CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

101901

The AOAC Research Institute hereby certifies the method known as

Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq

manufactured by

BioControl Systems, Inc./MilliporeSigma
12822 SE 32nd St.
Bellevue, WA 98005 USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*℠ 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*℠ 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, AOAC Research Institute Senior Director

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SUBMITTING COMPANY
BioControl Systems, Inc./MilliporeSigma
12822 SE 32nd St.
Bellevue, WA 98005

METHODS NAME
Assurance® GDS EHEC ID for *E. coli O*157:H7 Tq

CATALOG NUMBER
71037-52 (52 tests)

INDEPENDENT LABORATORY
Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204

APPLICABILITY OF METHOD
Target Organism – *Escherichia coli* O157:H7; *E. coli* O157:NM

Matrixes – raw ground beef (375 g, ~85% lean), raw beef trim (375 g, ~85% lean), frozen finely textured beef (375 g, 97% lean), carcass cloths

Performance claims – The Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq (analyzed by either hand processing or automated PIPETMAX) is considered equivalent to the reference method, the U. S. Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) *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 raw beef, finely textured beef, and carcass cloths.

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

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

AOAC International, *Evaluation of the Assurance® GDS for E. coli O157:H7 method and Assurance® GDS for shiga toxin genes method in selected foods: collaborative study.*, Journal of AOAC International, 88, 1334, (4)

ORIGINAL CERTIFICATION DATE
October 21, 2019

CERTIFICATION RENEWAL RECORD
Renewed through December 2027.

METHOD MODIFICATION RECORD
1. **January 2020 Level 2**
2. **January 2021 Level 1**
3. **November 2021 Level 1**
4. **January 2023 Level 1**

SUMMARY OF MODIFICATION
1. **mEHEC media and mEHEC+novobiocin manufacturing location change to Darmstadt, Germany and rebranding to Merck format.**
2. **Editorial changes for clarity.**
3. **Editorial changes and update to software.**
4. **Editorial changes.**

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PRINCIPLE OF THE METHOD (1)
The Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq (EHEC ID) is a test method used in conjunction with combination of both an upfront PCR assay which detects the *eae* gene of *Escherichia coli* O157:H7, such as Assurance GDS for *E. coli* O157:H7 Tq (GDS O157) method and an antibody capturing system to concentrate the targeted organisms. EHEC ID provides specific identification of *E. coli* O157:H7 by recognizing the presence of four gene targets: O157, H7, and the two Shiga toxin genes. The assay is based on DNA amplification and detection by real-time PCR. The automatic nucleic acid amplification system is used for the genetic confirmation of pathogenic *E. coli* O157:H7. The system consists of antibody coated magnetic particles to capture the target organisms from the enriched sample (IMS). The PickPen® device quickly and easily collects the magnetic IMS particles and transfers the concentrated target (maximum 8 samples at a time). With the use of the antibody coated magnetic particles and the PickPen device, the target organism is concentrated to makes more DNA available for analysis, allowing for a shorter enrichment. The patented probes and primers utilize highly conserved target gene sequences.
The EHEC ID assay is a secondary assay designed to detect *E. coli* O157:H7 based on U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) Microbiology Laboratory Guidebook (MLG) definitions (2) and U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) definitions (3) for genetic confirmation following a positive result using previously validated GDS O157 assay, AOAC® OMA℠ **2005.04** (4).
The Assurance GDS Rotor-Gene® is a centrifugal air-exchange rotary thermal PCR cycler that allows constant rotary motion and overcomes the heat equilibrium issues inherent in conventional Peltier block systems. It also eliminates dwell time, resulting in faster results.
The Assurance GDS PIPETMAX® (PPMX) is an automated pipetting solution for the efficient processing of high-throughput biological assays. The PPMX machine will robotically capture target organisms from the enrichment using the PickPen IMS device and then aliquot directly into the amplification tubes.

DISCUSSION OF THE VALIDATION STUDY (1)

Results of these studies confirmed that EHEC ID method is inclusive and specific for *E*. *coli* O157:H7. In the internal method comparison study, EHEC ID and the reference culture methods are statistically comparable for the detection of *E*. *coli* O157:H7 in raw beef trim and raw ground beef. POD statistical analysis of the samples indicated that for all foods analyzed, there was no statistical difference between the EHEC ID presumptive compared to the EHEC ID confirmed. The external method comparison study yielded similar results for raw ground beef. The alternative method (enrichment media) recovered statistically better for frozen finely textured beef and for carcass cloths. The mEHEC broth is more selective compared to the mTSB media. This greater selectivity by mEHEC may be the reason that the mEHEC broth recovered more *E*. *coli* O157:H7 positive samples compared to the reference method.

In this study, we observed 100% inclusivity among the 50 strains of *E*. *coli* O157:H7 and 100% exclusivity for the 100 strains of non- *E*. *coli* O157:H7 analyzed. These results demonstrate that EHEC ID is an effective method for the detection of *E*. *coli* O157:H7 in raw beef trim, raw ground beef, frozen finely textured beef, and on carcass cloths. During the robustness evaluation, the change to the operational parameters of the method indicated the method is robust and had no negative impact on results.

The EHEC ID may also be used to confirm the presence of an *E*. *coli* O157:H7 organism from an isolated colony. Fifty different *E*. *coli* O157:H7 organisms were plated onto isolation agars and a non-selective plating agar. From all the agars tested, the EHEC ID kit was able to properly identify all 50 *E*. *coli* O157:H7 organisms. All non-target organisms were properly determined not to be an *E*. *coli* O157:H7 isolate. The Independent Laboratory study yielded similar results. These results demonstrate EHEC ID can be used as a confirmation procedure for *E*. *coli* O157:H7 isolates from plating agar.

The Assurance GDS PPMX is an automated pipetting solution for the efficient processing of high-throughput samples. The PPMX machine will robotically capture target organisms from the enrichment using the PickPen device and then aliquot directly into the amplification tubes. POD statistical analyses of the samples indicated that for all foods analyzed, there was no statistical difference between the EHEC ID presumptive positives using the PPMX compared to the EHEC ID confirmed positives. These results demonstrate the PPMX machine may be used instead of manual (hand) processing of samples in preparation for PCR analysis.

GDS samples may also be IMS-concentrated and plated onto selective agar for *E*. *coli* O157:H7 using the PickPen® device and O157 Concentration Reagent. Results of these confirmation samples matched the reference samples, except for FTB, where three additional samples were confirmed to contain for *E*. *coli* O157:H7 using the PickPen device compared to the reference method.

Table 1: EHEC ID Inclusivity Results for *E. coli* O157:H7 – from mEHEC Broth (1)

INCLUSIVITY					Genotype			
					GDS EHEC [g]	GDS EHEC ID [h]		
N°	Organism	Serotype	Source	Origin	*eae*	O157	H7	*stx1* / *stx2*
1	*Escherichia coli*	O157:H7	ATCC 35150 [a]	Clinical isolate	+ [i]	+	+	+
2	*Escherichia coli*	O157:H7	ATCC 43895	Beef	+	+	+	+
3	*Escherichia coli*	O157:H7	ATCC 43890	Clinical isolate	+	+	+	+
4	*Escherichia coli*	O157:H7	ATCC 43894	Clinical isolate	+	+	+	+
5	*Escherichia coli*	O157:H7	OPHD [b]	Clinical isolate	+	+	+	+
6	*Escherichia coli*	O157:H7	OPHD	Clinical isolate	+	+	+	+
7	*Escherichia coli*	O157:H7	OPHD	Clinical isolate	+	+	+	+
8	*Escherichia coli*	O157:H7	OPHD	Clinical isolate	+	+	+	+
9	*Escherichia coli*	O157:H7	OPHD	Clinical isolate	+	+	+	+
10	*Escherichia coli*	O157:H7	PSU 93.0058 [c]	beef	+	+	+	+
11	*Escherichia coli*	O157:H7	PSU 88.0222	Clinical isolate	+	+	+	+
12	*Escherichia coli*	O157:H7	PSU 93.0054	beef	+	+	+	+
13	*Escherichia coli*	O157:H7	PSU Deca 3B	Clinical isolate	+	+	+	+
14	*Escherichia coli*	O157:H7	Es 190 [d]	Unknown	+	+	+	+
15	*Escherichia coli*	O157:H7	Es 191	Unknown	+	+	+	+
16	*Escherichia coli*	O157:H7	Es 192	Unknown	+	+	+	+
17	*Escherichia coli*	O157:H7	Es 194	Unknown	+	+	+	+
18	*Escherichia coli*	O157:H7	Es 195	Unknown	+	+	+	+
19	*Escherichia coli*	O157:H7	Es 189	Beef	+	+	+	+
20	*Escherichia coli*	O157:H7	Es 537	Beef	+	+	+	+
21	*Escherichia coli*	O157:H7	Es 538	Beef	+	+	+	+
22	*Escherichia coli*	O157:H7	Es 567	Beef	+	+	+	+
23	*Escherichia coli*	O157:H7	Es 568	Beef	+	+	+	+
24	*Escherichia coli*	O157:H7	Es 579	Unknown	+	+	+	+
25	*Escherichia coli*	O157:H7	Es 620	Beef	+	+	+	+
26	*Escherichia coli*	O157:H7	Es 621	Beef	+	+	+	+
27	*Escherichia coli*	O157:H7	Es 684	Beef	+	+	+	+
28	*Escherichia coli*	O157:H7	Es 685	Beef	+	+	+	+
29	*Escherichia coli*	O157:H7	Es 216	Unknown	+	+	+	+
30	*Escherichia coli*	O157:H7	Es 219	Beef	+	+	+	+
31	*Escherichia coli*	O157:H7	Es 242	Beef	+	+	+	+
32	*Escherichia coli*	O157:H7	Es 333	Beef	+	+	+	+
33	*Escherichia coli*	O157:H7	Es334	Beef	+	+	+	+
34	*Escherichia coli*	O157:H7	Es 355	Beef	+	+	+	+
35	*Escherichia coli*	O157:H7	Es 545	Beef	+	+	+	+
36	*Escherichia coli*	O157:H7	FDA [e]	Unknown	+	+	+	+
37	*Escherichia coli*	O157:H7 (NM)	ATCC 700376	Clinical isolate	+	+	+	+
38	*Escherichia coli*	O157:H7 (NM)	ATCC 700377	Clinical isolate	+	+	+	+
39	*Escherichia coli*	O157:H7 (NM)	ATCC 700378	Clinical isolate	+	+	+	+
40	*Escherichia coli*	O157:H7 (NM)	PSU DR3	Beef	+	+	+	+
41	*Escherichia coli*	O157:H7 (NM)	Es 351	Beef	+	+	+	+
42	*Escherichia coli*	O157:H7 (NM)	USDA-ARS 55AC1 [f]	Meat	+	+	+	+
43	*Escherichia coli*	O157:H7 (NM)	USDA-ARS 131AC1	Meat	+	+	+	+
44	*Escherichia coli*	O157:H7 (NM)	USDA-ARS 237AC1	Meat	+	+	+	+
45	*Escherichia coli*	O157:H7 (NM)	USDA-ARS 299AB3	Meat	+	+	+	+
46	*Escherichia coli*	O157:H7 (rough)	FDA MA6	Meat	+	+	+	+
47	*Escherichia coli*	O157:H7 (non-STX producing)	ATCC 43888	Clinical isolate	+	+	+	-
48	*Escherichia coli*	O157:H7 (non-STX producing)	ATCC 700728	Unknown	+	+	+	-
49	*Escherichia coli*	O157:H7 (non-STX producing)	ES 340	Unknown	+	+	+	-
50	*Escherichia coli*	O157:H7 (non-STX producing)	USDA-ARS 114AC1	Meat	+	+	+	-

[a] ATCC = American Type Culture collection (Manassas, VA).
[b] OPHD = Oregon Public Health Division (Portland, OR).
[c] PSU = *E. coli* Reference Center (Penn State University, University Park, PA).
[d] "ES" is the identification for individual *E. coli* strains in MilliporeSigma culture collection (Bellevue, WA).
[e] FDA = Food and Drug Administration (White Oak, MD).
[f] USDA-ARS = U.S. Department of Agriculture – Agricultural Research Service (Beltsville, MD).
[g] GDS EHEC = Assurance GDS for *E. coli* O157:H7 Tq (*eae*).
[h] GDS EHEC ID = Assurance GDS EHEC ID for *E. coli* O157:H7 Tq (O157, H7, *stx*1/*stx*2).
[i] '+' is GDS assay positive and '-' is GDS assay negative. These PCR results match the previously characterized biochemical and genetic profiles.

Table 2: EHEC ID Exclusivity Results for Non- *E. coli* O157:H7 Organisms – from non-Selective BHI Broth (1)

EXCLUSIVITY					Genotype			
					GDS EHEC [k]	GDS EHEC ID [l]		
N°	Organism	Serotype	Source	Origin	*eae*	O157	H7	*stx1* / *stx2*
— 25 strains from non-target family, [SORTED BY GENUS]								
1	*Aerococcus viridans*		Ao 7 [a]	shrimp	-	-	-	-
2	*Aeromonas hydrophila*		ATCC 35654 [b]	water	-	-	-	-
3	*Alcaligenes faecalis*		ATCC 15246	unknown	-	-	-	-
4	*Bacillus cereus*		ATCC 14579	air	-	-	-	-
5	*Bacillus pumilus*		University of Washington	unknown	-	-	-	-
6	*Bacillus subtilis*		ATCC 6051	unknown	-	-	-	-
7	*Brevibacterium linens*		ATCC 9172	cheese	-	-	-	-
8	*Candida albicans*		ATCC 24433	clinical isolate	-	-	-	-
9	*Enterococcus faecalis*		ATCC 33186	unknown	-	-	-	-
10	*Enterococcus faecium*		ATCC 19434	unknown	-	-	-	-
11	*Jonesia denitrificans*		ATCC 14870	unknown	-	-	-	-
12	*Kurthia zopfii*		ATCC 33403	poultry	-	-	-	-
13	*Lactobacillus rhamnosus*		ATCC 7469	unknown	-	-	-	-
14	*Listeria innocua*	6b	ATCC 33091	clinical isolate	-	-	-	-
15	*Listeria monocytogenes*	1/2a	ATCC 51772	dairy isolate	-	-	-	-
16	*Listeria monocytogenes*	4b	ATCC 49594	clinical isolate	-	-	-	-
17	*Macrococcus caseolyticus*		ATCC 29750	dairy isolate	-	-	-	-
18	*Micrococcus luteus*		ATCC 383	dairy isolate	-	-	-	-
19	*Pseudomonas aeruginosa*		ATCC 27853	clinical isolate	-	-	-	-
20	*Staphylococcus aureus*		ATCC 12600	clinical isolate	-	-	-	-
21	*Staphylococcus haemolyticus*		ATCC 29970	clinical isolate	-	-	-	-
22	*Staphylococcus saprophyticus*		ATCC 15305	unknown	-	-	-	-
23	*Streptococcus pyogenes*		ATCC 19615	clinical isolate	-	-	-	-
24	*Vibrio albensis*		ATCC 14547	fish	-	-	-	-
25	*Vibrio parahaemolyticus*		Vi 2	pork	-	-	-	-
— 25 strains from target family or target genus (apart from the target species), [SORTED BY GENUS]								
26	*Citrobacter braakii*		Ci 48	Unknown	-	-	-	-
27	*Citrobacter freundii*	O157	WSDA [c]	Unknown	-	-	-	-
28	*Citrobacter freundii*	O157	DATCP [d]	Unknown	-	-	-	-
29	*Cronobacter dublinensis* subsp. *lactaridi*		DSMZ 18707 [e]	Dairy isolate	-	-	-	-
30	*Cronobacter sakazakii*		ATCC 29544	Clinical isolate	-	-	-	-
31	*Edwardsiella tarda*	O1483:H1	ATCC 15947	Clinical isolate	-	-	-	-
32	*Enterobacter cloacae*		ATCC 33457	water	-	-	-	-
33	*Escherichia vulneris*		Esp 1	Pork isolate	-	-	-	-
34	*Escherichia hermanii*		ATCC 33650	Clinical isolate	-	-	-	-
35	*Escherichia fergusonii*		Esp 9	Unknown	-	-	-	-
36	*Hafnia alvei*		Ha 5	Beef isolate	-	-	-	-
37	*Klebsiella oxytoca*		ATCC 43086	Unknown	-	-	-	-
38	*Klebsiella pneumoniae* subsp. *pneumoniae*		ATCC 31488	Environmental isolate	-	-	-	-
39	*Morganella morganii*		ATCC 9237	Clinical isolate	-	-	-	-
40	*Proteus vulgaris*		ATCC 33420	Clinical isolate	-	-	-	-
41	*Salmonella enterica* subsp. *enterica*	Matopeni, O157	CDC [f]	Clinical isolate	-	-	-	-
42	*Salmonella enterica* subsp. *enterica*	Infantis	CDC	Clinical isolate	-	-	-	-
43	*Salmonella enterica* subsp. *enterica*	Typhimurium	ATCC 14028	Poultry isolate	-	-	-	-
44	*Salmonella enterica* subsp. *enterica*	Arizonae	ATCC 13314	Unknown	-	-	-	-
45	*Salmonella enterica* subsp. *enterica*	Enteritidis	ATCC 13076	Unknown	-	-	-	-
46	*Shigella flexneri*	2a	ATCC 700930	Unknown	-	-	-	-
47	*Shigella dysenteriae*		ATCC 13313	Clinical isolate	-	-	-	+
48	*Shigella boydii*		ATCC 49348	Animal isolate	-	-	-	-
49	*Yersinia ruckeri*		ATCC 29473	Seafood isolate	-	-	-	-
50	*Yersinia entercolitica*		Ye 8	Unknown	-	-	-	-

BioControl Systems, Inc / MilliporeSigma Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq, AOAC *Performance Tested Methods℠* Certification Number 101901

					Genotype			
EXCLUSIVITY					GDS EHEC	GDS EHEC ID		
N°	Organism	Serotype	Source	Origin	*eae*	O157	H7	*stx1* / *stx2*
— 50 strains from non-target serotypes, [SORTED BY serotype]								
51	*Escherichia coli*	O8:H16	Es 555	Beef isolate	-	-	-	+
52	*Escherichia coli*	O8:H19	Es 624	Beef isolate	-	-	-	+
53	*Escherichia coli*	O19:H7	Es 235	Food isolate	-	-	+	-
54	*Escherichia coli*	O22:H7	PSU 88.1674 [g]	Beef isolate	-	-	+	-
55	*Escherichia coli*	O26:H11	ATCC BAA-2196	Clinical isolate	-	-	-	+
56	*Escherichia coli*	O26:H46	MSU TW07705 [h]	Clinical isolate	-	-	-	+
57	*Escherichia coli*	O35:NM	MSU TW05144	Clinical isolate	-	-	-	-
58	*Escherichia coli*	O45:H2	ATCC BAA-2193	Clinical isolate	-	-	-	+
59	*Escherichia coli*	O45:H2	MSU TW01589	Clinical isolate	-	-	-	+
60	*Escherichia coli*	O45:NM	MSU TW07947	Clinical isolate	-	-	-	+
61	*Escherichia coli*	O55:H6	MSU TW00588	Clinical isolate	-	-	-	-
62	*Escherichia coli*	O55:H7	MSU TW07620	Clinical isolate	+	-	+	+
63	*Escherichia coli*	O55:H7	MSU TW04052	Clinical isolate	-	-	-	-
64	*Escherichia coli*	O55:H7	PSU 88.1004	Rabbit isolate	+	-	+	-
65	*Escherichia coli*	O55:H8	Es 633	Beef isolate	-	-	-	+
66	*Escherichia coli*	O55:NM	UM DEC2B	Clinical isolate	-	-	-	-
67	*Escherichia coli*	O76:H19	Es 553	Beef isolate	-	-	-	+
68	*Escherichia coli*	O91:H10	Es 547	Beef isolate	-	-	-	+
69	*Escherichia coli*	O91:H21	ATCC 51434	Unknown	-	-	-	+
70	*Escherichia coli*	O103:H11	ATCC BAA-2215	Unknown	-	-	-	+
71	*Escherichia coli*	O103:H2	USDA-MARC MAR125B [i]	Beef isolate	-	-	-	+
72	*Escherichia coli*	O103:H25	ATCC BAA-2199	Unknown	-	-	-	+
73	*Escherichia coli*	O103:H7	PSU 88.0161	Rodent isolate	-	-	-	-
74	*Escherichia coli*	O103:K:H8	ATCC 23982	Clinical isolate	-	-	-	-
75	*Escherichia coli*	O111:H2	MSU TW06296	Clinical isolate	-	-	-	-
76	*Escherichia coli*	O111:H8	ATCC 700840	Clinical isolate	-	-	-	+
77	*Escherichia coli*	O111:NM	CDC	Clinical isolate	-	-	-	+
78	*Escherichia coli*	O111:NM	CDC	Clinical isolate	-	-	-	+
79	*Escherichia coli*	O113:H21	ATCC BAA-183	Clinical isolate	-	-	-	+
80	*Escherichia coli*	O118:H16	MSU TW06410	Clinical isolate	-	-	-	+
81	*Escherichia coli*	O118:H8	MSU TW04166	Clinical isolate	-	-	-	-
82	*Escherichia coli*	O119:H6	MSU TW07894	Clinical isolate	-	-	-	-
83	*Escherichia coli*	O121:H19	MSU TW08023	Clinical isolate	-	-	-	+
84	*Escherichia coli*	O121:H7	USDA-MARC Apr 035A	Beef isolate	-	-	+	+
85	*Escherichia coli*	O128:H7	UM DEC13B [j]	Clinical isolate	-	-	+	-
86	*Escherichia coli*	O128:NM	UM DEC14D	Clinical isolate	-	-	-	-
87	*Escherichia coli*	O145:H28	ATCC BAA-1652	Clinical/dairy isolate	+	-	-	+
88	*Escherichia coli*	O145:H28	USDA-MARC May 109	Beef isolate	+	-	-	+
89	*Escherichia coli*	O145:NM	MSU TW08087	Clinical isolate	+	-	-	+
90	*Escherichia coli*	O156:H25	MSU TW01680	Clinical isolate	-	-	-	+
91	*Escherichia coli*	O157:H11	PSU 85.1867	Unknown	-	+	-	-
92	*Escherichia coli*	O157:H16	PSU 75.0140	Pork isolate	-	+	-	-
113	*Escherichia coli*	O157:H29	Es 321	Beef isolate	-	+	-	-
94	*Escherichia coli*	O157:H19	PSU 88.1649	Pork isolate	-	+	-	-
95	*Escherichia coli*	O157:H29	Es 550	Beef isolate	-	+	-	-
96	*Escherichia coli*	O157:H43	PSU 80.0792	Pork isolate	-	+	-	-
97	*Escherichia coli*	O157:H45	Es 237	Unknown	-	+	-	-
98	*Escherichia coli*	O172:H28	Es 542	Beef isolate	+	-	-	-
99	*Escherichia coli*	O172:H7	Es 546	Beef isolate	+	-	-	-
100	*Escherichia coli*	O174:H21	Es 644	Beef isolate	-	-	-	+

[a] "Ao", "Vi", "Ci", "Esp", "Ha", "Ye", and "Es" are the identification for individual culture collection strains in MilliporeSigma.

[b] ATCC = American Type Culture collection (Manassas, VA).

[c] WSDA = Washington State Department of Agriculture (Olympia, WA).

[d] DATCP = Wisconsin Department of Agriculture, Trade and Consumer Protection (Madison, WI).

[e] DSMZ = German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).

[f] CDC = Center for Disease Control (Atlanta, GA).

[g] PSU = *E. coli* Reference Center (Penn State University, University Park, PA).

[h] MSU = STEC Center at Michigan State University (East Lansing, MI).

[i] USDA-MARC = U.S. Department of Agriculture Meat Animal Research Center (Clay Center, NE).

[j] UM = University of Michigan (Ann Arbor , Michigan)

culture collection (Bellevue, WA).

[k] GDS EHEC = Assurance GDS for *E. coli* O157:H7 Tq (*eae*).
[l] GDS EHEC ID = Assurance GDS EHEC ID for *E. coli* O157:H7 Tq (O157, H7, *stx*1/*stx*2).

Table 3: EHEC ID Inclusivity Results for *E. coli* O157:H7- from Isolated Colonies [a] (1)

INCLUSIVITY				GDS EHEC ID					
N°	Organism	Serotype	Source [b]	mRBA [d]	RA O157 [e]	RF [f]	TC-SMAC [g]	SBA [h]	TSA-YE [i]
1	*Escherichia coli*	O157:H7	ATCC 35150 [c]	+ [j]	+	+	+	+	+
2	*Escherichia coli*	O157:H7	ATCC 43895	+	+	+	+	+	+
3	*Escherichia coli*	O157:H7	ATCC 43890	+	+	+	+	+	+
4	*Escherichia coli*	O157:H7	ATCC 43894	+	+	+	+	+	+
5	*Escherichia coli*	O157:H7	OPHD	+	+	+	+	+	+
6	*Escherichia coli*	O157:H7	OPHD	+	+	+	+	+	+
7	*Escherichia coli*	O157:H7	OPHD	+	+	+	+	+	+
8	*Escherichia coli*	O157:H7	OPHD	+	+	+	+	+	+
9	*Escherichia coli*	O157:H7	OPHD	+	+	+	+	+	+
10	*Escherichia coli*	O157:H7	PSU 93.0058	+	+	+	+	+	+
11	*Escherichia coli*	O157:H7	PSU 88.0222	+	+	+	+	+	+
12	*Escherichia coli*	O157:H7	PSU 93.0054	+	+	+	+	+	+
13	*Escherichia coli*	O157:H7	PSU Deca 3B	+	+	+	+	+	+
14	*Escherichia coli*	O157:H7	Es 190	+	+	+	+	+	+
15	*Escherichia coli*	O157:H7	Es 191	+	+	+	+	+	+
16	*Escherichia coli*	O157:H7	Es 192	+	+	+	+	+	+
17	*Escherichia coli*	O157:H7	Es 194	+	+	+	+	+	+
18	*Escherichia coli*	O157:H7	Es 195	+	+	+	+	+	+
19	*Escherichia coli*	O157:H7	Es 189	+	+	+	+	+	+
20	*Escherichia coli*	O157:H7	Es 537	+	+	+	+	+	+
21	*Escherichia coli*	O157:H7	Es 538	+	+	+	+	+	+
22	*Escherichia coli*	O157:H7	Es 567	+	+	+	+	+	+
23	*Escherichia coli*	O157:H7	Es 568	+	+	+	+	+	+
24	*Escherichia coli*	O157:H7	Es 579	+	+	+	+	+	+
25	*Escherichia coli*	O157:H7	Es 620	+	+	+	+	+	+
26	*Escherichia coli*	O157:H7	Es 621	+	+	+	+	+	+
27	*Escherichia coli*	O157:H7	Es 684	+	+	+	+	+	+
28	*Escherichia coli*	O157:H7	Es 685	+	+	+	+	+	+
29	*Escherichia coli*	O157:H7	Es 216	+	+	+	+	+	+
30	*Escherichia coli*	O157:H7	Es 219	+	+	+	+	+	+
31	*Escherichia coli*	O157:H7	Es 242	+	+	+	+	+	+
32	*Escherichia coli*	O157:H7	Es 333	+	+	+	+	+	+
33	*Escherichia coli*	O157:H7	Es334	+	+	+	+	+	+
34	*Escherichia coli*	O157:H7	Es 355	+	+	+	+	+	+
35	*Escherichia coli*	O157:H7	Es 545	+	+	+	+	+	+
36	*Escherichia coli*	O157:H7	FDA	+	+	+	+	+	+
37	*Escherichia coli*	O157:H7 (NM)	ATCC 700376	+	+	+	+	+	+
38	*Escherichia coli*	O157:H7 (NM)	ATCC 700377	+	+	+	+	+	+
39	*Escherichia coli*	O157:H7 (NM)	ATCC 700378	+	+	+	+	+	+
40	*Escherichia coli*	O157:H7 (NM)	PSU DR3	+	+	+	+	+	+
41	*Escherichia coli*	O157:H7 (NM)	Es 351	+	+	+	+	+	+
42	*Escherichia coli*	O157:H7 (NM)	USDA-ARS 55AC1	+	+	+	+	+	+
43	*Escherichia coli*	O157:H7 (NM)	USDA-ARS 131AC1	+	+	+	+	+	+
44	*Escherichia coli*	O157:H7 (NM)	USDA-ARS 237AC1	+	+	+	+	+	+
45	*Escherichia coli*	O157:H7 (NM)	USDA-ARS 299AB3	+	+	+	+	+	+
46	*Escherichia coli*	O157:H7 (rough)	FDA MA6	+	+	+	+	+	+
47	*Escherichia coli*	O157:H7 (non-STX producing)	ATCC 43888	+	+	+	+	+	+
48	*Escherichia coli*	O157:H7 (non-STX producing)	ATCC 700728	+	+	+	+	+	+
49	*Escherichia coli*	O157:H7 (non-STX producing)	ES 340	+	+	+	+	+	+
50	*Escherichia coli*	O157:H7 (non-STX producing)	USDA-ARS 114AC1	+	+	+	+	+	+

[a] An isolated colony from all plates that contained growth was suspended in 500 µL of Assurance® GDS Wash Buffer. Using a sterile 1 µL loop, a small amount of the suspension was transferred to 100 µL of Assurance® GDS Resuspension Buffer Tq in a resuspension plate well and gently stirred with loop.

[b] Refer to Table 1 for strain source. "ES" is the identification for individual *E. coli* strains in BioControl/MilliporeSigma culture collection (Bellevue, WA).

[c] ATCC = American Type Culture collection (Manassas, VA).

[d] mRBA = Rainbow® Agar O157 (Biolog) containing 5.0 mg/L novobiocin, 0.05 mg/L cefixime trihydrate and 0.15 mg/L potassium tellurite.

[e] RA O157 = Rainbow® Agar O157 (Biolog) containing 10.0 mg/L novobiocin and 0.8 mg/L potassium tellurite.
[f] RF = R&F® *E. coli* O157:H7 Agar.
[g] TC-SMAC = Tellurite Cefixime – Sorbitol MacConkey Agar.
[h] SBA = Tryptic Soy Agar with 5% Sheep Blood.
[i] TSA-YE = Trypticase Soy Agar with Yeast Extract.
[j] Growth on plate. '+' is GDS assay positive and '-' is GDS assay negative.

Table 4: Colony Exclusivity Results for Non-*Escherichia coli* O157:H7 Organisms including other EHECS - from Isolated Colonies [a] (1)

EXCLUSIVITY				GDS EHEC ID					
N°	Organism	Serotype	Source [b]	mRBA [c]	RA O157 [d]	RF [e]	TC-SMAC [f]	SBA [g]	TSA-YE [h]
— 25 strains from non-target family, [SORTED BY GENUS]									
1	*Aerococcus viridans*		Ao 7	- [i]	-	NG [j]	NG	-	-
2	*Aeromonas hydrophila*		ATCC 35654 [b]	-	-	-	-	-	-
3	*Alcaligenes faecalis*		ATCC 15246	-	-	-	-	-	-
4	*Bacillus cereus*		ATCC 14579	NG	-	NG	NG	-	-
5	*Bacillus pumilus*		University of Washington	-	-	NG	NG	-	-
6	*Bacillus subtilis*		ATCC 6051	NG	-	NG	NG	-	-
7	*Brevibacterium linens*		ATCC 9172	-	-	-	-	-	-
8	*Candida albicans*		ATCC 24433	-	-	-	NG	-	-
9	*Enterococcus faecalis*		ATCC 33186	-	-	NG	NG	-	-
10	*Enterococcus faecium*		ATCC 19434	-	-	NG	NG	-	-
11	*Jonesia denitrificans*		ATCC 14870	-	-	-	-	-	-
12	*Kurthia zopfii*		ATCC 33403	NG	-	NG	NG	-	-
13	*Lactobacillus rhamnosus*		ATCC 7469	NG	-	NG	NG	-	-
14	*Listeria innocua*	6b	ATCC 33091	NG	-	NG	NG	-	-
15	*Listeria monocytogenes*	1/2a	ATCC 51772	NG	-	NG	NG	-	-
16	*Listeria monocytogenes*	4b	ATCC 49594	NG	-	NG	NG	-	-
17	*Macrococcus caseolyticus*		ATCC 29750	-	-	-	-	-	-
18	*Micrococcus luteus*		ATCC 383	NG	-	NG	NG	-	-
19	*Pseudomonas aeruginosa*		ATCC 27853	-	-	-	-	-	-
20	*Staphylococcus aureus*		ATCC 12600	NG	-	NG	NG	-	-
21	*Staphylococcus haemolyticus*		ATCC 29970	NG	-	NG	NG	-	-
22	*Staphylococcus saprophyticus*		ATCC 15305	NG	-	NG	NG	-	-
23	*Streptococcus pyogenes*		ATCC 19615	-	-	NG	NG	-	-
24	*Vibrio albensis*		ATCC 14547	NG	-	NG	NG	-	-
25	*Vibrio parahaemolyticus*		Vi 2	NG	NG	NG	-	-	-
— 25 strains from target family or target genus (apart from the target species), [SORTED BY GENUS]									
26	*Citrobacter braakii*		Ci 48	NG	-	-	-	-	-
27	*Citrobacter freundii*	O157	WSDA	NG	-	-	-	-	-
28	*Citrobacter freundii*	O157	DATCP	NG	-	-	-	-	-
29	*Cronobacter dublinensis* subsp. *lactaridi*		DSMZ 18707	NG	-	-	-	-	-
30	*Cronobacter sakazakii*		ATCC 29544	NG	-	-	-	-	-
31	*Edwardsiella tarda*	O1483:H1	ATCC 15947	NG	-	NG	-	-	-
32	*Enterobacter cloacae*		ATCC 33457	NG	-	-	-	-	-
33	*Escherichia vulneris*		Esp 1	NG	-	NG	-	-	-
34	*Escherichia hermanii*		ATCC 33650	-	-	-	-	-	-
35	*Escherichia fergusonii*		Esp 9	NG	-	-	-	-	-
36	*Hafnia alvei*		Ha 5	NG	-	-	-	-	-
37	*Klebsiella oxytoca*		ATCC 43086	NG	-	-	-	-	-
38	*Klebsiella pneumoniae subsp. pneumoniae*		ATCC 31488	NG	-	-	-	-	-
39	*Morganella morganii*		ATCC 9237	-	-	-	-	-	-
40	*Proteus vulgaris*		ATCC 33420	-	-	-	-	-	-
41	*Salmonella enterica* subsp. *enterica*	Matopeni O157	CDC	NG	-	-	-	-	-
42	*Salmonella enterica* subsp. *enterica*	Infantis	CDC	NG	-	-	-	-	-
43	*Salmonella enterica* subsp. *enterica*	Typhimurium	ATCC 14028	NG	-	-	-	-	-
44	*Salmonella enterica* subsp. *enterica*	Arizonae	ATCC 13314	NG	-	-	-	-	-
45	*Salmonella enterica* subsp. *enterica*	Enteritidis	ATCC 13076	NG	-	-	-	-	-
46	*Shigella flexneri*	2a	ATCC 700930	NG	-	-	-	-	-
47	*Shigella dysenteriae*		ATCC 13313	NG	-	-	-	-	-
48	*Shigella boydii*		ATCC 49348	NG	-	-	-	-	-
49	*Yersinia ruckeri*		ATCC 29473	NG	NG	NG	NG	-	-
50	*Yersinia entercolitica*		Ye 8	NG	-	NG	NG	-	-

EXCLUSIVITY				EHEC ID					
N°	Organism	Serotype	BEL code (ES)	mRBA [1]	RA O157 [2]	RF [3]	TC-SMAC [4]	SBA [5]	TSA-YE [6]
— 50 strains from non-target serotypes, [SORTED BY serotype]									
51	*Escherichia coli*	O8:H16	Es 555	NG	-	-	-	-	-
52	*Escherichia coli*	O8:H19	Es 624	NG	-	-	-	-	-
53	*Escherichia coli*	O19:H7	Es 235	NG	-	-	-	-	-
54	*Escherichia coli*	O22:H7	PSU 88.1674	NG	-	-	-	-	-
55	*Escherichia coli*	O26:H11	ATCC BAA-2196	-	-	-	-	-	-
56	*Escherichia coli*	O26:H46	MSU TW07705	-	-	-	-	-	-
57	*Escherichia coli*	O35:NM	MSU TW05144	-	-	-	-	-	-
58	*Escherichia coli*	O45:H2	ATCC BAA-2193	-	-	-	-	-	-
59	*Escherichia coli*	O45:H2	MSU TW01589	-	-	-	-	-	-
60	*Escherichia coli*	O45:NM	MSU TW07947	-	-	-	-	-	-
61	*Escherichia coli*	O55:H6	MSU TW00588	-	-	-	-	-	-
62	*Escherichia coli*	O55:H7	MSU TW07620	NG	-	-	-	-	-
63	*Escherichia coli*	O55:H7	MSU TW04052	-	-	-	-	-	-
64	*Escherichia coli*	O55:H7	PSU 88.1004	NG	-	-	-	-	-
65	*Escherichia coli*	O55:H8	Es 633	NG	-	-	-	-	-
66	*Escherichia coli*	O55:NM	UM DEC2B	-	-	-	-	-	-
67	*Escherichia coli*	O76:H19	Es 553	NG	-	-	-	-	-
68	*Escherichia coli*	O91:H10	Es 547	NG	-	-	-	-	-
69	*Escherichia coli*	O91:H21	ATCC 51434	-	-	-	-	-	-
70	*Escherichia coli*	O103:H11	ATCC BAA-2215	-	-	-	-	-	-
71	*Escherichia coli*	O103:H2	USDA-MARC MAR125B	-	-	-	-	-	-
72	*Escherichia coli*	O103:H25	ATCC BAA-2199	-	-	-	-	-	-
73	*Escherichia coli*	O103:H7	PSU 88.0161	-	-	-	-	-	-
74	*Escherichia coli*	O103:K:H8	ATCC 23982	-	-	-	-	-	-
75	*Escherichia coli*	O111:H2	MSU TW06296	-	-	-	-	-	-
76	*Escherichia coli*	O111:H8	ATCC 700840	-	-	-	-	-	-
77	*Escherichia coli*	O111:NM	CDC	-	-	-	-	-	-
78	*Escherichia coli*	O111:NM	CDC	-	-	-	-	-	-
79	*Escherichia coli*	O113:H21	ATCC BAA-183	-	-	-	-	-	-
80	*Escherichia coli*	O118:H16	MSU TW06410	-	-	-	-	-	-
81	*Escherichia coli*	O118:H8	MSU TW04166	NG	-	-	-	-	-
82	*Escherichia coli*	O119:H6	MSU TW07894	NG	-	-	-	-	-
83	*Escherichia coli*	O121:H19	MSU TW08023	-	-	-	-	-	-
84	*Escherichia coli*	O121:H7	USDA-MARC Apr 035A	NG	-	-	-	-	-
85	*Escherichia coli*	O128:H7	UM DEC13B	NG	-	-	-	-	-
86	*Escherichia coli*	O128:NM	UM DEC14D	-	-	-	-	-	-
87	*Escherichia coli*	O145:H28	ATCC BAA-1652	-	-	-	-	-	-
88	*Escherichia coli*	O145:H28	USDA-MARC May 109	-	-	-	-	-	-
89	*Escherichia coli*	O145:NM	MSU TW08087	-	-	-	-	-	-
90	*Escherichia coli*	O156:H25	MSU TW01680	-	-	-	-	-	-
91	*Escherichia coli*	O157:H11	PSU 85.1867	-	-	-	-	-	-
92	*Escherichia coli*	O157:H16	PSU 75.0140	-	-	-	-	-	-
113	*Escherichia coli*	O157:H29	Es 321	-	-	-	-	-	-
94	*Escherichia coli*	O157:H19	PSU 88.1649	-	-	-	-	-	-
95	*Escherichia coli*	O157:H29	Es 550	-	-	-	-	-	-
96	*Escherichia coli*	O157:H43	PSU 80.0792	-	-	-	-	-	-
97	*Escherichia coli*	O157:H45	Es 237	-	-	-	-	-	-
98	*Escherichia coli*	O172:H28	Es 542	-	-	-	-	-	-
99	*Escherichia coli*	O172:H7	Es 546	-	-	-	-	-	-
100	*Escherichia coli*	O174:H21	Es 644	-	-	-	-	-	-

[a] An isolated colony from all plates that contained growth was suspended in 500 µL of Assurance® GDS Wash Buffer. Using a sterile 1 µL loop, a small amount of the suspension was transferred to 100 µL of Assurance® GDS Resuspension Buffer Tq in a resuspension plate well and gently stirred with loop.

[b] Refer to Table 2 for strain source. "ES" is the identification for individual *E. coli* strains in BioControl/MilliporeSigma culture collection (Bellevue, WA).

[c] mRBA = Rainbow® Agar O157 (Biolog) containing 5.0 mg/L novobiocin, 0.05 mg/L cefixime trihydrate and 0.15 mg/L potassium tellurite.

[d] RA O157 = Rainbow® Agar O157 (Biolog) containing 10.0 mg/L novobiocin and 0.8 mg/L potassium tellurite.

[e] RF = R&F® *E. coli* O157:H7 Agar.

[f] TC-SMAC = Tellurite Cefixime – Sorbitol MacConkey Agar.

[g] SBA = Tryptic Soy Agar with 5% Sheep Blood.

[h] TSA-YE = Trypticase Soy Agar with Yeast Extract.

[i] Growth on plate. '+' is GDS assay positive and '-' is GDS assay negative.

[j] No growth on agar plate.

Table 5. Assurance® GDS for EHEC ID Results, Inclusivity/Exclusivity (Independent Laboratory) (1)

Strain	Source	Origin	Assurance® GDS for EHEC ID					
			mRBA[a]	SBA[b]	TC-SMAC[c]	RA[d]	RF[e]	TSA-YE[f]
E. coli O157:H7	ATCC[g] 35150	Human Feces	+	+	+	+	+	+
E. coli O157:H7	ATCC 43895	Raw Hamburger Meat	+	+	+	+	+	+
E. coli O157:H7	ATCC 51657	Clinical Isolate	+	+	+	+	+	+
E. coli O157:H7	ATCC 700376	Human Feces	+	+	+	+	+	+
E. coli O157:H7	ATCC 700927	Not Available	+	+	+	+	+	+
E. coli O157:H7	ATCC BAA-460	Radish Sprouts	+	+	+	+	+	+
E. coli O157:H7	MSU[h] DEC3C	Human	+	+	+	+	+	+
E. coli O157:H7	MSU TW00116	Human	+	+	+	+	+	+
E. coli O157:H7	NCTC[i] 13125	Human Diarrhea Stool	+	+	+	+	+	+
E. coli O157:H7	NCTC 13126	Not Available	+	+	+	+	+	+
E. coli O157:H7	QL[j] 14077.4	Apple Cider	+	+	+	+	+	+
E. coli O157:H7	QL 164673	Beef Trim	+	+	+	+	+	+
E. coli O157:H7	QL 2-207	Meat	+	+	+	+	+	+
E. coli O157:H7	QL 2-370	Meat	+	+	+	+	+	+
E. coli O157:H7	QL 2-704	Beef	+	+	+	+	+	+
E. coli O157:H7	USDA[k] 465-97	Not Available	+	+	+	+	+	+
Citrobacter freundii	QL 100813-2A	Sliced Deli Turkey	-	-	-	-	-	-
Escherichia vulneris	ATCC 29943	Human Wound	NG[n]	-	-	-	NG	-
Hafnia alvei	ATCC 51815	Milk	-	-	NG	-	-	-
Salmonella enterica	UPENN[l] STS 5	Not Available	-	-	-	-	-	-
E. coli O26	ATCC BAA-1653	Not Available	-	-	-	-	-	-
E. coli O55	MSU TW00585	Infant Feces	-	-	-	-	-	-
E. coli O91	NCTC 9091	Not Available	-	-	-	-	-	-
E. coli O121	PSU[m] 5.0959	Not Available	-	-	-	-	-	-

[a] Modified Rainbow® Agar, [b] Sheep's Blood Agar, [c] Tellurite Cefixime – Sorbitol MacConkey Agar, [d] Rainbow® Agar, [e] R&F® *E. coli* O157:H7 Agar,.
[f] Trypticase Soy Agar with Yeast Extract, [g] American Type Culture Collection, Manassas, VA, [h] Michigan State Culture Collection, Lansing, MI, [i] National Collection Type Cultures, Salisbury, UK, [j] Q Laboratories Culture Collection, Cincinnati, OH, [k] United States Department of Agriculture Culture Collection, Athens, GA. [l] University of Pennsylvania Culture Collection, Philadelphia, PA, [m] Pennsylvania State Culture Collection, University Park, PA, [n] No Growth.

Table 7: Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq, Candidate vs. Reference – POD Results: MANUAL SAMPLE PROCESSING (1)

Matrix	Inoculating strain	MPN[a]/ Test Portion or CFU[b]/Cloth	N[c]	Candidate			Reference			dPOD$_C$[g]	95% CI[h]
				x[d]	POD$_C$[e]	95% CI	X	POD$_R$[f]	95% CI		
Ground beef 375 g	*E. coli* O157:H7 (ATCC[i] 35150)	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.21 (0.81, 1.93)	20	12	0.60	0.39, 0.78	14	0.70	0.48, 0.85	-0.10	-0.36, 0.18
		4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef[k]	*E. coli* O157:H7 (ATCC 43895)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.50 (0.25, 0.86)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		3.70 (1.52, 9.02)	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	*E. coli* O157:NM (ATCC 700376)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.42 (0.90, 2.47)	20	13	0.65	0.43, 0.82	16	0.80	0.58, 0.92	-0.15	-0.40, 0.12
		2.93 (1.38, 6.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Frozen Finely Textured Beef 375 g	*E. coli* O157:H7 (ATCC 43888)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.00 (0.00, 0.09)	20	9	0.45	0.26, 0.66	0	0.00	0.16, 0.61	0.45	0.20, 0.66
		0.46 (0.20, 1.02)	5	5	1.00	0.57, 1.00	2	0.40	0.18, 0.77	0.60	0.03, 0.88
Carcass cloth 1 cloth[l]	*E. coli* O157:H7 (ATCC 43890)	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.80	20	10	0.50	0.30, 0.70	2	0.10	0.03, 0.30	0.40	0.12, 0.61
		3.00	5	3	0.60	0.23, 0.88	1	0.20	0.03, 0.64	0.40	-0.16, 0.75

[a] MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval.
[b] CFU/Cloth = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate.
[c] N = Number of test portions.
[d] x = Number of positive test portions.
[e] POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[f] POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials.
[g] dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i] ATCC = American Type Culture Collection, Manassas, VA.
[j] N/A = Not applicable.
[k] Results from Independent Laboratory analysis of raw ground beef.
[l] MPN not available for carcass cloth. Low and high inoculation levels cfu/cloth indicated.

Table 8: Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq, Presumptive vs. Alternative and Traditional Confirmed – POD Result: MANUAL SAMPLE PROCESSING (1)

Matrix	Inoculating strain	MPN[a]/ Test Portion or CFU[b]/Cloth	N[c]	Presumptive			Confirmed			dPOD$_{CP}$[g]	95% CI[h]
				x[d]	POD$_{CP}$[e]	95% CI	X	POD$_{CC}$[f]	95% CI		
Ground beef 375 g	*E. coli* O157:H7 (ATCC[i] 35150)	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.21 (0.81, 1.93)	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.21, 0.11
		4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef[k]	*E. coli* O157:H7 (ATCC 43895)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.50 (0.25, 0.86)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	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
Beef trim 375 g	*E. coli* O157:NM (ATCC 700376)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.42 (0.90, 2.47)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.13, 0.13
		2.93 (1.38, 6.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Frozen Finely Textured Beef 375 g	*E. coli* O157:H7 (ATCC 43888)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.00 (0.00, 0.09)	20	12	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.16, 0.26
		0.46 (0.20, 1.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Carcass cloth 1 cloth[l]	*E. coli* O157:H7 (ATCC 43890)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.80	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		3.00	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.47, 0.47

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator, with 95% confidence interval.
[b]CFU/Cloth = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate.
[c]N = Number of test portions.
[d]x = Number of positive test portions.
[e]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[f]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[g]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i]ATCC = American Type Culture Collection, Manassas, VA.
[j]N/A = Not applicable.
[k]Results from Independent Laboratory analysis of raw ground beef.
[l]MPN not available for carcass cloth. Low and high inoculation levels CFU/cloth indicated.

Table 9: Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq, Candidate vs. Reference – POD Results: PIPETMAX SAMPLE PROCESSING (1)

Matrix	Inoculating strain	MPN[a]/ Test Portion or CFU[b]/Cloth	N[c]	Candidate			Reference			dPOD$_{C}$[g]	95% CI[h]
				x[d]	POD$_{C}$[e]	95% CI	X	POD$_{R}$[f]	95% CI		
Ground beef 375 g	*E. coli* O157:H7 (ATCC[i] 35150)	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.21 (0.81, 1.93)	20	12	0.60	0.39, 0.78	14	0.70	0.48, 0.85	-0.10	-0.36, 0.18
		4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ground beef[k]	*E. coli* O157:H7 (ATCC 43895)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.50 (0.25, 0.86)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		3.70 (1.52, 9.02)	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	*E. coli* O157:NM (ATCC 700376)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.42 (0.90, 2.47)	20	13	0.65	0.43, 0.82	16	0.80	0.58, 0.92	-0.15	-0.40, 0.12
		2.93 (1.38, 6.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Frozen Finely Textured Beef 375 g	*E. coli* O157:H7 (ATCC 43888)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.00 (0.00, 0.09)	20	8	0.40	0.22, 0.61	0	0.00	0.16, 0.61	0.40	0.16, 0.61
		0.46 (0.20, 1.02)	5	5	1.00	0.57, 1.00	2	0.40	0.18, 0.77	0.60	0.03, 0.88
Carcass cloth 1 cloth[l]	*E. coli* O157:H7 (ATCC 43890)	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.80	20	10	0.50	0.30, 0.70	2	0.10	0.03, 0.30	0.40	0.12, 0.61
		3.00	5	3	0.60	0.23, 0.88	1	0.20	0.03, 0.64	0.40	-0.16, 0.75

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval.
[b]CFU/Cloth = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate .
[c]N = Number of test portions.
[d]x = Number of positive test portions.
[e]POD$_{C}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]POD$_{R}$ = Reference method confirmed positive outcomes divided by the total number of trials.
[g]dPOD$_{C}$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i]ATCC = American Type Culture Collection, Manassas, VA.
[j]N/A = Not applicable.
[k]Results from Independent Laboratory analysis of raw ground beef.
[l]MPN not available for carcass cloth. Low and high inoculation levels cfu/cloth indicated.

Table 10: Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq, Presumptive vs. Alternative and Traditional Confirmed – POD Result: PIPETMAX SAMPLE PROCESSING (1)

Matrix	Inoculating strain	MPN[a]/ Test Portion or CFU[b]/Cloth	N[c]	Presumptive			Confirmed			dPOD$_{CP}$[g]	95% CI[h]
				x[d]	POD$_{CP}$[e]	95% CI	X	POD$_{CC}$[f]	95% CI		
Ground beef 375 g	*E. coli* O157:H7 (ATCC[i] 35150)	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.21 (0.81, 1.93)	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.21, 0.11
		4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Ground beef[k]	*E. coli* 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
		0.50 (0.25, 0.86)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	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
Beef trim 375 g	*E. coli* O157:NM (ATCC[1] 700376)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.42 (0.90, 2.47)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.13, 0.13
		2.93 (1.38, 6.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Frozen Finely Textured Beef 375 g	*E. coli* O157:H7 (ATCC 43888)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.00 (0.00, 0.09)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.16, 0.26
		0.46 (0.20, 1.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Carcass cloth 1 cloth[l]	*E. coli* O157:H7 (ATCC 43890)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.80	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		3.00	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.47, 0.47

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator, with 95% confidence interval.
[b]CFU/Cloth = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate.
[c]N = Number of test portions.
[d]x = Number of positive test portions.
[e]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[f]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[g]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i]N/A = Not applicable.
[j]Results from Independent Laboratory analysis of raw ground beef.
[k]MPN not available for carcass cloth. Low and high inoculation levels CFU/cloth indicated.

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