



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.

071303

The AOAC Research Institute hereby certifies the method known as

Assurance[®] GDS STEC (*eae*) Tq with Assurance[®] GDS Shiga Toxin Genes (Top 7) Tq

manufactured by

BioControl Systems, Inc. / MilliporeSigma

12822 SE 32nd Street

Bellevue, WA 98005 USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

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

Bradley A. Stawick, AOAC Research Institute Senior Director

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AUTHORS Philip Feldsine, Andrew H. Lienau, Megan Montgomery-Fullerton, David Kerr, and Marcus Jucker	SUBMITTING COMPANY BioControl Systems, Inc. 12822 SE 32 nd St. Bellevue, WA 98005 USA	CURRENT SPONSOR BioControl Systems, Inc. / MilliporeSigma 12822 SE 32 nd St. Bellevue, WA 98005 USA
METHOD NAME Assurance® GDS Top 7 STEC (<i>eae</i>) Tq with Assurance® GDS Shiga Toxin Genes (Top 7) Tq	CATALOG NUMBERS 71017-100, 71018-100	
INDEPENDENT LABORATORY Silliker Inc, Food Science Center South Holland, IL USA		
APPLICABILITY OF METHOD Analytes – Top 7 Shiga Toxin-producing <i>Escherichia coli</i> (STEC), serotypes O26, O45, O103, O111, O121, O145, and O157 Matrixes – 375 g -Raw beef trim, raw ground beef 25 g - mixed greens and spinach Performance claims – The method proved comparable to the reference methods.	REFERENCE METHODS U.S. Department of Agriculture, Food Safety and Inspection Service. 2011.Microbiological Laboratory Guidelines (2) U.S. Food and Drug Administration. Bacteriological Analytical Manual, Chapter 4A (3)	
ORIGINAL CERTIFICATION DATE July 24, 2013	CERTIFICATION RENEWAL RECORD Renewed through December 2027.	
METHOD MODIFICATION RECORD 1. December 2017 Level 1 2. November 2018 Level 1 3. January 2020 Level 2 4. November 2021 Level 1 5. January 2023 Level 1	SUMMARY OF MODIFICATION 1. Editorial changes 2. Editorial changes to insert 71017-100. Added insert for Shiga Toxins 71018-100. 3. mEHEC media and mEHEC+novobiocin manufacturing location change to Darmstadt, Germany and rebranding to Merck format. 4. Editorial changes and update to software. 5. Editorial changes.	
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PRINCIPLE OF THE METHOD (1)

BioControl has developed a method for detection of Top 7 STEC using the Assurance® GDS pathogen detection system, utilizing proprietary probes and primers directed against a highly conserved DNA sequence of the target organism. In addition, the sample preparation procedure utilizes a proprietary device and reagents that concentrate populations of target microorganisms, thereby providing increased sensitivity and elimination of potential competitive microflora. To date, five Assurance GDS methods utilizing this platform have already been validated either as AOAC Official Methods – for *E. coli* O157:H7 (OMA 2005.04), for Shiga Toxin Genes (O157) (OMA 2005.05) and for *Salmonella* (OMA 2009.03) – or as a Harmonized AOAC and PTM Method – for *Listeria* spp. (Cert #070701) and for *Listeria monocytogenes* (Cert #070702).

Top 7 STEC are detected from the combined use of two assays, Assurance GDS Top 7 STEC (*eae*) and the Assurance GDS Shiga Toxin Genes (Top 7). The sample preparation procedure allows the screening for the 7 serogroups of interest. Top 7 STEC are defined as containing the *eae* gene, one of the *stx* genes and belonging to the following O serogroups of *E. coli*: O103, O111, O121, O145, O26, O45 and O157. The Assurance GDS Top 7 STEC (*eae*) assay detects the presence of the *eae* gene. The Assurance GDS Shiga Toxin Genes (Top 7) assay detects and differentiates the presence of *stx1* and *stx2*. The advantage of using the combined Assurance GDS Top 7 STEC (*eae*) assay with the Assurance GDS Shiga Toxin Genes (Top 7) assay is that the identified adulterant is positive for both genes (*eae* and *stx*). An organism having only one (*stx*) but not the other gene (*eae*) does not satisfy the above regulatory definition. Therefore, the assays should be run in combination, first Assurance GDS Top 7 STEC (*eae*) and then Assurance GDS Shiga Toxin Genes (Top 7). When used together, the analyst can determine whether a sample contains Top STEC. Assurance GDS *eae/stx* has been designed to provide detection of the Top 7 STEC from a sample. From the same sample, GDS *eae/stx* will provide separate results for the presence of *E. coli* O157:H7 and other Top STEC (non-O157). The sample preparation procedure allows the screening for the 7 serogroups of interest. The GDS *eae/stx* probes and primers detect separately and simultaneously the presence of the *eae*. In addition, the presence of *E. coli* O157:H7 is also distinctly detected.

DISCUSSION OF THE VALIDATION STUDY (1)

Results of these studies validated that Assurance GDS *eae/stx* is robust, inclusive and specific for Top 7 STEC. In the internal method comparison study, GDS *eae/stx* and the reference culture methods are statistically not different for the detection of Top 7 STEC in raw beef trim, raw ground beef, spinach and mixed greens. The external method comparison study yielded similar results for raw beef trim. In this study, we observed 100% inclusivity among the 67 Top 7 STEC and 100% exclusivity for the 31 non-Top 7 STEC organisms analyzed. These results demonstrate that GDS EAE is an effective method for the detection of Top 7 STEC in raw beef trim, raw ground beef, mixed greens and spinach. In the internal method comparison study, for spinach (low and high level), results indicated that GDS *eae/stx* and reference methods are statistically different, with GDS *eae/stx* results recovering higher number of Top 7 STEC confirmed positive samples than reference culture method. In general, a lower recovery of STEC from mixed produce by the reference method compared to GDS *eae/stx* method was observed. The reference method for leafy produce employs a rinse off step of the leaves, followed by incubation of the rinse material. By comparison, the GDS *eae/stx* preparation methodology specifies mastication of the leaves and incubation of the entire sample. The incubation of a rinsate compared to the entire sample may account for the lower recovery for the reference method.

Table 1. Validation of Inclusivity of Assurance GDS Top 7 STEC (*eae*) Assay with the Assurance GDS Shiga Toxin Genes (Top 7) Assay (1)

				Assurance GDS Top 7 STEC (<i>eae</i>) with Shiga Toxin Genes (Top 7)				
	Organism	Serogroup	Source	<i>E. coli</i> O157:H7	<i>eae</i>	<i>stx</i> ₁	<i>stx</i> ₂	Top STEC
1	<i>E. coli</i>	O26	MSU, TW08570	-	+	-	+	+
2	<i>E. coli</i>	O26	MSU, TW07814	-	+	+	+	+
3	<i>E. coli</i>	O26	MSU, TW07705	-	+	+	+	+
4	<i>E. coli</i>	O26	MSU, TW05512	-	+	+	-	+
5	<i>E. coli</i>	O26	MSU, TW08998	-	+	+	-	+
6	<i>E. coli</i>	O26	MSU, TW05555	-	+	+	-	+
7	<i>E. coli</i>	O26	MSU, TW09156	-	+	+	-	+
8	<i>E. coli</i>	O26	MSU, TW04270	-	+	+	-	+
9	<i>E. coli</i>	O26	MSU, TW07936	-	+	+	-	+
10	<i>E. coli</i>	O26	MSU, TW07948	-	+	+	-	+
11	<i>E. coli</i>	O103	MSU, TW07697	-	+	+	-	+
12	<i>E. coli</i>	O103	MSU, TW07971	-	+	+	-	+
13	<i>E. coli</i>	O103	MSU, TW08101	-	+	+	-	+
14	<i>E. coli</i>	O103	MSU, TW08103	-	+	+	-	+
15	<i>E. coli</i>	O103	MSU, TW01675	-	+	+	-	+
16	<i>E. coli</i>	O103	Industry isolate	-	+	+	-	+
17	<i>E. coli</i>	O103	Industry isolate	-	+	+	-	+
18	<i>E. coli</i>	O103	MSU, TW07881	-	+	+	-	+
19	<i>E. coli</i>	O103	MSU, TW07975	-	+	+	-	+
20	<i>E. coli</i>	O103	MSU, TW07990	-	+	+	-	+
21	<i>E. coli</i>	O103	MSU, TW04162	-	+	+	-	+
22	<i>E. coli</i>	O103	MSU, TW03051	-	+	+	-	+
23	<i>E. coli</i>	O121	MSU, TW07931	-	+	-	+	+
24	<i>E. coli</i>	O121	MSU, TW08974	-	+	-	+	+
25	<i>E. coli</i>	O121	MSU, TW08023	-	+	-	+	+
26	<i>E. coli</i>	O121	MSU, TW07927	-	+	-	+	+
27	<i>E. coli</i>	O121	MSU, TW08004	-	+	-	+	+
28	<i>E. coli</i>	O121	MSU, TW08039	-	+	-	+	+
29	<i>E. coli</i>	O121	MSU, TW07972	-	+	-	+	+
30	<i>E. coli</i>	O121	MSU, TW08969	-	+	-	+	+
31	<i>E. coli</i>	O121	MSU, TW08970	-	+	-	+	+
32	<i>E. coli</i>	O111	MSU, TW07601	-	+	+	+	+
33	<i>E. coli</i>	O111	MSU, TW07926	-	+	+	-	+
34	<i>E. coli</i>	O111	MSU, TW07813	-	+	+	+	+
35	<i>E. coli</i>	O111	MSU, TW05608	-	+	+	-	+
36	<i>E. coli</i>	O111	MSU, TW04257	-	+	+	+	+
37	<i>E. coli</i>	O111	MSU, TW07870	-	+	+	-	+
38	<i>E. coli</i>	O111	ATCC 700840	-	+	+	+	+
39	<i>E. coli</i>	O111	MSU, TW05150	-	+	+	+	+
40	<i>E. coli</i>	O111	Industry Isolate	-	+	+	-	+
41	<i>E. coli</i>	O111	Industry Isolate	-	+	+	-	+
42	<i>E. coli</i>	O111	Industry Isolate	-	+	+	-	+
43	<i>E. coli</i>	O45	MSU, TW07947	-	+	+	-	+
44	<i>E. coli</i>	O45	MSU, TW00965	-	+	+	-	+
45	<i>E. coli</i>	O45	MSU, TW03068	-	+	+	-	+
46	<i>E. coli</i>	O45	MSU, TW09066	-	+	+	-	+
47	<i>E. coli</i>	O45	MSU, TW03070	-	+	+	-	+
48	<i>E. coli</i>	O45	MSU, TW01589	-	+	+	-	+
49	<i>E. coli</i>	O45	MSU, TW07947	-	+	+	-	+
50	<i>E. coli</i>	O45	MSU, TW09183	-	+	+	-	+
51	<i>E. coli</i>	O145	MSU, TW07596	+	+	+	-	+
52	<i>E. coli</i>	O145	MSU, TW01664	-	+	+	-	+
53	<i>E. coli</i>	O145	MSU, TW08087	+	+	+	-	+
54	<i>E. coli</i>	O145	MSU, TW09153	-	+	-	+	+
55	<i>E. coli</i>	O145	MSU, TW03064	+	+	-	+	+
56	<i>E. coli</i>	O145	MSU, TW07860	-	+	+	-	+
57	<i>E. coli</i>	O145	Industry Isolate	+	+	+	-	+
58	<i>E. coli</i>	O145	Industry Isolate	-	+	-	+	+
59	<i>E. coli</i>	O145	MSU, TW09356	+	+	-	+	+
60	<i>E. coli</i>	O157:H7	ATCC 35150	+	+	+	+	+
61	<i>E. coli</i>	O157:H7	ATCC 43890	+	+	+	-	+
62	<i>E. coli</i>	O157:H7	Industry Isolate	+	+	+	-	+
63	<i>E. coli</i>	O157:H7	Industry Isolate	+	+	+	-	+
64	<i>E. coli</i>	O157:H7	Government Isolate	+	+	-	+	+
65	<i>E. coli</i>	O157:H7	Government Isolate	+	+	-	+	+
66	<i>E. coli</i>	O157:H7	Government Isolate	+	+	-	+	+
67	<i>E. coli</i>	O157:H7	Government Isolate	+	+	+	+	+

^aMSU = Michigan State University STEC Center; ATCC = American Type Culture Collection

Table 2. Validation of Exclusivity of Assurance GDS Top 7 STEC (*eae*) Assay with the Assurance GDS Shiga Toxin Genes (Top 7) Assay (1)

	Organism	Serogroup	Source	Assurance GDS Top 7 STEC (<i>eae</i>) with Shiga Toxin Genes (Top 7)				
				<i>E. coli</i> O157:H7	<i>eae</i>	<i>stx</i> ₁	<i>stx</i> ₂	Top STEC
1	<i>Shigella flexneri</i>	N/A	Industry isolate	-	-	-	-	-
2	<i>Citrobacter freundii</i>	N/A	Government isolate	-	-	-	-	-
3	<i>Enterobacter gergoviae</i>	N/A	ATCC 33028	-	-	-	-	-
4	<i>Salmonella enterica</i> subsp. <i>tranoroa</i>	O103	Industry isolate	-	-	-	-	-
5	<i>Salmonella enterica</i> subsp. <i>adelaide</i>	O111	ATCC 10718	-	-	-	-	-
6	<i>Pseudomonas aeruginosa</i>	N/A	ATCC 15442	-	-	-	-	-
7	<i>Escherichia hermannii</i>	N/A	ATCC 33650	-	-	-	-	-
8	<i>Klebsiella oxytoca</i>	N/A	ATCC 43086	-	-	-	-	-
Non-Target Serogroups (<i>eae</i> - / <i>stx</i> -)								
9	<i>Escherichia coli</i>	N/A	ATCC 11775	-	-	-	-	-
10	<i>Escherichia coli</i>	N/A	ATCC 11229	-	-	-	-	-
11	<i>Escherichia coli</i>	N/A	ATCC 25922	-	-	-	-	-
12	<i>Escherichia coli</i>	N/A	ATCC 29194	-	-	-	-	-
13	<i>Escherichia coli</i>	N/A	ATCC 15223	-	-	-	-	-
14	<i>Escherichia coli</i>	N/A	ATCC 8677	-	-	-	-	-
15	<i>Escherichia coli</i>	N/A	ATCC 8739	-	-	-	-	-
16	<i>Escherichia coli</i>	N/A	ATCC 15939	-	-	-	-	-
Non-Target Serogroups (<i>eae</i> + / <i>stx</i> +)								
17	<i>Escherichia coli</i>	O156	MSU, TW01407	-	-	-	-	-
Non-Target Serogroups (<i>eae</i> + / <i>stx</i> -)								
18	<i>Escherichia coli</i>	O172	Industry isolate	-	-	-	-	-
19	<i>Escherichia coli</i>	O55	U of MI, TW07620	-	-	-	-	-
20	<i>Escherichia coli</i>	O91	U of MI, TW01674	-	-	-	-	-
Non-Target Serogroups (<i>eae</i> - / <i>stx</i> +)								
21	<i>Escherichia coli</i>	O8	Industry isolate	-	-	-	-	-
22	<i>Escherichia coli</i>	O76	Industry isolate	-	-	-	-	-
23	<i>Escherichia coli</i>	O18	MSU, TW07933	-	-	-	-	-
Target Serogroup (<i>eae</i> + / <i>stx</i> -)								
24	<i>Escherichia coli</i>	O26	MSU, TW01208	-	+	-	-	-
25	<i>Escherichia coli</i>	O111	MSU, TW03810	-	+	-	-	-
26	<i>Escherichia coli</i>	O45	MSU, TW03036	-	+	-	-	-
27	<i>Escherichia coli</i>	O121	MSU, TW08980	-	+	-	-	-
28	<i>Escherichia coli</i>	O145	MSU, TW07865	-	+	-	-	-
Target Serogroup (<i>eae</i> - / <i>stx</i> -)								
29	<i>Escherichia coli</i>	O26	MSU, TW04081	-	-	-	-	-
30	<i>Escherichia coli</i>	O26	ATCC 12795	-	-	-	-	-
31	<i>Escherichia coli</i>	O111	MSU, TW06296	-	-	-	-	-

^aMSU = Michigan State University STEC Center; ATCC = American Type Culture Collection

Table 4. Comparison of Assurance GDS Top 7 STEC (eae) Assay with the Assurance GDS Shiga Toxin Genes (Top 7) Assay to Reference: Assurance GDS Presumptive vs. Confirmed – POD (1)

Matrix	Concentration		N ^b	Assurance GDS Presumptive			Assurance GDS Confirmed			dPOD _{CP} ^f	95% CI ^g
	MPN ^a /25g	LCL, UCL		X ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Raw ground beef	0.00		5	0	0.00	0.00, 0.43 ^h	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.68	0.40, 1.12	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.28), 0.28
	5.08	1.90, 3.58	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
Raw beef trim	0.00		5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.85	0.49, 1.49	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	(-0.26), 0.26
	3.37	1.38, 8.21	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
	0.99 ⁱ	0.68, 1.46	20	6	0.30	0.15, 0.52	5	0.25	0.11, 0.47	0.05	(-0.22), 0.31
	4.38 ⁱ	1.72, 11.15	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	(-0.46), 0.46
Spinach	0.00		5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.30	0.12, 0.55	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.28), 0.28
	1.13	0.53, 2.39	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
Mixed greens	0.00		5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.29	0.13, 0.51	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	(-0.28), 0.28
	1.64	0.60, 3.0	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 across labs using the AOAC 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^h– = This indicates the range of lowest to highest values, with a comma between the two valuesⁱ = analysis by Independent Lab

Table 5. Comparison of Assurance GDS Top 7 STEC (*eae*) Assay with the Assurance GDS Shiga Toxin Genes (Top 7) to Reference: Method Comparison Results – POD (1)

Matrix	Concentration		N ^b	Assurance GDS Presumptive			FDA/USDA Reference			dPOD _c ^f	95% CI ^g
	MPN ^a /25g	LCL, UCL		X ^c	POD _{CP} ^d	95% CI	x	POD _R ^e	95% CI		
Raw ground beef	0.00		5	0	0.00	0.00, 0.43 ^h	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.68	0.40, 1.12	20	13	0.65	0.43, 0.82	8	0.40	0.22, 0.61	0.25	(-0.05), 0.50
	5.08	1.90, 3.58	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43), 0.43
Raw beef trim	0.00		5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.85	0.49, 1.49	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	(-0.13), 0.40
	3.37	1.38, 8.21	5	5	1.00	0.57, 1.00	4	0.80	0.38, 0.96	0.00	(-0.45), 0.45
	0.00 ⁱ		5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.99 ⁱ	0.68, 1.46	20	5	0.25	0.11, 0.47	9	0.45	0.26, 0.66	-0.20	(-0.45), 0.09
	4.38 ⁱ	1.72, 11.15	5	3	0.60	0.23, 0.88	5	1.00	0.57, 1.00	-0.40	(-0.77), 0.12
Spinach	0.00		5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.30	0.12, 0.55	20	13	0.65	0.43, 0.82	6	0.30	0.15, 0.52	0.35	(0.04), 0.58
	1.13	0.53, 2.39	5	5	1.00	0.57, 1.00	2	0.40	0.12, 0.77	0.60	(0.03), 0.88
Mixed greens	0.00		5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43), 0.43
	0.29	0.13, 0.51	20	9	0.45	0.26, 0.66	4	0.20	0.08, 0.42	0.20	(-0.26), 0.62
	1.64	0.60, 3.0	5	5	1.00	0.57, 1.00	4	0.80	0.38, 0.96	0.20	(-0.26), 0.62

^aMPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC 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^h– = This indicates the range of lowest to highest values, with a comma between the two valuesⁱ = analysis by Independent Lab**REFERENCES CITED**

1. Feldsine, P., Lienau, A.H., Montgomery-Fullerton, M., Kerr, D., and Jucker, M. Evaluation of the Assurance® GDS Top 7 STEC (*eae*) Tq with Assurance® GDS Shiga Toxin Genes (Top 7) Tq in Selected Foods., AOAC *Performance Tested Methods*SM certification number 071303.
2. U.S. Department of Agriculture, Food Safety and Inspection Service. 2011. *Microbiological Laboratory Guidelines*, http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp, Chapter 5B.01
3. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*, <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm>, Chapter 4A