



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
022204

The AOAC Research Institute hereby certifies the method known as:

Pathotrak Next Generation Enrichment

manufactured by

Pathotrak Inc.

4467 Technology Drive

College Park, MD 20742 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, Senior Director
Signature for AOAC Research Institute

Issue Date
Expiration Date

January 30, 2025
December 31, 2025

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MODIFICATION MARCH 2023: Andrew Deterding*, Wesley Thompson*, M. Joseph Benzinger, Jr.*, Benjamin Bastin*, Javier Atencia**, Alexa Grace Baldwin**, Sena Martinos**

MODIFICATION DECEMBER 2023: Andrew Deterding*, Wesley Thompson*, M. Joseph Benzinger, Jr.*, Benjamin Bastin*, Erin Crowley*, Sean Martinos**, Alexa Grace Baldwin**, Javier Atencia**

*Q Laboratories

**Pathotrak

SUBMITTING COMPANY

Pathotrak Inc.
4467 Technology Drive
College Park, MD 20742 USA

METHOD NAME

Pathotrak Next Generation Enrichment

CATALOG NUMBER

0375RLH001

INDEPENDENT LABORATORY

Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204

APPLICABILITY OF METHOD

Target Organisms – *Salmonella* spp. and Shiga-toxin producing *Escherichia coli* (STEC).

Matrixes

Original Validation (BAM 4A and BAM 5) – Romaine hearts (375 g, 1,500 g).

MODIFICATION DECEMBER 2023 (MLG 4.14 and MLG 5C.03) – Beef trim sampling cloths.

Performance claims

ORIGINAL VALIDATION – No statistically significant differences detected between the Pathotrak Next Generation Enrichment and the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM), Chapter 4A, Diarrheagenic *Escherichia coli* (3) and FDA/BAM Chapter 5, *Salmonella* (2) reference methods for Romaine hearts.

MODIFICATION MARCH 2023 – The study data unable to find a statistically detectable difference from 0 between the Pathotrak Next Generation Enrichment method and the U.S. Food and Drug Administration Bacteriological Analytical Manual (BAM) Chapter 4A: *Diarrheagenic Escherichia coli* (2) and Chapter 5: *Salmonella* (3) reference methods for romaine hearts using *Performance Tested Methods*SM (PTM) Bio-Rad iQ-check PCR kits (iQ-Check *Salmonella* II Real-Time PCR, PTM 100803 (5) and iQ-Check STEC VirX, iQ-Check STEC SerO, iQ-Check Ser O II, PTM 121203 (6), Hygiena BAX system PCR assays (BAX[®] System Real-Time PCR Assay for *Salmonella*, PTM 081201 (7), BAX[®] System Real-Time PCR Assay Suite for STEC, PTM 091301 (8), and BAX[®] System Real-Time PCR Assay for *E. coli* O157:H7 Exact, PTM 100203 (9), and Eurofins BACGene PCR assays (BACGene *Salmonella* spp., PTM 121501 (10), and BACGene STEC TOP7 Workflow, PTM 022003 (11).

MODIFICATION DECEMBER 2023 – The study data were unable to detect a statistical difference in results between the Pathotrak Next Generation Enrichment for beef trim sampling cloths and the U.S. Department of Agriculture Food Safety and Inspection Services Microbiology Laboratory Guidebook (MLG) 4.14 and 5C.03 reference methods for detection of *Salmonella* and STEC Groups O157:H7, O26, O45, O103, O111, O121, O145, and O45(13,14).

ORIGINAL CERTIFICATION DATE February 25, 2022	CERTIFICATION RENEWAL RECORD Renewed through December 2025.
METHOD MODIFICATION RECORD 1. March 2023 Level 2 2. December 2023 Level 3 3. January 2025 Level 1	SUMMARY OF MODIFICATION 1. Evaluation of (1) new configuration to the enrichment bag, (2) extend the matrix claim to 1,500 g Romaine hearts, and (3) add the Eurofins BACGene PCR assays (BACGene <i>Salmonella</i> spp., PTM 121501, and BACGene STEC TOP7 Workflow, PTM 022003) for detection of <i>Salmonella</i> spp. and Shiga toxin-producing <i>Escherichia coli</i> . 2. Matrix extension to include beef trim sampling cloths using new enrichment paired with Hygiena's BAX System Real-Time PCR Assay for <i>Salmonella</i> (PTM 081201) (15), STEC (PTM 091301) (16), and <i>E. coli</i> O157:H7 (PTM 102003) (17). 3. Addition of a new sampling procedure for using 100 mL subsample for the 1500 g Romaine lettuce.
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PRINCIPLE OF THE METHOD (1)

The Pathotrak Next Generation Enrichment method employs a specialized enrichment bag to concentrate the bacteria that are present in an enriched food sample. Following a 5.5-6 h incubation period, the bags are loaded into a pressure vessel and attached to waste lines. The vessel is then sealed and pressurized using an air pump. The increase in pressure forces 500 mL of enrichment through a Pathotrak concentrating unit attached to the Next Generation Enrichment bag. The concentrating unit is removed and then backwashed using sterile saline to extract the bacteria, which are then further concentrated by centrifugation. A portion of the concentrated sample can then be tested by a qualified PCR method for *Salmonella* and STEC, providing a significantly faster time to result compared to methods with traditional enrichments.

DISCUSSION OF THE VALIDATION STUDY (1)

The Pathotrak Next Generation Enrichment was able to detect 106 out of 106 *Salmonella* spp. and 50 out of 50 STEC, while not detecting any organisms from the exclusivity panels. The Pathotrak Next Generation Enrichment successfully detected STEC and *Salmonella* spp. in Romaine hearts. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods and the reference method for all portions tested.

The Pathotrak Next Generation Enrichment significantly reduces enrichment time allowing for quick of *Salmonella* and STEC organisms from a single enrichment when compared to other enrichment methods. The bacterial extraction can then be simply and quickly tested using either Bio-Rad iQ-Check kits or Hygiena BAX System PCR assays for detection of *Salmonella* spp. and STEC.

Table 1. Summary of Salmonella spp. Inclusivity Panel Results (1)

No.	Salmonella species and subspecies	Serovar (if applicable)	Antigenic Formula or O group	Source ^a	Origin	Results
1	<i>S. bongori</i>	Not applicable	Group V 66:z41:-	ATCC 43975	Not Available	Detected
2	<i>S. bongori</i>	Not applicable	Group V 66:z41:-	NCTC 10946	Frog	Detected
3	<i>S. bongori</i>	Not applicable	Group V 66:z41:-	NCTC 12419	Not Available	Detected
4	<i>S. enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa 51:z4,z23:-	ATCC 13314	Not Available	Detected
5	<i>S. enterica</i> subsp. <i>arizonae</i>	Not applicable	Not Available	ATCC BAA-1577	Not Available	Detected
6	<i>S. enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa: 43:g,z51:-	QL11007-4	Veterinary	Detected
7	<i>S. enterica</i> subsp. <i>diarizonae</i>	Not applicable	Not Available	ATCC BAA-1579	Not Available	Detected
8	<i>S. enterica</i> subsp. <i>diarizonae</i>	Not applicable	Group IIIb 35:i:z	ATCC BAA-216	Human blood	Detected
9	<i>S. enterica</i> subsp. <i>diarizonae</i>	Not applicable	Not Available	ATCC BAA-639	Human feces	Detected
10	<i>S. enterica</i> subsp. <i>houtenae</i>	Halmstad	Group IV 3,15:g,m,s:-	QL024.1	Clinical isolate	Detected
11	<i>S. enterica</i> subsp. <i>houtenae</i>	Harmelen	Group IV 51:z4,z23:-	ATCC 15783	Boa constrictor	Detected
12	<i>S. enterica</i> subsp. <i>houtenae</i>	Ochsenzoll	Group IV 16:z4,z23:-	ATCC 29932	Not Available	Detected
13	<i>S. enterica</i> subsp. <i>indica</i>	Ferlac	Group VI 1,6,14,25:a:e,n,x	ATCC 43976	Not Available	Detected
14	<i>S. enterica</i> subsp. <i>indica</i>	Ferlac	H(1,6,14,25); a; e,n,x; k, z6	NCTC 10458	Ceylonese desiccated coconut	Detected
15	<i>S. enterica</i> subsp. <i>indica</i>	Not applicable	Group VI 45:a:e,n,x	ATCC BAA-1578	India	Detected
16	<i>S. enterica</i> subsp. <i>salamae</i>	Artis	Group II 56:b:-	ATCC 700149	Not Available	Detected
17	<i>S. enterica</i> subsp. <i>salamae</i>	Basel	Group II 58:z13,z28:1,5	ATCC 700151	Not Available	Detected
18	<i>S. enterica</i> subsp. <i>salamae</i>	Not applicable	Group II: 4,12:b:1,5	QL02415	Not Available	Detected
19	<i>S. enterica</i> subsp. <i>enterica</i>	Abaetetuba	Group I 11:k:1,5	ATCC 35640	Creek water	Detected
20	<i>S. enterica</i> subsp. <i>enterica</i>	Abortusequi	Group I 4,12:-:e,n,x	ATCC 9842	Not Available	Detected
21	<i>S. enterica</i> subsp. <i>enterica</i>	Abortusovis	Group I 4,12:c:1,6	NCTC 10241	Not Available	Detected
22	<i>S. enterica</i> subsp. <i>enterica</i>	Abony	Group I 1,4,[5],12,[27]:b:e,n,x	NCTC 6017	Not Available	Detected
23	<i>S. enterica</i> subsp. <i>enterica</i>	Adelaide	Group I 35:f,g:-:[z27]	UPenn STS 2	Not Available	Detected
24	<i>S. enterica</i> subsp. <i>enterica</i>	Agona	Group I 1,4,[5],12:f,g,s:[1,2]:[z27],[z45]	ATCC 51957	Not Available	Detected
25	<i>S. enterica</i> subsp. <i>enterica</i>	Agama	Group I 4,12:i:1,6	UPenn STS 3	Not Available	Detected
26	<i>S. enterica</i> subsp. <i>enterica</i>	Agoueve	Group I 13,22:z29:-	UPenn STS 5	Not Available	Detected
27	<i>S. enterica</i> subsp. <i>enterica</i>	Alachua	Group I 35:z4,z23:-:[z37],[z45]	UPenn STS 6	Not Available	Detected
28	<i>S. enterica</i> subsp. <i>enterica</i>	Albany	Group I 8,20:z4,z24:-:[z45]	UPenn STS 7	Not Available	Detected
29	<i>S. enterica</i> subsp. <i>enterica</i>	Anatum	Group I 3,{10}{15}{15,34}:e,h:1,6:[z64]	ATCC 9270	Pork liver	Detected
30	<i>S. enterica</i> subsp. <i>enterica</i>	Arkansas	Group I 3,{10}{15}{15,34}:e,h:1,5	UPenn STS 11	Not Available	Detected
31	<i>S. enterica</i> subsp. <i>enterica</i>	Bareilly	Group I 6,7,14:y:1,5	FDA 1206H	Not Available	Detected
32	<i>S. enterica</i> subsp. <i>enterica</i>	Berta	Group I 1,9,12:[f],g,[t]:-	UPenn STS 13	Not Available	Detected
33	<i>S. enterica</i> subsp. <i>enterica</i>	Binza	Group I 3,{10}{15}{15,34}:y:1,5	UPenn STS 14	Not Available	Detected
34	<i>S. enterica</i> subsp. <i>enterica</i>	Bovis-Morbificans	Group I 6,8,20:r:[i]:1,5:[R1...]	UPenn STS 16	Not Available	Detected
35	<i>S. enterica</i> subsp. <i>enterica</i>	Brandenburg	Group I 4,[5],12:l,v:e,n,z15	UPenn STS 18	Not Available	Detected
36	<i>S. enterica</i> subsp. <i>enterica</i>	Bredeney	Group I 1,4,12,27:l,v:1,7:[z40]	NCTC 5731	Not Available	Detected
37	<i>S. enterica</i> subsp. <i>enterica</i>	California	Group I 4,12:g,m,t:[z67]	NCTC 6018	Not Available	Detected
38	<i>S. enterica</i> subsp. <i>enterica</i>	Cerro	Group I 6,14,18:z4,z23:[1,5]:[z45],[z82]	UPenn STS 22	Not Available	Detected
39	<i>S. enterica</i> subsp. <i>enterica</i>	Choleraesuis	Group I 6,7:c:1,5	ATCC 10708	Equine isolate	Detected
40	<i>S. enterica</i> subsp. <i>enterica</i>	Choleraesuis var Kunzendorf	Group I 6,7:[c]:1,5	ATCC 12011	Not available	Detected
41	<i>S. enterica</i> subsp. <i>enterica</i>	Cubana	Group I 1,13,23:z29:-:[z37],[z43]	UPenn STS 24	Not available	Detected
42	<i>S. enterica</i> subsp. <i>enterica</i>	Derby	Group I 1,4,[5],12:f,g:[1,2]	NCTC 5721	Not available	Detected
43	<i>S. enterica</i> subsp. <i>enterica</i>	Drypool	Group I 3,{10}{15}{15,34}:g,m,s:-	UPenn STS 26	Not available	Detected
44	<i>S. enterica</i> subsp. <i>enterica</i>	Dublin	Group I 1,9,12,[Vi]:g,p:-	UPenn STS 27	Not available	Detected
45	<i>S. enterica</i> subsp. <i>enterica</i>	Eastbourne	Group I 1,9,12:e,h:1,5	FDA 4017H	Not available	Detected
46	<i>S. enterica</i> subsp. <i>enterica</i>	Enteritidis	Group I 1,9,12:g,m:-	ATCC 13076	Not available	Detected
47	<i>S. enterica</i> subsp. <i>enterica</i>	Galiema	Group I 6,7,14:k:1,2	QL024.2	Environmental isolate	Detected
48	<i>S. enterica</i> subsp. <i>enterica</i>	Give	Group I 3,{10}{15}{15,34}:l,v:1,7:[d]	UPenn STS 42	Not available	Detected
49	<i>S. enterica</i> subsp. <i>enterica</i>	Haardt	Group I 8:k:1,5	UPenn STS 44	Not available	Detected
50	<i>S. enterica</i> subsp. <i>enterica</i>	Hadar	Group I 6,8:z10:e,n,x	ATCC 51956	Not available	Detected
51	<i>S. enterica</i> subsp. <i>enterica</i>	Havana	Group I 1,13,23:f,g,[s]:-[z79]	UPenn STS 47	Not available	Detected
52	<i>S. enterica</i> subsp. <i>enterica</i>	Heidelberg	Group I 1,4,[5],12:r:1,2	ATCC 8326	Not available	Detected
53	<i>S. enterica</i> subsp. <i>enterica</i>	Illinois	E3(3,15,34); z10; 1,5	ATCC 11646	Not available	Detected
54	<i>S. enterica</i> subsp. <i>enterica</i>	Indiana	Group I 1,4,12:z:1,7	NCTC 11304	Turkey	Detected
55	<i>S. enterica</i> subsp. <i>enterica</i>	Infantis	Group I 6,7,14:r:1,5:[R1...],[z37],[z45],[z49]	ATCC 51741	Pasta	Detected
56	<i>S. enterica</i> subsp. <i>enterica</i>	Javiana	Group I 1,9,12:l,z28:1,5:[R1...]	ATCC 10721	Not available	Detected

57	<i>S. enterica</i> subsp. <i>enterica</i>	Jerusalem	Group I 6,7,14:z10:l,w	QL024.12	Pet Food	Detected
58	<i>S. enterica</i> subsp. <i>enterica</i>	Johannesburg	Group I 1,40:b:e,n,x	UPenn STS 56	Not available	Detected
59	<i>S. enterica</i> subsp. <i>enterica</i>	Kahla	Group I 1,42:z35:1,6	ATCC 17980	Not available	Detected
60	<i>S. enterica</i> subsp. <i>enterica</i>	Kaitaan	Group I 1,6,14,25:m,t:-	QL024.7	Pet Food	Detected
61	<i>S. enterica</i> subsp. <i>enterica</i>	Kentucky	Group I 8,20:i:z6	ATCC 9263	Not available	Detected
62	<i>S. enterica</i> subsp. <i>enterica</i>	Krefeld	Group I 1,3,19:y:l,w	UPenn STS 58	Not available	Detected
63	<i>S. enterica</i> subsp. <i>enterica</i>	Lille	Group I 6,7,14:z38:-:[z82]	UPenn STS 59	Not available	Detected
64	<i>S. enterica</i> subsp. <i>enterica</i>	Livingstone	Group I 6,7,14:d:l,w	UPenn STS 63	Not available	Detected
65	<i>S. enterica</i> subsp. <i>enterica</i>	London	Group I 3,{10}{15}:l,v:1,6	UPenn STS 64	Not available	Detected
66	<i>S. enterica</i> subsp. <i>enterica</i>	Manhattan	Group I 6,8:d:1,5:[z58]	UPenn STS 65	Not available	Detected
67	<i>S. enterica</i> subsp. <i>enterica</i>	Mbankaka	Group I 6,7,14:z10:e,n,z15:[z37],[z45]	FDA 37N	Low moisture ingredient	Detected
68	<i>S. enterica</i> subsp. <i>enterica</i>	Menden	Group I 6,7:z10:1,2	ATCC 15992	Environmental isolate	Detected
69	<i>S. enterica</i> subsp. <i>enterica</i>	Meleagridis	Group I 3,{10}{15}{15,34}:e,h:l,w	QL12074-1	Feces	Detected
70	<i>S. enterica</i> subsp. <i>enterica</i>	Menhaden	Group I 3,15,34: 1,v:1,7	QL024.20	Pet food	Detected
71	<i>S. enterica</i> subsp. <i>enterica</i>	Minnesota	L(21); b; e,n,x	UPenn STS 70	Not available	Detected
72	<i>S. enterica</i> subsp. <i>enterica</i>	Montevideo	Group I 6,7,14,[54]:g,m,[p],s:[1,2,7]	ATCC 8387	Not available	Detected
73	<i>S. enterica</i> subsp. <i>enterica</i>	Muenchen	Group I 6,8:d:1,2:[z67]	ATCC BAA-1594	Human stool	Detected
74	<i>S. enterica</i> subsp. <i>enterica</i>	Newington	Group I 3,{10}{15}:e,h:1,6	QL024.8	Fish oil	Detected
75	<i>S. enterica</i> subsp. <i>enterica</i>	Newport	Group I 6,8,20:e,h:1,2:[z67],[z78]	ATCC 6962	Food poisoning	Detected
76	<i>S. enterica</i> subsp. <i>enterica</i>	Ohio	Group I 6,7,14:b:l,w:[z59]	UPenn STS 81	Not available	Detected
77	<i>S. enterica</i> subsp. <i>enterica</i>	Oranienburg	Group I 6,7,14:m,t:[z57]	ATCC 9239	Not available	Detected
78	<i>S. enterica</i> subsp. <i>enterica</i>	Orthmarshen	Group I 6,7,14:g,m,[t]:-	QL024.13	Pet kibble	Detected
79	<i>S. enterica</i> subsp. <i>enterica</i>	Paratyphi A	Group I 1,2,12:a:[1,5]	ATCC 9150	Not available	Detected
80	<i>S. enterica</i> subsp. <i>enterica</i>	Paratyphi B	Group I 1,4,[5],12:b:1,2:[z5],[z33]	ATCC 10719	Not available	Detected
81	<i>S. enterica</i> subsp. <i>enterica</i>	Paratyphi C	C1(6,7,[Vi]); c; 1,5	ATCC 13428	Not available	Detected
82	<i>S. enterica</i> subsp. <i>enterica</i>	Pomona	M(28); y; 1,7	ATCC 10729	Clinical isolate	Detected
83	<i>S. enterica</i> subsp. <i>enterica</i>	Poona	Group I 1,13,22:z:1,6:[z44],[z59]	NCTC 4840	Infant enteritis	Detected
84	<i>S. enterica</i> subsp. <i>enterica</i>	Potsdam	C ₁	QL15091-1A	Pet food	Detected
85	<i>S. enterica</i> subsp. <i>enterica</i>	Preston	Group I 1,4,12:z:l,w	QL024.16	Environmental isolate	Detected
86	<i>S. enterica</i> subsp. <i>enterica</i>	Pullorum	Group I 9,12:-:-	ATCC 13036	Egg	Detected
87	<i>S. enterica</i> subsp. <i>enterica</i>	Rubislaw	Group I 11:r:e,n,x	UPenn STS 92	Not available	Detected
88	<i>S. enterica</i> subsp. <i>enterica</i>	Saintpaul	Group I 1,4,[5],12:e,h:1,2	ATCC 9712	Cystitis, Panama	Detected
89	<i>S. enterica</i> subsp. <i>enterica</i>	San-Diego	Group I 1,4,[5],12:e,h:e,n,z15	UPenn STS 94	Not available	Detected
90	<i>S. enterica</i> subsp. <i>enterica</i>	Schwarzengrund	Group I 1,4,12,27:d:1,7	UPenn STS 95	Not available	Detected
91	<i>S. enterica</i> subsp. <i>enterica</i>	Senftenberg	Group I 1,3,19:g,[s],t:-	ATCC 43845	Not available	Detected
92	<i>S. enterica</i> subsp. <i>enterica</i>	Stanley	B(4,5,12); d; 1,2	ATCC 7308	Not available	Detected
93	<i>S. enterica</i> subsp. <i>enterica</i>	Sylvania	H([1],6,14,[25]); g,p; -	QL091313.4	PG Isolate	Detected
94	<i>S. enterica</i> subsp. <i>enterica</i>	Tallahassee	Group I 6,8:z4,z32:-	ATCC 12002	Not available	Detected
95	<i>S. enterica</i> subsp. <i>enterica</i>	Tennessee	Group I 6,7,14:z29:[1,2,7]	QL024.6	Clinical Isolate	Detected
96	<i>S. enterica</i> subsp. <i>enterica</i>	Thompson	Group I 6,7,14:k:1,5:[R1...]	FDA 2051H	Not available	Detected
97	<i>S. enterica</i> subsp. <i>enterica</i>	Tranoroa	55(55); k; z39	NCTC 10252	Not available	Detected
98	<i>S. enterica</i> subsp. <i>enterica</i>	Typhi	Group I 9,12[Vi]:d:-:[j],[z66]	ATCC 6539	Not available	Detected
99	<i>S. enterica</i> subsp. <i>enterica</i>	Typhimurium	Group I 1,4,[5],12:i:1,2	ATCC 14028	Tissue, animal	Detected
100	<i>S. enterica</i> subsp. <i>enterica</i>	Utrecht	Group I 52:d:1,5	NCTC 10077	Not available	Detected
101	<i>S. enterica</i> subsp. <i>enterica</i>	Urbana	N(30); b; e,n,x	UPenn STS 110	Not available	Detected
102	<i>S. enterica</i> subsp. <i>enterica</i>	Virchow	Group I 6,7,14:r:1,2	ATCC 51955	Not available	Detected
103	<i>S. enterica</i> subsp. <i>enterica</i>	Volta	Group I 11:r:l,z13,z28	QL024.9	Raw material	Detected
105	<i>S. enterica</i> subsp. <i>enterica</i>	Westhampton	Group I 3,{10}{15}{15,34}:g,s,t:-[z37]	QL024.14	Dog kibble	Detected
106	<i>S. enterica</i> subsp. <i>enterica</i>	Worthington	Group I 1,13,23:z:l,w:[z43]	UPenn STS 114	Not available	Detected

*ATCC = American Type Culture Collection, Manassas, VA; QL = Q Laboratories, Cincinnati, OH; FDA = US Food and Drug Administration, College Park, MD; NCTC = National Collection of Type Cultures, Porton Down, Salisbury, UK; UPenn = University of Pennsylvania *Salmonella* Reference Center, Kennett Square, PA.

Table 2: Summary of Salmonella spp. Exclusivity Panel Results (1)

No.	Species	Source ^a	Origin	Results
1	<i>Acinetobacter baumannii</i>	ATCC 19606	Urine	Not detected
2	<i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	ATCC 8750	Not available	Not detected
3	<i>Aeromonas hydrophila</i>	ATCC 49140	Clinical isolate	Not detected
4	<i>Citrobacter braakii</i>	ATCC 43162	Clinical isolate	Not detected
5	<i>Citrobacter farmeri</i>	ATCC 51633	Human feces	Not detected
6	<i>Citrobacter freundii</i>	QL11007-10	Clinical hospital	Not detected
7	<i>Cronobacter sakazakii</i>	ATCC 29544	Child's throat	Not detected
8	<i>Edwardsiella tarda</i>	ATCC 15947	Human feces	Not detected
9	<i>Enterobacter aerogenes</i>	ATCC 35029	Not available	Not detected
10	<i>Enterobacter cloacae</i>	ATCC 13047	Spinal fluid	Not detected
11	<i>Escherichia coli</i>	ATCC 8739	Feces	Not detected
12	<i>Escherichia coli</i> O157	ATCC 43895	Raw hamburger meat	Not detected
13	<i>Escherichia fergusonii</i>	ATCC 35469	Human feces	Not detected
14	<i>Escherichia hermannii</i>	ATCC 33650	Mouse brain	Not detected
15	<i>Escherichia vulneris</i>	ATCC 29943	Human wound	Not detected
16	<i>Hafnia alvei</i>	ATCC 51815	Milk	Not detected
17	<i>Haemophilus influenzae</i>	ATCC 19418	Not available	Not detected
18	<i>Klebsiella oxytoca</i>	ATCC 43165	Clinical isolate	Not detected
19	<i>Klebsiella pneumoniae</i> subsp. <i>pneumonia</i>	ATCC 4352	Cow's milk	Not detected
20	<i>Morganella morganii</i>	ATCC 25829	Human	Not detected
21	<i>Mycobacterium smegmatis</i>	ATCC 19420	Not available	Not detected
22	<i>Pantoea agglomerans</i>	ATCC 19552	Sewage	Not detected
23	<i>Proteus mirabilis</i>	ATCC 7002	Urine	Not detected
24	<i>Providencia rettgeri</i>	ATCC 14505	Not available	Not detected
25	<i>Pseudomonas aeruginosa</i>	ATCC 9027	Outer ear infection	Not detected
26	<i>Rahnella aquatilis</i>	ATCC 55046	Soil, Wisconsin	Not detected
27	<i>Serratia marcescens</i>	ATCC 13880	Human	Not detected
28	<i>Shigella boydii</i>	ATCC 9207	Pork liver	Not detected
29	<i>Shimwellia blattae</i>	ATCC 29907	Insect	Not detected
30	<i>Vibrio vulnificus</i>	QL02111-1A	Seafood product	Not detected

^aATCC = American Type Culture Collection, Manassas, VA; QL = Q laboratories, Cincinnati, OH**Table 3: Summary of STEC Inclusivity Panel Results (1)**

No.	Species	Serotype	Virulence	Source ^a	Origin	Results
1	<i>E. coli</i>	O3	stx1	MSU TW01413	Cow	Detected
2	<i>E. coli</i>	O5	stx1, eae	MSU TW05090	Cow	Detected
3	<i>E. coli</i>	O5	stx1, eae, b-eae	MSU TW07864	Calf Feces	Detected
4	<i>E. coli</i>	O26	stx1, eae	MSU TW04270	Human	Detected
5	<i>E. coli</i>	O26	stx1, eae	MSU TW04284	Human Child	Detected
6	<i>E. coli</i>	O26	stx1, eae	MSU TW02295	Human Infant	Detected
7	<i>E. coli</i>	O26	stx1, eae,	MSU TW07862	Calf, Cow	Detected
8	<i>E. coli</i>	O26	stx1, eae	MSU TW07814	Human	Detected
9	<i>E. coli</i>	O26	stx1, eae	MSU TW09183	Human	Detected
10	<i>E. coli</i>	O45	stx1, eae	MSU TW10121	Human	Detected
11	<i>E. coli</i>	O45	stx1, eae	MSU TW14003	Human	Detected
12	<i>E. coli</i>	O45	stx1, eae	MSU TW07947	Human	Detected
13	<i>E. coli</i>	O45	stx1, eae	MSU TW09183	Not available	Detected
14	<i>E. coli</i>	O45	stx, eae	PSU 1.2622	Not available	Detected
15	<i>E. coli</i>	O45	stx	PSU 2.0164	Not available	Detected
16	<i>E. coli</i>	O55	stx1, eae	MSU TW00585	Human	Detected
17	<i>E. coli</i>	O80	stx1, eae, E-hly (ehxA), und	MSU TW07875	Calf feces	Detected
18	<i>E. coli</i>	O103	stx1, eae	MSU TW08101	Human	Detected
19	<i>E. coli</i>	O103	stx1, eae	MSU TW07971	Human	Detected
20	<i>E. coli</i>	O103	stx1, eae	MSU TW11239	Human, child	Detected
21	<i>E. coli</i>	O103	stx1, eae	MSU TW07697	Human	Detected
22	<i>E. coli</i>	O103	stx1, eae	MSU TW05997	Human	Detected
23	<i>E. coli</i>	O103	stx, eae	MSU TW04162	Human	Detected

24	<i>E. coli</i>	O111	<i>stx1, stx2, eae</i>	MSU TW00186	Human	Detected
25	<i>E. coli</i>	O111	<i>stx1, eae</i>	MSU TW14960	Human	Detected
26	<i>E. coli</i>	O111	<i>stx1, eae</i>	MSU TW01387	Human	Detected
27	<i>E. coli</i>	O111	<i>stx1, stx2, eae</i>	MSU TW05614	Human	Detected
28	<i>E. coli</i>	O111	<i>stx2</i>	MSU TW06296	Human child	Detected
29	<i>E. coli</i>	O111	<i>stx1, eae</i>	MSU TW07502	Human	Detected
30	<i>E. coli</i>	O113	<i>stx2</i>	MSU TW01678	Human	Detected
31	<i>E. coli</i>	O115	<i>stx1, stx2, eae</i>	CCUG 38087	Not available	Detected
32	<i>E. coli</i>	O118	<i>stx1</i>	MSU TW08644	Human	Detected
33	<i>E. coli</i>	O121	<i>stx2, eae</i>	MSU TW07614	Human	Detected
34	<i>E. coli</i>	O121	<i>stx2, eae</i>	MSU TW08039	Human	Detected
35	<i>E. coli</i>	O121	<i>stx2, eae</i>	MSU TW07931	Human	Detected
36	<i>E. coli</i>	O121	<i>stx2, eae</i>	PSU 5.0959	Not available	Detected
37	<i>E. coli</i>	O121	<i>stx1, eae</i>	PSU 7.1686	Not available	Detected
38	<i>E. coli</i>	O121	<i>stx1, eae</i>	PSU 7.1709	Not available	Detected
39	<i>E. coli</i>	O145	<i>stx1, eae</i>	MSU TW01664	Meat powder	Detected
40	<i>E. coli</i>	O145	<i>stx1, eae</i>	MSU TW09356	Not available	Detected
41	<i>E. coli</i>	O145	<i>stx1, eae</i>	MSU TW09153	Human	Detected
42	<i>E. coli</i>	O145	<i>stx1, eae</i>	MSU TW07596	Human	Detected
43	<i>E. coli</i>	O145	<i>stx1, eae</i>	PSU 10.0707	Not available	Detected
44	<i>E. coli</i>	O145	<i>stx, eae</i>	PSU 10.0708	Not available	Detected
45	<i>E. coli</i>	O157	<i>stx1, eae</i>	ATCC BAA-460	Human feces	Detected
46	<i>E. coli</i>	O157	<i>stx1, eae</i>	ATCC 35150b	Human feces	Detected
47	<i>E. coli</i>	O157	<i>stx1, eae</i>	ATCC 43895	Raw hamburger meat	Detected
48	<i>E. coli</i>	O157	<i>stx1, stx2, eae</i>	MSU TW02302	Hamburger	Detected
49	<i>E. coli</i>	O157	<i>stx2, eae</i>	MSU TW00116	Human	Detected
50	<i>E. coli</i>	O157	<i>stx1, stx2, eae</i>	MSU TW02302	Hamburger	Detected

*ATCC = American Type Culture Collection, Manassas, VA; MSU = Michigan State University STEC Center, East Lansing, MI; PSU = Pennsylvania State University Culture Collection, University Park, PA; CCUG = Culture Collection University of Gothenburg, Gothenburg, Sweden.

Table 4: Summary of STEC Exclusivity Panel Results (1)

No.	Organism	Source ^a	Origin	Results
1	<i>Alcaligenes faecalis</i>	ATCC 8750	Not Available	Not detected
2	<i>Aeromonas hydrophila</i>	ATCC 49140	Clinical Isolate	Not detected
3	<i>Citrobacter braakii</i>	ATCC 43162	Clinical Isolate	Not detected
4	<i>Citrobacter farmeri</i>	ATCC 51633	Human Feces	Not detected
5	<i>Cronobacter sakazakii</i>	QL 17031.4	Infant Formula	Not detected
6	<i>Edwardsiella tarda</i>	ATCC 15947	Human Feces	Not detected
7	<i>Klebsiella aerogenes</i>	ATCC 13048	Sputum	Not detected
8	<i>Enterococcus faecalis</i>	ATCC 29212	Urine	Not detected
9	<i>Escherichia blattae</i>	ATCC 29907	Insect	Not detected
10	<i>Escherichia fergusonii</i>	ATCC 35469	Human Feces	Not detected
11	<i>Escherichia hermannii</i>	ATCC 33650	Mouse Brain	Not detected
12	<i>Escherichia vulneris</i>	ATCC 29943	Human Wound	Not detected
13	<i>Hafnia alvei</i>	ATCC 51815	Milk	Not detected
14	<i>Haemophilus influenzae</i>	ATCC 19418	Not Available	Not detected
15	<i>Klebsiella pneumoniae</i>	ATCC 4352	Cow Milk	Not detected
16	<i>Kocuria rhizophila</i>	ATCC 9341	Soil	Not detected
17	<i>Morganella morganii</i>	ATCC 25829	Human	Not detected
18	<i>Mycobacterium smegmatis</i>	ATCC 19420	Not Available	Not detected
19	<i>Pantoea agglomerans</i>	ATCC 19552	Sewage	Not detected
20	<i>Proteus mirabilis</i>	ATCC 7002	Urine	Not detected
21	<i>Providencia rettgeri</i>	ATCC 14505	Not Available	Not detected
22	<i>Pseudomonas aeruginosa</i>	ATCC 9027	Ear Infection	Not detected
23	<i>Pseudomonas fluorescens</i>	QL 17041.3	Raw Milk	Not detected
24	<i>Pseudomonas gessardii</i>	QL 17041.12	Raw Milk	Not detected
25	<i>Rahnella aquatilis</i>	ATCC 55046	Soil	Not detected
26	<i>Salmonella Typhimurium</i>	ATCC 14028	Animal Tissue	Not detected
27	<i>Serratia marcescens</i>	ATCC 13880	Human	Not detected
28	<i>Shigella boydii</i>	ATCC 9290	Pork liver	Not detected
29	<i>Stenotrophomonas maltophilia</i>	ATCC 13637	Patient with mouth cancer	Not detected
30	<i>Vibrio vulnificus</i>	QL 02111-1A	Seafood product	Not detected

^aATCC = American Type Culture Collection, Manassas, VA; QL = Q laboratories, Cincinnati, OH

Table 5. Pathotrak Next Generation Enrichment – *Salmonella* spp., Candidate vs. Reference – POD Results (1)

Matrix	MPN ^a / Test Portion	N ^b	Candidate ^h			Reference			dPOD _c ^f	95% CI ^g
			x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Romaine Hearts (375 g)	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
<i>S. Typhimurium</i> (NCTC ^h 12023)/ <i>E. coli</i> O157:H7 (ATCC ⁱ 43895)	0.73 (0.44, 1.20)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
	2.23 (1.18, 4.72)	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 calculated using the LCF MPN calculator ver. 2.0, 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.^hNCTC = National Collection of Type Cultures, Porton Down, Salisbury, UK.ⁱATCC = American Type Culture Collection, Manassas, VA.^jN/A = Not applicable.**Table 6. Pathotrak Next Generation Enrichment – *Salmonella* spp., Presumptive vs. Confirmed – POD Results (1)**

Matrix	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{cp} ^f	95% CI ^g
			x ^c	POD _{cp} ^d	95% CI	X	POD _{cc} ^e	95% CI		
Romaine Hearts (375 g)	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
<i>S. Typhimurium</i> (NCTC ^h 12023)/ <i>E. coli</i> O157:H7 (ATCC ⁱ 43895)	0.73 (0.44, 1.20)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
	2.23 (1.18, 4.72)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0, 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.^hNCTC = National Collection of Type Cultures, Porton Down, Salisbury, UK.ⁱATCC = American Type Culture Collection, Manassas, VA.^jN/A = Not applicable.**Table 7. Pathotrak Next Generation Enrichment – STEC, Candidate vs. Reference – POD Results (1)**

Matrix	MPN ^a / Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
			x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Romaine Hearts (375 g)	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
<i>S. Typhimurium</i> (NCTC ^h 12023)/ <i>E. coli</i> O157:H7 (ATCC ⁱ 43895)	0.70 (0.41, 1.12)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
	2.60 (1.12, 5.74)	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 calculated using the LCF MPN calculator ver. 2.0, 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.^hNCTC = National Collection of Type Cultures, Porton Down, Salisbury, UK.ⁱATCC = American Type Culture Collection, Manassas, VA.^jN/A = Not applicable.

Table 8. Pathotrak Next Generation Enrichment – STEC, Presumptive vs. Confirmed – POD Results (1)

Matrix	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD ^c	95% CI ^g
			x ^c	POD ^d	95% CI	X	POD ^e	95% CI		
Romaine Hearts (375 g)	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
<i>S. Typhimurium</i> (NCTC ^h 12023)/ <i>E. coli</i> O157:H7 (ATCC ⁱ 43895)	0.70 (0.41, 1.12)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
	2.60 (1.12, 5.74)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0, 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.^hNCTC = National Collection of Type Cultures, Porton Down, Salisbury, UK.ⁱATCC = American Type Culture Collection, Manassas, VA.^jN/A = Not applicable.**DISCUSSION OF MODIFICATION APPROVED MARCH 2023 (4)**

The Pathotrak Next Generation Enrichment successfully detected STEC and *Salmonella* spp. from romaine hearts (375 g and 1,500 g). Using POD analysis, the study data were unable to find a statistically detectable difference from 0 between the candidate method and the reference methods for all portions tested. The Pathotrak Next Generation Enrichment significantly reduces enrichment time for *Salmonella* spp. and STEC, allowing for quick results when compared to other enrichment methods including those used in real-time PCR methods. The bacterial extraction can be simply and quickly tested using either Bio-Rad iQ-Check kits, Hygiena BAX system PCR kits, and Eurofins BACGene PCR kits for detection of *Salmonella* spp. and STEC.

Table 1. Pathotrak Next Generation Enrichment Results – *Salmonella* spp., Candidate vs. Reference – POD Results (4)

Matrix	MPN ^a / Test Portion	N ^b	Pathotrak results ^c			BAM Ch. 5 results			dPOD ^c	95% CI ^h
			X ^d	POD ^e	95% CI	X	POD ^f	95% CI		
Romaine Hearts ^j (375 g)	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	0.86 (0.52, 1.36)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
	4.45 (2.37, 9.26)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Romaine Hearts ^k (1500 g)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	1.02 (0.64, 1.63)	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.37, 0.19
	6.45 (3.91, 14.45)	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 calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval.^bN = Number of test portions.^cPathotrak results = Outcomes were identical for Bio-Rad iQ-Check, Hygiena BAX System, and Eurofins BACGene PCR kits.^dx = Number of positive test portions.^ePOD_c = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.^fPOD_R = Reference method positive outcomes divided by the total number of trials.^gdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values.^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.^j375 g Romaine hearts inoculated with *S. Typhimurium* American Type Culture Collection (ATCC) 14028 and *E. coli* O157:H7 ATCC 43895.^kN/A = Not applicable.^l1500 g Romaine hearts inoculated with *E. coli* O26:H11 ATCC BAA-2196, *Salmonella enterica* Enteritidis ATCC 13076.**Table 2. Pathotrak Next Generation Enrichment Results – *Salmonella* spp., Presumptive vs. Confirmed – POD Results (4)**

Matrix	MPN ^a / Test Portion	N ^b	Pathotrak Presumptive			Pathotrak Confirmed			dPOD ^c	95% CI ^g
			x ^c	POD ^d	95% CI	X	POD ^e	95% CI		
Romaine Hearts ^j (375 g)	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.86 (0.52, 1.36)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
	4.45 (2.37, 9.26)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Romaine Hearts ^k (1500 g)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	1.02 (0.64, 1.63)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
	6.45 (3.91, 14.45)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval.^bN = Number of test portions.^cPathotrak presumptive results = Outcomes were identical for Bio-Rad iQ-Check, Hygiena BAX System, and Eurofins BACGene PCR kits.^dx = Number of positive test portions.^ePOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.^fPOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.^gdPOD_c = Difference between the presumptive candidate method results and the confirmed candidate method result POD values.^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.^j375 g Romaine hearts inoculated with *S. Typhimurium* American Type Culture Collection (ATCC) 14028 and *E. coli* O157:H7 ATCC 43895.^kN/A = Not applicable.^l1500 g Romaine hearts inoculated with *E. coli* O26:H11 ATCC BAA-2196, *Salmonella enterica* Enteritidis ATCC 13076.

Table 3. Pathotrak Next Generation Enrichment Results – STEC, Candidate vs. Reference – POD Results (4)

Matrix	MPN ^a / Test Portion	Pathotrak results ^c				BAM Ch. 4A results				
		N ^b	X ^d	POD _c ^e	95% CI	X	POD _R ^f	95% CI	dPOD _c ^g	95% CI ^h
Romaine Hearts (375 g) ATCC ⁱ	N/A ^j	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	0.47 (0.27, 0.73)	20	5	0.25	0.11, 0.47	7	0.35	0.18, 0.57	-0.10	-0.36, 0.18
	4.56 (2.40, 9.41)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Romaine Hearts (1500 g)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	0.56 (0.32, 0.91)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
	3.69 (2.02, 7.43)	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 calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval.^bN = Number of test portions.^cPathotrak results = Outcomes were identical for Bio-Rad iQ-Check, Hygiena BAX System, and Eurofins BACGene PCR kits.^dx = Number of positive test portions.^ePOD_c = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.^fPOD_R = Reference method positive outcomes divided by the total number of trials.^gdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values.^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.ⁱ375 g Romaine hearts inoculated with *S.Typhimurium* American Type Culture Collection (ATCC) 14028 and *E. coli* O157:H7 ATCC 43895.^jN/A = Not applicable.^k1500 g Romaine hearts inoculated with *E. coli* O26:H11 ATCC BAA-2196, *Salmonella enterica* Enteritidis ATCC 13076.**Table 4. Pathotrak Next Generation Enrichment Results – STEC, Presumptive vs. Confirmed – POD Results (4)**

Matrix	MPN ^a / Test Portion	N ^b	Pathotrak Presumptive results ^c			Pathotrak Confirmed results			dPOD _c ^g	95% CI ^h
			x ^d	POD _{CP} ^e	95% CI	x	POD _{CC} ^f	95% CI		
Romaine Hearts (375 g)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.47 (0.27, 0.73)	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.13, 0.13
	4.56 (2.40, 9.41)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Romaine Hearts (1500 g)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.56 (0.32, 0.91)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
	3.69 (2.02, 7.43)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval.^bN = Number of test portions.^cPathotrak presumptive results = Outcomes were identical for Bio-Rad iQ-Check, Hygiena BAX System, and Eurofins BACGene PCR kits.^dx = Number of positive test portions^ePOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.^fPOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.^gdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.ⁱ375 g Romaine hearts inoculated with *S.Typhimurium* American Type Culture Collection (ATCC) 14028 and *E. coli* O157:H7 ATCC 43895.^jN/A = Not applicable.^k1500 g Romaine hearts inoculated with *E. coli* O26:H11 ATCC BAA-2196, *Salmonella enterica* Enteritidis ATCC 13076.

DISCUSSION OF MODIFICATION APPROVED DECEMBER 2023 (12)

In the inclusivity and exclusivity studies, all 106 *Salmonella* spp., 50 *E. coli* O157:H7, and 50 non-O157 STEC isolates were correctly detected, while all 15 exclusivity isolates were correctly excluded. In the matrix study there were no statistically significant differences between the presumptive and confirmed results or between the candidate and reference methods results for the beef trim sampling cloths.

Table 1. <i>Salmonella</i> spp. Inclusivity Panel and Results (12)						
No.	<i>Salmonella</i> species and subspecies	Serovar (if applicable)	Antigenic Formula or O group	Source ^a	Origin	Result
1	<i>S. bongori</i>	Not applicable	Group V 66:z41:-	ATCC 43975	Not Available	Detected
2	<i>S. bongori</i>	Not applicable	Group V 66:z41:-	NCTC 10946	Frog	Detected
3	<i>S. bongori</i>	Not applicable	Group V 66:z41:-	NCTC 12419	Not Available	Detected
4	<i>S. bongori</i>	Not applicable	Group V 48:l-	FDA 94-2708	Not Available	Detected
5	<i>S. bongori</i>	Not applicable	Group V 40:z35	FDA 95-0123	Not Available	Detected
4	<i>S. enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa 51:z4,z23:-	ATCC 13314	Not Available	Detected
5	<i>S. enterica</i> subsp. <i>arizonae</i>	Not applicable	Not Available	ATCC BAA-1577	Not Available	Detected
6	<i>S. enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa: 43:g,z51:-	QL11007-4	Veterinary	Detected
7	<i>S. enterica</i> subsp. <i>diarizonae</i>	Not applicable	Not Available	ATCC BAA-1579	Not Available	Detected
8	<i>S. enterica</i> subsp. <i>diarizonae</i>	Not applicable	Group IIIb 35:i:z	ATCC BAA-216	Human blood	Detected
9	<i>S. enterica</i> subsp. <i>diarizonae</i>	Not applicable	Not Available	ATCC BAA-639	Human feces	Detected
10	<i>S. enterica</i> subsp. <i>houtenae</i>	Halmstad	Group IV 3,15:g,m,s:-	QL024.1	Clinical isolate	Detected
11	<i>S. enterica</i> subsp. <i>houtenae</i>	Harmelen	Group IV 51:z4,z23:-	ATCC 15783	Boa constrictor	Detected
12	<i>S. enterica</i> subsp. <i>houtenae</i>	Ochsenzoll	Group IV 16:z4,z23:-	ATCC 29932	Not Available	Detected
13	<i>S. enterica</i> subsp. <i>indica</i>	Ferlac	Group VI 1,6,14,25:a:e,n,x	ATCC 43976	Not Available	Detected
14	<i>S. enterica</i> subsp. <i>indica</i>	Ferlac	H(1,6,14,25); a; e,n,x; k, z6	NCTC 10458	Ceylonese desiccated coconut	Detected
15	<i>S. enterica</i> subsp. <i>indica</i>	Not applicable	Group VI 45:a:e,n,x	ATCC BAA-1578	India	Detected
16	<i>S. enterica</i> subsp. <i>salamae</i>	Artis	Group II 56:b:-	ATCC 700149	Not Available	Detected
17	<i>S. enterica</i> subsp. <i>salamae</i>	Basel	Group II 58:z13,z28:1,5	ATCC 700151	Not Available	Detected
18	<i>S. enterica</i> subsp. <i>salamae</i>	Not applicable	Group II: 4,12:b:1,5	QL02415	Not Available	Detected
19	<i>S. enterica</i> subsp. <i>enterica</i>	Abaetetuba	Group I 11:k:1,5	ATCC 35640	Creek water	Detected
20	<i>S. enterica</i> subsp. <i>enterica</i>	Abortusequi	Group I 4,12:-:e,n,x	ATCC 9842	Not Available	Detected
21	<i>S. enterica</i> subsp. <i>enterica</i>	Abortusovis	Group I 4,12:c:1,6	NCTC 10241	Not Available	Detected
22	<i>S. enterica</i> subsp. <i>enterica</i>	Abony	Group I 1,4,[5],12,[27]:b:e,n,x	NCTC 6017	Not Available	Detected
23	<i>S. enterica</i> subsp. <i>enterica</i>	Adelaide	Group I 35:f,g:-:[z27]	UPenn STS 2	Not Available	Detected
24	<i>S. enterica</i> subsp. <i>enterica</i>	Agona	Group I 1,4,[5],12:f,g,s:[1,2]:[z27],[z45]	ATCC 51957	Not Available	Detected
25	<i>S. enterica</i> subsp. <i>enterica</i>	Agama	Group I 4,12:i:1,6	UPenn STS 3	Not Available	Detected
26	<i>S. enterica</i> subsp. <i>enterica</i>	Agoueve	Group I 13,22:z29:-	UPenn STS 5	Not Available	Detected
27	<i>S. enterica</i> subsp. <i>enterica</i>	Alachua	Group I 35:z4,z23:-:[z37],[z45]	UPenn STS 6	Not Available	Detected
28	<i>S. enterica</i> subsp. <i>enterica</i>	Albany	Group I 8,20:z4,z24:-:[z45]	UPenn STS 7	Not Available	Detected
29	<i>S. enterica</i> subsp. <i>enterica</i>	Anatum	Group I 3,{10}{15}{15,34}:e,h:1,6:[z64]	ATCC 9270	Pork liver	Detected
30	<i>S. enterica</i> subsp. <i>enterica</i>	Arkansas	Group I 3,{10}{15}{15,34}:e,h:1,5	UPenn STS 11	Not Available	Detected
31	<i>S. enterica</i> subsp. <i>enterica</i>	Bareilly	Group I 6,7,14:y:1,5	FDA 1206H	Not Available	Detected
32	<i>S. enterica</i> subsp. <i>enterica</i>	Berta	Group I 1,9,12:[f],g,[t]:-	UPenn STS 13	Not Available	Detected
33	<i>S. enterica</i> subsp. <i>enterica</i>	Binza	Group I 3,{10}{15}{15,34}:y:1,5	UPenn STS 14	Not Available	Detected
34	<i>S. enterica</i> subsp. <i>enterica</i>	Bovismorbificans	Group I 6,8,20:r,[i]:1,5:[R1...]	UPenn STS 16	Not Available	Detected
35	<i>S. enterica</i> subsp. <i>enterica</i>	Brandenburg	Group I 4,[5],12:l,v:e,n,z15	UPenn STS 18	Not Available	Detected
36	<i>S. enterica</i> subsp. <i>enterica</i>	Bredeney	Group I 1,4,12,27:l,v:1,7:[z40]	NCTC 5731	Not Available	Detected
37	<i>S. enterica</i> subsp. <i>enterica</i>	California	Group I 4,12:g,m,t:[z67]	NCTC 6018	Not Available	Detected

38	<i>S. enterica</i> subsp. <i>enterica</i>	Cerro	Group I 6,14,18:z4,z23:[1,5]:[z45],[z82]	UPenn STS 22	Not Available	Detected
39	<i>S. enterica</i> subsp. <i>enterica</i>	Choleraesuis	Group I 6,7:c:1,5	ATCC 10708	Equine isolate	Detected
40	<i>S. enterica</i> subsp. <i>enterica</i>	Choleraesuis var Kunzendorf	Group I 6,7:[c]:1,5	ATCC 12011	Not available	Detected
41	<i>S. enterica</i> subsp. <i>enterica</i>	Cubana	Group I 1,13,23:z29:-:[z37],[z43]	UPenn STS 24	Not available	Detected
42	<i>S. enterica</i> subsp. <i>enterica</i>	Derby	Group I 1,4,[5],12:f,g:[1,2]	NCTC 5721	Not available	Detected
43	<i>S. enterica</i> subsp. <i>enterica</i>	Drypool	Group I 3,{10}{15}{15,34}:g,m,s:-	UPenn STS 26	Not available	Detected
44	<i>S. enterica</i> subsp. <i>enterica</i>	Dublin	Group I 1,9,12,[Vi]:g,p:-	UPenn STS 27	Not available	Detected
45	<i>S. enterica</i> subsp. <i>enterica</i>	Eastbourne	Group I 1,9,12:e,h:1,5	FDA 4017H	Not available	Detected
46	<i>S. enterica</i> subsp. <i>enterica</i>	Enteritidis	Group I 1,9,12:g,m:-	ATCC 13076	Not available	Detected
47	<i>S. enterica</i> subsp. <i>enterica</i>	Galiema	Group I 6,7,14:k:1,2	QL024.2	Environmental isolate	Detected
48	<i>S. enterica</i> subsp. <i>enterica</i>	Give	Group I 3,{10}{15}{15,34}:l,v:1,7:[d]	UPenn STS 42	Not available	Detected
49	<i>S. enterica</i> subsp. <i>enterica</i>	Haardt	Group I 8:k:1,5	UPenn STS 44	Not available	Detected
50	<i>S. enterica</i> subsp. <i>enterica</i>	Hadar	Group I 6,8:z10:e,n,x	ATCC 51956	Not available	Detected
51	<i>S. enterica</i> subsp. <i>enterica</i>	Havana	Group I 1,13,23:f,g,[s]:-[z79]	UPenn STS 47	Not available	Detected
52	<i>S. enterica</i> subsp. <i>enterica</i>	Heidelberg	Group I 1,4,[5],12:r:1,2	ATCC 8326	Not available	Detected
53	<i>S. enterica</i> subsp. <i>enterica</i>	Illinois	E3(3,15,34); z10; 1,5	ATCC 11646	Not available	Detected
54	<i>S. enterica</i> subsp. <i>enterica</i>	Indiana	Group I 1,4,12:z:1,7	NCTC 11304	Turkey	Detected
55	<i>S. enterica</i> subsp. <i>enterica</i>	Infantis	Group I 6,7,14:r:1,5:[R1...],[z37],[z45],[z49]	ATCC 51741	Pasta	Detected
56	<i>S. enterica</i> subsp. <i>enterica</i>	Javiana	Group I 1,9,12:l,z28:1,5:[R1...]	ATCC 10721	Not available	Detected
57	<i>S. enterica</i> subsp. <i>enterica</i>	Jerusalem	Group I 6,7,14:z10:l,w	QL024.12	Pet Food	Detected
58	<i>S. enterica</i> subsp. <i>enterica</i>	Johannesburg	Group I 1,40:b:e,n,x	UPenn STS 56	Not available	Detected
59	<i>S. enterica</i> subsp. <i>enterica</i>	Kahla	Group I 1,42:z35:1,6	ATCC 17980	Not available	Detected
60	<i>S. enterica</i> subsp. <i>enterica</i>	Kaitaan	Group I 1,6,14,25:m,t:-	QL024.7	Pet Food	Detected
61	<i>S. enterica</i> subsp. <i>enterica</i>	Kentucky	Group I 8,20:i:z6	ATCC 9263	Not available	Detected
62	<i>S. enterica</i> subsp. <i>enterica</i>	Krefeld	Group I 1,3,19:y:l,w	UPenn STS 58	Not available	Detected
63	<i>S. enterica</i> subsp. <i>enterica</i>	Lille	Group I 6,7,14:z38:-:[z82]	UPenn STS 59	Not available	Detected
64	<i>S. enterica</i> subsp. <i>enterica</i>	Livingstone	Group I 6,7,14:d:l,w	UPenn STS 63	Not available	Detected
65	<i>S. enterica</i> subsp. <i>enterica</i>	London	Group I 3,{10}{15}:l,v:1,6	UPenn STS 64	Not available	Detected
66	<i>S. enterica</i> subsp. <i>enterica</i>	Manhattan	Group I 6,8:d:1,5:[z58]	UPenn STS 65	Not available	Detected
67	<i>S. enterica</i> subsp. <i>enterica</i>	Mbandaka	Group I 6,7,14:z10:e,n,z15:[z37],[z45]	FDA 37N	Low moisture ingredient	Detected
68	<i>S. enterica</i> subsp. <i>enterica</i>	Menden	Group I 6,7:z10:1,2	ATCC 15992	Environmental isolate	Detected
69	<i>S. enterica</i> subsp. <i>enterica</i>	Meleagridis	Group I 3,{10}{15}{15,34}:e,h:l,w	QL12074-1	Feces	Detected
70	<i>S. enterica</i> subsp. <i>enterica</i>	Menhaden	Group I 3,15,34: 1,v:1,7	QL024.20	Pet food	Detected
71	<i>S. enterica</i> subsp. <i>enterica</i>	Minnesota	L(21); b; e,n,x	UPenn STS 70	Not available	Detected
72	<i>S. enterica</i> subsp. <i>enterica</i>	Montevideo	Group I 6,7,14,[54]:g,m,[p],s:[1,2,7]	ATCC 8387	Not available	Detected
73	<i>S. enterica</i> subsp. <i>enterica</i>	Muenchen	Group I 6,8:d:1,2:[z67]	ATCC BAA-1594	Human stool	Detected
74	<i>S. enterica</i> subsp. <i>enterica</i>	Newington	Group I 3,{10}{15}:e,h:1,6	QL024.8	Fish oil	Detected
75	<i>S. enterica</i> subsp. <i>enterica</i>	Newport	Group I 6,8,20:e,h:1,2:[z67],[z78]	ATCC 6962	Food poisoning	Detected
76	<i>S. enterica</i> subsp. <i>enterica</i>	Ohio	Group I 6,7,14:b:l,w:[z59]	UPenn STS 81	Not available	Detected
77	<i>S. enterica</i> subsp. <i>enterica</i>	Oranienburg	Group I 6,7,14:m,t:[z57]	ATCC 9239	Not available	Detected
78	<i>S. enterica</i> subsp. <i>enterica</i>	Orthmarshen	Group I 6,7,14:g,m,[t]:-	QL024.13	Pet kibble	Detected
79	<i>S. enterica</i> subsp. <i>enterica</i>	Paratyphi A	Group I 1,2,12:a:[1,5]	ATCC 9150	Not available	Detected
80	<i>S. enterica</i> subsp. <i>enterica</i>	Paratyphi B	Group I 1,4,[5],12:b:1,2:[z5],[z33]	ATCC 10719	Not available	Detected
81	<i>S. enterica</i> subsp. <i>enterica</i>	Paratyphi C	C1(6,7,[Vi]; c; 1,5	ATCC 13428	Not available	Detected
82	<i>S. enterica</i> subsp. <i>enterica</i>	Pomona	M(28); y; 1,7	ATCC 10729	Clinical isolate	Detected
83	<i>S. enterica</i> subsp. <i>enterica</i>	Poona	Group I 1,13,22:z:1,6:[z44],[z59]	NCTC 4840	Infant enteritis	Detected
84	<i>S. enterica</i> subsp. <i>enterica</i>	Potsdam	C ₁	QL15091-1A	Pet food	Detected

85	<i>S. enterica</i> subsp. <i>enterica</i>	Preston	Group I 1,4,12:z:l,w	QL024.16	Environmental isolate	Detected
86	<i>S. enterica</i> subsp. <i>enterica</i>	Pullorum	Group I 9,12:-:-	ATCC 13036	Egg	Detected
87	<i>S. enterica</i> subsp. <i>enterica</i>	Rubislaw	Group I 11:r:e,n,x	UPenn STS 92	Not available	Detected
88	<i>S. enterica</i> subsp. <i>enterica</i>	Saintpaul	Group I 1,4,[5],12:e,h:1,2	ATCC 9712	Cystitis, Panama	Detected
89	<i>S. enterica</i> subsp. <i>enterica</i>	San-Diego	Group I 1,4,[5],12:e,h:e,n,z15	UPenn STS 94	Not available	Detected
90	<i>S. enterica</i> subsp. <i>enterica</i>	Schwarzengrund	Group I 1,4,12,27:d:1,7	UPenn STS 95	Not available	Detected
91	<i>S. enterica</i> subsp. <i>enterica</i>	Senftenberg	Group I 1,3,19:g,[s],t:-	ATCC 43845	Not available	Detected
92	<i>S. enterica</i> subsp. <i>enterica</i>	Stanley	B(4,5,12); d; 1,2	ATCC 7308	Not available	Detected
93	<i>S. enterica</i> subsp. <i>enterica</i>	Sylvania	H([1],6,14,[25]); g,p; -	QL091313.4	PG Isolate	Detected
94	<i>S. enterica</i> subsp. <i>enterica</i>	Tallahassee	Group I 6,8:z4,z32:-	ATCC 12002	Not available	Detected
95	<i>S. enterica</i> subsp. <i>enterica</i>	Tennessee	Group I 6,7,14:z29:[1,2,7]	QL024.6	Clinical Isolate	Detected
96	<i>S. enterica</i> subsp. <i>enterica</i>	Thompson	Group I 6,7,14:k:1,5:[R1...]	FDA 2051H	Not available	Detected
97	<i>S. enterica</i> subsp. <i>enterica</i>	Tranoroa	55(55); k; z39	NCTC 10252	Not available	Detected
98	<i>S. enterica</i> subsp. <i>enterica</i>	Typhi	Group I 9,12[Vi]:d:-:[j],[z66]	ATCC 6539	Not available	Detected
99	<i>S. enterica</i> subsp. <i>enterica</i>	Typhimurium	Group I 1,4,[5],12:i:1,2	ATCC 14028	Tissue, animal	Detected
100	<i>S. enterica</i> subsp. <i>enterica</i>	Utrecht	Group I 52:d:1,5	NCTC 10077	Not available	Detected
101	<i>S. enterica</i> subsp. <i>enterica</i>	Urbana	N(30); b; e,n,x	UPenn STS 110	Not available	Detected
102	<i>S. enterica</i> subsp. <i>enterica</i>	Virchow	Group I 6,7,14:r:1,2	ATCC 51955	Not available	Detected
103	<i>S. enterica</i> subsp. <i>enterica</i>	Volta	Group I 11:r:l,z13,z28	QL024.9	Raw material	Detected
105	<i>S. enterica</i> subsp. <i>enterica</i>	Westhampton	Group I 3,[10]{15}{15,34}:g,s,t:-:[z37]	QL024.14	Dog kibble	Detected
106	<i>S. enterica</i> subsp. <i>enterica</i>	Worthington	Group I 1,13,23:z:l,w:[z43]	UPenn STS 114	Not available	Detected

*ATCC = American Type Culture Collection, Manassas, VA; QL = Q Laboratories, Cincinnati, OH; FDA = US Food and Drug Administration, College Park, MD; NCTC = National Collection of Type Cultures, Porton Down, Salisbury, UK; UPenn = University of Pennsylvania *Salmonella* Reference Center, Kennett Square, PA.

Table 2. O157:H7 STEC Inclusivity Panel and Results (12)

No.	Organism	Serotype	Source ^a	Strain Number	Origin	Virulence Factors			Result
						<i>stx1</i>	<i>stx2</i>	<i>eae</i>	
1	<i>Escherichia coli</i>	O157:H7	MSU	TW00116	Human; Washington, USA	-	+	+	Detected
2	<i>Escherichia coli</i>	O157:H7	QL	12298-2	Deli meat (ham)	+	+	+	Detected
3	<i>Escherichia coli</i>	O157:H7	QL	12298-1	Not Available	+	+	+	Detected
4	<i>Escherichia coli</i>	O157:H7	ATCC	35150	Human Feces	+	+	+	Detected
5	<i>Escherichia coli</i>	O157:H7	QL	2-709	Beef	+	+	+	Detected
6	<i>Escherichia coli</i>	O157:H7	ATCC	43889	Not Available	+	+	+	Detected
7	<i>Escherichia coli</i>	O157:H7	ATCC	43890	Not Available	+	+	+	Detected
8	<i>Escherichia coli</i>	O157:H7	ATCC	43894	Not Available	+	+	+	Detected
9	<i>Escherichia coli</i>	O157:H7	ATCC	43895	Raw hamburger meat implicated in hemorrhagic colitis outbreak	+	+	+	Detected
10	<i>Escherichia coli</i>	O157:H7	ATCC	51657	Clinical Isolate	+	+	+	Detected
11	<i>Escherichia coli</i>	O157:H7	QL	14077-1	Meat	+	+	+	Detected
12	<i>Escherichia coli</i>	O157:H7	ATCC	51658	Clinical Isolate	+	+	+	Detected
13	<i>Escherichia coli</i>	O157:H7	ATCC	51659	Clinical Isolate	+	+	+	Detected
14	<i>Escherichia coli</i>	O157:H7	ATCC	700531	Clinical Isolate	+	+	+	Detected
15	<i>Escherichia coli</i>	O157:H7	ATCC	700599	Salami	+	+	+	Detected
16	<i>Escherichia coli</i>	O157:H7	MSU	TW00975	Human; California, USA	+	-	+	Detected
17	<i>Escherichia coli</i>	O157:H7	MSU	TW02302	Hamburger meat	+	+	+	Detected
18	<i>Escherichia coli</i>	O157:H7	MSU	TW04863	Human, Washington, USA	+	+	+	Detected
19	<i>Escherichia coli</i>	O157:H7	MSU	TW05356	Human Washington, USA	+	+	+	Detected

20	<i>Escherichia coli</i>	O157:H7	MSU	TW07587	Human, Okayama, Japan	+	+	+	Detected
21	<i>Escherichia coli</i>	O157:H7	ATCC	BAA-460	Human Feces	+	+	+	Detected
22	<i>Escherichia coli</i>	O157:H7	MSU	DEC3A	Human Washington, USA	+	+	+	Detected
23	<i>Escherichia coli</i>	O157:H7	MSU	DEC3B	Human Washington, USA	+	+	+	Detected
24	<i>Escherichia coli</i>	O157:H7	MSU	DEC3C	Human, New Mexico, USA	+	+	+	Detected
25	<i>Escherichia coli</i>	O157:H7	MSU	DEC3D	Human, Michigan, USA	+	+	+	Detected
26	<i>Escherichia coli</i>	O157:H7	MSU	DEC3E	Human Canada	+	+	+	Detected
27	<i>Escherichia coli</i>	O157:H7	QL	14077-3	Salami	+	+	+	Detected
28	<i>Escherichia coli</i>	O157:H7	MSU	DEC4B	Human Denmark	+	+	+	Detected
29	<i>Escherichia coli</i>	O157:H7	QL	14077-4	Apple Cider	+	+	+	Detected
30	<i>Escherichia coli</i>	O157:H7	QL	14077-8	Hamburger meat	+	+	+	Detected
31	<i>Escherichia coli</i>	O157:H7	MSU	DEC4E	Human Denmark	+	+	+	Detected
32	<i>Escherichia coli</i>	O157:H7	QL	14077-5	Hamburger meat	+	+	+	Detected
33	<i>Escherichia coli</i>	O157:H7	QL	2-202	Meat	+	+	+	Detected
34	<i>Escherichia coli</i>	O157:H7	QL	2-203	Meat	+	+	+	Detected
35	<i>Escherichia coli</i>	O157:H7	QL	2-204	Meat	+	+	+	Detected
36	<i>Escherichia coli</i>	O157:H7	QL	2-205	Meat	+	+	+	Detected
37	<i>Escherichia coli</i>	O157:H7	QL	2-206	Meat	+	+	+	Detected
38	<i>Escherichia coli</i>	O157:H7	QL	2-207	Meat	+	+	+	Detected
39	<i>Escherichia coli</i>	O157:H7	QL	2-214	Meat	+	+	+	Detected
40	<i>Escherichia coli</i>	O157:H7	QL	2-370	Meat	+	+	+	Detected
41	<i>Escherichia coli</i>	O157:H7	QL	2-701	Beef	+	+	+	Detected
42	<i>Escherichia coli</i>	O157:H7	QL	2-704	Beef	+	+	+	Detected
43	<i>Escherichia coli</i>	O157:H7	QL	2-705	Beef	+	+	+	Detected
44	<i>Escherichia coli</i>	O157:H7	QL	2-706	Beef	+	+	+	Detected
45	<i>Escherichia coli</i>	O157:H7	QL	2-707	Beef	+	+	+	Detected
46	<i>Escherichia coli</i>	O157:H7	QL	2-708	Beef	+	+	+	Detected
47	<i>Escherichia coli</i>	O157:H7	QL	2-710	Beef	+	+	+	Detected
48	<i>Escherichia coli</i>	O157:H7	QL	14077-6	Food	+	+	+	Detected
49	<i>Escherichia coli</i>	O157:H7???	NCTC	13125	Human diarrhea stool, York 1995	+	+	+	Detected
50	<i>Escherichia coli</i>	O157:H7	QL	14077-7	Hamburger meat	+	+	+	Detected

* ATCC = American Type Culture Collection, Manassas, VA; MSU = Michigan State University STEC Center, East Lansing, MI; PSU = Pennsylvania State University Culture Collection, State College, PA. NCTC = National Collection of Type Cultures, United Kingdom.

Table 3. Non-O157:H7 STEC Inclusivity Panel and Results (12)

No.	Organism	Serotype	Source ^a	Strain Number	Origin	Virulence Factors			Result
						<i>stx1</i>	<i>stx2</i>	<i>eae</i>	
1	<i>Escherichia coli</i>	O26	MSU	DEC10E	Human	+	-	+	Detected
2	<i>Escherichia coli</i>	O26	MSU	TW07814	Human	+	+	+	Detected
3	<i>Escherichia coli</i>	O26	MSU	TW00971	Human	+	-	+	Detected
4	<i>Escherichia coli</i>	O26	MSU	TW04270	Human	+	-	+	Detected
5	<i>Escherichia coli</i>	O26	MSU	TW04284	Human Child	+	-	+	Detected
6	<i>Escherichia coli</i>	O26	MSU	TW00971	Human	+	-	+	Detected
7	<i>Escherichia coli</i>	O26	MSU	TW05992	Human	+	-	+	Detected
8	<i>Escherichia coli</i>	O26	MSU	TW08031	Human	+	-	+	Detected
9	<i>Escherichia coli</i>	O26	MSU	TW01188	Human	+	-	+	Detected
10	<i>Escherichia coli</i>	O45	PSU	1.2622	Cow	+	+	-	Detected
11	<i>Escherichia coli</i>	O45	PSU	1.2635	Cow	+	+	-	Detected
12	<i>Escherichia coli</i>	O45	PSU	11.1079	Not Available	+	+	+	Detected
13	<i>Escherichia coli</i>	O45	MSU	TW09183	N/A	+	-	+	Detected

14	<i>Escherichia coli</i>	O45	MSU	TW10121	Human	+	-	+	Detected
15	<i>Escherichia coli</i>	O45	ATCC	BAA-2193	Human	+	-	+	Detected
16	<i>Escherichia coli</i>	O45	QL	23208-2	Beef Trim	+	-	+	Detected
17	<i>Escherichia coli</i>	O45	QL	23208-3	Beef Trim	+	-	+	Detected
18	<i>Escherichia coli</i>	O103	MSU	TW11239	Human Child	+	-	-	Detected
19	<i>Escherichia coli</i>	O103	QL	23094-1	Raw Flour	+	-	+	Detected
20	<i>Escherichia coli</i>	O103	ATCC	BAA-2215	Not Available	+	-	+	Detected
21	<i>Escherichia coli</i>	O103	MSU	TW08101	Human	+	-	+	Detected
22	<i>Escherichia coli</i>	O103	MSU	TW07697	Human	+	-	+	Detected
23	<i>Escherichia coli</i>	O103	MSU	TW05997	Human	+	-	+	Detected
24	<i>Escherichia coli</i>	O103	MSU	TW07971	Human	+	-	+	Detected
25	<i>Escherichia coli</i>	O103	PSU	9.0036	Not Available	+	+	+	Detected
26	<i>Escherichia coli</i>	O111	MSU	TW06315	Human	+	+	+	Detected
27	<i>Escherichia coli</i>	O111	MSU	TW06296	Human Child	-	+	-	Detected
28	<i>Escherichia coli</i>	O111	MSU	TW14960	Human	+	-	+	Detected
29	<i>Escherichia coli</i>	O111	MSU	TW00073	Human	+	-	+	Detected
30	<i>Escherichia coli</i>	O111	ATCC	BAA-2440	Human	+	+	+	Detected
31	<i>Escherichia coli</i>	O111	QL	23208-7	Beef Trim	+	+	+	Detected
32	<i>Escherichia coli</i>	O111	QL	12289-3A	Not Available	+	+	+	Detected
33	<i>Escherichia coli</i>	O111	MSU	TW07926	Human	+	+	+	Detected
34	<i>Escherichia coli</i>	O121	MSU	TW07614	Human	-	+	+	Detected
35	<i>Escherichia coli</i>	O121	MSU	TW07931	Human	-	+	+	Detected
36	<i>Escherichia coli</i>	O121	PSU	5.0959	Not Available	+	+	+	Detected
37	<i>Escherichia coli</i>	O121	ATCC	BAA-2219	Human	-	+	+	Detected
38	<i>Escherichia coli</i>	O121	QL	23208-1	Beef Trim	-	+	+	Detected
39	<i>Escherichia coli</i>	O121	QL	23208-5	Beef Trim	-	+	+	Detected
40	<i>Escherichia coli</i>	O121	QL	23208-8	Beef Trim	-	+	+	Detected
41	<i>Escherichia coli</i>	O121	MSU	TW08023	Human	-	+	+	Detected
42	<i>Escherichia coli</i>	O121	MSU	TW08039	Human	-	+	+	Detected
43	<i>Escherichia coli</i>	O145	ATCC	BAA-2192	Human	+	+	+	Detected
44	<i>Escherichia coli</i>	O145	MSU	TW09153	Human	-	+	+	Detected
45	<i>Escherichia coli</i>	O145	QL	23208-6	Beef Trim	+	+	+	Detected
46	<i>Escherichia coli</i>	O145	MSU	TW09356	Human	+	-	+	Detected
47	<i>Escherichia coli</i>	O145	MSU	TW09153	Human	-	+	+	Detected
48	<i>Escherichia coli</i>	O145	MSU	TW08087	Human	+	-	+	Detected
49	<i>Escherichia coli</i>	O145	MSU	TW07596	Human	+	-	+	Detected
50	<i>Escherichia coli</i>	O45	QL	23208-4	Beef Trim	+	-	+	Detected

^a ATCC = American Type Culture Collection, Manassas, VA; MSU = Michigan State University STEC Center, East Lansing, MI; PSU = Pennsylvania State University Culture Collection, State College, PA, QL = Q Laboratories, Cincinnati, OH.

Table 4. *Salmonella* spp., O157:H7 STEC, and Non-O157:H7 STEC Exclusivity Panel and Results (12)

No.	Organism	Source ^a	Origin	Results
1	<i>Alcaligenes faecalis</i>	ATCC 8750	Not Available	Not Detected
2	<i>Citrobacter braakii</i>	ATCC 43162	Clinical Isolate	Not Detected
3	<i>Cronobacter sakazakii</i>	QL 17031.4b	Infant Formula	Not Detected
4	<i>Edwardsiella tarda</i>	ATCC 15947	Human Feces	Not Detected
5	<i>Klebsiella aerogenes</i>	ATCC 13048	Sputum	Not Detected
6	<i>Escherichia hermannii</i>	ATCC 33650	Mouse Brain	Not Detected
7	<i>Escherichia vulneris</i>	ATCC 29943	Human Wound	Not Detected
8	<i>Hafnia alvei</i>	ATCC 51815	Milk	Not Detected
9	<i>Klebsiella pneumoniae</i>	ATCC 4352	Cow Milk	Not Detected

10	<i>Morganella morganii</i>	ATCC 25829	Human	Not Detected
11	<i>Proteus mirabilis</i>	ATCC 7002	Urine	Not Detected
12	<i>Providencia rettgeri</i>	ATCC 14505	Not Available	Not Detected
13	<i>Pseudomonas aeruginosa</i>	ATCC 9027	Ear Infection	Not Detected
14	<i>Serratia marcescens</i>	ATCC 13880	Human	Not Detected
15	<i>Shigella boydii</i>	ATCC 9290	Pork Liver	Not Detected

^aATCC = American Type Culture Collection, Manassas, VA; QL = Q Laboratories, Cincinnati, OH.

Table 5. Pathotrak NGE with BAX® System Real-Time PCR Assays for *E. coli* O157:H7 EXACT, STEC and *Salmonella* presumptive vs. confirmed results (12)

Matrix ^a	BAX kit	Strain	cfu ^b /cloth	N ^c	NGE + BAX presumptive result			NGE + BAX confirmed result			dPOD _{CP} ^g	95% CI ^h
					X ^d	POD _{CP} ^e	95% CI	x	POD _{CC} ^f	95% CI		
Beef trim sampling cloth	BAX RT <i>Salmonella</i>	<i>S. Typhimurium</i> (ATCC 14028)	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.6	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.43, 0.43
			6.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim sampling cloth	BAX RT <i>E. coli</i> O157:H7 Exact	<i>E. coli</i> O157:H7 (ATCC 43895)	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.8	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
			7.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef trim sampling cloth	BAX RT STEC Suite	<i>E. coli</i> O26 (MSU TW07814)	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.4	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
			4.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMatrix = Each cloth was inoculated with all three target organisms simultaneously. Results are separated by target.

^bcfu/cloth = Number of organisms applied to the sampling cloth prior to equilibration period.

^cN = Number of test portions.

^dx = Number of positive test portions.

^ePOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

^fPOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

^gdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 6. Pathotrak NGE with BAX® System Real-Time PCR Assays for *E. coli* O157:H7 EXACT, STEC and *Salmonella* vs. Reference method results (12)

Matrix ^a	BAX kit	Strain	cfu ^b /cloth	N ^c	NGE + BAX results			Reference method ^d results			dPOD _{CP} ^h	95% CI ⁱ
					X ^d	POD _C ^e	95% CI	x	POD _R ^g	95% CI		
Beef trim sampling cloth	BAX RT <i>Salmonella</i>	<i>S. Typhimurium</i> (ATCC 14028)	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.6	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
			6.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim sampling cloth	BAX RT <i>E. coli</i> O157:H7 Exact	<i>E. coli</i> O157:H7 (ATCC 43895)	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.8	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			7.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Beef trim sampling cloth	BAX RT STEC Suite	<i>E. coli</i> O26 (MSU TW07814)	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.4	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			4.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMatrix = Each cloth was inoculated with all three target organisms simultaneously. Results are separated by target.

^bcfu/cloth = Number of organisms applied to the sampling cloth prior to equilibration period.

^cN = Number of test portions.

^dx = Number of positive test portions.

^ePOD_C = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

^fReference method = MLG 4.14 for *Salmonella* or MLG 5C.03 for *E. coli* O157:H7 and *E. coli* O26.

^gPOD_R = Reference method positive outcomes divided by the total number of trials.

^hdPOD_C = Difference between the candidate method and reference method POD values.

ⁱ95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

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