



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.

082104

The AOAC Research Institute hereby certifies the method known as

GENE-UP[®] QUANT *Salmonella*

manufactured by

bioMérieux SA

1201 Normandy Pl. Suite 200

Philadelphia, PA 19112 USA

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

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

Bradley A. Stawick, AOAC Research Institute Senior Director

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AUTHORS ORIGINAL VALIDATION: Adam C. Joelsson, Darryl Barkhouse, Kristin Kahle, and Nicholas A. Siciliano MODIFICATION AUGUST 2021: Ronald Johnson and Adam Joelsson MODIFICATION DECEMBER 2022: Adam Joelsson, Nikki Taylor, Ronald Johnson, John Mills, and Patrick Bird		MANUFACTURED BY bioMérieux SA 1201 Normandy Pl. Suite 200 Philadelphia, PA 19112	CURRENT SPONSOR bioMérieux 595 Anglum Road Hazelwood, MO 63042
METHOD NAME GENE-UP® QUANT <i>Salmonella</i> Approval based on validation data from PTM 061801 GENE-UP® EnviroPRO™		CATALOG NUMBER IS1085	
ORIGINAL VALIDATION INDEPENDENT LABORATORY Q Laboratories, Inc. 1400 Harrison Avenue Cincinnati, OH 45214 USA		MODIFICATION JULY 2021 TEQ Analytical 12635 E. Montview Blvd #175 Aurora, CO 80042 USA	
APPLICABILITY OF METHOD Target organisms – <i>Salmonella</i> species. MODIFICATION AUGUST 2021 – (MLG 2.05 and MLG 4.10) – raw ground turkey (100 g), chicken carcass rinsate (40 mL), raw beef trim (100 g), carcass sampling cloth (MicroTally® or equivalent) MODIFICATION DECEMBER 2022 – MLG 4.12, 325 g) quantitation of <i>Salmonella</i> as low as 1 CFU/g in breaded and stuffed raw chicken Performance claims – The study data detected no statistical difference between the GENE-UP® QUANT <i>Salmonella</i> method and the reference methods.		REFERENCE METHODS U.S. Department of Agriculture-Food Safety and Inspection Service Microbiology Laboratory Guidebook (MLG), 2.05, Most Probable Number Procedure and Tables. (4) U. S. Department of Agriculture-Food Safety and Inspection Service Microbiology Laboratory Guidebook (MLG), 4.10, Isolation and Identification of <i>Salmonella</i> from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges. (5) U. S. Department of Agriculture-Food Safety and Inspection Service Microbiology Laboratory Guidebook (MLG), 4.12, Isolation and Identification of <i>Salmonella</i> from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges. (7)	
ORIGINAL CERTIFICATION DATE June 02, 2018		CERTIFICATION RENEWAL RECORD Renewed through December 2026.	
METHOD MODIFICATION RECORD 1. August 2021 Level 3 2. January 2022 Level 1 3. December 2022 Level 2		SUMMARY OF MODIFICATION 1. Method modification for <i>Salmonella</i> quantification in raw ground turkey, chicken carcass rinsate, raw beef trim and carcass sampling cloth (MicroTally® or equivalent) and certification of PTM 082104 GENE-UP® QUANT <i>Salmonella</i> . 2. Rebranding, reformatting, and editorial/clerical changes. 3. Matrix extension to detect <i>Salmonella</i> as low as 1 CFU/g in breaded and stuffed raw chicken.	
Under this AOAC <i>Performance Tested Methods</i> SM License Number, 082104 this method is distributed by: NONE		Under this AOAC <i>Performance Tested Methods</i> SM License Number, 082104 this method is distributed as: NONE	

The following validation information supports PTM 082104 GENE-UP® QUANT *Salmonella*.

PRINCIPLE OF THE METHOD (1)

GENE-UP QUANT *Salmonella* (part of enviroPRO™ suite of assays) is a real-time PCR assay for the rapid quantification of *Salmonella* species in a variety of matrix types. The assay utilizes both an enrichment free and abbreviated enrichment-based sample prep paired with a multiplex detection method which targets highly conserved pan-*Salmonella* genetic signatures on the Cy5 channel and an internal amplification control (IAC) on the HEX channel.

This method is intended for the detection and enumeration of *Salmonella* as low as 1 CFU per gram contamination level in ready-to-cook or ready-to-heat poultry products.

DISCUSSION OF THE VALIDATION STUDY (1)

The initial validation included the inclusivity and exclusivity testing, which indicated that the GENE UP QUANT *Salmonella* was able to accurately detect the *Salmonella* inclusivity isolate panel while correctly excluding all non-specific bacteria tested. The robustness and lot-to-lot stability data also indicated that the assay is reproducible and rugged and that it can be manufactured uniformly and consistently.

Table 3. Inclusivity Strain Results *Salmonella* BPW for 18 h at 35°C (1)

No.	Organism (Serogroup)	Source	Origin	Result	No.	Organism (Serogroup)	Source	Origin	Result
1	<i>Salmonella bongori</i> (N/A) ^a	NCTC ^b 12419	Not Available	+	26	<i>Salmonella</i> Bovis-Morbificans (C2)	UPENN ^c STS 16	Not Available	+
2	<i>Salmonella bongori</i> (N/A)	ATCC ^d 43975	Not Available	+	27	<i>Salmonella</i> Brandenburg (B)	UPENN STS 18	Not Available	+
3	<i>Salmonella bongori</i> (N/A)	NCTC 10946	Amphibian, Frog	+	28	<i>Salmonella</i> Bredeney (B)	NCTC 5731	Not Available	+
4	<i>Salmonella</i> Artis (56)	ATCC 700149	Not Available	+	29	<i>Salmonella</i> California (B)	NCTC 6018	Not Available	+
5	<i>Salmonella arizonae</i> (N/A)	ATCC 13314	Not Available	+	30	<i>Salmonella</i> Cerro (K)	UPENN STS 22	Not Available	+
6	<i>Salmonella arizonae</i> (N/A)	ATCC BAA-1577	Not Available	+	31	<i>Salmonella</i> Choleraesuis (C1)	ATCC 10708	Not Available	+
7	<i>Salmonella arizonae</i> (N/A)	QL ^e 11007-4	Veterinary	+	32	<i>Salmonella</i> Choleraesuis var Kunzendorf (C1)	ATCC 12011	Not Available	+
8	<i>Salmonella. diarizonae</i> (N/A)	ATCC BAA-1579	Not Available	+	33	<i>Salmonella</i> Cubana (G)	UPENN STS 24	Not Available	+
9	<i>Salmonella diarizonae</i> (N/A)	ATCC BAA-216	Human Blood	+	34	<i>Salmonella</i> Derby (B)	NCTC 5721	Not Available	+
10	<i>Salmonella. diarizonae</i> (N/A)	ATCC BAA-639	Human Feces	+	35	<i>Salmonella</i> Drypool (E2)	UPENN STS 26	Not Available	+
11	<i>Salmonella</i> Abaetetuba (F)	ATCC 35640	Creek Water	+	36	<i>Salmonella</i> Dublin (D1)	UPENN STS 27	Not Available	+
12	<i>Salmonella</i> Abortusequi (B)	FDA/ 9842	Not Available	+	37	<i>Salmonella</i> Eastbourne (D1)	FDA 4017H	Not Available	+
13	<i>Salmonella</i> Abortusovis (B)	NCTC10241	Not Available	+	38	<i>Salmonella</i> Enteritidis (D1)	ATCC 13076	Not Available	+
14	<i>Salmonella</i> Abony (B)	NCTC 6017	Not Available	+	39	<i>Salmonella</i> Galiema (C1)	QL 024.2	Clinical Isolate	+
15	<i>Salmonella</i> Adelaide (O)	UPENN STS 2	Not Available	+	40	<i>Salmonella</i> Give (E1)	UPENN STS 42	Not Available	+
16	<i>Salmonella</i> Agona (B)	ATCC 51957	Not Available	+	41	<i>Salmonella</i> Haardt (C2)	UPENN STS 44	Not Available	+
17	<i>Salmonella</i> Agama (B)	UPENN STS 3	Not Available	+	42	<i>Salmonella</i> Hadar (C2)	ATCC 51956	Not Available	+
18	<i>Salmonella</i> Agoueve (G)	UPENN STS 5	Not Available	+	43	<i>Salmonella</i> Havana (G)	UPENN STS 47	Not Available	+
19	<i>Salmonella</i> Alachua (O)	UPENN STS 6	Not Available	+	44	<i>Salmonella</i> Heidelberg (B)	ATCC 8326	Not Available	+
20	<i>Salmonella</i> Albany (C2)	UPENN STS 7	Not Available	+	45	<i>Salmonella</i> Indiana (B)	NCTC 11304	Turkey	+
21	<i>Salmonella</i> Anatum (E1)	ATCC 9270	Pork Liver	+	46	<i>Salmonella</i> Infantis (C1)	ATCC 51741	Pasta	+
22	<i>Salmonella</i> Arkansas (E3)	UPENN STS 11	Not Available	+	47	<i>Salmonella</i> Javiana (D1)	ATCC 10721	Not Available	+
23	<i>Salmonella</i> Bareilly (C1)	FDA 1206H	Not Available	+	48	<i>Salmonella</i> Jerusalem (C1)	QL 024.12	Not Available	+
24	<i>Salmonella</i> Berta (D1)	UPENN STS 13	Not Available	+	49	<i>Salmonella</i> Johannesburg (R)	UPENN STS 56	Not Available	+
25	<i>Salmonella</i> Binza (E2)	UPENN STS 14	Not Available	+	50	<i>Salmonella</i> Kahla (T)	ATCC 17980	Human Feces	+
51	<i>Salmonella</i> Kaitaan (H)	QL1 024.7	Clinical Isolate	+	76	<i>Salmonella</i> Saintpaul (B)	ATCC 9712	Cystitis	+
52	<i>Salmonella</i> Kentucky (C2)	ATCC2 9263	Not Available	+	77	<i>Salmonella</i> San-Diego (B)	UPENN STS 94	Not Available	+
53	<i>Salmonella</i> Krefeld (E4)	UPENN3 STS 58	Not Available	+	78	<i>Salmonella</i> Schalkwijk (H)	QL 024.10	Clinical Isolate	+

54	<i>Salmonella</i> Lille (C1)	UPENN STS 59	Not Available	+	79	<i>Salmonella</i> Schwarzengrund (B)	UPENN STS 95	Not Available	+
55	<i>Salmonella</i> Livingstone (C1)	UPENN STS 63	Not Available	+	80	<i>Salmonella</i> Senftenberg (E4)	ATCC 43845	Not Available	+
56	<i>Salmonella</i> London (E1)	UPENN STS 64	Not Available	+	81	<i>Salmonella</i> Tallahassee (C2)	ATCC 12002	Not Available	+
57	<i>Salmonella</i> Manhattan (C2)	UPENN STS 65	Not Available	+	82	<i>Salmonella</i> Tennessee (C1)	QL 024.6	Clinical Isolate	+
58	<i>Salmonella</i> Mbankaka (C1)	FDA4 37N	Not Available	+	83	<i>Salmonella</i> Thompson (C1)	FDA 2051H	Not Available	+
59	<i>Salmonella</i> Menden (C1)	ATCC 15992	Human Feces	+	84	<i>Salmonella</i> Typhi (D1)	ATCC 6539	Not Available	+
60	<i>Salmonella</i> Meleagridis (E1)	QL 12074-1	Not Available	+	85	<i>Salmonella</i> Typhimurium (B)	ATCC 14028	Animal Tissue	+
61	<i>Salmonella</i> Menhaden (E3)	QL 024.20	Clinical Isolate	+	86	<i>Salmonella</i> Salamae (C1)	QL 02415	Clinical Isolate	+
62	<i>Salmonella</i> Montevideo (C1)	ATCC 8387	Not Available	+	87	<i>Salmonella</i> Basel (58)	ATCC 700151	Not Available	+
63	<i>Salmonella</i> Muenchen (C2)	ATCC BAA-1594	Roma Tomatoes	+	88	<i>Salmonella</i> Utrecht (52)	NCTC 10077	Not Available	+
64	<i>Salmonella</i> Neasden (D1)	QL 024.4	Clinical Isolate	+	89	<i>Salmonella</i> Urbana (N)	UPENN STS 110	Not Available	+
65	<i>Salmonella</i> Newington (E2)	QL 0248	Clinical Isolate	+	90	<i>Salmonella</i> Vellore (B)	ATCC 15611	Rectal Swab	+
66	<i>Salmonella</i> Newport (C2)	ATCC 6962	England	+	91	<i>Salmonella</i> Virchow (C1)	ATCC 51955	Not Available	+
67	<i>Salmonella</i> Ohio (C1)	UPENN STS 81	Illinois Hospital	+	92	<i>Salmonella</i> Volta (F)	QL 024.9	Clinical Isolate	+
68	<i>Salmonella</i> Oranienburg (C1)	ATCC 9239	Not Available	+	93	<i>Salmonella</i> Westhampton (E1)	QL 024.14	Clinical Isolate	+
69	<i>Salmonella</i> Orthmarshen (C1)	QL 024.13	Clinical Isolate	+	94	<i>Salmonella</i> Worthington (G)	UPENN STS 114	Not Available	+
70	<i>Salmonella</i> Paratyphi A (A)	ATCC 9150	Not Available	+	95	<i>Salmonella</i> Halmstad (E2)	QL 024.1	Ground Beef	+
71	<i>Salmonella</i> Paratyphi B (B)	ATCC 10719	Not Available	+	96	<i>Salmonella</i> Harmelen (51)	ATCC 15783	Not Available	+
72	<i>Salmonella</i> Poona (G)	NCTC5 4840	Infant	+	97	<i>Salmonella</i> Ochsenzoll (I)	ATCC 29932	Not Available	+
73	<i>Salmonella</i> Preston (B)	QL 024.16	Clinical Isolate	+	98	<i>Salmonella indica</i> (N/A)	ATCC BAA-1578	India	+
74	<i>Salmonella</i> Pullorum (D1)	ATCC 13036	Egg	+	99	<i>Salmonella indica</i> (N/A)	ATCC 43976	Not Available	+
75	<i>Salmonella</i> Rubislaw (F)	UPENN STS 92	Not Available	+	100	<i>Salmonella</i> Ferlac (H)	NCTC 10458	Not Available	+

^aN/A – Not applicable.

^bNCTC – National Culture Type Collection, Porton Down, Salisbury, UK.

^cUPENN – University of Pennsylvania Culture Collection, Philadelphia, PA.

^dATCC – American Type Culture Collection, Manassas, VA.

^eQL – Q Laboratories Culture Collection, Cincinnati, OH.

^fFDA – US Food and Drug Administration Culture Collection, College Park, MD.

Table 5. Exclusivity Strain Results for *Salmonella* Cy5 Channel BPW for 18 h at 35°C (1)

No	Organism	Source	Origin	Result	No	Organism	Source	Origin	Result
1	<i>Alcaligenes faecalis</i>	ATCC ^a 8750	Unknown	-	19	<i>Lactobacillus acidophilus</i>	ATCC 314	Unknown	-
2	<i>Bacillus coagulans</i>	ATCC 7050	Dairy Products	-	20	<i>Lactobacillus kefir</i>	ATCC 35411	Kefir	-
3	<i>Campylobacter jejuni</i>	ATCC 33560	Herring Gull cloacal swab	-	21	<i>Lactobacillus lactis</i>	ATCC 4797	Unknown	-
4	<i>Campylobacter lari</i>	ATCC BAA-1060	Bovine Feces	-	22	<i>Lactobacillus plantarum</i>	ATCC 8014	Unknown	-
5	<i>Candida albicans</i>	ATCC 24433	Nail Infection	-	23	<i>Morganella morganii</i>	ATCC 25829	Human	-
6	<i>Carnobacterium maltaromaticum</i>	ATCC 43224	Vacuum Packed Beef	-	24	<i>Proteus mirabilis</i>	ATCC 7002	Urine	-
7	<i>Citrobacter freundii</i>	ATCC 8090	Unknown	-	25	<i>Proteus vulgaris</i>	ATCC 6380	Clinical Isolate	-
8	<i>Edwardsiella tarda</i>	ATCC 15947	Feces, Human	-	26	<i>Pseudomonas aeruginosa</i>	ATCC 27853	Clinical Isolate	-
9	<i>Enterobacter aerogenes</i>	ATCC 13048	Sputum	-	27	<i>Pseudomonas extremorientalis</i>	QL ^b 17041.1	Raw Milk	-
10	<i>Enterobacter cloacae</i>	ATCC 23355	Unknown	-	28	<i>Pseudomonas fluorescens</i>	QL 17041-3	Raw Milk	-
11	<i>Enterococcus faecalis</i>	ATCC 29212	Urine	-	29	<i>Pseudomonas gessardii</i>	QL 17041-12	Raw Milk	-
12	<i>Enterococcus faecium</i>	ATCC 19434	Unknown	-	30	<i>Shigella sonnei</i>	ATCC 29930	Unknown	-
13	<i>Escherichia coli</i>	ATCC 25922	Clinical Isolate	-	31	<i>Staphylococcus aureus</i>	ATCC 10832	Unknown	-
14	<i>Escherichia coli</i>	BEI NR ^c -4356	Unknown	-	32	<i>Staphylococcus epidermidis</i>	ATCC 12228	Unknown	-
15	<i>Escherichia coli</i>	BEI NR-12	Unknown	-	33	<i>Staphylococcus haemolyticus</i>	ATCC 29970	Human Skin	-
16	<i>Hafnia alvei</i>	ATCC 51815	Unknown	-	34	<i>Staphylococcus hominis</i>	ATCC 27844	Human Skin	-
17	<i>Klebsiella pneumoniae</i>	ATCC 13883	Unknown	-	35	<i>Streptococcus pneumoniae</i>	ATCC 6302	Unknown	-
18	<i>Kocuria rhizophila</i>	ATCC 9341	Soil	-					

^aATCC – American Type Culture Collection, Manassas, VA.^bQL – Q Laboratories Culture Collection, Cincinnati, OH.^cBEI NR – Invisible Sentinel Culture Collection, Philadelphia, PA.**DISCUSSION OF MODIFICATION APPROVED AUGUST 2021 (3)**

For each matrix evaluated, all mean differences between methods were <0.30 Log₁₀ for each contamination level (except the high level during the independent laboratory study which was 0.36). A majority of the contamination for each matrix met acceptance criteria for statistical equivalence (90% confidence intervals fell within the -0.5, 0.5). In the instances where a level was slightly outside the equivalence evaluation, no statistically significant difference (95% confidence interval containing the point 0) was observed. Based on the results of the equivalence tests, statistically significant tests and low mean difference between methods, the data demonstrated in this study indicate the candidate method performed as similar or better than the reference method for the matrices evaluated.

Results of the inclusivity and exclusivity study supplemented the results of the initial validation study and indicated the method is highly specific for *Salmonella* species and selective against closely related non-target organisms.

The AOAC Performance Tested Methods Level 3 modification study evaluated the GENE-UP EnviroPro assay for the quantification of *Salmonella* in select foods matrices and was granted PTM 082104 GENE-UP QUANT *Salmonella*. The GENE-UP QUANT *Salmonella* method allows the user to obtain results in as little as 3 h (8.5 h for carcass sampling cloths) with an accurate estimation of the amount of *Salmonella* in the test product. The sample preparation is streamlined for use in any laboratory. The procedure is easy to follow, allowing for a technician at any level of training to perform the method and obtain accurate results.

Table 1 Inclusivity List: *Salmonella* strains (3)

No.	Organism	Species/ Subspecies	Serotype	Source	GENE-UP enviroPRO Results ^a
1	<i>S. enterica</i>	<i>arizonae</i>	IIIa 41:z4,z23:-	SAFE 19 ^b	+
2	<i>S. enterica</i>	<i>arizonae</i>	IIIa 40:z4,z23:-	SAFE 20	+
3	<i>S. enterica</i>	<i>arizonae</i>	IIIa 48:g,z51:-	SAFE 21	+
4	<i>S. enterica</i>	<i>arizonae</i>	IIIa 21:g,z51:-	SAFE 22	+
5	<i>S. enterica</i>	<i>arizonae</i>	IIIa 51:g,z51:-	SAFE 23	+
6	<i>S. enterica</i>	<i>arizonae</i>	IIIa 62:g,z51:-	SAFE 24	+
7	<i>S. enterica</i>	<i>arizonae</i>	IIIa 48:z4,z23,z32:-	SAFE 25	+
8	<i>S. enterica</i>	<i>arizonae</i>	IIIa 48:z4,z23:-	SAFE 26	+
9	<i>S. enterica</i>	<i>diarizonae</i>	IIIb 60:r:e,n,x,z15	SAFE 27	+
10	<i>S. enterica</i>	<i>diarizonae</i>	IIIb 48:i:z	SAFE 28	+
11	<i>S. enterica</i>	<i>diarizonae</i>	IIIb 61:k:1,5,(7)	SAFE 29	+
12	<i>S. enterica</i>	<i>diarizonae</i>	IIIb 61:l,v:1,5,7	SAFE 30	+
13	<i>S. enterica</i>	<i>diarizonae</i>	IIIb 48: z10: e,n,x,z15	SAFE 31	+
14	<i>S. enterica</i>	<i>diarizonae</i>	IIIb 38:z10:z53	SAFE 32	+
15	<i>S. enterica</i>	<i>diarizonae</i>	IIIb 60:r:z	SAFE 33	+
16	<i>S. enterica</i>	<i>diarizonae</i>	IIIb 50:i:z	SAFE 34	+
17	<i>S. enterica</i>	<i>enterica</i>	Newport	SAFE 1	+
18	<i>S. enterica</i>	<i>enterica</i>	Enteritidis	SAFE 2	+
19	<i>S. enterica</i>	<i>enterica</i>	Heidelberg	SAFE 3	+
20	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 4	+

21	<i>S. enterica</i>	<i>enterica</i>	Typhi	SAFE 5	+
22	<i>S. enterica</i>	<i>enterica</i>	4,5,12:b:-	SAFE 6	+
23	<i>S. enterica</i>	<i>enterica</i>	Hadar	SAFE 7	+
24	<i>S. enterica</i>	<i>enterica</i>	Virchow	SAFE 8	+
25	<i>S. enterica</i>	<i>enterica</i>	Brandenburg	SAFE 9	+
26	<i>S. enterica</i>	<i>enterica</i>	Typhimurium / DT104b	SAFE 56	+
27	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 57	+
28	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 58	+
29	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 59	+
30	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 60	+
31	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 61	+
32	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 62	+
33	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 63	+
34	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 64	+
35	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 65	+
36	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 66	+
37	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 67	+
38	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 68	+
39	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	SAFE 69	+
40	<i>S. enterica</i>	<i>enterica</i>	Typhimurium / DT104	SAFE 70	+
41	<i>S. enterica</i>	<i>enterica</i>	Typhimurium / DT104	SAFE 71	+
42	<i>S. enterica</i>	<i>enterica</i>	Typhimurium / DT104	SAFE 72	+
43	<i>S. enterica</i>	<i>enterica</i>	14,[5],12:i:-	SAFE 73	+
44	<i>S. enterica</i>	<i>enterica</i>	14,[5],12:i:-	SAFE 74	+
45	<i>S. enterica</i>	<i>enterica</i>	14,[5],12:i:-	SAFE 75	+
46	<i>S. enterica</i>	<i>enterica</i>	14,[5],12:i:-	SAFE 76	+
47	<i>S. enterica</i>	<i>enterica</i>	14,[5],12:i:-	SAFE 77	+
48	<i>S. enterica</i>	<i>enterica</i>	14,[5],12:i:-	SAFE 78	+
49	<i>S. enterica</i>	<i>enterica</i>	Enteritidis	SAFE 79	+
50	<i>S. enterica</i>	<i>enterica</i>	Enteritidis	SAFE 80	+
51	<i>S. enterica</i>	<i>enterica</i>	Enteritidis	SAFE 81	+
52	<i>S. enterica</i>	<i>enterica</i>	Saphra	SAFE 82	+
53	<i>S. enterica</i>	<i>enterica</i>	Rubislaw	SAFE 83	+
54	<i>S. enterica</i>	<i>enterica</i>	Michigan	SAFE 84	+
55	<i>S. enterica</i>	<i>enterica</i>	Urbana	SAFE 85	+
56	<i>S. enterica</i>	<i>enterica</i>	Vietnam	SAFE 86	+
57	<i>S. enterica</i>	<i>enterica</i>	Tornow	SAFE 87	+
58	<i>S. enterica</i>	<i>enterica</i>	Gera	SAFE 88	+
59	<i>S. enterica</i>	<i>enterica</i>	Fresno	SAFE 89	+
60	<i>S. enterica</i>	<i>enterica</i>	Brisbane	SAFE 90	+
61	<i>S. enterica</i>	<i>enterica</i>	Agona	SAFE 91	+
62	<i>S. enterica</i>	<i>enterica</i>	Muenchen	SAFE 92	+
63	<i>S. enterica</i>	<i>enterica</i>	Senftenberg	SAFE 93	+
64	<i>S. enterica</i>	<i>enterica</i>	Muenster	SAFE 94	+
65	<i>S. enterica</i>	<i>enterica</i>	Montevideo	SAFE 95	+
66	<i>S. enterica</i>	<i>enterica</i>	Johannesburg	SAFE 96	+
67	<i>S. enterica</i>	<i>enterica</i>	Javiana	SAFE 97	+
68	<i>S. enterica</i>	<i>enterica</i>	Inverness	SAFE 98	+
69	<i>S. enterica</i>	<i>enterica</i>	Cubana	SAFE 99	+
70	<i>S. enterica</i>	<i>enterica</i>	Cerro	SAFE 100	+
71	<i>S. enterica</i>	<i>enterica</i>	Alachua	SAFE 101	+
72	<i>S. enterica</i>	<i>enterica</i>	Albany	BMX ^c 10955307	+
73	<i>S. enterica</i>	<i>enterica</i>	Bareilly	BMX 115977	+
74	<i>S. enterica</i>	<i>enterica</i>	Choleraesuis	BMX 115461	+
75	<i>S. enterica</i>	<i>enterica</i>	Florida	BMX 105801	+
76	<i>S. enterica</i>	<i>enterica</i>	Gallinarum	BMX 106305	+
77	<i>S. enterica</i>	<i>enterica</i>	Gaminara	BMX 105802	+
78	<i>S. enterica</i>	<i>enterica</i>	Havana	BMX 10923346	+
79	<i>S. enterica</i>	<i>enterica</i>	Illinois	BMX 105804	+
80	<i>S. enterica</i>	<i>enterica</i>	Infantis	BMX 10956559	+
81	<i>S. enterica</i>	<i>enterica</i>	Kentucky	BMX 105805	+
82	<i>S. enterica</i>	<i>enterica</i>	Liverpool	BMX 10958507	+
83	<i>S. enterica</i>	<i>enterica</i>	Livingstone	BMX 111561	+
84	<i>S. enterica</i>	<i>enterica</i>	London	BMX 10954513	+
85	<i>S. enterica</i>	<i>enterica</i>	Mbandaka	BMX 10957707	+
86	<i>S. enterica</i>	<i>enterica</i>	Meleagridis	BMX 10955583	+
87	<i>S. enterica</i>	<i>enterica</i>	Minnesota	BMX 110274	+
88	<i>S. enterica</i>	<i>enterica</i>	Paratyphi A	BMX 105807	+
89	<i>S. enterica</i>	<i>enterica</i>	Paratyphi B	BMX 110425	+

90	<i>S. enterica</i>	<i>enterica</i>	Paratyphi C	BMX 102229	+
91	<i>S. enterica</i>	<i>enterica</i>	Pomona	BMX 10956398	+
92	<i>S. enterica</i>	<i>enterica</i>	Pullorum	BMX 106346	+
93	<i>S. enterica</i>	<i>enterica</i>	Reading	BMX 10955282	+
94	<i>S. enterica</i>	<i>enterica</i>	Schwarzengrund	BMX 10952675	+
95	<i>S. enterica</i>	<i>enterica</i>	Soerenga	BMX 10936318	+
96	<i>S. enterica</i>	<i>enterica</i>	Tennessee	BMX 112762	+
97	<i>S. enterica</i>	<i>enterica</i>	Uganda	BMX 10955111	+
98	<i>S. enterica</i>	<i>enterica</i>	Waycross	BMX 115142	+
99	<i>S. enterica</i>	<i>enterica</i>	Worthington	BMX 10958916	+
100	<i>S. enterica</i>	<i>enterica</i>	Yoruba	BMX 10879323	+
101	<i>S. enterica</i>	<i>enterica</i>	Albany	BMX 10955307	+
102	<i>S. enterica</i>	<i>houtenae</i>	IV 50:g,z51:-	SAFE 35	+
103	<i>S. enterica</i>	<i>houtenae</i>	IV 48:g,z51:-	SAFE 36	+
104	<i>S. enterica</i>	<i>houtenae</i>	IV 44:z4,z23:-	SAFE 37	+
105	<i>S. enterica</i>	<i>houtenae</i>	IV 45:g,z51:-	SAFE 38	+
106	<i>S. enterica</i>	<i>houtenae</i>	IV 16:z4,z32:-	SAFE 39	+
107	<i>S. enterica</i>	<i>houtenae</i>	IV 11:z4,z23:-	SAFE 40	+
108	<i>S. enterica</i>	<i>houtenae</i>	IV 6,7:z36:-	SAFE 41	+
109	<i>S. enterica</i>	<i>houtenae</i>	IV 16:z4,z32:-	SAFE 42	+
110	<i>S. enterica</i>	<i>houtenae</i>	IV 40:g,z51:-	SAFE 43	+
111	<i>S. enterica</i>	<i>houtenae</i>	IV 40:z4,z24:-	SAFE 44	+
112	<i>S. enterica</i>	<i>indica</i>	VI 6,14,25:z10:1,(2),7	SAFE 51	+
113	<i>S. enterica</i>	<i>indica</i>	VI 11:b:1,7	SAFE 52	+
114	<i>S. enterica</i>	<i>indica</i>	VI 6,7:z41:1,7	SAFE 53	+
115	<i>S. enterica</i>	<i>indica</i>	VI 11:a:1,5	SAFE 54	+
116	<i>S. enterica</i>	<i>indica</i>	VI 6,14,25:a:e,n,x	SAFE 55	+
117	<i>S. enterica</i>	<i>salamae</i>	II 58:l,z13,z28:z6	SAFE 10	+
118	<i>S. enterica</i>	<i>salamae</i>	II 47:d:z39	SAFE 11	+
119	<i>S. enterica</i>	<i>salamae</i>	II 48:d:z6	SAFE 12	+
120	<i>S. enterica</i>	<i>salamae</i>	II 50:b:z6	SAFE 13	+
121	<i>S. enterica</i>	<i>salamae</i>	II 53:lz28:z39	SAFE 14	+
122	<i>S. enterica</i>	<i>salamae</i>	II 39:lz28:enx	SAFE 15	+
123	<i>S. enterica</i>	<i>salamae</i>	II 13,22:z29:enx	SAFE 16	+
124	<i>S. enterica</i>	<i>salamae</i>	II 4,12:b:-	SAFE 17	+
125	<i>S. enterica</i>	<i>salamae</i>	II 18:z4,z23:-	SAFE 18	+
126	<i>S. bongori</i>		V 48:i:-	SAFE 45	+
127	<i>S. bongori</i>		V 40:z35:-	SAFE 46	+
128	<i>S. bongori</i>		V 44:z39:-	SAFE 47	+
129	<i>S. bongori</i>		V 60:z41:-	SAFE 48	+
130	<i>S. bongori</i>		V 66:z41:-	SAFE 49	+

^aResult: “+” = strain detected, “-” = strain not detected; ^bSAFE – US FDA CFSAN Culture Collection

^cBMX – bioMérieux Culture Collection

Table 2. Exclusivity List: Non-target strains (3)

No.	Organism	Source	Origin	GENE-UP enviroPRO Result ^a
1	<i>Alcaligenes faecalis</i>	ATCC ^b 8750	Unknown	-
2	<i>Bacillus cereus</i>	ATCC 14579	Unknown	-
3	<i>Bacillus coagulans</i>	White Labs BC ^c	Unknown	-
4	<i>Campylobacter coli</i>	ATCC 33560	Bovine Feces	-
5	<i>Campylobacter jejuni</i>	ATCC 33560	Herring Gull cloacal swab	-
6	<i>Campylobacter lari</i>	ATCC BAA-1060	Bovine Feces	-
7	<i>Candida albicans</i>	ATCC 24433	Nail Infection	-
8	<i>Citrobacter freundii</i>	ATCC 8090	Unknown	-
9	<i>Edwardsiella tarda</i>	ATCC 15947	Feces, Human	-
10	<i>Enterobacter aerogenes</i>	ATCC 13048	Sputum	-
11	<i>Enterobacter cloacae</i>	ATCC 23355	Unknown	-
12	<i>Enterococcus faecalis</i>	ATCC 29212	Urine	-
13	<i>Enterococcus faecium</i>	ATCC 19434	Unknown	-
14	<i>Escherichia coli</i> O6	ATCC 25922	Clinical Isolate	-
15	<i>Escherichia coli</i> O157:H7	BEI NR2-4356 ^d	Unknown	-
16	<i>Escherichia coli</i> O157:H7	BEI NR-12	Unknown	-
17	<i>Hafnia alvei</i>	ATCC 51815	Unknown	-
18	<i>Klebsiella pneumoniae</i>	ATCC 13883	Unknown	-
19	<i>Kocuria rhizophila</i>	ATCC 9341	Soil	-

20	<i>Lactobacillus acidophilus</i>	ATCC 314	Unknown	-
21	<i>Lactobacillus brevis</i>	VTT 991190 ^e	Unknown	-
22	<i>Lactobacillus buchneri</i>	VTT E-54	Unknown	-
23	<i>Lactobacillus plantarum</i>	ATCC 8014	Unknown	-
24	<i>Listeria monocytogenes</i>	ATCC 7644	Human	-
25	<i>Morganella morganii</i>	ATCC 25829	Human	-
26	<i>Proteus mirabilis</i>	ATCC 7002	Urine	-
27	<i>Proteus vulgaris</i>	ATCC 6380	Clinical Isolate	-
28	<i>Pseudomonas aeruginosa</i>	ATCC 27853	Clinical Isolate	-
29	<i>Shigella sonnei</i>	ATCC 29930	Unknown	-
30	<i>Staphylococcus aureus</i>	ATCC 10832	Unknown	-
31	<i>Staphylococcus epidermidis</i>	ATCC 700296	Unknown	-
32	<i>Staphylococcus galinarum</i>	ATCC 49148	Unknown	-
33	<i>Staphylococcus warneri</i>	ATCC 49454	Unknown	-
34	<i>Streptococcus agalactiae</i>	ATCC 12386	Unknown	-
35	<i>Vibrio parahaemolyticus</i>	ATCC 17802	Food	-

^aResult: "+" = strain detected, "-" = strain not detected; ^bATCC – American Type Culture Collection, Manassas, VA; ^c White Labs Library Strain, San Diego, CA;

^dBEI NR – Biodefense and Emerging Infections Research Resources Repository, Manassas, VA; ^e VTT Culture Collection, VTT, Finland

Table 3: Raw Ground Turkey Method Comparison Results of GENE-UP QUANT *Salmonella* vs. USDA/FSIS MLG 2.05 (3)

Inoculation Level	Sample Replicate	GENE-UP enviroPRO					USDA/FSIS MLG 2.05 ^a					Mean Difference ^d	90% CI ^e	95% CI ^f
		CFU/g	Log ₁₀	Log ₁₀ Mean	SD ^b (S _r)	RSD _r ^c	MPN/g	Log ₁₀	Log ₁₀ Mean	SD (S _r)	RSD _r			
Low	1	6	0.80	1.42	0.51	35.93	11	1.04	1.19	0.16	13.25	0.23	-0.13, 0.59	-0.22, 0.67
	2	59	1.77				11	1.04						
	3	32	1.51				24	1.38						
	4	18	1.26				15	1.17						
	5	56	1.75				21	1.32						
Medium	1	55	1.74	1.92	0.18	9.28	75	1.88	1.87	0.17	8.99	0.05	-0.16, 0.25	-0.11, 0.20
	2	76	1.88				93	1.97						
	3	160	2.21				93	1.97						
	4	86	1.94				38	1.58						
	5	68	1.83				93	1.97						
High	1	930	2.97	2.68	0.17	6.31	430	2.63	2.80	0.39	14.00	-0.11	-0.47, 0.24	-0.26, 0.04
	2	440	2.64				930	2.97						
	3	390	2.59				2400	3.38						
	4	340	2.53				430	2.63						
	5	480	2.68				230	2.36						

^aUnpaired analysis following the USDA/FSIS-MLG 2.05 reference method.

^bSD = Standard deviation.

^cRSD_r = Relative Standard deviation.

^dMean Difference = Candidate Log Mean - Reference Log Mean.

^e90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent.

^f90% CI = If the confidence interval does not contain the point 0, then the methods would be considered statistically different.

Table 4: Poultry Carcass Rinsate Method Comparison Results of GENE-UP QUANT *Salmonella* vs. USDA/FSIS MLG 2.05 (3)

Inoculation Level	Sample Replicate	GENE-UP enviroPRO					USDA/FSIS MLG 2.05 ^a					Mean Difference ^d	90% CI ^e	95% CI ^f
		CFU/mL	Log ₁₀	Log ₁₀ Mean	SD ^b (S _r)	RSD _r ^c	MPN/mL	Log ₁₀	Log ₁₀ Mean	SD (S _r)	RSD _r			
Low	1	120	2.09	1.96	0.51	26.01	46	1.66	1.68	0.24	14.0	0.28	-0.01, 0.56	-0.07, 0.62
	2	37	1.57				24	1.38						
	3	80	1.90				110	2.04						
	4	110	2.05				46	1.66						
	5	150	2.19				46	1.66						
Medium	1	510	2.71	2.63	0.06	2.39	240	2.38	2.44	0.22	8.84	0.19	-0.01, 0.37	-0.05, 0.42
	2	450	2.65				150	2.18						
	3	430	2.63				460	2.66						
	4	350	2.54				210	2.32						
	5	400	2.60				460	2.66						
High	1	1300	3.11	3.54	0.70	19.73	930	2.97	3.39	0.58	16.9	0.14	-0.61, 0.89	-0.79, 1.08
	2	1100	3.03				11000	4.04						
	3	890	2.95				430	2.63						
	4	21,000	4.32				4600	3.66						
	5	19000	4.28				4600	3.66						

^aUnpaired analysis following the USDA/FSIS-MLG 2.05 reference method.

^bSD = Standard deviation.

^cRSD_r = Relative Standard deviation.

^dMean Difference = Candidate Log Mean - Reference Log Mean.

^e90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent.

^f90% CI = If the confidence interval does not contain the point 0, then the methods would be considered statistically different.

Table 5: Raw Beef Trim Method Comparison Results of GENE-UP QUANT *Salmonella* vs. USDA/FSIS MLG 2.05 (3)

Inoculation Level	Sample Replicate	GENE-UP enviroPRO					USDA/FSIS MLG 2.05 ^a					Mean Difference ^d	90% CI ^e	95% CI ^f
		CFU/g	Log ₁₀	Log ₁₀ Mean	SD ^b (S _r)	RSD _r ^c	MPN/g	Log ₁₀	Log ₁₀ Mean	SD (S _r)	RSD _r			
Low	1	42	1.62	1.85	0.22	11.6	43	1.63	1.66	0.21	13.0	0.19	-0.06, 0.44	-0.12, 0.50
	2	42	1.62				23	1.36						
	3	82	1.91				93	1.97						
	4	108	2.03				46	1.66						
	5	113	2.05				46	1.66						
Medium	1	220	2.33	2.15	0.23	10.7	430	2.63	2.42	0.21	8.52	-0.27	-0.52, -0.01	-0.59, 0.05
	2	57	1.75				210	2.32						
	3	160	2.20				430	2.63						
	4	150	2.17				210	2.32						
	5	190	2.28				150	2.18						
High	1	1100	3.03	2.95	0.11	3.78	2400	3.38	3.13	0.35	11.3	-0.18	-0.48, 0.13	-0.56, 0.20
	2	820	2.92				430	2.63						
	3	590	2.77				2400	3.38						
	4	1100	3.03				2400	3.38						
	5	1000	3.02				750	2.88						

^aUnpaired analysis following the USDA/FSIS-MLG 2.05 reference method.

^bSD = Standard deviation.

^cRSD_r = Relative Standard deviation.

^dMean Difference = Candidate Log Mean - Reference Log Mean.

^e90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent.

^f90% CI = If the confidence interval does not contain the point 0, then the methods would be considered statistically different.

Table 6: Carcass Sampling Cloth Method Comparison Results of GENE-UP QUANT *Salmonella* vs. USDA/FSIS MLG 2.05 (3)

Inoculation Level	Sample Replicate	GENE-UP enviroPRO					USDA/FSIS MLG 2.05 ^a					Mean Difference ^d	90% CI ^e	95% CI ^f
		CFU/swab	Log ₁₀	Log ₁₀ Mean	SD ^b (S _r)	RSD _r ^c	MPN/swab	Log ₁₀	Log ₁₀ Mean	SD (S _r)	RSD _r			
Low	1	140	2.13	2.01	0.23	11.6	86	1.93	1.77	0.15	8.40	0.24	0.01, 0.47	-0.04, 0.53
	2	150	2.19				86	1.93						
	3	47	1.67				46	1.66						
	4	160	2.20				46	1.66						
	5	75	1.88				46	1.66						
Medium	1	290	2.47	2.76	0.23	8.27	300	2.48	2.78	0.30	10.9	-0.02	-0.34, 0.30	-0.41, 0.37
	2	670	2.82				860	2.93						
	3	1100	3.03				300	2.48						
	4	400	2.60				720	2.86						
	5	790	2.90				1500	3.18						
High	1	3300	3.51	3.48	0.28	7.98	4800	3.68	3.51	0.22	6.37	-0.03	-0.32, 0.27	-0.40, 0.34
	2	2700	3.44				2400	3.38						
	3	7900	3.90				4200	3.62						
	4	2700	3.43				1500	3.18						
	5	1300	3.12				4800	3.68						

^aUnpaired analysis following the USDA/FSIS-MLG 2.05 reference method.

^bSD = Standard deviation.

^cRSD_r = Relative Standard deviation.

^dMean Difference = Candidate Log Mean - Reference Log Mean.

^e90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent.

^f90% CI = If the confidence interval does not contain the point 0, then the methods would be considered statistically different.

Table 7: Poultry Carcass Rinsate Method Comparison Results of GENE-UP QUANT *Salmonella* vs. USDA/FSIS MLG 2.05 – Independent Laboratory Study (3)

Inoculation Level	Sample Replicate	GENE-UP enviroPRO					USDA/FSIS MLG 2.05 ^a					Mean Difference ^d	90% CI ^e	95% CI ^f
		CFU/mL	Log ₁₀	Log ₁₀ Mean	SD ^b (S _r)	RSD _r ^c	MPN/mL	Log ₁₀	Log ₁₀ Mean	SD (S _r)	RSD _r			
Low	1	260	2.42	2.14	0.204	9.54	93	1.97	2.05	0.28	12.9	0.09	-0.20, 0.37	-0.27, 0.44
	2	190	2.27				240	2.38						
	3	110	2.05				120	2.08						
	4	83	1.92				150	2.18						
	5	100	2.02				43	1.63						
Medium	1	590	2.77	2.73	0.205	7.50	430	2.63	2.55	0.34	12.56	0.18	-0.15, 0.51	-0.23, 0.59
	2	280	2.45				430	2.63						
	3	540	2.73				92	1.96						
	4	1000	3.02				750	2.88						
	5	470	2.67				430	2.63						
High	1	1200	3.08	3.27	0.157	4.80	4300	3.63	3.63	0.00	0.00	-0.36	-0.49, -0.23	-0.52, -0.20
	2	1400	3.16				4300	3.63						
	3	2900	3.46				4300	3.63						
	4	1900	3.27				4300	3.63						
	5	2400	3.39				4300	3.63						

^aUnpaired analysis following the USDA/FSIS-MLG 2.05 reference method.

^bSD = Standard deviation.

^cRSD_r = Relative Standard deviation.

^dMean Difference = Candidate Log Mean - Reference Log Mean.

^e90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent.

^f90% CI = If the confidence interval does not contain the point 0, then the methods would be considered statistically different.

DISCUSSION OF MODIFICATION APPROVED DECEMBER 2022 (6)

In the matrix study, mean differences between the GENE-UP QUANT *Salmonella* assay and the MLG reference method were less than 0.5 log₁₀ at each contamination level, demonstrating comparable results between the two methods.

The GENE-UP QUANT *Salmonella* method allows the user to obtain accurate results after 4 h of enrichment as low as 1 cfu/g contamination. The sample preparation is streamlined for use in any laboratory, and is easy to follow, allowing for a technician at any level of training to perform the method and obtain accurate results.

Table 1: Breaded and stuffed raw chicken Comparison Results of GENE-UP QUANT *Salmonella* vs. SDA/FSIS MLG 2.05 (6)

Inoculation Level	Sample Replicate	GENE-UP QUANT				MLG 2.05 ^a				Difference of means ^c	90% CI ^d	95% CI ^e
		CFU/g	Log ₁₀	Log ₁₀ Mean	SD ^b (S _r)	MPN/g	Log ₁₀	Log ₁₀ Mean	SD (S _r)			
Control	1	0	-1.00	0.00	0.00	0	-1.00	0.00	0.00	0.00	0.00, 0.00	0.00, 0.00
	2	0	-1.00			0	-1.00					
	3	0	-1.00			0	-1.00					
	4	0	-1.00			0	-1.00					
	5	0	-1.00			0	-1.00					
Low	1	4.43	0.66	0.56	0.32	1.50	0.20	0.25	0.49	0.31	-0.16, 0.79	-0.28, 0.90
	2	8.19	0.92			0.36	-0.34					
	3	3.29	0.53			2.30	0.38					
	4	3.82	0.59			0.92	0.01					
	5	1.11	0.08			9.30	0.97					
Medium	1	275	2.44	1.78	0.44	21	1.32	1.52	0.44	0.26	-0.27, 0.79	-0.40, 0.92
	2	91	1.96			23	1.36					
	3	23	1.36			9.3	0.97					
	4	52	1.72			93	1.97					
	5	26	1.42			93	1.97					
High	1	575	2.76	2.82	0.25	230	2.63	2.62	0.44	0.20	-0.22, 0.62	-0.32, 0.73
	2	431	2.64			230	2.63					
	3	394	2.60			430	2.63					
	4	1577	3.20			2400	3.38					
	5	859	2.93			230	2.36					

^aUnpaired analysis following the MLG 2.05 reference method.

^bSD = Standard deviation.

^cDifference of means = Candidate Log Mean - Reference Log Mean.

^d90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent.

^e95 CI = If the confidence interval does not contain the point 0, then the methods would be considered statistically different.

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