<i>E. coli</i> O157:H7 QL 14077.4	N/A 0.34 (0.14, 0.61)	5 20	0 10	0.00 0.50	0.00, 0.43 0.30, 0.70	0 9	0.00 0.45	0.00, 0.43 0.26, 0.66	0.00 0.05	-0.47, 0.47 -0.11, 0.21
h)		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw milk (25 g 2X BPW, 24 h)	<i>E. coli</i> O157:H7 QL 14077.4	N/A 0.34 (0.14, 0.61)	5 20	0 9	0.00 0.45	0.00, 0.43 0.26, 0.66	0 9	0.00 0.45	0.00, 0.43 0.26, 0.66	0.00 0.00	-0.47, 0.47 -0.13, 0.13
h)		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>x = Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>CC</sub> = Candidate method presumptive positive outcomes confirmed by BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact assay divided by the total number of trials.

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>ATCC = American Type Culture Collection, Manassas, VA.

<sup>i</sup>Not applicable.

<sup>j</sup>QL = Q Laboratories, Cincinnati, OH.

<sup>k</sup>Matrix conducted in the method developer laboratory, Hygiena, New Castle, DE. All other matrixes were conducted by the independent laboratory, Q Laboratories, Cincinnati, OH.

<sup>l</sup>DD = Qualicon Culture Collection Number, Hygiena Culture Collection, New Castle, DE.

<sup>m</sup>Repeat Beef trim study completed by Independent Laboratory.

**Table 9. BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact results (confirmed by BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact) vs. Reference Method results (1)**

Matrix <sup>a</sup>	Strain	MPN <sup>b</sup> /test portion	N <sup>c</sup>	BAX Exact results confirmed			Reference <sup>f</sup> method results			dPOD <sub>CP</sub> <sup>h</sup>	95% CI <sup>i</sup>
				X <sup>d</sup>	POD <sub>CC</sub> <sup>e</sup>	95% CI	x	POD <sub>R</sub> <sup>g</sup>	95% CI		
Ground beef (25 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A <sup>k</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (25 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (25 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef <sup>o</sup> (25 g, mTSB, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef <sup>o</sup> (25 g, mTSB, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.21, 0.11
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

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Ground beef <sup>a</sup> (25 g, mTSB, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef (375 g, mTSB, 8 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (375 g, mTSB, 10 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef (375 g, mTSB, 24 h)	<i>E. coli</i> O157:H7 ATCC 43895	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.39 (0.17, 0.68)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
		4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.68 (0.38, 1.14)	20	0	0.00	0.00, 0.16	7	0.35	0.18, 0.57	-0.35	-0.57, -0.12
		6.53 (2.30, 18.54)	5	1	0.20	0.00, 0.62	5	1.00	0.57, 1.00	-0.80	-1.00, -0.19
Beef trim (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.68 (0.38, 1.14)	20	0	0.00	0.00, 0.16	7	0.35	0.18, 0.57	-0.35	-0.57, -0.12
		6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.68 (0.38, 1.14)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim <sup>m</sup> (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 DD13078 <sup>n</sup>	N/A	5	0	0.00	0.00, 1.00	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.83 (0.50, 1.4)	20	10	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		1.5 (0.75, 2.75)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim <sup>m</sup> (375 g, BAX MP media, 10 h)	<i>E. coli</i> O157:H7 DD13078	N/A	5	0	0.00	0.00, 1.00	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.83 (0.50, 1.4)	20	10	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		1.5 (0.75, 2.75)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim <sup>m</sup> (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 DD13078	N/A	5	0	0.00	0.00, 1.00	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.83 (0.50, 1.4)	20	10	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		1.5 (0.75, 2.75)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim <sup>o</sup> (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 QL <sup>n</sup> 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.62 (0.37, 0.94)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		2.10 (1.13, 4.99)	5	5	0.20	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim <sup>o</sup> (375 g, BAX MP media, 10 h) <sup>o</sup>	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.62 (0.37, 0.94)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		2.10 (1.13, 4.99)	5	5	0.20	0.57, 1.00	5	1.00	0.57, 1.00	-0.80	-1.00, -0.19
Beef trim <sup>o</sup> (375 g, BAX MP media, 12 h) <sup>o</sup>	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.62 (0.37, 0.94)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		2.10 (1.13, 4.99)	5	5	0.20	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim <sup>o</sup> (375 g, BAX MP media, 24 h) <sup>o</sup>	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.62 (0.37, 0.94)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		2.10 (1.13, 4.99)	5	5	0.20	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim (375 g, mTSB, QL 164673	<i>E. coli</i> O157:H7 QL 164673	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.68 (0.38, 1.14)	20	3	0.15	0.05, 0.36	7	0.35	0.18, 0.57	-0.20	-0.44, 0.07



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8 h)		6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim (375 g, mTSB, 10 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.68 (0.38, 1.14) 6.53 (2.30, 18.54)	5 20	0 5	0.00 0.25	0.00, 0.43 0.11, 0.47	0 7	0.00 0.35	0.00, 0.43 0.18, 0.57	0.00 -0.10	-0.43, 0.43 -0.36, 0.18
Beef trim (375 g, mTSB, 24 h)	<i>E. coli</i> O157:H7 QL 164673	N/A 0.68 (0.38, 1.14) 6.53 (2.30, 18.54)	5 20	0 6	0.00 0.30	0.00, 0.43 0.15, 0.52	0 7	0.00 0.35	0.00, 0.43 0.18, 0.57	0.00 -0.05	-0.43, 0.43 -0.32, 0.23
Leafy greens (375 g, BAX MP media, 6 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A 0.45 (0.22, 0.78) 3.70 (1.52, 9.02)	5 20	0 9	0.00 0.45	0.00, 0.43 0.26, 0.66	0 8	0.00 0.40	0.00, 0.43 0.22, 0.61	0.00 0.05	-0.43, 0.43 -0.24, 0.33
Leafy greens (375 g, BAX MP media, 8 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A 0.45 (0.22, 0.78) 3.70 (1.52, 9.02)	5 20	0 9	0.00 0.45	0.00, 0.43 0.26, 0.66	0 8	0.00 0.40	0.00, 0.43 0.22, 0.61	0.00 0.05	-0.43, 0.43 -0.24, 0.33
Leafy greens (375 g, BAX MP media, 24 h)	<i>E. coli</i> O157:H7 ATCC BAA-460	N/A 0.45 (0.22, 0.78) 3.70 (1.52, 9.02)	5 20	0 9	0.00 0.45	0.00, 0.43 0.26, 0.66	0 8	0.00 0.40	0.00, 0.43 0.22, 0.61	0.00 0.05	-0.43, 0.43 -0.24, 0.33
Raw milk (25 g 2X BPW, 12 h)	<i>E. coli</i> O157:H7 QL 14077.4	N/A 0.34 (0.14, 0.61) 4.12 (1.58, 10.72)	5 20	0 9	0.00 0.45	0.00, 0.43 0.26, 0.66	0 7	0.00 0.35	0.00, 0.43 0.18, 0.57	0.00 0.10	-0.43, 0.43 -0.19, 0.37
Raw milk (25 g 2X BPW, 20 h)	<i>E. coli</i> O157:H7 QL 14077.4	N/A 0.34 (0.14, 0.61) 4.12 (1.58, 10.72)	5 20	0 9	0.00 0.45	0.00, 0.43 0.26, 0.66	0 7	0.00 0.35	0.00, 0.43 0.18, 0.57	0.00 0.10	-0.43, 0.43 -0.19, 0.37
Raw milk (25 g 2X BPW, 24 h)	<i>E. coli</i> O157:H7 QL 14077.4	N/A 0.34 (0.14, 0.61) 4.12 (1.58, 10.72)	5 20	0 9	0.00 0.45	0.00, 0.43 0.26, 0.66	0 7	0.00 0.35	0.00, 0.43 0.18, 0.57	0.00 0.10	-0.43, 0.43 -0.19, 0.37

<sup>a</sup>Results of the matrix study were analyzed as an unpaired study, with the exception of 25 g ground beef in mTSB, which was analyzed as a paired study.

<sup>b</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

<sup>c</sup>N = Number of test portions.

<sup>d</sup>x = Number of positive test portions.

<sup>e</sup>POD<sub>c</sub> = Candidate method presumptive positive outcomes confirmed positive by BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact assay divided by the total number of trials.

<sup>f</sup>Reference method = MLG 5C.00 for ground beef and beef trim, BAM 4A for leafy greens and ISO 16654 for raw milk.

<sup>g</sup>Reference method = MLG 5C.00 for ground beef and beef trim, BAM 4A for leafy greens and ISO 16654 for raw milk.

<sup>h</sup>POD<sub>R</sub> = Confirmed reference method positive outcomes divided by the total number of trials.

<sup>i</sup>dPOD<sub>c</sub> = Difference between the candidate method and reference method POD values.

<sup>j</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>k</sup>ATCC = American Type Culture Collection, Manassas, VA.

<sup>l</sup>Not applicable.

<sup>m</sup>QL = Q Laboratories, Cincinnati, OH.

<sup>n</sup>Matrix conducted in the method developer laboratory, Hygiena, New Castle, DE. All other matrixes were conducted by the independent laboratory, Q Laboratories, Cincinnati, OH.

<sup>o</sup>DD = Qualicon Culture Collection Number, <sup>p</sup>Hygiena Culture Collection, New Castle, DE.

<sup>q</sup>Repeat Beef trim study completed by Independent Laboratory.

**DISCUSSION OF MODIFICATION APPROVED JANUARY 2022 (5)**

The BAX System Real-time PCR Assay successfully detected the target STEC species in dried cannabis flower and dried hemp flower at a 10 g sample size. Difference in POD analysis for the presumptive versus confirmed positives showed no statistically significant differences, with all ranges of the 95% confidence intervals containing the zero point.

According to independent laboratory feedback, processing samples for these assays was very user friendly with a standard heat dependent lysis step and transfer into pre-aliquoted lyophilized pellets in PCR wells. Short run times on the instrument helped improve throughput for processing samples.

**Table 2. Matrix study: *E. coli* O157:H7 Exact presumptive vs. confirmed results in dried cannabis flower (>0.3% THC) and dried hemp flower (≤0.3% THC) (5)**

Matrix and Inoculum	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	x <sup>c</sup>	Presumptive		x	Confirmed		dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
				POD <sub>cp</sub> <sup>d</sup>	95% CI		POD <sub>cc</sub> <sup>e</sup>	95% CI		
Dried cannabis flower	NA <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
10 g ( <i>E. coli</i> O157:H7	0.88 (0.40, 2.02)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
ATCC <sup>h</sup> 43895)	2.96 (1.54, 9.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Dried hemp Flower	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
10 g ( <i>E. coli</i> O157:H7	1.48 (0.77, 3.74)	20	14	0.70	0.48, 0.86	14	0.70	0.48, 0.86	0.00	(-0.13, 0.13)
ATCC 43890)	6.77 (3.95, 16.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>x = Number of positive test portions.

<sup>d</sup>POD<sub>cp</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>cc</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.

<sup>f</sup>dPOD<sub>cp</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>American Type Culture Collection, Manassas, VA.

<sup>i</sup>Not applicable.

**DISCUSSION OF THE MODIFICATION STUDY APPROVED APRIL 2023 (8)**

The BAX System Real-time PCR Assay for *E. coli* O157:H7 Exact successfully detected the target STEC species in beef trim sampling cloths at a 375 g test portion size. Although the initial study MP media data set showed 9 presumptive positives at 8 h but 10 presumptive positives at 10 and 24 h, subsequent repeat studies demonstrated the method's ability to detect all presumptive positives at 8, 10 and 24 h. All presumptive positives in these second sets of data also confirmed positive. The study data were unable to find a statistical difference between *E. coli* O157:H7 Exact methods' presumptive and confirmed results, nor between the *E. coli* O157:H7 Exact and the MLG 5C.03 reference method results with 95% confidence.

**Table 3. Matrix study: BAX Real-time PCR Assay for *E. coli* O157:H7 Exact presumptive vs. confirmed results in beef trim (375 g) sampling cloths (8)**

Matrix and Inoculum	cfu <sup>a</sup> / Test Portion	N <sup>b</sup>	x <sup>c</sup>	Presumptive		x	Confirmed		dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
				POD <sub>cp</sub> <sup>d</sup>	95% CI		POD <sub>cc</sub> <sup>e</sup>	95% CI		
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 ATCC 43895 <sup>h</sup> ) 8 h, MP media	NA <sup>i</sup>	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	(-0.47, 0.47)
	0.72	20	9	0.45	0.26, 0.66	10	0.50	0.30, 0.70	-0.05	(-0.21, 0.11)
	5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 ATCC 43895 <sup>h</sup> ) 10 h, MP media	NA	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	(-0.47, 0.47)
	0.72	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
	5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 ATCC 43895 <sup>h</sup> ) 24 h, MP media	NA	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	(-0.47, 0.47)
	0.72	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
	5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

<sup>a</sup>cfu/test portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>x = Number of positive test portions.

<sup>d</sup>POD<sub>cp</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>cc</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.

<sup>f</sup>dPOD<sub>cp</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>American Type Culture Collection, Manassas, VA.

<sup>i</sup>Not applicable.

**Table 4. BAX Real-time PCR Assay for *E. coli* O157:H7 Exact method vs. reference method results in beef trim (375 g size) sampling cloths (8)**

Matrix and Inoculum	cfu <sup>a</sup> / Test Portion	N <sup>b</sup>	x <sup>c</sup>	BAX Method		x	Reference Method		dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
				POD <sub>c</sub> <sup>d</sup>	95% CI		POD <sub>R</sub> <sup>e</sup>	95% CI		
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 ATCC 43895 <sup>h</sup> ) 8 h, MP media <sup>i</sup>	NA <sup>i</sup>	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	(-0.43, 0.43)
	0.72	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	(-0.24, 0.33)
	5	5	5	1.00	0.57, 1.00	4	0.80	0.38, 0.96	0.20	(-0.26, 0.62)
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 ATCC 43895 <sup>h</sup> ) 10 h, MP media	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43, 0.43)
	0.72	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	(-0.19, 0.37)
	5	5	5	1.00	0.57, 1.00	4	0.80	0.38, 0.96	0.20	(-0.28, 0.62)
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 ATCC 43895 <sup>h</sup> ) 24 h, MP media	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43, 0.43)
	0.72	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	(-0.19, 0.37)
	5	5	5	0.80	0.38, 0.96	4	0.80	0.38, 0.96	0.20	(-0.28, 0.62)
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 ATCC 43895 <sup>h</sup> ) 8 h, mTSB+caa <sup>k</sup>	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
	0.72	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	(-0.13, 0.13)
	5	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.20	(-0.47, 0.47)
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 ATCC 43895 <sup>h</sup> ) 24 h, mTSB+caa	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
	0.72	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	(-0.13, 0.13)
	5	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.20	(-0.47, 0.47)

<sup>a</sup>cfu/portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>X = Number of positive test portions.

<sup>d</sup>POD<sub>c</sub> = Confirmed candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

<sup>e</sup>POD<sub>R</sub> = Confirmed reference method positive outcomes divided by the total number of trials.

<sup>f</sup>dPOD<sub>c</sub> = Difference between the candidate method and reference method POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>American Type Culture Collection, Manassas, VA

<sup>i</sup>Results calculated using unpaired POD statistical analysis.

<sup>j</sup>Not applicable.

<sup>k</sup>Results calculated using paired POD statistical analysis.

**Table 5. Additional Matrix study 1: BAX Real-time PCR Assay for *E. coli* O157:H7 Exact presumptive vs. confirmed results in beef trim (375 g) sampling cloths (8)**

Matrix and Inoculum	cfu <sup>a</sup> / Test Portion	N <sup>b</sup>	x <sup>c</sup>	Presumptive		x	Confirmed	
				POD <sub>cp</sub> <sup>d</sup>	95% CI		POD <sub>cc</sub> <sup>e</sup>	95% CI
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 DD1980 <sup>h</sup> ) 8 h, MP media	NA <sup>i</sup> 0.63 5.42	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.00 0.26, 0.66 0.57, 1.00	0 9 5	0.00 0.45 1.00	0.00, 0.00 0.26, 0.66 0.57, 1.00
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 DD1980) 10 h, MP media	NA 0.63 5.42	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.00 0.26, 0.66 0.57, 1.00	0 9 5	0.00 0.45 1.00	0.00, 0.00 0.26, 0.66 0.57, 1.00
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 DD1980) 24 h, MP media	NA 0.63 5.42	5 20 5	0 9 5	0.00 0.45 1.00	0.00, 0.00 0.26, 0.66 0.57, 1.00	0 9 5	0.00 0.45 1.00	0.00, 0.00 0.26, 0.66 0.57, 1.00

<sup>a</sup>cfu/test portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.<sup>b</sup>N = Number of test portions.<sup>c</sup>x = Number of positive test portions.<sup>d</sup>POD<sub>cp</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.<sup>e</sup>POD<sub>cc</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.<sup>f</sup>dPOD<sub>cp</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.<sup>h</sup>Hygiene Culture Collection, New Castle, DE, USA<sup>i</sup>Not applicable.**Table 6. Additional Matrix study 2: BAX Real-time PCR Assay for *E. coli* O157:H7 Exact presumptive vs. confirmed results in beef trim (375 g size) sampling cloths (8)**

Matrix and Inoculum	cfu <sup>a</sup> / Test Portion	N <sup>b</sup>	x <sup>c</sup>	Presumptive		x	Confirmed		dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
				POD <sub>cp</sub> <sup>d</sup>	95% CI		POD <sub>cc</sub> <sup>e</sup>	95% CI		
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 DD8873 <sup>h</sup> ) 8 h, MP media	NA <sup>i</sup> 0.62 5.3	5 20 5	0 7 5	0.00 0.35 1.00	0.00, 0.00 0.18, 0.57 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00 0.18, 0.57 0.57, 1.00	0.00	(-0.47, 0.47) (-0.13, 0.13) (-0.47, 0.47)
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 DD8873) 10 h, MP media	NA 0.62 5.3	5 20 5	0 7 5	0.00 0.35 1.00	0.00, 0.00 0.18, 0.57 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00 0.18, 0.57 0.57, 1.00	0.00	(-0.47, 0.47) (-0.13, 0.13) (-0.47, 0.47)
Beef trim Sampling cloth ( <i>E. coli</i> O157:H7 DD8873) 24 h, MP media	NA 0.62 5.3	5 20 5	0 7 5	0.00 0.35 1.00	0.00, 0.00 0.18, 0.57 0.57, 1.00	0 7 5	0.00 0.35 1.00	0.00 0.18, 0.57 0.57, 1.00	0.00	(-0.47, 0.47) (-0.13, 0.13) (-0.47, 0.47)

<sup>a</sup>cfu/test portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.<sup>b</sup>N = Number of test portions.<sup>c</sup>x = Number of positive test portions.<sup>d</sup>POD<sub>cp</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.<sup>e</sup>POD<sub>cc</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.<sup>f</sup>dPOD<sub>cp</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.<sup>h</sup>Hygiene Culture Collection, New Castle, DE, USA<sup>i</sup>Not applicable.**REFERENCES CITED**

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