



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
101502

The AOAC Research Institute hereby certifies the method known as

Assurance® GDS MPX for Top STEC (MPX ID)

manufactured by

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

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A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, AOAC Research Institute Senior Director

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METHOD NAME Assurance® GDS MPX ID for Top STEC (MPX ID)		CATALOG NUMBER 71019-52 (52 tests)	
INDEPENDENT LABORATORY Vanguard Sciences North Sioux City, South Dakota USA			
APPLICABILITY OF METHOD Analytes – Top 6 Shiga Toxin-producing <i>Escherichia coli</i> (STEC), serotypes O26, O45, O103, O111, O121, and O145 Matrixes – USDA-FSIS MLG – raw ground beef 20% fat (375 g), raw beef trim 45% fat (375 g), stainless steel (4” x 4” sponge) FDA BAM – raw spinach (375 g) Performance claims – The test method performed comparable to the reference method.		REFERENCE METHODS U.S. Department of Agriculture, Food Safety and Inspection Service <i>Microbiological Laboratory Guidelines</i> , 5B.05, 2014, (2) U.S. Food and Drug Administration. <i>Bacteriological Analytical Manual</i> , Chapter 4A (3) U.S. Department of Agriculture, Food Safety and Inspection Service <i>Microbiological Laboratory Guidelines</i> , 5.09, 2015 (4)	
ORIGINAL CERTIFICATION DATE October 09, 2015		CERTIFICATION RENEWAL RECORD Renewed through December 2027.	
METHOD MODIFICATION RECORD 1. December 2017 Level 1 2. January 2020 Level 2 3. November 2021 Level 1 4. January 2023 Level 1		SUMMARY OF MODIFICATION 1. Editorial changes for clarity. 2. mEHEC media and mEHEC+novobiocin manufacturing location change to Darmstadt, Germany and rebranding to Merck format. 3. Editorial changes and update to software. 4. Editorial changes.	
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PRINCIPLE OF THE METHOD (1)

The MPX ID is designed to detect specific "Top 6" non-O157 Shiga Toxigenic *E. coli* serogroups following a positive result using previously validated Assurance GDS MPX Top 7 STEC assay (PTM No. 071301). The MPX ID utilizes a proprietary IMS-based sample preparation procedure to capture organisms belonging to six specific Top STEC serogroups (O26, O45, O103, O111, O121, O145) prior to genetic analysis for the associated pathogenicity genes. MPX ID utilizes a complex mix of multiple markers to successfully discriminate the Top 6 STEC. The PCR reactions have been divided between two Amplification Tubes. Each tube contains an internal control and all necessary PCR reagents. Results will automatically be combined between the two tubes via Assurance GDS Rotor-Gene software.

DISCUSSION OF THE VALIDATION STUDY (1)

Results of these studies confirmed that Assurance GDS MPX ID for Top STEC is inclusive and specific for Top 6 STEC. In the internal method comparison study, MPX ID and the reference culture methods are statistically not different for the detection of Top 6 STEC in raw beef trim, raw ground beef and raw spinach and on stainless steel surfaces. POD statistical analyses of the samples indicated that for all foods analyzed, there was no statistical difference between the MPX ID presumptive compared to the MPX ID confirmed.

The external method comparison study yielded similar results for raw ground beef and on stainless steel surfaces. In this study, we observed 100% inclusivity among the 50 Top 6 STEC and 100% exclusivity for the 30 non-Top 6 STEC organisms analyzed. These results demonstrate that MPX ID is an effective method for the detection of Top 6 STEC in raw beef trim, raw ground beef and raw spinach and on stainless steel surface.

The GDS MPX ID for Top STEC also may be used to confirm the presence of a Top STEC organism from an isolated colony. Fifty different Top 6 STEC organisms were plated onto isolation agars and a non-selective plating agar. From all the agars tested, the MPX ID was able to properly identify all 50 Top 6 STEC organisms.

All non-target organisms were properly determined not to be a Top STEC. The Independent Laboratory study yielded similar results, except for two Top 6 STEC organisms which were not properly identified from L-EMB agar only, although these two strains were successfully identified on the other four agars and in the Method Developer study. These results demonstrate MPX ID can be used as a confirmation procedure for Top 6 STEC isolates from plating agar.

Finally, the PickPen device was compared to the OctoMACS separator for IMS isolation of Top 6 STEC from enriched samples. Across all samples, the PickPen and OctoMACS were able to IMS isolate all of the presumptive STEC organisms from the samples, except for one sample for *E. coli* O26, in which the OctoMACS did not isolate the organism while it was confirmed positive by the PickPen.

The OctoMACS procedure also requires an additional acid-treatment of the IMS isolation beads and re-plating of the sample on Rainbow agar, a procedure which is not required for PickPen. These data demonstrate that the PickPen procedure is equivalent to the OctoMACS procedure and the acid treatment protocol for isolation of Top 7 STEC. These findings are in agreement with a previous study that found the PickPen method to be advantageous for the isolation of *E. coli* O157:H7 from cattle hide and fecal samples (7). The PickPen device may be used as a substitute for the OctoMACS column system for IMS isolation of Top 7 STEC.

Table 1. Validation of Inclusivity of Assurance GDS MPX ID for Top STEC (1)

No.	Organism	Source	Origin	Serotype	Genotype ^a			MPX Top 7 STEC Result	MPX ID Result
					eae	stx ₁	stx ₂		
1	<i>Escherichia coli</i>	MSU ^b , TW04270	human	O26:H2	+	+	-	+	O26
2	<i>Escherichia coli</i>	MSU, TW05555	human	O26:HNT	+	+	-	+	O26
3	<i>Escherichia coli</i>	MSU, TW07705	stool, human	O26:H46	+	+	+	+	O26
4	<i>Escherichia coli</i>	MSU, TW07814	human	O26:H11	+	+	+	+	O26
5	<i>Escherichia coli</i>	MSU, TW07948	human	O26:NM	+	+	-	+	O26
6	<i>Escherichia coli</i>	MSU, TW08570	HUS, human	O26:H11	+	-	+	+	O26
7	<i>Escherichia coli</i>	ATCC <i>c</i> , BAA-2204	human	O26:H11	+	+	-	+	O26
8	<i>Escherichia coli</i>	ATCC, BAA-2205	stool, human	O26:H11	+	+	-	+	O26
9	<i>Escherichia coli</i>	MSU, TW00965	HC, human	O45:H2	+	+	-	+	O45
10	<i>Escherichia coli</i>	MSU, TW01589	human	O45:H2	+	+	-	+	O45
11	<i>Escherichia coli</i>	MSU, TW03068	cow	O45:H2	+	+	-	+	O45
12	<i>Escherichia coli</i>	MSU, TW07947	diarrhea, human	O45:NM	+	+	-	+	O45
13	<i>Escherichia coli</i>	MSU, TW09066	cow	O45:NM	+	+	-	+	O45
14	<i>Escherichia coli</i>	MSU, TW09183	human	O45:NM	+	+	-	+	O45
15	<i>Escherichia coli</i>	ATCC, BAA-2185	stool, human	O45:H2	+	+	-	+	O45
16	<i>Escherichia coli</i>	ATCC, BAA-2193	human	O45:H2	+	+	-	+	O45
17	<i>Escherichia coli</i>	MSU, TW04162	diarrhea, human	O103:H6	+	+	-	+	O103
18	<i>Escherichia coli</i>	MSU, TW07881	BD, human	O103:H2	+	+	-	+	O103
19	<i>Escherichia coli</i>	MSU, TW07971	human	O103:H6	+	+	-	+	O103
20	<i>Escherichia coli</i>	MSU, TW07975	human	O103:HNT	+	+	-	+	O103
21	<i>Escherichia coli</i>	MSU, TW07990	human	O103:NM	+	+	-	+	O103
22	<i>Escherichia coli</i>	MSU, TW08101	human	O103:H2	+	+	-	+	O103
23	<i>Escherichia coli</i>	MSU, TW08103	human	O103:H25	+	+	-	+	O103
24	<i>Escherichia coli</i>	ATCC, BAA-2199	stool, human	O103:H25	+	+	-	+	O103
25	<i>Escherichia coli</i>	ATCC, BAA-2215	human	O103:H11	+	+	-	+	O103
26	<i>Escherichia coli</i>	MSU, TW04257	BD, human	O111:NM	+	+	+	+	O111
27	<i>Escherichia coli</i>	MSU, TW05150	diarrhea, cow	O111:NM	+	+	+	+	O111
28	<i>Escherichia coli</i>	MSU, TW06296	human	O111:H2	+	+	+	+	O111
29	<i>Escherichia coli</i>	MSU, TW07870	diarrhea, cow	O111:H2	+	+	-	+	O111
30	<i>Escherichia coli</i>	ATCC 700840	stool, human	O111:H8	+	+	+	+	O111
31	<i>Escherichia coli</i>	ATCC, BAA-2180	stool, human	O111:H8	+	+	+	+	O111
32	<i>Escherichia coli</i>	ATCC, BAA-2201	stool, human	O111:H8	+	+	-	+	O111
33	<i>Escherichia coli</i>	CDC <i>d</i> , E05	human	O111:HNT	+	+	+	+	O111
34	<i>Escherichia coli</i>	MSU, TW07927	diarrhea, human	O121:NM	+	-	+	+	O121
35	<i>Escherichia coli</i>	MSU, TW07931	human	O121:NM	+	-	+	+	O121
36	<i>Escherichia coli</i>	MSU, TW08004	human	O121:NM	+	-	+	+	O121
37	<i>Escherichia coli</i>	MSU, TW08023	human	O121:H19	+	-	+	+	O121
38	<i>Escherichia coli</i>	MSU, TW08039	human	O121:HNT	+	-	+	+	O121

39	<i>Escherichia coli</i>	MSU, TW08970	HC, human	O121:HNT	+	-	+	+	O121
40	<i>Escherichia coli</i>	MSU, TW08974	HC, human	O121:HNT	+	-	+	+	O121
41	<i>Escherichia coli</i>	ATCC, BAA-2187	unknown	O121:H19	+	-	+	+	O121
42	<i>Escherichia coli</i>	ATCC, BAA-2219	stool, human	O121:H19	+	-	+	+	O121
43	<i>Escherichia coli</i>	MSU, TW03064	cow	O145:NM	+	-	+	+	O145
44	<i>Escherichia coli</i>	MSU, TW07596	diarrhea, human	O145:NM	+	+	-	+	O145
45	<i>Escherichia coli</i>	MSU, TW07865	cow	O145:H28	+	-	+	+	O145
46	<i>Escherichia coli</i>	MSU, TW09356	HUS, human	O145:H28	+	+	+	+	O145
47	<i>Escherichia coli</i>	ATCC, BAA-1652	stool, human	O145:H28	+	+	-	+	O145
48	<i>Escherichia coli</i>	ATCC, BAA-2211	stool, human	O145:H25	+	+	-	+	O145
49	<i>Escherichia coli</i>	ATCC, BAA-2222	stool, human	O145:NM	+	+	-	+	O145
50	<i>Escherichia coli</i>	CDC, 2010C-4330	human	O145:NM	+	+	+	+	O145

^a Gene profile determined from originating source.

^b MSU = Michigan State University STEC Center, Lansing, MI.

^c ATCC = American Type Culture Collection, Manassas, VI.

^d CDC = Center for Disease Control and Prevention, Atlanta, GA.

Table 2. Validation of Exclusivity of Assurance GDS MPX ID for Top STEC (1)

				Genotype ^a			MPX Top 7 STEC Result	MPX ID Result
No.	Organism	Serogroup	Source	eae	stx ₁	stx ₂		
	Non- <i>E. coli</i>							
1	<i>Citrobacter freundii</i>	N/A	ATCC 8090 ^b	-	-	-	-	-
2	<i>Enterobacter gergoviae</i>	N/A	ATCC 33028	-	-	-	-	-
3	<i>Escherichia hermannii</i>	N/A	ATCC, 33650	-	-	-	-	-
4	<i>Klebsiella oxytoca</i>	N/A	ATCC 43086	-	-	-	-	-
5	<i>Pseudomonas aeruginosa</i>	N/A	ATCC 15442	-	-	-	-	-
6	<i>Salmonella enterica</i> subsp <i>adelaide</i>	N/A	ATCC 10718	-	-	-	-	-
7	<i>Shigella flexneri</i>	N/A	ATCC 700930	-	-	-	-	-
	Non-Target Serogroups (eae- / stx-)							
8	<i>Escherichia coli</i>	unk	ATCC 8677	-	-	-	-	-
9	<i>Escherichia coli</i>	unk	ATCC 8739	-	-	-	-	-
10	<i>Escherichia coli</i>	unk	ATCC 11229	-	-	-	-	-
11	<i>Escherichia coli</i>	O1	ATCC 11775	-	-	-	-	-
12	<i>Escherichia coli</i>	unk	ATCC 25922	-	-	-	-	-
13	<i>Escherichia coli</i>	unk	ATCC 29194	-	-	-	-	-
	Non-Target Serogroups (eae+ / stx-)							
14	<i>Escherichia coli</i>	O55:H7	MSU, TW04052	+	-	-	-	-
15	<i>Escherichia coli</i>	O118:H8	MSU, TW04166	+	-	-	-	-
16	<i>Escherichia coli</i>	O119:H6	MSU, TW07894	+	-	-	-	-
17	<i>Escherichia coli</i>	O172	Natural isolate ^c	+	-	-	-	-
	Non-Target Serogroups (eae- / stx+)							
18	<i>Escherichia coli</i>	O8:H19	Natural isolate ^c	-	+	+	-	-
19	<i>Escherichia coli</i>	O76:H19	Natural isolate ^c	-	+	-	-	-
20	<i>Escherichia coli</i>	O91:H21	ATCC 51434	-	-	+	-	-
	Non-Target Serogroups (eae+ / stx +)							
21	<i>Escherichia coli</i>	O69:NM	MSU, TW07942	+	+	-	+	-
22	<i>Escherichia coli</i>	O118:H16	MSU, TW06410	+	+	-	+	-
	Target Serogroup (eae- / stx -)							
23	<i>Escherichia coli</i>	O26	ATCC 12795	-	-	-	-	-
24	<i>Escherichia coli</i>	O103	Natural isolate ^c	-	-	-	-	-
25	<i>Escherichia coli</i>	O111	MSU, TW06296	-	-	-	-	-
	Target Serougrouop (eae+ / stx -)							
26	<i>Escherichia coli</i>	O26:H11	MSU, TW07143	+	-	-	-	-
27	<i>Escherichia coli</i>	O45	MSU, TW09066	+	-	-	-	-
28	<i>Escherichia coli</i>	O111:H11	MSU, TW03810	+	-	-	-	-
29	<i>Escherichia coli</i>	O145:NM	MSU, TW05149	+	-	-	-	-
	Target Serougrouop (eae- / stx +)							
30	<i>Escherichia coli</i>	O121	USDA, Apr035A	-	+	-	-	-

^a Genetic profile determined from originating source.^b ATCC = American Type Culture Collection, Manassas, VI^c Michigan State University STEC Center, Lansing, MI.^d Serotype determined by Penn State.^e USDA = U.S. Department of Agriculture, Washington, DC.

Table 4. Comparison of Assurance GDS MPX ID for Top STEC: GDS Presumptive vs. Confirmed – POD (1)

Matrix	Concentration	N ^b	MPX ID Presumptive			MPX ID Confirmed			dPOD _{CP} ^f	95% CI ^g
	MPN ^a /25g		x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Raw beef trim	0.00	5	0	0.00	0.00, 0.43 ^h	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.79	20	11	0.55	0.34, 0.74	12	0.60	0.39, 0.78	-0.05	(-0.33), 0.24
	4.33	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
Raw ground beef	0.00	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.51	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	(-0.28), 0.28
	2.80	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
	0.00 ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.09 ⁱ	20	13	0.65	0.43, 0.82	16	0.80	0.58, 0.92	-0.15	(-0.40), 0.12
	0.44 ⁱ	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
Raw Spinach	0.00	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	2.02	20	16	0.80	0.58, 0.92	17	0.85	0.64, 0.95	-0.05	(-0.29), 0.19
	3.58	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
Steel	NA ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	NA	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.28), 0.28
	NA	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
	NA ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	NA ⁱ	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	(-0.28), 0.28
	NA ⁱ	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43

^aMPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.^bN = Number of test portions.^cx = Number of positive test portions.^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.^hThis indicates the range of lowest to highest values, with a comma between the two values.ⁱAnalysis by Independent Laboratory.^jNA = Not applicable.**Table 5. Comparison of Assurance GDS MPX ID for Top STEC: GDS Presumptive to Reference – POD (1)**

Matrix	Concentration	N ^b	MPX ID Presumptive			FDA/USDA Reference			dPOD _C ^f	95% CI ^g
	MPN ^a /25g		x ^c	POD _{CP} ^d	95% CI	x	POD _R ^e	95% CI		
Raw beef trim	0.00	5	0	0.00	0.00, 0.43 ^h	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.79	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	(-0.28), 0.28
	4.33	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
Raw ground beef	0.00	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.32
	0.51	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.58	-0.05	(-0.23), 0.28
	2.80	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
	0.00 ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.09 ⁱ	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.28), 0.28
	0.44 ⁱ	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
Raw Spinach	0.00	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	2.02	20	16	0.80	0.58, 0.92	17	0.85	0.64, 0.95	-0.05	(-0.29), 0.19
	3.58	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
Steel	NA ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	NA	20	13	0.65	0.43, 0.82	15	0.75	0.53, 0.89	-0.10	(-0.36), 0.28
	NA	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
	NA ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	NA ⁱ	20	8	0.40	0.22, 0.61	13	0.65	0.43, 0.82	-0.25	(-0.05), 0.50
	NA ⁱ	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43

^aMPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.^bN = Number of test portions.^cx = Number of positive test portions.^dPOD_C = Confirmed candidate method positive outcomes divided by the total number of trials.^ePOD_R = Confirmed reference method positive outcomes divided by the total number of trials.^fdPOD_C = Difference between the candidate method and reference method POD values.^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.^hThis indicates the range of lowest to highest values, with a comma between the two values.ⁱAnalysis by Independent Laboratory.^jNA = Not applicable.

Table 6. Validation of Assurance GDS MPX ID for Top STEC as Colony Confirmatory Method – Inclusivity Study (1)

No.	Organism	Source	Origin	Serotype	TSA-YE	Confirmation of Top 6 STEC by MPX ID PLATE MEDIA ^a			
						L-EMB	mRBA	RA-NC	BVCC
3	<i>Escherichia coli</i>	MSU, TW07705	stool, human	O26:H46	+ ^c	+ ^c	+ ^c	+ ^c	+ ^c
4	<i>Escherichia coli</i>	MSU, TW07814	human	O26:H11	+	+	+	+	+
5	<i>Escherichia coli</i>	MSU, TW07948	human	O26:NM	+	+	+	+	+
6	<i>Escherichia coli</i>	MSU, TW08570	HUS, human	O26:H11	+ ^c	+ ^c	+ ^c	+ ^c	+ ^c
7	<i>Escherichia coli</i>	ATCC d, BAA-2196	human	O26:H11	+	+	+	+	+
8	<i>Escherichia coli</i>	ATCC, BAA-2204	stool, human	O26:H11	+	+	+	+	+
9	<i>Escherichia coli</i>	MSU, TW00965	HC, human	O45:H2	+ ^c	+ ^c	+ ^c	+ ^c	+ ^c
10	<i>Escherichia coli</i>	MSU, TW01589	human	O45:H2	+	+	+	+	+
11	<i>Escherichia coli</i>	MSU, TW03068	cow	O45:H2	+ ^c	+ ^c	+ ^c	+ ^c	+ ^c
12	<i>Escherichia coli</i>	MSU, TW07947	diarrhea, human	O45:NM	+	+	+	+	+
13	<i>Escherichia coli</i>	MSU, TW09066	cow	O45:NM	+	+	+	+	+
14	<i>Escherichia coli</i>	MSU, TW09183	human	O45:NM	+	+	+	+	+
15	<i>Escherichia coli</i>	ATCC, BAA-2185	stool, human	O45:H2	+	+	+	+	+
16	<i>Escherichia coli</i>	ATCC, BAA-2193	human	O45:H2	+	+	+	+	+
17	<i>Escherichia coli</i>	MSU, TW04162	diarrhea, human	O103:H6	+ ^c	+ ^c	+ ^c	+ ^c	+ ^c
18	<i>Escherichia coli</i>	MSU, TW07881	BD, human	O103:H2	+	+	+	+	+
19	<i>Escherichia coli</i>	MSU, TW07971	human	O103:H6	+	+	+	+	+
20	<i>Escherichia coli</i>	MSU, TW07975	human	O103:HNT	+	+	+	+	+
21	<i>Escherichia coli</i>	MSU, TW07990	human	O103:NM	+	+	+	+	+
22	<i>Escherichia coli</i>	MSU, TW08101	human	O103:H2	+	+	+	+	+
23	<i>Escherichia coli</i>	MSU, TW08103	human	O103:H25	+	+	+	+	+
24	<i>Escherichia coli</i>	ATCC, BAA-2199	stool, human	O103:H25	+ ^c	+ ^e	+ ^c	+ ^c	+ ^c
25	<i>Escherichia coli</i>	ATCC, BAA-2215	human	O103:H11	+	+	+	+	+
26	<i>Escherichia coli</i>	MSU, TW04257	BD, human	O111:NM	+	+	+	+	+
27	<i>Escherichia coli</i>	MSU, TW05150	diarrhea, cow	O111:NM	+	+	+	+	+
28	<i>Escherichia coli</i>	MSU, TW06296	human	O111:H2	+	+	+	+	+
29	<i>Escherichia coli</i>	MSU, TW07870	diarrhea, cow	O111:H2	+ ^c	+ ^c	+ ^c	+ ^c	+ ^c
30	<i>Escherichia coli</i>	ATCC 700840	stool, human	O111:H8	+ ^c	+ ^e	+ ^c	+ ^c	+ ^c
31	<i>Escherichia coli</i>	ATCC, BAA-2180	stool, human	O111:H8	+	+	+	+	+
32	<i>Escherichia coli</i>	ATCC, BAA-2201	stool, human	O111:H8	+	+	+	+	+
33	<i>Escherichia coli</i>	CDC f, E05	human	O111:HNT	+	+	+	+	+
34	<i>Escherichia coli</i>	MSU, TW07927	diarrhea, human	O121:NM	+ ^c	+ ^c	+ ^c	+ ^c	+ ^b
35	<i>Escherichia coli</i>	MSU, TW07931	human	O121:NM	+	+	+	+	+
36	<i>Escherichia coli</i>	MSU, TW08004	human	O121:NM	+	+	+	+	+
37	<i>Escherichia coli</i>	MSU, TW08023	human	O121:H19	+ ^c	+ ^c	+ ^c	+ ^c	+ ^c
38	<i>Escherichia coli</i>	MSU, TW08039	human	O121:HNT	+	+	+	+	+
39	<i>Escherichia coli</i>	MSU, TW08970	HC, human	O121:HNT	+	+	+	+	+
40	<i>Escherichia coli</i>	MSU, TW08974	HC, human	O121:HNT	+	+	+	+	+
41	<i>Escherichia coli</i>	ATCC, BAA-2187	unknown	O121:H19	+	+	+	+	+
42	<i>Escherichia coli</i>	ATCC, BAA-2219	stool, human	O121:H19	+	+	+	+	+
43	<i>Escherichia coli</i>	MSU, TW03064	cow	O145:NM	+	+	+	+	+
44	<i>Escherichia coli</i>	MSU, TW07596	diarrhea, human	O145:NM	+	+	+	+	+
45	<i>Escherichia coli</i>	MSU, TW07865	cow	O145:H28	+	+	+	+	+
46	<i>Escherichia coli</i>	MSU, TW09356	HUS, human	O145:H28	+ ^c	+ ^c	+ ^c	+ ^c	+ ^c
47	<i>Escherichia coli</i>	ATCC, BAA-1652	stool, human	O145:H28	+	+	+	+	+
48	<i>Escherichia coli</i>	ATCC, BAA-2211	stool, human	O145:H25	+	+	+	+	+
49	<i>Escherichia coli</i>	ATCC, BAA-2222	stool, human	O145:NM	+ ^c	+ ^c	+ ^c	+ ^c	+ ^c
50	<i>Escherichia coli</i>	CDC, 2010C-4330	human	O145:NM	+	+	+	+	+

^a mRBA = modified rainbow agar; L-EMB = Levine's eosin-methylene blue agar; TSA-YE = trypticase soy agar with yeast; RANC = rainbow agar with novobiocin and cefixime; BVCC = enterohemolysin agar with selective agents.

^b Michigan State University STEC Center, Lansing, MI.

^c Strain analyzed also by Independent Laboratory. MPX ID Results were the same for Method Developer and Independent Laboratory for all isolates on each individual plate type.

^d ATCC = American Type Culture Collection, Manassas, VA.

^e Strain analyzed also by Independent Laboratory. MPX ID Results were positive for Method Developer; Independent Laboratory results were negative from L-EMB agar only.

^f CDC = Center for Disease Control and Prevention, Atlanta, GA.

Table 7. Validation of Assurance GDS MPX ID for Top STEC as Colony Confirmatory Method – Exclusivity Study (1)

No.	Organism	<i>E. coli</i>			Confirmation of Top 6 STEC by MPX ID			
		Serotype	Source	TSA-YE	PLATE MEDIA ^a			
					L-EMB	mRBA	RA-NC	BVCC
Non-<i>E. coli</i>								
1	<i>Citrobacter freundii</i>	N/A	ATCC b 6879	- ^c	- ^c	- ^c	- ^c	- ^c
2	<i>Enterobacter gergoviae</i>	N/A	ATCC 33028	- ^c	- ^c	- ^c	- ^c	- ^c
3	<i>Escherichia hermannii</i>	N/A	ATCC 33650	- ^c	- ^c	- ^c	- ^c	- ^c
4	<i>Klebsiella oxytoca</i>	N/A	ATCC 43086	- ^c	- ^c	- ^c	- ^c	- ^c
5	<i>Pseudomonas aeruginosa</i>	N/A	ATCC 15442	-	-	-	-	-
6	<i>Salmonella enterica</i> subsp typhimurium	N/A	ATCC 51812	- ^c	- ^c	- ^c	- ^c	- ^c
7	<i>Shigella flexneri</i>	N/A	ATCC 700930	-	-	-	-	-
Non-Target Serogroup (eae- / stx-)								
8	<i>Escherichia coli</i>	unk	ATCC 8677	-	-	-	-	-
9	<i>Escherichia coli</i>	unk	ATCC 8739	-	-	-	-	-
10	<i>Escherichia coli</i>	unk	ATCC 11229	-	-	-	-	-
11	<i>Escherichia coli</i>	O1	ATCC 11775	- ^c	- ^c	- ^c	- ^c	- ^c
12	<i>Escherichia coli</i>	O6	ATCC 25922	-	-	-	-	-
13	<i>Escherichia coli</i>	unk	ATCC 29194	-	-	-	-	-
Non-Target Serogroup (eae+ / stx-)								
14	<i>Escherichia coli</i>	O55:H7	MSU d, TW04052	-	-	-	-	-
15	<i>Escherichia coli</i>	O118:H8	MSU, TW04166	- ^c	- ^c	- ^c	- ^c	- ^c
16	<i>Escherichia coli</i>	O119:H6	MSU, TW07894	-	-	-	-	-
17	<i>Escherichia coli</i>	O172	Natural isolate	-	-	-	-	-
Non-Target Serogroup (eae- / stx+)								
18	<i>Escherichia coli</i>	O8:H19	Natural isolate	-	-	-	-	-
19	<i>Escherichia coli</i>	O76:H19	Natural isolate	-	-	-	-	-
20	<i>Escherichia coli</i>	O91:H21	ATCC 51434	-	-	-	-	-
Non-Target Serogroup (eae+ / stx+)								
21	<i>Escherichia coli</i>	O69:NM	MSU, TW07942	-	-	-	-	-
22	<i>Escherichia coli</i>	O118:H16	MSU, TW06410	-	-	-	-	-
	<i>Escherichia coli</i>	O157:H7	ATCC 43890	- ^e	- ^e	- ^e	- ^e	- ^e
Target Serogroup (eae- / stx-)								
23	<i>Escherichia coli</i>	O26	ATCC 12795	-	-	-	-	-
24	<i>Escherichia coli</i>	O103	Natural isolate, beef	-	-	-	-	-
25	<i>Escherichia coli</i>	O111	MSU, TW06296	-	-	-	-	-
Target Serogroup (eae+ / stx-)								
26	<i>Escherichia coli</i>	O26:H11	MSU, TW01208	-	-	-	-	-
27	<i>Escherichia coli</i>	O45	MSU, TW09066	-	-	-	-	-
28	<i>Escherichia coli</i>	O111:H11	MSU, TW03810	-	-	-	-	-
29	<i>Escherichia coli</i>	O145:NM	MSU, TW05149	-	-	-	-	-
Target Serogroup (eae- / stx+)								
30	<i>Escherichia coli</i>	O121	USDA f Apr035A	-	-	-	-	-

^a mRBA = modified rainbow agar; L-EMB = Levine's eosin-methylene blue agar; TSA-YE = trypticase soy agar with yeast; RANC = rainbow agar with novobiocin and cefixime; BVCC = enterohemolysin agar with selective agents

^b ATCC = American Type Culture Collection, Manassas, VI.

^c Strain analyzed also by Independent Laboratory. MPX ID Results were the same for Method Developer and Independent Laboratory for all isolates on each individual plate type.

^d Michigan State University STEC Center, Lansing, MI.

^e Strain analyzed by Independent Laboratory only.

^f USDA = U.S. Department of Agriculture, Washington, DC.

Table 8: Comparison of Confirmation of Top STEC by OctoMACS and PickPen IMS procedures from mTSB (1)

Food Type	Contaminating Organism	Source	MLG	Sample Size	CFU/g	mTSB (mTSB+n)			
						Total tested	Presump	PickPen confirmed	OctoMACS confirmed
Ground Beef	<i>E. coli</i> O45:H2	ATCC BAA-2185	5B.05	325 g	LOW	20	7	7	7
					High	5	5	5	5
	-	-			Uninoculated	5	0	0	0
Ground Beef ^a	<i>E. coli</i> O45:H2	ATCC BAA-2185	5B.05		Low	20	13	13	13
					High	5	5	5	5
	-	-			Uninoculated	5	0	0	0
Ground Beef	<i>E. coli</i> O157:H7	ATCC 35150	5.06		High	30	30	30	30
Raw beef trim	<i>E. coli</i> O121:H19	ATCC BAA-2219	5B.05	325 g	Low	20	10	10	10
	<i>E. coli</i> O26	natural					1	1	1
	<i>E. coli</i> O121:H19	ATCC BAA-2219			High	5	4	4	4
	<i>E. coli</i> O26	natural					5	5	4
	-	-			Uninoculated	5	0	0	0
Raw beef trim	<i>E. coli</i> O157:H7	ATCC 35150	5.06		Low	10	6	6	6
	-	-			Uninoculated	2	0	0	0
Stainless Steel	<i>E. coli</i> O26:H11	ATCC BAA-1653	5B.05	-	Low	20	15	15	15
					High	5	5	5	5
	-	-			Uninoculated	10	0	0	0
Stainless Steel	<i>E. coli</i> O26:H11	ATCC BAA-1653	5B.05	-	Low	20	13	13	13
					High	5	5	5	5
	-	-			Uninoculated	10	0	0	0

^a - analyzed by Independent Lab

Table 9: Comparison of Confirmation of Top STEC by OctoMACS and PickPen II procedures in mEHEC (1)

Food Type	Contaminating Organism	Source	MLG	Sample Size	CFU/g	mEHEC			
						Total tested	Presump	PickPen confirmed	OctoMACS confirmed
Ground Beef	<i>E. coli</i> O45:H2	ATCC BAA-2185	5B.05	375 g	Low	20	8	8	8
	-	-			High	5	5	5	5
	-	-			Uninoculated	5	0	0	0
Ground Beef ^a	<i>E. coli</i> O45:H2	ATCC BAA-2185	5B.05	375 g	LOW	20	16	16	16
	-	-			High	5	5	5	5
	-	-			Uninoculated	5	0	0	0
Raw beef trim	<i>E. coli</i> O121:H19	ATCC BAA-2219	5B.05	375 g	Low	20	12	12	12
	<i>E. coli</i> O26	natural					1	1	1
	<i>E. coli</i> O121:H19	ATCC BAA-2219			High	5	5	5	5
	<i>E. coli</i> O26	natural					5	5	5
	-	-			Uninoculated	5	0	0	0
Stainless steel	<i>E. coli</i> O26:H11	ATCC BAA-1653	5B.05	-	Low	20	13	13	13
	-	-			High	5	5	5	5
	-	-			Uninoculated	10	0	0	0
Stainless steel ^a	<i>E. coli</i> O26:H11	ATCC BAA-1653	5B.05	-	Low	20	8	8	8
	-	-			High	5	5	5	5
	-	-			Uninoculated	10	0	0	0

^a - analyzed by Independent Lab

REFERENCES CITED

1. Feldsine, P., Lienau, A. H., Shah K., Immermann, A., Soliven, K., Kaur, M., Kerr, D.E., and Jucker, M, Comparison of Assurance® GDS MPX ID for Top STEC with Reference Culture Methods for the Detection of *E. coli* Top 6 STEC; Direct Confirmation of Top 6 STEC from Isolation Plates and Determination of Equivalence of PickPen and FSIS OctoMACS Concentrations Protocols, AOAC Performance Tested MethodsSM certification number 101502.
2. U.S. Department of Agriculture, Food Safety and Inspection Service *Microbiological Laboratory Guidelines*, 5B.05, 2014, <http://www.fsis.usda.gov/wps/wcm/connect/7ffc02b5-3d33-4a79-b50c-81f208893204/MLG-5B.pdf?MOD=AJPERES>
3. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*, Chapter 4A <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm>
4. U.S. Department of Agriculture, Food Safety and Inspection Service *Microbiological Laboratory Guidelines*, 5.09, 2015 <http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5.pdf?MOD=AJPERES>