



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.

022203

The AOAC Research Institute hereby certifies the method known as

GENE-UP[®] Pathogenic *E. coli* (PEC)

manufactured by

bioMérieux, SA

Cheemin de l'Orme

69280 Marcy-l'Etoile, France

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

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

Bradley A. Stawick, AOAC Research Institute Senior Director

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METHOD NAME GENE-UP® Pathogenic <i>E. coli</i> (PEC)		CATALOG NUMBER 424143	
INDEPENDENT LABORATORY Q Laboratories 1930 Radcliff Drive Cincinnati, OH 45204			
APPLICABILITY OF METHOD Target Organisms – Shiga-toxin producing <i>E. coli</i> (STEC) containing highly correlated co-localized virulence genes <i>stx</i> and <i>eae</i> . Matrixes – MLG 5C.02 and BAM Ch 4A – Raw ground beef (80% lean, 375 g), raw beef trim (375 g), Romaine lettuce (375 g), and carcass sampling cloth (MicroTally® or equivalent) from pork trim , carcass sampling cloth (MicroTally® or equivalent) from beef trim Performance claims – The GENE-UP PEC method is comparable to the USDA Food Safety Inspection Service <i>Microbiological Laboratory Guidebook</i> 5C.02 <i>Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia Coli (STECs) from Meat Products and Carcass and Environmental Sponges</i> [2] and the FDA Bacteriological Analytical Manual Chapter 4A: <i>Diarrheagenic Escherichia coli</i> [3] for the detection of STEC containing highly correlated co-localized virulence genes <i>stx</i> and <i>eae</i> .		REFERENCES U.S. Department of Agriculture Food Safety and Inspection Service (2021) <i>Microbiology Laboratory Guidebook, Revision 5C.02 Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponges</i> (2) U.S. Food and Drug Administration (2020) Bacteriological Analytical Manual, Chapter 4A: <i>Diarrheagenic Escherichia coli</i> . (3)	
ORIGINAL CERTIFICATION DATE February 23, 2022		CERTIFICATION RENEWAL RECORD Renewed through December 2026.	
METHOD MODIFICATION RECORD 1. December 2022 Level 1 2. January 2023 Level 2 3. August 2024 Level 1 4. October 2024 Level 1		SUMMARY OF MODIFICATION 1. Editorial changes. 2. Matrix extension to include carcass sampling cloth (MicroTally® or equivalent) from beef trim 3. New color and cap to PCR tube of the assay. 4. Software update from version 3.2 to 4.0 for minor updates.	
Under this AOAC <i>Performance Tested Methods</i> SM License Number, 022103 this method is distributed by: NONE		Under this AOAC <i>Performance Tested Methods</i> SM License Number, 022103 this method is distributed as: NONE	

PRINCIPLE OF THE METHOD (1)

The GENE-UP® Pathogenic *E. coli* kit is to be used with the GENE-UP Thermocycler. This kit contains all of the necessary components for PCR, including sample-specific primers and probes and an internal amplification control. The GENE-UP Thermocycler detects fluorescence at several wavelengths (channels) to allow multitarget detection in the same reaction vessel. The fluorescent signal of the target is recorded in channel 640.

Both the assay for the target and the internal amplification control utilizes dual Fluorescence Resonance Energy Transfer (FRET) hybridization probes. These probes consist of two different, short oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the reaction cycle. The first probe for the sample assay is labeled at the 3' end with fluorescein; the second probe is labeled at the 5' end with LC Red 640. FRET occurs only after the two probes come in close proximity from hybridizing to the template DNA.

The resulting fluorescent signal from the FRET interaction, which forms a real-time amplification curve, is how the amplified target is detected by the GENE-UP Thermocycler. After the PCR cycling program finishes, the PCR product(s) are melted to determine the presence of the target DNA. The software interprets data for each sample and gives a positive, negative, or inhibited result.

The internal amplification control, contained in the freeze-dried pellets, validates that the reaction conditions are sustainable for PCR to occur, thus validating a negative outcome for the sample. The internal amplification control is amplified by the same primer set but uses a different set of hybridization FRET probes to allow detection in the 705 channel.

Color dyes have been added to each reaction component. The sample lysis buffer is colored in red, and the reagent PCR master mix is colored in blue.

DISCUSSION OF THE VALIDATION STUDY (1)

The GENE-UP PRO PEC successfully detected pathogenic *E. coli* (PEC) containing co-localized *stx* and *eae* virulence genes. No statistical difference was observed between the candidate and confirmed results, or between the candidate and reference method for each of the 4 matrices evaluated. In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified, and all exclusivity organisms were correctly excluded. The robustness study demonstrated that small changes in the method parameters had no negligible impact on the method's performance.

The GENE-UP PEC method is quick and simple to perform, providing results in less than 1.5 hours post incubation of the selective enrichment. The GENE-UP software is user friendly with the ability to track lot information and sample identification quickly and with ease. GENE-UP software and instrument also offer the ability to utilize an open platform and set up unique runs before a run is completed. The software also provides the user to analyze each individual CP curve and melting curve as well aid in troubleshooting issues within an individual reaction. The small footprint is also desirable when working in laboratories with a limited amount of space or having only a small area available for PCR amplification.

Table 1: Results for inclusivity of the GENE-UP PEC method (1)

#	Organism	Source	Origin	Results ^a
1	<i>E. coli</i> O26	CRA 1301128 ^b	Clinical isolate	+
2	<i>E. coli</i> O26	CRA 1301130	Clinical isolate	+
3	<i>E. coli</i> O26	GRE 1551002 ^c	Foodborne isolate	+
4	<i>E. coli</i> O26:H11	CRA 1301123	Clinical isolate	+
5	<i>E. coli</i> O26:H11	CRA 1301124	Clinical isolate	+
6	<i>E. coli</i> O26:H11	CRA 1301125	Clinical isolate	+
7	<i>E. coli</i> O26:H11	CRA 1301126	Clinical isolate	+
8	<i>E. coli</i> O26:H11	CRA 1301127	Clinical isolate	+
9	<i>E. coli</i> O26:H11	CRA 1301129	Clinical isolate	+
10	<i>E. coli</i> O26:H11	CRA 1301131	Foodborne isolate	+
11	<i>E. coli</i> O45:H2	CRA 1301132	Clinical isolate	+
12	<i>E. coli</i> O45:H2	CRA 1301133	Foodborne isolate	+
13	<i>E. coli</i> O45:H2	CRA 1301134	Clinical isolate	+
14	<i>E. coli</i> O45:H2	CRA 1301135	Clinical isolate	+
15	<i>E. coli</i> O45:H2	CRA 1301136	Clinical isolate	+
16	<i>E. coli</i> O103:H11	CRA 1301139	Foodborne isolate	+
17	<i>E. coli</i> O103:H2	CRA 1301137	Foodborne isolate	+
18	<i>E. coli</i> O103:H2	CRA 1301138	Foodborne isolate	+
19	<i>E. coli</i> O103:H25	CRA 1301140	Foodborne isolate	+
20	<i>E. coli</i> O103:H25	CRA 1301141	Foodborne isolate	+
21	<i>E. coli</i> O111	CRA 1301145	Clinical isolate	+
22	<i>E. coli</i> O111	CRA 1301151	Clinical isolate	+
23	<i>E. coli</i> O111	CRA 1301152	Clinical isolate	+
24	<i>E. coli</i> O111:H8	CRA 090513	Clinical isolate	+
25	<i>E. coli</i> O111:H8	CRA 1301142	Clinical isolate	+
26	<i>E. coli</i> O111:H8	CRA 1301143	Clinical isolate	+
27	<i>E. coli</i> O111:H8	CRA 1301144	Clinical isolate	+
28	<i>E. coli</i> O111:H8	CRA 1301146	Clinical isolate	+
29	<i>E. coli</i> O111:H8	CRA 1301147	Clinical isolate	+
30	<i>E. coli</i> O111:nonmotile	CRA 1301147	Clinical isolate	+
31	<i>E. coli</i> O111:nonmotile	CRA 1301150	Clinical isolate	+
32	<i>E. coli</i> O121:H19	CRA 1301153	Clinical isolate	+
33	<i>E. coli</i> O121:H19	CRA 1301154	Clinical isolate	+
34	<i>E. coli</i> O121:H19	CRA 1301155	Clinical isolate	+
35	<i>E. coli</i> O121:H19	CRA 1301156	Clinical isolate	+
36	<i>E. coli</i> O145	CRA 1301162	Clinical isolate	+
37	<i>E. coli</i> O145	CRA 1301163	Clinical isolate	+
38	<i>E. coli</i> O145	CRA 1301166	Foodborne isolate	+
39	<i>E. coli</i> O145:H25	CRA 1301157	Clinical isolate	+
40	<i>E. coli</i> O145:H28	CRA 1301159	Clinical isolate	+
41	<i>E. coli</i> O145:H48	CRA 1301158	Clinical isolate	+
42	<i>E. coli</i> O145:nonmotile	CRA 1301160	Clinical isolate	+
43	<i>E. coli</i> O145:nonmotile	CRA 1301161	Clinical isolate	+
44	<i>E. coli</i> O145:nonmotile	CRA 1301164	Clinical isolate	+
45	<i>E. coli</i> O145:nonmotile	CRA 1301165	Clinical isolate	+
46	<i>E. coli</i> O157:H7	CRA 9405025	Clinical isolate	+
47	<i>E. coli</i> O157:H7	CRA 9405026	Foodborne isolate	+
48	<i>E. coli</i> O157:H7	CRA 9405027	Clinical isolate	+
49	<i>E. coli</i> O157:H7	GRE 0758001	Clinical isolate	+
50	<i>E. coli</i> O157:H7	GRE 1360001	Ground Beef	+

^a + = Positive Result; - = Negative Result; ^bCRA = Crappone Stock Culture Collection, Crappone, France; ^cGRE = Grenoble Stock Culture Collection, Grenoble, France.

Table 2. Exclusivity Results for the GENE-UP PEC (1)

#	Organism	Source	Origin	Results ^a
1	<i>Aeromonas hydrophila</i>	CRA 0603112 ^b	Clinical Isolate	-
2	<i>Arthobacter</i> spp.	GRE 0756008 ^c	Not Available	-
3	<i>Bacillus cereus</i>	CRA 0204002	Foodborne Isolate	-
4	<i>Bordetella avium</i>	GRE 1558146	Not Available	-
5	<i>Citrobacter freundii</i>	CRA 1902622	Foodborne Isolate	-
6	<i>Cronobacter sakazakii</i>	CRA 2106626	Infant formula	-
7	<i>Enterobacter hermannii</i>	GRE 8501042	Not Available	-
8	<i>Enterobacter cloacae</i>	CRA 1902634	Milk Product	-
9	<i>Erwinia</i> spp.	GRE 2006121	Environmental Isolate	-
10	<i>Escherichia coli</i> O109:H25	GRE 1055159	Cheese	-
11	<i>Escherichia coli</i> O113:H3	GRE 1360020	Foodborne Isolate	-
12	<i>Escherichia coli</i> O146	CRA 1301169	Foodborne Isolate	-
13	<i>Escherichia coli</i> O16:NM	ATCC 23511 ^d	Clinical Isolate	-
14	<i>Escherichia coli</i> O166:H7	GRE 1360019	Foodborne Isolate	-
15	<i>Escherichia coli</i> O174:H8	GRE 1055160	Cheese	-
16	<i>Escherichia coli</i> O55:H7	GRE 1452001	Clinical Isolate	-
17	<i>Escherichia gergusonii</i>	GRE 1654003	Not Available	-
18	<i>Escherichia hermannii</i>	CRA 1905632	Not Available	-
19	<i>Escherichia vulneris</i>	GRE 0356010	Foodborne Isolate	-
20	<i>Hafnia alvei</i>	CRA 0906209	Duck	-
21	<i>Klebsiella oxytoca</i>	CRA 0906216	Clinical Isolate	-
22	<i>Pantoea agglomerans</i>	CRA 8406031	Not Available	-
23	<i>Proteus vulgaris</i>	CRA 0906227	Not Available	-
24	<i>Pseudomonas aeruginosa</i>	CRA 2010606	Clinical Isolate	-
25	<i>Rahnella aquatilis</i>	GRE 03560002	Water	-
26	<i>Salmonella</i> Typhimurium	CRA 1710094	Foodborne Isolate	-
27	<i>Serratia marcescens</i>	CRA 906262	Milk Product	-
28	<i>Shigella sonnei</i>	CRA 906241	Clinical Isolate	-
29	<i>Shimwellia blattae</i>	GRE 1558005	Clinical Isolate	-
30	<i>Yersenia enterocolitica</i>	CRA 0906243	Carrot	-

^a + = Positive Result; - = Negative Result; ^bCRA = Crappone Stock Culture Collection, Crappone, France; ^cGRE = Grenoble Stock Culture Collection, Grenoble, France. ^d American Type Culture Collection, Manassas, VA.

Table 3: GENE-UP PEC Presumptive vs Confirmed Results (Paired) – POD Results (1)

Matrix	Time Point	MPN ^a or CFU / Test Portion	N ^b	x ^c	Presumptive		x	Confirmed		dPOD _{cp} ^f	95% CI ^g
					POD _{cp} ^d	95% CI		POD _{cc} ^e	95% CI		
Raw ground beef (80% lean) ^h (375 g)	10 h	NA ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw beef trim ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Romaine lettuce ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Carcass sampling cloth from pork trim (MicroTally or equivalent) ^h	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.70	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.70	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Romaine lettuce ^h (375 g) Independent Laboratory	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		2.58 (1.51, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		2.58 (1.51, 5.78)	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 based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval; CFU = Colony forming unit is based on plate counts of the inoculum

^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; ^aConfirmed results were identical between all three protocols (VIDAS ESP2, direct streak, traditional confirmation); ^hNA – Not Applicable

Table 4: GENE-UP PEC Confirmed vs Reference Results (Paired) – POD Results (1)

Matrix	Time Point	MPNs / Test Portion	N ^b	x ^c	Candidate		x	Reference		dPOD ^f	95% CI ^g
					POD ^d	95% CI		POD ^e	95% CI		
Raw ground beef (80% lean) ^h (375 g)	10 h	NA ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw beef trim ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Romaine lettuce ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.28, 0.28
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.28, 0.28
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Carcass sampling cloth from pork trim (MicroTally or equivalent) ^h	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.70	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.70	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Romaine lettuce ^h (375 g) Independent Laboratory	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		2.58 (1.51, 5.78)	5	5	1.00	0.57, 0.100	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		2.58 (1.51, 5.78)	5	5	1.00	0.57, 0.100	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval; CFU = Colony forming unit is based on plate counts of the inoculum

^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; ^hConfirmed results were identical between all three protocols (VIDAS ESP2, direct streak, traditional confirmation); ⁱNA – Not Applicable

Table 5: GENE-UP STEC – *stx* & *eae* Presumptive vs Confirmed Results (Paired) – POD Results (1)

Matrix	Time Point	MPN _a / Test Portion	N ^b	x ^c	Presumptive		x	Confirmed		dPOD _{cp} ^f	95% CI ^g
					POD _{cp} ^d	95% CI		POD _{cc} ^e	95% CI		
Raw ground beef (80% lean) ^h (375 g)	10 h	NA ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw beef trim ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Romaine lettuce ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Carcass sampling cloth from pork trim (MicroTally or equivalent) ^h	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.70	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.70	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Romaine lettuce ^h (375 g) Independent Laboratory	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		2.58 (1.51, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		2.58 (1.51, 5.78)	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 based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval; CFU = Colony forming unit is based on plate counts of the inoculum; ^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; ^hConfirmed results were identical between all three protocols (VIDAS ESP2, direct streak, traditional confirmation); ⁱNA – Not Applicable

Table 6: GENE-UP STEC – *stx* & *eae* Confirmed vs Reference Results (Paired) – POD Results (1)

Matrix	Time Point	MPN _a / Test Portion	N ^b	x ^c	Candidate		x	Reference		dPOD _c ^f	95% CI ^g
					POD _c ^d	95% CI		POD _r ^e	95% CI		
Raw ground beef (80% lean) ^h (375 g)	10 h	NA ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw beef trim ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Romaine lettuce ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.28, 0.28
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.28, 0.28
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

Carcass sampling cloth from pork trim (MicroTally or equivalent) ^h	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.70	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Romaine lettuce ^h (375 g) Independent Laboratory	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.70	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		2.58 (1.51, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		2.58 (1.51, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval; CFU = Colony forming unit is based on plate counts of the inoculum

^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; ^hConfirmed results were identical between all three protocols (VIDAS ESP2, direct streak, traditional confirmation); ⁱNA – Not Applicable

Table 7: GENE-UP STEC – Top 6 Presumptive vs Confirmed Results (Paired) – POD Results (1)

Matrix	Time Point	MPN _a / Test Portion	N ^b	x ^c	Presumptive		x	Confirmed		dPOD _{CP} ^f	95% CI ^g
					POD _{CP} ^d	95% CI		POD _{CC} ^e	95% CI		
Raw ground beef (80% lean) ^h (375 g)	10 h	NA ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw beef trim ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Romaine lettuce ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Carcass sampling cloth from pork trim (MicroTally or equivalent) ^h	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.70	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.70	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Romaine lettuce ^h (375 g) Independent Laboratory	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		2.58 (1.51, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		2.58 (1.51, 5.78)	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 based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval; CFU = Colony forming unit is based on plate counts of the inoculum; ^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; ^hConfirmed results were identical between all three protocols (VIDAS ESP2, direct streak, traditional confirmation); ⁱNA – Not Applicable

Table 8: GENE-UP STEC – Top 6 Confirmed vs Reference Results (Paired) – POD Results (1)

Matrix	Time Point	MPN _a / Test Portion	N ^b	x ^c	Candidate		x	Reference		dPOD _c ^f	95% CI ^g
					POD _c ^d	95% CI		POD _R ^e	95% CI		
Raw ground beef (80% lean) ^h (375 g)	10 h	NA ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.90 (0.54, 1.51)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw beef trim ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.67 (0.38, 1.12)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		4.38 (2.29, 9.37)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Romaine lettuce ^h (375 g)	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.28, 0.28
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.51 (0.27, 0.84)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.28, 0.28
		>5.10 (>2.70, >8.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Carcass sampling cloth from pork trim (MicroTally or equivalent) ^h	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.70	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.70	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		6.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Romaine lettuce ^h (375 g) Independent Laboratory	10 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		2.58 (1.51, 5.78)	5	5	1.00	0.57, 0.100	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.56 (0.30, 0.94)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		2.58 (1.51, 5.78)	5	5	1.00	0.57, 0.100	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval; CFU = Colony forming unit is based on plate counts of the inoculum ^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; ^hConfirmed results were identical between all three protocols (VIDAS ESP2, direct streak, traditional confirmation); ⁱNA – Not Applicable

DISCUSSION OF MODIFICATION APPROVED JANUARY 2023 (4)

Results of the matrix extension studies demonstrate that the GENE-UP PEC method successfully detected STEC using both MicroTally sampling cloth from beef trim using either BPW or mTSB. No differences were observed when test portions were prepared using a direct lysis protocol or using the VIDAS ESPT protocol. Results from the two alternative confirmation protocols and the reference method confirmation protocol were in alignment with no deviations observed. In the method comparison study, the candidate method test portions enriched in BPW had statistically greater recovery than test portions enriched in mTSB in the recovery of the target analyte. The validation study indicates that end users can employ the candidate method using traditional BPW enrichment with a shortened incubation period of 8 h or can follow traditional MLG enrichment with mTSB and detection of STEC at 15 h of incubation.

Table 1: GENE-UP PEC Presumptive vs Confirmed Results – POD Results (4)

Matrix/ Enrichment	Time Point	cfu ^a / Test Portion	N ^b	x ^c	Presumptive		x	Confirmed		dPOD _{cp} ^f	95% CI ^g
					POD _{cp} ^d	95% CI		POD _{cc} ^e	95% CI		
Carcass sampling cloth from beef trim (MicroTally or equivalent)/ BPW ^{h,i}	8 h	NA ^j	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		2	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		2	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Carcass sampling cloth from beef trim (MicroTally or equivalent)/ mTSB ^k	15 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		2	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0.00	-0.13, 0.13
		9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		2	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0.00	-0.13, 0.13
		9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aCFU = Colony forming unit is based on plate counts of the inoculum; ^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; ^hResults identical between direct lysis and VIDAS ESPT; ⁱConfirmed results were identical between all three protocols (VIDAS ESP2, direct streak, traditional confirmation); ^jNA – Not Applicable; ^kConfirmation of test portions following candidate method confirmation procedure.

Table 2: GENE-UP PEC Confirmed vs Reference Results – POD Results (4)

Matrix	Time Point	cfu ^a / Test Portion	N ^b	x ^c	Candidate		x	Reference		dPOD _c ^f	95% CI ^g
					POD _c ^d	95% CI		POD _r ^e	95% CI		
Carcass sampling cloth from beef trim (MicroTally or equivalent) BPW ^{h,i}	8 h	NA ^j	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		2	20	11	0.55	0.34, 0.74	4	0.20	0.08, 0.42	0.35	0.05, 0.58
		9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		2	20	11	0.55	0.34, 0.74	4	0.20	0.08, 0.42	0.35	0.05, 0.58
		9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Carcass sampling cloth from beef trim (MicroTally or equivalent) / mTSB ^k	15 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		2	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0.00	-0.13, 0.13
		9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	24 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		2	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0.00	-0.13, 0.13
		9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aCFU = Colony forming unit is based on plate counts of the inoculum; ^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 candidate method confirmed 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; ^hResults identical between direct lysis and VIDAS ESPT; ⁱConfirmed results were identical between all three protocols (VIDAS ESP2, direct streak, traditional confirmation); ^jNA – Not Applicable;

^kConfirmation of test portions following reference method confirmation procedure.

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