CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

071301

The AOAC Research Institute hereby certifies the method known as

Assurance® GDS for MPX Top 7 STEC

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*℠ 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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METHOD NAME
Assurance® GDS for MPX Top 7 STEC

CATALOG NUMBER
71015-100

INDEPENDENT LABORATORY
Silliker Inc,
Food Science Center
South Holland, IL USA

APPLICABILITY OF METHOD
Target organism – Top 7 Shiga Toxin-producing *Escherichia coli* (STEC), serotypes O26, O45, O103, O111, O121, O145, and O157

Matrixes – (USDA MLG 5B.01) – Raw beef trim (375 g), raw ground beef (375 g), stainless steel (4" x 4" sponge)
(FDA BAM Ch 4A) – 375 g spinach, 25 g - leafy greens and spinach

Performance claims – In this study, 100% inclusivity among the 67 Top 7 STEC strains and 100% exclusivity for the 31 potential cross-reacting non-Top 7 STEC organisms was achieved. In method comparison studies, GDS MPX test results showed no statistically significant differences to the reference culture methods for raw beef trim, raw ground beef, stainless steel, leafy greens and spinach.

REFERENCE METHODS
U.S. Department of Agriculture, Food Safety and Inspection Service. 2011.Microbiological Laboratory Guidelines, Chapter 5B.01 (2)

U.S. Food and Drug Administration. Bacteriological Analytical Manual, Chapter 4A (3)

ORIGINAL CERTIFICATION DATE
July 23, 2013

CERTIFICATION RENEWAL RECORD
Renewed through December 2027.

METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. January 2016 Level 2	1. Hold time change from 15 to 20 minutes.
2. December 2017 Level 1	2. Editorial changes for clarity.
3. November 2018 Level 1	3. Editorial changes to update version number; labels discontinued.
4. January 2020 Level 2	4. mEHEC media and mEHEC+novobiocin manufacturing location change to Darmstadt, Germany and rebranding to Merck format.
5. June 2020 Level 1	5. Addition of spinach (375 g) and stainless steel (4" x 4" sponge). Linked to PTM 101502 Assurance® GDS MPX ID for Top STEC (MPX ID).
6. January 2021 Level 1	6. Editorial changes and rebranding to BioControl Systems Inc. / MilliporeSigma.
7. November 2021 Level 1	7. Editorial changes and update to software.
8. January 2023 Leve 1	8. Editorial changes

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PRINCIPLE OF THE METHOD
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).
Assurance GDS MPX Top 7 STEC has been designed to provide detection of the Top 7 STEC from a sample in one combined test. From the same sample, GDS MPX 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 MPX probes and primers detect separately and simultaneously the presence of the *eae*, *stx1* and *stx2*. In addition, the presence of *E. coli* O157:H7 is also distinctly detected.

DISCUSSION OF THE VALIDATION STUDY

Results of these studies validated that Assurance® GDS MPX Top 7 STEC is robust, inclusive and specific for Top 7 STEC. In the internal method comparison study, GDS MPX 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 leafy 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 MPX is an effective method for the detection of Top 7 STEC in raw beef trim, raw ground beef, leafy greens and spinach.

In the internal method comparison study, for spinach (high level), results indicated that GDS MPX and reference methods are statistically different, with GDS MPX results recovering higher number of Top 7 STEC confirmed positive samples than reference culture method. In general, a lower recovery of STEC from leafy produce by the reference method compared to GDS MPX 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 MPX 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. Organisms Included in Validation of Inclusivity of Assurance GDS MPX Top 7 STEC (1)

				Assurance GDS MPX Top 7 STEC				
	Organism	Serogroup	Source	*E. coli* O157:H7	*eae*	*stx*$_1$	*stx*$_2$	Top STEC
1	*E. coli*	O26	MSU, TW08570	-	+	-	+	+
2	*E. coli*	O26	MSU, TW07814	-	+	+	+	+
3	*E. coli*	O26	MSU, TW07705	-	+	+	+	+
4	*E. coli*	O26	MSU, TW05512	-	+	+	-	+
5	*E. coli*	O26	MSU, TW08998	-	+	+	-	+
6	*E. coli*	O26	MSU, TW05555	-	+	+	-	+
7	*E. coli*	O26	MSU, TW09156	-	+	+	-	+
8	*E. coli*	O26	MSU, TW04270	-	+	+	-	+
9	*E. coli*	O26	MSU, TW07936	-	+	+	-	+
10	*E. coli*	O26	MSU, TW07948	-	+	+	-	+
11	*E. coli*	O103	MSU, TW07697	-	+	+	-	+
12	*E. coli*	O103	MSU, TW07971	-	+	+	-	+
13	*E. coli*	O103	MSU, TW08101	-	+	+	-	+
14	*E. coli*	O103	MSU, TW08103	-	+	+	-	+
15	*E. coli*	O103	MSU, TW01675	-	+	+	-	+
16	*E. coli*	O103	Industry isolate	-	+	+	-	+
17	*E. coli*	O103	Industry isolate	-	+	+	-	+
18	*E. coli*	O103	MSU, TW07881	-	+	+	-	+
19	*E. coli*	O103	MSU, TW07975	-	+	+	-	+
20	*E. coli*	O103	MSU, TW07990	-	+	+	-	+
21	*E. coli*	O103	MSU, TW04162	-	+	+	-	+
22	*E. coli*	O103	MSU, TW03051	-	+	+	-	+
23	*E. coli*	O121	MSU, TW07931	-	+	-	+	+
24	*E. coli*	O121	MSU, TW08974	-	+	-	+	+
25	*E. coli*	O121	MSU, TW08023	-	+	-	+	+
26	*E. coli*	O121	MSU, TW07927	-	+	-	+	+
27	*E. coli*	O121	MSU, TW08004	-	+	-	+	+
28	*E. coli*	O121	MSU, TW08039	-	+	-	+	+
29	*E. coli*	O121	MSU, TW07972	-	+	-	+	+
30	*E. coli*	O121	MSU, TW08969	-	+	-	+	+
31	*E. coli*	O121	MSU, TW08970	-	+	-	+	+
32	*E. coli*	O111	MSU, TW07601	-	+	+	+	+
33	*E. coli*	O111	MSU, TW07926	-	+	+	-	+
34	*E. coli*	O111	MSU, TW07813	-	+	+	+	+
35	*E. coli*	O111	MSU, TW05608	-	+	+	-	+
36	*E. coli*	O111	MSU, TW04257	-	+	+	+	+
37	*E. coli*	O111	MSU, TW07870	-	+	+	-	+
38	*E. coli*	O111	ATCC 700840	-	+	+	+	+
39	*E. coli*	O111	MSU, TW05150	-	+	+	+	+
40	*E. coli*	O111	Industry Isolate	-	+	+	-	+
41	*E. coli*	O111	Industry Isolate	-	+	+	-	+
42	*E. coli*	O111	Industry Isolate	-	+	+	-	+
43	*E. coli*	O45	MSU, TW07947	-	+	+	-	+
44	*E. coli*	O45	MSU, TW00965	-	+	+	-	+
45	*E. coli*	O45	MSU, TW03068	-	+	+	-	+
46	*E. coli*	O45	MSU, TW09066	-	+	+	-	+
47	*E. coli*	O45	MSU, TW03070	-	+	+	-	+
48	*E. coli*	O45	MSU, TW01589	-	+	+	-	+
49	*E. coli*	O45	MSU, TW07947	-	+	+	-	+
50	*E. coli*	O45	MSU, TW09183	-	+	+	-	+
51	*E. coli*	O145	MSU, TW07596	+	+	+	-	+
52	*E. coli*	O145	MSU, TW01664	-	+	+	-	+
53	*E. coli*	O145	MSU, TW08087	+	+	+	-	+
54	*E. coli*	O145	MSU, TW09153	-	+	-	+	+
55	*E. coli*	O145	MSU, TW03064	+	+	-	+	+
56	*E. coli*	O145	MSU, TW07860	-	+	+	-	+
57	*E. coli*	O145	Industry Isolate	+	+	+	-	+
58	*E. coli*	O145	Industry Isolate	-	+	-	+	+
59	*E. coli*	O145	MSU, TW09356	+	+	-	+	+
60	*E. coli*	O157:H7	ATCC 35150	+	+	+	+	+
61	*E. coli*	O157:H7	ATCC 43890	+	+	+	-	+
62	*E. coli*	O157:H7	Industry Isolate	+	+	+	-	+
63	*E. coli*	O157:H7	Industry Isolate	+	+	+	-	+
64	*E. coli*	O157:H7	Government Isolate	+	+	-	+	+
65	*E. coli*	O157:H7	Government Isolate	+	+	-	+	+
66	*E. coli*	O157:H7	Government Isolate	+	+	-	+	+
67	*E. coli*	O157:H7	Government Isolate	+	+	+	+	+

Table 2. Organisms Included in Validation of Exclusivity of Assurance GDS MPX Top 7 STEC (1)

				Assurance GDS MPX Top 7 STEC				
	Organism	Serogroup	Source	*E. coli* O157:H7	*eae*	*stx*$_1$	*stx*$_2$	Top STEC
1	*Shigella flexneri*	N/A	Industry isolate	-	-	-	-	-
2	*Citrobacter freundii*	N/A	Government isolate	-	-	-	-	-
3	*Enterobacter gergoviae*	N/A	ATCC 33028	-	-	-	-	-
4	*Salmonella enterica* subsp. *tranoroa*	O103	Industry isolate	-	-	-	-	-
5	*Salmonella enterica* subsp. *adelaide*	O111	ATCC 10718	-	-	-	-	-
6	*Pseudomonas aeruginosa*	N/A	ATCC 15442	-	-	-	-	-
7	*Escherichia hermannii*	N/A	ATCC 33650	-	-	-	-	-
8	*Klebsiella oxytoca*	N/A	ATCC 43086	-	-	-	-	-
	Non-Target Serogroups (*eae* - / *stx* -)							
9	*Escherichia coli*	N/A	ATCC 11775	-	-	-	-	-
10	*Escherichia coli*	N/A	ATCC 11229	-	-	-	-	-
11	*Escherichia coli*	N/A	ATCC 25922	-	-	-	-	-
12	*Escherichia coli*	N/A	ATCC 29194	-	-	-	-	-
13	*Escherichia coli*	N/A	ATCC 15223	-	-	-	-	-
14	*Escherichia coli*	N/A	ATCC 8677	-	-	-	-	-
15	*Escherichia coli*	N/A	ATCC 8739	-	-	-	-	-
16	*Escherichia coli*	N/A	ATCC 15939	-	-	-	-	-
	Non-Target Serogroups (*eae* + / *stx* +)							
17	*Escherichia coli*	O156	MSU, TW01407	-	-	-	-	-
	Non-Target Serogroups (*eae* + / *stx* -)							
18	*Escherichia coli*	O172	Industry isolate	-	-	-	-	-
19	*Escherichia coli*	O55	U of MI, TW07620	-	-	-	-	-
20	*Escherichia coli*	O91	U of MI, TW01674	-	-	-	-	-
	Non-Target Serogroups (*eae* - / *stx* +)							
21	*Escherichia coli*	O8	Industry isolate	-	-	-	-	-
22	*Escherichia coli*	O76	Industry isolate	-	-	-	-	-
23	*Escherichia coli*	O18	MSU, TW07933	-	-	-	-	-
	Target Serougroup (*eae* + / *stx* -)							
24	*Escherichia coli*	O26	MSU, TW01208	-	+	-	-	-
25	*Escherichia coli*	O111	MSU, TW03810	-	+	-	-	-
26	*Escherichia coli*	O45	MSU, TW03036	-	+	-	-	-
27	*Escherichia coli*	O121	MSU, TW08980	-	+	-	-	-
28	*Escherichia coli*	O145	MSU, TW07865	-	+	-	-	-
	Target Serogroup (*eae* - / *stx* -)							
29	*Escherichia coli*	O26	MSU, TW04081	-	-	-	-	-
30	*Escherichia coli*	O26	ATCC 12795	-	-	-	-	-
31	*Escherichia coli*	O111	MSU, TW06296	-	-	-	-	-

Table 4. Comparison Assurance GDS MPX Top 7 STEC 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	14	0.70	0.48, 0.85	13	0.65	0.43, 0.82	0.05	(-0.23), 0.32
	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	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	(-0.32), 0.23
	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
Leafy 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

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator, with 95% confidence interval
[b]N = Number of test potions
[c]x = Number of positive test portions
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]95% 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 values
[i] = analysis by Independent Lab

Table 5. Comparison Assurance GDS MPX Top 7 STEC 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	12	0.60	0.39, 0.78	6	0.30	0.15, 0.52	0.30	(-0.01), 0.54
	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
Leafy 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

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator, with 95% confidence interval
[b]N = Number of test potions
[c]x = Number of positive test portions
[d]POD$_C$ = Confirmed candidate method positive outcomes divided by the total number of trials
[e]POD$_R$ = Confirmed reference method positive outcomes divided by the total number of trials
[f]dPOD$_C$ = Difference between the candidate method and reference method POD values
[g]95% 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 values
[i] = analysis by Independent Lab

REFERENCES CITED

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