



CERTIFICATION

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022002

The AOAC Research Institute hereby certifies the method known as

BACGene *E. coli* O157:H7 Workflow

manufactured by

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

Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date	December 19, 2025
Expiration Date	December 31, 2026

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METHOD NAME BACGene <i>E. coli</i> O157:H7 Workflow	CATALOG NUMBERS 1. BACGene <i>E. coli</i> O157:H7: 5123222701, 5123222711 2. BACGene Mplex STEC Screen: 5123223001, 5123223011
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INDEPENDENT LABORATORY Q Laboratories 1930 Radcliff Drive Cincinnati, OH 45204 USA	APPLICABILITY OF METHOD Target organisms – <i>Escherichia coli</i> O157:H7 Matrixes – USDA MLG Ch. 5C.00 – Fresh raw ground beef (375 g), fresh raw beef trim (375 g), beef carcass sampling cloth (4" x 4"), stainless steel (4" x 4", 18-gauge 300 series, brush finish, NSF-certified), unglazed ceramic tile (1" x 1") FDA BAM Ch. 4A – Romaine lettuce (375 g), all-purpose flour (25 g) Performance claims – The BACGene <i>E. coli</i> O157:H7 workflow is comparable to the U. S. Department of Agriculture Food Safety and Inspection Service (FSIS) <i>Microbiology Laboratory Guidebook</i> (MLG), 5C.00, <i>Detection, Isolation And Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponges</i> (2) (fresh raw ground beef (73% lean), fresh raw beef trim, beef carcass sampling cloth, stainless steel environmental sponges and ceramic tile environmental swabs) and the Food and Drug Administration (FDA) <i>Bacteriological Analytical Manual</i> (BAM), Chapter 4A, <i>Diarrheagenic Escherichia coli</i> (3) reference methods (Romaine lettuce and all-purpose flour).
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ORIGINAL CERTIFICATION DATE February 08, 2020	CERTIFICATION RENEWAL RECORD Renewed through December 2026.
METHOD MODIFICATION RECORD 1. November 2020 Level 2 2. January 2021 Level 2 3. December 2022 Level 1 4. January 2024 Level 2 5. February 2025 Level 1	SUMMARY OF MODIFICATION 1. Manufacturing location addition of Eurofins Technologies Hungary Kft. 2. Detergent replacement required by European Chemicals Agency. 3. Company rebranded to Gold Standard Diagnostics Freiburg GmbH. 4. Addition of PURE software. 5. Editorial changes.
Under this AOAC <i>Performance Tested Methods</i>SM License Number, 022002 this method is distributed by: NONE	Under this AOAC <i>Performance Tested Methods</i>SM License Number, 022002 this method is distributed as: NONE

PRINCIPLE OF THE METHOD (1)

The BACGene *E. coli* O157:H7 workflow utilizes the BACGene *E. coli* O157:H7 and BACGene Mplex STEC Screen test kits for the detection of *E. coli* O157:H7. The BACGene technology is based on the detection of amplified fragments using FAM™ fluorescence-labeled hybridization probe quenched by non-fluorescent Tide Quencher™ 2 or by non-fluorescent Quencher MGBEQ; R6G™ fluorescence-labeled hybridization probe quenched by non-fluorescent Tide Quencher™ 2; or ROX™ fluorescence-labeled hybridization probe quenched by non-fluorescent Tide Quencher™ 3.

Each test kit that is part of the BACGene *E. coli* O157:H7 and BACGene Mplex STEC workflows utilizes Uracil-DNA Glycosylase (UNG) where Deoxyuridine triphosphate (dUTP) substitutes for deoxythymidine triphosphate (dTTP) in the PCR master mix. This substitution causes newly synthesized amplicons to contain deoxyuridine instead of deoxythymidine. The heat labile UNG cleaves hydrolytically at the N-glycosylic bond of uracil and deoxyribose where it is inactivated by the initial denaturation of the PCR. A PCR preceded by this step exclusively amplifies from native, i.e. thymine containing DNA (e.g. bacterial DNA). Thus, reducing the risk of false positive signals due to carry over contamination from previous analyses.

The BACGene *E. coli* O157:H7 workflow consists of a screening step with the BACGene *E. coli* O157:H7 test kit followed with the BACGene Mplex STEC Screen test kit. A test sample is deemed a presumptive positive for *E. coli* O157:H7 if the sample receives a positive result from both test kits. Since this procedure allows for the detection of *E. coli* O157:H7 first and then the detection of the virulence genes, the user would only need to be concerned with *E. coli* O157:H7 contamination. The BACGene *E. coli* O157:H7 workflow was validated for use with the Agilent AriaMx™ and/or Bio-Rad CFX96 Touch™ Deep Well PCR platforms. All run files were then downloaded and analyzed by either the BACGene Evaluation Sheet or the FastFinder software. Additionally, there is an option of conducting a sample pre-treatment using PRERaser BACGene to eliminate free DNA from enrichments prior to the lysis procedure.

All test portions are enriched in pre-warmed modified Tryptone Soya Broth (mTSB) for 10-24 h at 41.5±1°C. After enrichment, aliquots of 30 µL is sampled and thermally and enzymatically lysed to release the DNA and 5 µL of the lysate is then analyzed by real-time PCR using Agilent AriaMx and/or Bio-Rad CFX96 Touch Deep Well PCR platforms.

DISCUSSION OF THE VALIDATION STUDY (1)

BACGene E. coli O157:H7 Workflow

The BACGene E. coli O157:H7 workflow successfully detected E. coli O157:H7 from fresh raw ground beef, fresh raw beef trim, beef carcass sampling cloth, romaine lettuce, all-purpose flour, stainless steel environmental sponges and ceramic tile environmental swabs after 10 and 24 hours of enrichment. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the BACGene E. coli O157:H7 workflow and the reference method for all samples tested for both timepoints regardless of PCR platform or the use of the PR_{Eraser} BACGene treatment.

The BACGene E. coli O157:H7 workflow is quick to perform, on the Agilent AriaMx and the Bio-Rad CFX96 Touch Deep Well, with or without the PR_{Eraser} BACGene pre-treatment. The Abraxis IMS beads for BACGene E. coli STEC Solution workflow utilizes a small footprint and less consumables than the reference method procedures. Confirmation results can be provided in less than 2 hours post-incubation for 94 sample replicates when using either workflow. By being capable of being ran on multiple PCR platforms and the optional use of a pre-treatment, the BACGene E. coli O157:H7 workflow is customizable to the end user.

NOTE: Data generated for the Inclusivity, Exclusivity, and Matrix studies were generated by Q Laboratories.

Data Tables for Inclusivity and Exclusivity (1)

Table 54. BACGene E. coli O157:H7 Kit, Inclusivity Results (1)

No.	Species	Serotype	Source	Origin	Result	
					Agilent AriaMx	Bio-Rad CFX96 Touch Deep Well
1	E. coli	O157:H7	MSU ¹ TW00116	Human	+	+
2	E. coli	O157:H7	MSU TW00975	Human	+	+
3	E. coli	O157:H7	MSU TW02302	Hamburger	+	+
4	E. coli	O157:H7	MSU TW04863	Human	+	+
5	E. coli	O157:H7	MSU TW05356	Human	+	+
6	E. coli	O157:H7	MSU TW07587	Human	+	+
7	E. coli	O157:H7	ATCC ² BAA-460	Human Feces	+	+
8	E. coli	O157:H7	ATCC 35150	Human Feces	+	+
9	E. coli	O157:H7	ATCC 43889	Human Feces	+	+
10	E. coli	O157:H7	ATCC 43895	Hamburger	+	+
11	E. coli	O157:H7	ATCC 51657	Clinical Isolate	+	+
12	E. coli	O157:H7	ATCC 51659	Clinical Isolate	+	+
13	E. coli	O157:H7	ATCC 700599	Salami	+	+
14	E. coli	O157:H7	ATCC 700927	Not Available	+	+
15	E. coli	O157:H7	MSU DEC3A	Human	+	+
16	E. coli	O157:H7	MSU DEC3B	Human	+	+
17	E. coli	O157:H7	MSU DEC3C	Human	+	+
18	E. coli	O157:H7	MSU DEC3D	Human	+	+
19	E. coli	O157:H7	MSU DEC3E	Human	+	+
20	E. coli	O157:H7	MSU DEC4B	Human	+	+
21	E. coli	O157:H7	MSU DEC4D	Cow, Calf	+	+
22	E. coli	O157:H7	MSU DEC4E	Human	+	+
23	E. coli	O157:H7	QL ³ 2-202	Meat	+	+
24	E. coli	O157:H7	QL 2-205	Meat	+	+
25	E. coli	O157:H7	QL 2-206	Meat	+	+
26	E. coli	O157:H7	QL 2-207	Meat	+	+
27	E. coli	O157:H7	QL 2-370	Meat	+	+
28	E. coli	O157:H7	QL 2-701	Beef	+	+
29	E. coli	O157:H7	QL 2-704	Beef	+	+
30	E. coli	O157:H7	QL 2-705	Beef	+	+
31	E. coli	O157:H7	QL 2-706	Beef	+	+
32	E. coli	O157:H7	QL 2-707	Beef	+	+
33	E. coli	O157:H7	USDA ⁴ 465-97	Beef	+	+
34	E. coli	O157:H7	QL 2-709	Beef	+	+
35	E. coli	O157:H7	QL 2-710	Beef	+	+
36	E. coli	O157:H7	QL 14077.2	Meat	+	+
37	E. coli	O157:H7	QL 14077.3	Salami	+	+
38	E. coli	O157:H7	QL 14077.5	Hamburger	+	+
39	E. coli	O157:H7	QL 14077.6	Food	+	+

Table 54. BACGene E. coli O157:H7 Kit, Inclusivity Results (Continued) (1)

No.	Species	Serotype	Source	Origin	Result	
					Agilent AriaMx	Bio-Rad CFX96 Touch Deep Well
40	E. coli	O157:H7	QL 14077.7	Ground Beef	+	+
41	E. coli	O157:H7	QL 14077.8	Ground Beef	+	+

42	<i>E. coli</i>	O157:H7	ATCC 700376	Human Feces	+	+
43	<i>E. coli</i>	O157:H7	BEI NR ⁵ -4356	Not Available	+	+
44	<i>E. coli</i>	O157:H7	ATCC 43890	Human Feces	+	+
45	<i>E. coli</i>	O157:H7	QL 12298-1	Not Available	+	+
46	<i>E. coli</i>	O157:H7	QL 12298-2	Deli Ham	+	+
47	<i>E. coli</i>	O157:H7	QL 14077.1	Meat	+	+
48	<i>E. coli</i>	O157:H7	QL 2-214	Meat	+	+
49	<i>E. coli</i>	O157:H7	BEI NR-12	Not Available	+	+
50	<i>E. coli</i>	O157:H7	QL 2-203	Meat	+	+

1. MSU – Michigan State University Culture Collection, 2. ATCC – American Type Culture Collection, 3. QL – Q Laboratories Culture Collection, 4. USDA – United States Department of Agriculture Culture Collection, 5. BEI-NR - BEI Resources Culture Collection

Table 55. BACGene E. coli O157:H7 Kit, Exclusivity Results (1)

No.	Species	Source	Origin	Result	
				Agilent AriaMx	Bio-Rad CFX96 Touch Deep Well
1	<i>Aeromonas hydrophila</i>	ATCC ¹ 49140	Clinical Isolate	-	-
2	<i>Citrobacter braakii</i>	ATCC 43162	Clinical Isolate	-	-
3	<i>Citrobacter farmeri</i>	ATCC 51633	Human Feces	-	-
4	<i>Cronobacter sakazakii</i>	QL ² 17031.4	Infant Formula	-	-
5	<i>E. coli</i> O103	MSU ³ TW08101	Human	-	-
6	<i>E. coli</i> O103	PSU ⁴ 9.0036	Not Available	-	-
7	<i>E. coli</i> O111	MSU DEC8D	Human Infant	-	-
8	<i>E. coli</i> O121	PSU 5.0959	Not Available	-	-
9	<i>E. coli</i> O121	PSU 7.1686	Not Available	-	-
10	<i>E. coli</i> O145	MSU TW07596	Human	-	-
11	<i>E. coli</i> O26	ATCC BAA-1653	Stool, Associated with Ice Cream	-	-
12	<i>E. coli</i> O26	MSU TW07814	Human	-	-
13	<i>E. coli</i> O45	MSU TW14003	Human	-	-
14	<i>E. coli</i> O45	MSU TW07947	Human	-	-
15	<i>Edwardsiella tarda</i>	ATCC 15947	Human Feces	-	-
16	<i>Enterobacter aerogenes</i>	ATCC 13048	Sputum	-	-
17	<i>Escherichia fergusonii</i>	ATCC 35469	Human Feces	-	-
18	<i>Escherichia hermanii</i>	ATCC 33650	Mouse Brain	-	-
19	<i>Escherichia vulneris</i>	ATCC 29943	Clinical Isolate, Human Wound	-	-
20	<i>Haemophilus influenzae</i>	ATCC 19418	Not Available	-	-
21	<i>Hafnia alvei</i>	ATCC 51815	Milk	-	-
22	<i>Klebsiella pneumoniae</i>	ATCC 4352	Dairy Product	-	-
23	<i>Morganella morganii</i>	ATCC 25829	Human	-	-
24	<i>Mycobacterium smegmatis</i>	ATCC 19420	Not Available	-	-
25	<i>Pantoea agglomerans</i>	ATCC 19552	Sewage	-	-
26	<i>Providencia rettgeri</i>	ATCC 14505	Not Available	-	-
27	<i>Pseudomonas aeruginosa</i>	ATCC 9027	Clinical Isolate, Outer Ear Infection	-	-
28	<i>Serratia marcescens</i>	ATCC 13880	Human	-	-
29	<i>Shigella boydii</i>	ATCC 9290	Human Feces	-	-
30	<i>Vibrio vulnificus</i>	ATCC 49140	Clinical Isolate	-	-

ATCC – American Type Culture Collection, 2. QL – Q Laboratories Culture Collection, 3. MSU – Michigan State University Culture Collection, 4. PSU - Pennsylvania State University Culture Collection

Table 56. BACGene Mplex STEC Screen Kit, Inclusivity Results (1)

No.	Species and Serotype	Source	Known Virulence Factors			Origin	Result					
							Agilent AriaMx			Bio-Rad CFX96 Touch Deep Well		
			stx1	stx2	eae		stx1	stx2	eae	stx1	stx2	eae
1	<i>E. coli</i> O103	MSU ¹ TW08101	+	-	+	Human	+	-	+	+	-	+
2	<i>E. coli</i> O103	MSU TW04162	+	-	+	Human	+	-	+	+	-	+
3	<i>E. coli</i> O103	MSU TW05997	+	-	+	Human	+	-	+	+	-	+
4	<i>E. coli</i> O103	MSU TW07697	+	-	+	Human	+	-	+	+	-	+
5	<i>E. coli</i> O103	MSU TW07971	+	-	+	Human	+	-	+	+	-	+
6	<i>E. coli</i> O103	MSU TW11239	+	-	+	Human	+	+ ^a / ^b	+	+	-	+
7	<i>E. coli</i> O103	NCTC ² 8196	/ ^c	/ ^c	+	Not Available	+	-	+	+	-	+
8	<i>E. coli</i> O103	PSU ³ 9.0036	/ ^c	/ ^c	+	Not Available	+	+	+	+	+	+
9	<i>E. coli</i> O111	MSU DEC 8D	+	+	+	Human Infant	+	+	+	+	+	+
10	<i>E. coli</i> O111	MSU DEC15A	/ ^c	/ ^c	+	Human	+	+	+	+	+	+

11	<i>E. coli</i> O111	MSU TW00186	+	+	+	Human	+	+	+	+	+	+
12	<i>E. coli</i> O111	MSU TW01387	+	-	+	Human	+	-	+	+	-	+
13	<i>E. coli</i> O111	MSU TW05614	+	+	+	Human	+	+	+	+	+	+
14	<i>E. coli</i> O111	MSU TW06315	+	+	+	Human	+	+	+	+	+	+
15	<i>E. coli</i> O111	MSU TW07502	+	-	+	Human	+	-	+	+	-	+
16	<i>E. coli</i> O111	MSU TW14960	+	-	+	Human	+	-	+	+	-	+
17	<i>E. coli</i> O111:H8	QL ⁴ 12289-3A	+	-	+	Not Available	+	-	+	+	-	+
18	<i>E. coli</i> O121	MSU MT#2	-	+	+	Human	+	+	+	+	+	+
19	<i>E. coli</i> O121	MSU TW07614	-	+	+	Human	+	+	+	+	+	+
20	<i>E. coli</i> O121	MSU TW07931	-	+	+	Human	-	+	+	+	+	+
21	<i>E. coli</i> O121	MSU TW08039	-	+	+	Human	-	+	+	-	+	+
22	<i>E. coli</i> O121	PSU 10.0709	/ ^c	/ ^c	+	Not Available	+	+ ^a / _{-b}	+	+ ^a / _{-b}	+	+
23	<i>E. coli</i> O121	PSU 5.0959	-	+	+	Not Available	-	+	+	-	+	+
24	<i>E. coli</i> O121	PSU 7.1686	/ ^c	/ ^c	+	Not Available	+	+	+	+	+	+
25	<i>E. coli</i> O121	PSU 7.1709	/ ^c	/ ^c	+	Not Available	+	+	+	+	+	+
26	<i>E. coli</i> O121	PSU 7.1732	/ ^c	/ ^c	+	Not Available	+	+	+	+	+	+
27	<i>E. coli</i> O145	MSU TW01664	+	-	+	Human	+	+	+	+	+	+
28	<i>E. coli</i> O145	MSU TW07596	+	-	+	Human	+	-	+	+	-	+
29	<i>E. coli</i> O145	MSU TW09153	+	-	+	Human	+	-	+	+	-	+
30	<i>E. coli</i> O145	MSU TW09356	+	-	+	Human	+ ^a / _{-b}	+	+	+	-	+
31	<i>E. coli</i> O145	PSU 10.0707	/ ^c	/ ^c	+	Not Available	+	+	+	+	+	+
32	<i>E. coli</i> O145	PSU 10.0708	/ ^c	/ ^c	+	Not Available	+	-	+	+	+	+
33	<i>E. coli</i> O145	PSU 7.1711	/ ^c	/ ^c	+	Not Available	-	+	+	-	+	+
34	<i>E. coli</i> O26	ATCC ⁵ BAA-1653	+	+	+	Human Feces	+	-	+	+	-	+
35	<i>E. coli</i> O26	MSU DEC 10B	+	-	+	Human	+	-	+	+	-	+
36	<i>E. coli</i> O26	MSU DEC 9F	+	-	+	Human	+	-	+	+	-	+
37	<i>E. coli</i> O26	MSU TW07862	+	-	+	Calf, Cow	+	-	+	+	-	+
38	<i>E. coli</i> O26	MSU TW00971	+	-	+	Human Feces	+	-	+	+	-	+
39	<i>E. coli</i> O26	MSU TW02295	+	-	+	Human Infant	+	-	+	+	-	+
40	<i>E. coli</i> O26	MSU TW04284	+	-	+	Human	+	-	+	+	-	+
41	<i>E. coli</i> O26	MSU TW07814	+	+	+	Human	+	+	+	+	+	+
42	<i>E. coli</i> O26	MSU TW08031	+	-	+	Human	+	-	+	+	-	+
43	<i>E. coli</i> O45	MSU DEC 11C	/ ^c	/ ^c	+	Human	+	-	+	+	-	+
44	<i>E. coli</i> O45	MSU TW07947	+	-	+	Human	+	-	+	+	-	+
45	<i>E. coli</i> O45	MSU TW09183	+	-	+	Human	+	-	+	+	-	+
46	<i>E. coli</i> O45	MSU TW10121	+	-	+	Human	+	-	+	+	-	+
47	<i>E. coli</i> O45	MSU TW14003	+	-	+	Human	+	-	+	+	-	+
48	<i>E. coli</i> O157:H7	BEI NR-4356	/ ^c	/ ^c	+	Not Available	+ ^a / _{-b}	+	+	-	+	+
49	<i>E. coli</i> O157:H7	MSU TW00116	-	+	+	Not Available	-	+	+	+ ^a / _{-b}	+	+
50	<i>E. coli</i> O157:H7 (NM)	ATCC 700376	+	-	+	Not Available	+	-	+	+	-	+

1. MSU – Michigan State University Culture Collection, 2. NCTC – National Collection of Type Cultures, 3. PSU - Pennsylvania State University Culture Collection, 4.

QL – Q Laboratories Culture Collection, 5. ATCC – American Type Culture Collection

*Results are positive when eae as well as one of the shigatoxins (stx1 or stx2) is positive

Table 57. BACGene Mplex STEC Screen Kit, Exclusivity Results (1)

No.	Species	Source	Origin	Result	
				Agilent AriaMx	Bio-Rad CFX96 Touch Deep Well
1	<i>Aeromonas hydrophila</i>	ATCC ¹ 49140	Clinical Isolate	-	-
2	<i>Alcaligenes faecalis</i>	ATCC 8750	Not Available	-	-
3	<i>Citrobacter braakii</i>	ATCC 43162	Clinical Isolate	-	-
4	<i>Citrobacter farmeri</i>	ATCC 51633	Human Feces	- ^a	-
5	<i>Cronobacter sakazakii</i>	QL ² 17031.4	Infant Formula	-	-
6	<i>Escherichia coli</i>	ATCC 8739	Feces	-	-
7	<i>Edwardsiella tarda</i>	ATCC 15947	Human Feces	-	-
8	<i>Enterobacter aerogenes</i>	ATCC 35029	Not Available	-	-
9	<i>Escherichia blattae</i>	ATCC 29907	Insect	-	-
10	<i>Escherichia fergusonii</i>	ATCC 35469	Human Feces	-	-
11	<i>Escherichia hermannii</i>	ATCC 33650	Mouse Brain	-	-

12	<i>Escherichia vulneris</i>	ATCC 29943	Clinical Isolate, Human Wound	-	-
13	<i>Haemophilus influenzae</i>	ATCC 19418	Not Available	-	-
14	<i>Hafnia alvei</i>	ATCC 51815	Milk	-	-
15	<i>Klebsiella pneumoniae</i>	ATCC 13440	Not Available	-	-
16	<i>Kluyvera intermedia</i>	ATCC 33110	Surface Water	-	-
17	<i>Morganella morganii</i>	ATCC 25829	Human	-	-
18	<i>Mycobacterium smegmatis</i>	ATCC 19420	Not Available	-	-
19	<i>Pantoea agglomerans</i>	ATCC 19552	Sewage	-	-
20	<i>Proteus mirabilis</i>	ATCC 7002	Urine	-	-
21	<i>Providencia rettgeri</i>	ATCC 14505	Not Available	-	-
22	<i>Pseudomonas aeruginosa</i>	ATCC 9027	Clinical Isolate, Outer Ear Infection	-	-
23	<i>Rahnella aquatilis</i>	ATCC 55046	Soil	-	-
24	<i>Salmonella Typhimurium</i>	ATCC 14028	Animal Tissue	-	-
25	<i>Serratia marcescens</i>	ATCC 8100	Not Available	-	-
26	<i>Shigella boydii</i>	ATCC 9207	Pork Liver	-	-
27	<i>Siccibacter turicensis</i>	ATCC 54945	Not Available	-	-
28	<i>Sphingomonas paucimobilis</i>	ATCC 29837	Hospital Respirator	- ^b	-
29	<i>Vibrio vulnificus</i>	QL 021111A	Seafood Product	-	-
30	<i>Yersinia enterocolitica</i>	ATCC 49397	Clinical Isolate	-	-

¹. ATCC – American Type Culture Collection, ². QL – Q Laboratories Culture Collection

^aIsolate produced a positive result for “stx2” when analyzed by the FastFinder Software. ^bIsolate produced a positive result for “eae” when analyzed by the FastFinder Software.

Data Tables for Matrix Studies (1)

Table 13. Fresh Raw Ground Beef Results: Analyzed on the Agilent AriaMX by the BACGene Evaluation Sheet¹, Candidate vs. Reference – POD Results (1)

STEC Strain	PREraser Treatment	Enrichment Time Point	MPN ^a / Test Portion	N ^b	Candidate ²			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	x ^c	POD _R ^e	95% CI		
<i>E. coli</i> O157:H7 (NM) NCTC ³ 13125	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.³NCTC – National Collection of Type Cultures^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval^bN = Number of test portions^cx = Number of positive test portions^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials^fdPOD_c = Difference between the confirmed candidate method result and reference 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% levelTable 14. Fresh Raw Ground Beef Results: Analyzed on the Agilent AriaMX by the BACGene Evaluation Sheet¹, Presumptive vs. Confirmed – POD Results (1)

STEC Strain	PREraser Treatment	Enrichment Time Point	MPN ^a / Test Portion	N ^b	Presumptive ²			Confirmed ³			dPOD _{cp} ^f	95% CI ^g
					x ^c	POD _{cp} ^d	95% CI	x ^c	POD _{cc} ^e	95% CI		
<i>E. coli</i> O157:H7 (NM) NCTC ⁴ 13125	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.39 (0.17, 0.68)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.³Alternative and traditional confirmation procedures produced the same results.⁴NCTC – National Collection of Type Cultures^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, 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

Table 17. Fresh Raw Beef Trim Results: Analyzed by the BACGene Evaluation Sheet¹, Candidate vs. Reference – POD Results (1)

STEC Strain	Thermocycler	Enrichment Time Point	MPN ^a / Test Portion	N ^b	Candidate ²			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	x ^c	POD _R ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ 35150	Agilent AriaMX™	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.68 (0.38, 1.14)	20	9	0.45	0.26, 0.66	11	0.55	0.34, 0.74	-0.10	-0.37, 0.19
			6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.68 (0.38, 1.14)	20	8	0.40	0.22, 0.61	11	0.55	0.34, 0.74	-0.15	-0.41, 0.14
			6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	Bio-Rad CFX96 Touch™ Deep Well	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.68 (0.38, 1.14)	20	9	0.45	0.26, 0.66	11	0.55	0.34, 0.74	-0.10	-0.37, 0.19
			6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.68 (0.38, 1.14)	20	9	0.45	0.26, 0.66	11	0.55	0.34, 0.74	-0.10	-0.37, 0.19
			6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.³ ATCC – American Type Culture Collection^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval^bN = Number of test portions^cx = Number of positive test portions^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials^fdPOD_c = Difference between the confirmed candidate method result and reference 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**Table 18. Fresh Raw Beef Trim Results: Analyzed by the BACGene Evaluation Sheet¹, Presumptive vs. Confirmed – POD Results (1)**

STEC Strain	Thermocycler	Enrichment Time Point	MPN ^a / Test Portion	N ^b	Presumptive ²			Confirmed ³			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	x ^c	POD _{CC} ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ⁴ 35150	Agilent AriaMX™	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.68 (0.38, 1.14)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	N/A
			6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.68 (0.38, 1.14)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.21, 0.11
			6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	Bio-Rad CFX96 Touch™ Deep Well	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.68 (0.38, 1.14)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	N/A
			6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.68 (0.38, 1.14)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	N/A
			6.53 (2.30, 18.54)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.³Alternative and traditional confirmation procedures produced the same results.⁴ ATCC – American Type Culture Collection^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, 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

Table 21. Beef Carcass Sampling Cloth Results: Analyzed on the Agilent AriaMX by the BACGene Evaluation Sheet¹, Candidate vs. Reference – POD Results (1)

Matrix	STEC Strain	Enrichment Time Point	CFU/Test Area	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	x ^c	POD _R ^e	95% CI		
Beef Carcass Sampling Cloths	<i>E. coli</i> O157:H7 USDA 465-97	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			56	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
			480	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			56	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
			480	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²MSU – Michigan State Culture Collection³The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.⁴MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval⁵N = Number of test portions⁶x = Number of positive test portions⁷POD_c = Candidate method confirmed positive outcomes divided by the total number of trials⁸POD_R = Reference method confirmed positive outcomes divided by the total number of trials⁹dPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values¹⁰95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level**Table 22. Beef Carcass Sampling Cloth Results: Analyzed on the Agilent AriaMX by the BACGene Evaluation Sheet¹, Presumptive vs. Confirmed – POD Results (1)**

Matrix	STEC Strain	Enrichment Time Point	CFU/Test Area	N ^b	Presumptive			Confirmed ²			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	x ^c	POD _{CC} ^e	95% CI		
Beef Carcass Sampling Cloths	<i>E. coli</i> O157:H7 USDA 465-97	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			56	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	N/A
			480	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			56	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	N/A
			480	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.² Alternative and traditional confirmation procedures produced the same results.³MSU – Michigan State Culture Collection⁴The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.⁵MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval⁶N = Number of test portions⁷x = Number of positive test portions⁸POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials⁹POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials¹⁰dPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values¹¹95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 23. Romaine Lettuce Results: Analyzed by the BACGene Evaluation Sheet¹, Candidate vs. Reference – POD Results (1)

STEC Strain	Thermocycler	Enrichment Time Point	MPN ^a / Test Portion	N ^b	Candidate ²			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	x ^c	POD _R ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ 43895	Agilent AriaMX™	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	N/A
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	N/A
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	Bio-Rad CFX96 Touch™ Deep Well	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	N/A
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	N/A
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.³ATCC – American Type Culture Collection^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval^bN = Number of test portions^cx = Number of positive test portions^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials^fdPOD_c = Difference between the confirmed candidate method result and reference 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**Table 24. Romaine Lettuce Results: Analyzed by the BACGene Evaluation Sheet¹, Presumptive vs. Confirmed – POD Results (1)**

STEC Strain	Thermocycler	Enrichment Time Point	MPN ^a / Test Portion	N ^b	Presumptive ²			Confirmed ³			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	x ^c	POD _{CC} ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ⁴ 43895	Agilent AriaMX™	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	N/A
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	N/A
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	Bio-Rad CFX96 Touch™ Deep Well	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	N/A
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	N/A
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.³Alternative and traditional confirmation procedures produced the same results.⁴ATCC – American Type Culture Collection^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, 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

Table 29. All Purpose Flour Results: Analyzed on the Bio-Rad CFX96 Touch Deep Well by the BACGene Evaluation Sheet¹, Candidate vs. Reference – POD Results (1)

STEC Strain	PREraser Treatment	Enrichment Time Point	MPN ^a / Test Portion	N ^b	Candidate ²			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	x ^c	POD _R ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ BAA-460	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	6	0.30	0.15, 0.52	0.20	-0.10, 0.45
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	6	0.30	0.15, 0.52	0.20	-0.10, 0.45
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	6	0.30	0.15, 0.52	0.20	-0.10, 0.45
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	6	0.30	0.15, 0.52	0.20	-0.10, 0.45
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.³ATCC – American Type Culture Collection^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval^bN = Number of test portions^cx = Number of positive test portions^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials^fdPOD_c = Difference between the confirmed candidate method result and reference 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**Table 30. All Purpose Flour Results: Analyzed on the Bio-Rad CFX96 Touch Deep Well by the BACGene Evaluation Sheet, Presumptive vs. Confirmed – POD Results (1)**

STEC Strain	PREraser Treatment	Enrichment Time Point	MPN ^a / Test Portion	N ^b	Presumptive ¹			Confirmed ²			dPOD _{cp} ^f	95% CI ^g
					x ^c	POD _{cp} ^d	95% CI	x ^c	POD _{cc} ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ BAA-460	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.77	10	0.50	0.30, 0.70	0.00	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.²Alternative and traditional confirmation procedures produced the same results.³ATCC – American Type Culture Collection^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, 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

Table 31. All Purpose Flour Results: Analyzed on the Bio-Rad CFX96 Touch Deep Well by the FastFinder Software, Presumptive vs. Confirmed – POD Results (1)

STEC Strain	PREraser Treatment	Enrichment Time Point	MPN ^a / Test Portion	N ^b	Presumptive ¹			Confirmed ²			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	x ^c	POD _{CC} ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ BAA-460	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			0.34 (0.14, 0.61)	20	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	N/A
			4.12 (1.58, 10.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.²Alternative and traditional confirmation procedures produced the same results.³ATCC – American Type Culture Collection^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, 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**Table 34. Stainless Steel Environmental Sponge Results: Analyzed on the Bio-Rad CFX96 Touch Deep Well by the BACGene Evaluation Sheet¹, Candidate vs. Reference – POD Results (1)**

STEC Strain	PREraser Treatment	Enrichment Time Point	CFU/Test Area	N ^b	Candidate ²			Reference			dPOD _C ^f	95% CI ^g
					x ^c	POD _C ^d	95% CI	x ^c	POD _R ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ 43895 with 10x <i>C. freundii</i> ATCC 8090	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			55 (<i>E. coli</i>) & 790 (<i>C. freundii</i>)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			400 (<i>E. coli</i>) & 7300 (<i>C. freundii</i>)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			55 (<i>E. coli</i>) & 790 (<i>C. freundii</i>)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			400 (<i>E. coli</i>) & 7300 (<i>C. freundii</i>)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			55 (<i>E. coli</i>) & 790 (<i>C. freundii</i>)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			400 (<i>E. coli</i>) & 7300 (<i>C. freundii</i>)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			55 (<i>E. coli</i>) & 790 (<i>C. freundii</i>)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			400 (<i>E. coli</i>) & 7300 (<i>C. freundii</i>)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.³ATCC – American Type Culture Collection^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval^bN = Number of test portions^cx = Number of positive test portions^dPOD_C = Candidate method confirmed positive outcomes divided by the total number of trials^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials^fdPOD_C = Difference between the confirmed candidate method result and reference 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

Table 35. Stainless Steel Environmental Sponge Results: Analyzed on the Bio-Rad CFX96 Touch Deep Well by the BACGene Evaluation Sheet¹, Presumptive vs. Confirmed – POD Results (1)

STEC Strain	PREraser Treatment	Enrichment Time Point	CFU/Test Area	N ^b	Presumptive ²			Confirmed ³			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	x ^c	POD _{CC} ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ⁴ 43895 with 10x <i>C. freundii</i> ATCC 8090	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			55 (<i>E. coli</i>) & 790 (<i>C. freundii</i>)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			400 (<i>E. coli</i>) & 7300 (<i>C. freundii</i>)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			55 (<i>E. coli</i>) & 790 (<i>C. freundii</i>)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			400 (<i>E. coli</i>) & 7300 (<i>C. freundii</i>)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			55 (<i>E. coli</i>) & 790 (<i>C. freundii</i>)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			400 (<i>E. coli</i>) & 7300 (<i>C. freundii</i>)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			55 (<i>E. coli</i>) & 790 (<i>C. freundii</i>)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	N/A
			400 (<i>E. coli</i>) & 7300 (<i>C. freundii</i>)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.³Alternative and traditional confirmation procedures produced the same results.⁴ATCC – American Type Culture Collection[®]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, 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**Table 39. Ceramic Tile Environmental Swabs Results: Analyzed on the Agilent AriaMx by the BACGene Evaluation Sheet¹, Candidate vs. Reference – POD Results (1)**

STEC Strain	Thermocycler	Enrichment Time Point	CFU/ Test Area	N ^b	Candidate ²			Reference			dPOD _C ^f	95% CI ^g
					x ^c	POD _C ^d	95% CI	x ^c	POD _R ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ 43895	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	-0.15, 0.41
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	8	0.40	0.22, 0.61	0.20	-0.10, 0.46
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene Evaluation Sheet and FastFinder software produced the same results.²The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.³ATCC – American Type Culture Collection⁴MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval^bN = Number of test portions^cx = Number of positive test portions^dPOD_C = Candidate method confirmed positive outcomes divided by the total number of trials^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials^fdPOD_C = Difference between the confirmed candidate method result and reference 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

Table 40. Ceramic Tile Environmental Swabs Results: Analyzed on the Bio-Rad CFX96 Touch Deep Well by the BACGene Evaluation Sheet, Candidate vs. Reference – POD Results (1)

STEC Strain	Thermocycler	Enrichment Time Point	CFU/ Test Area	N ^b	Candidate ¹			Reference			dPOD ^c	95% CI ^g
					x ^c	POD _C ^d	95% CI	x ^c	POD _R ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ² 43895	Without PRE ³ Eraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	8	0.40	0.22, 0.61	0.20	-0.10, 0.46
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PRE ³ Eraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			570	5	4	0.80	0.38, 1.00	5	1.00	0.57, 1.00	-0.20	-0.62, 0.28
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	8	0.40	0.22, 0.61	0.20	-0.10, 0.46
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.²ATCC – American Type Culture Collection³MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval^bN = Number of test portions^cx = Number of positive test portions^dPOD_C = Candidate method confirmed positive outcomes divided by the total number of trials^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials^fdPOD_C = Difference between the confirmed candidate method result and reference 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**Table 41. Ceramic Tile Environmental Swabs Results: Analyzed on the Bio-Rad CFX96 Touch Deep Well by the FastFinder software, Candidate vs. Reference – POD Results (1)**

STEC Strain	Thermocycler	Enrichment Time Point	CFU/ Test Area	N ^b	Candidate ¹			Reference			dPOD ^c	95% CI ^g
					x ^c	POD _C ^d	95% CI	x ^c	POD _R ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ² 43895	Without PRE ³ Eraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			570	5	4	0.80	0.38, 1.00	5	1.00	0.57, 1.00	-0.20	-0.62, 0.28
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	8	0.40	0.22, 0.61	0.20	-0.10, 0.46
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PRE ³ Eraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	8	0.40	0.22, 0.61	0.20	-0.10, 0.46
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.²ATCC – American Type Culture Collection³MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval^bN = Number of test portions^cx = Number of positive test portions^dPOD_C = Candidate method confirmed positive outcomes divided by the total number of trials^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials^fdPOD_C = Difference between the confirmed candidate method result and reference 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

Table 42. Ceramic Tile Environmental Swabs Results: Analyzed on the Agilent AriaMx by the BACGene Evaluation Sheet, Presumptive vs. Confirmed – POD Results (1)

STEC Strain	Thermocycler	Enrichment Time Point	CFU/ Test Area	N ^b	Presumptive ¹			Confirmed ²			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	x ^c	POD _{CC} ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ 43895	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	N/A
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.28, 0.08
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	N/A
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.²Alternative and traditional confirmation procedures produced the same results.³ATCC – American Type Culture Collection⁴MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval⁵N = Number of test portions⁶x = Number of positive test portions⁷POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials⁸POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials⁹dPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values¹⁰95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level**Table 43. Ceramic Tile Environmental Swabs Results: Analyzed on the Agilent AriaMx by the FastFinder software, Presumptive vs. Confirmed – POD Results (1)**

STEC Strain	Thermocycler	Enrichment Time Point	CFU/ Test Area	N ^b	Presumptive ¹			Confirmed ²			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	x ^c	POD _{CC} ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ 43895	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			60	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			60	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	-0.11, 0.21
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			60	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.28, 0.08
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			60	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
			570	5	4	1.00	0.80, 1.00	5	1.00	0.57, 1.00	-0.20	-0.76, 0.36

¹The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.²Alternative and traditional confirmation procedures produced the same results.³ATCC – American Type Culture Collection⁴MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval⁵N = Number of test portions⁶x = Number of positive test portions⁷POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials⁸POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials⁹dPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values¹⁰95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 44. Ceramic Tile Environmental Swabs Results: Analyzed on the Bio-Rad CFX96 Touch Deep Well by the BACGene Evaluation Sheet, Presumptive vs. Confirmed – POD Results (1)

STEC Strain	Thermocycler	Enrichment Time Point	CFU/ Test Area	N ^b	Presumptive ¹			Confirmed ²			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	x ^c	POD _{CC} ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ 43895	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	N/A
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	-0.11, 0.21
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.28, 0.08
			570	5	4	1.00	0.80, 1.00	5	1.00	0.57, 1.00	-0.20	-0.76, 0.36
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	N/A
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.²Alternative and traditional confirmation procedures produced the same results.³ATCC – American Type Culture Collection⁴MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval⁵N = Number of test portions⁶x = Number of positive test portions⁷POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials⁸POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials⁹dPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values¹⁰95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level**Table 45. Ceramic Tile Environmental Swabs Results: Analyzed on the Bio-Rad CFX96 Touch Deep Well by the Fast Finder software, Presumptive vs. Confirmed – POD Results (1)**

STEC Strain	Thermocycler	Enrichment Time Point	CFU/ Test Area	N ^b	Presumptive ¹			Confirmed ²			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	x ^c	POD _{CC} ^e	95% CI		
<i>E. coli</i> O157:H7 ATCC ³ 43895	Without PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	N/A
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	-0.11, 0.21
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A
	With PREraser	10 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.28, 0.08
			570	5	4	1.00	0.80, 1.00	5	1.00	0.57, 1.00	-0.20	-0.76, 0.36
		24 Hour	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	N/A
			60	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	N/A
			570	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	N/A

¹The BACGene STEC workflow and the BACGene *E. coli* O157:H7 workflow produced the same results.²Alternative and traditional confirmation procedures produced the same results.³ATCC – American Type Culture Collection⁴MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval⁵N = Number of test portions⁶x = Number of positive test portions⁷POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials⁸POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials⁹dPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values¹⁰95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

DISCUSSION OF MODIFICATION APPROVED JANUARY 2021 (5)

For the BACGene real-time PCR kits for the detection of bacterial pathogens in food and feed, as well as in environmental samples, the two tested alternative non-ionic detergents, Ecosurf™ SA-9 and Tween® 20, show a very similar lysis efficiency in comparison to the reference detergent Triton® X-100.

The product consistency study showed good agreement between the three lysis buffer formulations each tested with three different lots, with consistent and reproducible results. Cq-values in spiked matrixes were constant and reproducible, with mean Cq-values CV < 5% and thus it does not indicate a systematic inconsistency from production of individual lots with the different detergents. For BACGene *Salmonella* spp. 2, two inhibited samples were observed for Lot 2 and one inhibited sample for Lot 3. For lysis efficiency comparison those three replicates were taken out. Since all other replicates for those two lots are fine, most probably wrong handling (taking the lysate not from the upper half) can be seen as the reason for that.

Additional to the product consistency study, a matrix test was performed with the three different lysis buffer types, each with three relevant food and environmental samples, which were inoculated with the respective target strains. Enrichment was performed according to the instructions and lysis was performed with the certified buffer containing Triton® X-100 in comparison to the two alternatives, Ecosurf™ SA-9 and Tween® 20.

In none of the tested samples for none of the kits there was a significant difference between the lysis efficiencies observed. Cq-values are very similar between the reference condition and both alternatives.

Therefore, it may be concluded that this study demonstrates a similar performance of both tested alternatives, Ecosurf™ SA-9 and Tween® 20, compared to Triton® X-100.

DISCUSSION OF MODIFICATION APPROVED JANUARY 2024 (6)

In total, 2457, 5103 and 3276 datasets have been re-evaluated for the BACGene *Salmonella* spp. method, the three BACGene *Listeria* methods and the BACGene *E. coli* O157:H7 and STEC TOP7 workflows, respectively. Within the tested inclusivity/exclusivity datasets, no deviation to the initial validation data was observed.

These results strongly indicate that there is no negative impact of the usage of PURE on the specificity of any tested method.

In matrix datasets, a total of 7 deviations regarding final results occurred: 3 for the BACGene *Salmonella* spp. method, 1 for the three BACGene *Listeria* methods and 3 for the BACGene *E. coli* O157:H7 and the STEC TOP7 workflows. In the deviating samples, a fluorescence increase at some point during the PCR reaction was visible in the raw data. The change in final scoring of these samples from presumptive negative to presumptive positive, was subsequently due to a different interpretation/processing of the respective data by either PURE or the real-time PCR cyclers software, which is the basis for the Excel-based data evaluation. For such borderline signals, it is not unexpected that different algorithms yield different results in data interpretation.

Most important, the observed deviations in single sample results, did not have a relevant negative impact on the overall performance characteristics of the evaluated methods.

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