



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

011404

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

VeriflowTM Salmonella species (SS)

manufactured by

Invisible Sentinel, Inc.

3711 Market Street, 8th Floor

Philadelphia, PA 19104

USA

This method has been evaluated in the AOAC[®] *Performance Tested MethodsSM* Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance TestedSM* certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (January 15, 2017 – December 31, 2017). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Deborah McKenzie

Deborah McKenzie, Senior Director
Signature for AOAC Research Institute

January 15, 2017

Date

METHOD AUTHORS

ORIGINAL VALIDATION: Amrita Puri, Adam C. Joelsson, Shawn P. Terkhorn, Ashley S. Brown, Benjamin J. Pascal, Zara E. Gaudio, and Nicholas A. Siciliano
MODIFICATION JULY 2015: Ken Huang and Adam C. Joelsson
MODIFICATION AUGUST 2015: Adam C. Joelsson, Ken Huang, and Nicholas A. Siciliano
MODIFICATION JANUARY 2016(BLACK PEPPER): Ken Huang and Adam Joelsson
MODIFICATION JANUARY 2016 (WHEY POWDER): Ken Huang and Adam Joelsson

SUBMITTING COMPANY

Invisible Sentinel, Inc.
 3711 Market Street, 8th Floor
 Philadelphia, PA 19104
 USA

KIT NAME(S)

Veriflow™ *Salmonella* species (SS)

CATALOG NUMBERS

IS 1005

INDEPENDENT LABORATORY (Original Validaiton)

Q Laboratories, Inc
 1400 Harrison Avenue
 Cincinnati, OH 45214
 USA

Silliker Inc.
 3600 Eagle Nest Drive, South Building
 Crete, IL 60417
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AOAC EXPERTS AND PEER REVIEWERS

Yi Chen¹, Michael Brodsky², Wayne Ziemer³
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APPLICABILITY OF METHOD

Target organism – *Salmonella* species
Matrices – beef hot dogs, raw ground beef (20% fat), chicken carcass rinse, pasteurized milk (2%), stainless steel, sealed concrete, ceramic tile, plastic
August 2015 extension: peanut butter
January 2016 Extension: ground black pepper (25g), whey protein
Performance claims - The Veriflow® SS system allows for the rapid presumptive detection of *Salmonella* species from environmental (stainless steel, sealed concrete, plastic, and ceramic tile), raw meat (20% fat ground beef), chicken carcasses, dairy (2% milk) and Ready-to-Eat (RTE) food (hot dogs) matrices in 18 hours after initiation of sample enrichment with equivalent performance as compared to the traditional cultured based United States Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guide (USDA/FSIS MLG) chapter 4.06 and Food and Drug Administration Bacterial Analytical Manual (FDA BAM) Chapter 5 reference methods (3,4).

REFERENCE METHODS

U.S. Department of Agriculture, Food Safety and Inspection Service (2012) Microbiology Laboratory Guidebook, Chapter 4.06 "*Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Catfish Products and Carcass and Environmental Sponges*". (3)
 Food and Drug Administration Bacterial Analytical Manual Chapter 5 (2011) "*Salmonella*". (4)
 U.S. Food and Drug Administration *Bacterial Analytical Manual* Chapter 4a (2014) "*Diarrheagenic Escherichia coli*" (12)

ORIGINAL CERTIFICATION DATE

January 27, 2014

CERTIFICATION RENEWAL RECORD

Renewed Annually through December 2016

METHOD MODIFICATION RECORD

1. July 2015
2. August 2015
3. January 2016
4. January 2016

SUMMARY OF MODIFICATION

1. Change in sample volume from 1 mL to 500 µL.
2. Matrix extension to include peanut butter
3. Matrix extension to include ground black pepper.
4. Matrix extension to include whey protein.

Under this AOAC® *Performance Tested*SM License Number, 011404 this method is distributed by:
 NONE

Under this AOAC® *Performance Tested*SM License Number, 011404 this method is distributed as:
 NONE

PRINCIPLE OF THE METHOD (1)

Veriflow® *Salmonella* species (SS) (Cat no. IS1005) is a molecular based test that detects *Salmonella* species in environmental (stainless steel, sealed concrete, plastic, and ceramic tile) dairy (2% milk) raw meat (20% fat ground beef), chicken carcasses and RTE (hot dogs) food matrices. The method combines Polymerase Chain Reaction (PCR) with a rapid, chromatographic vertical flowthrough system that provides specific and highly sensitive detection of target associated molecular signatures coupled with rapid, easy-to-interpret results.

In this study, artificially inoculated environmental surfaces (stainless steel, sealed concrete, plastic, and ceramic tile), dairy (2% milk), raw meat (20% fat ground beef) and RTE food (hot dogs) matrices, with naturally contaminated chicken carcasses, were sampled, enriched and subjected to PCR amplification leading to the generation of a *Salmonella* species-specific analyte. For final analysis, the PCR generated analyte is applied directly to the sample window of the assay cassette, and the signal is allowed to develop for a total of 3 minutes, after which the cassette switch is retracted to remove the conjugate pad and reveal the underlying test membrane and results. In the event of a positive sample, the target analyte is captured and immobilized on the nitrocellulose test membrane and detected by a colloidal gold-protein conjugate, which generates a visual signal at the test line. The aggregation of the colloidal gold results in a distinct red line in the area indicated as "T" on the test cassette. A control line will also develop, indicated as "C" on the test cassette, and reacts only with the colloidal gold conjugate providing the user an indication that the test was run properly. The appearance of two distinct red lines is indicative of a positive sample for *Salmonella* species; whereas appearance of just the control line indicates a negative sample.

Summary of Results

To satisfy AOAC-RI Performance-Tested MethodSM (PTM) unpaired and paired method comparison validation requirements, replicate samples of environmental surfaces (stainless steel, sealed concrete, plastic, and ceramic tile), chicken carcasses, RTE food (hot dogs), and raw meat (20% fat ground beef) matrices were inoculated at a low and high level with an additional un-inoculated control set, in duplicate, and sampled according to directions outlined in either the IS Veriflow® SS assay insert or the USDA/FSIS MLG 4.06 or BAM chapter 5 reference methods (3,4,5). Probability of detection (POD) analysis indicated that there was no significant difference in performance between the reference methods and the Veriflow® SS rapid assay. All 104 tested strains of *Salmonella* species were detected in the inclusivity study, while 35 non-specific organisms went undetected in the exclusivity study. Additionally, robustness testing and lot-to-lot stability results indicate that the Veriflow® SS system is stable, rugged and is uniformly manufactured.

DISCUSSION OF THE ORIGINAL VALIDATION STUDY (1)

The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow® SS assay as compared to the traditional FDA BAM Chapter 5 and USDA/FSIS MLG chapter 4.06 culture based reference methods (3,4) for the detection of *Salmonella* species on environmental surfaces (stainless steel, sealed concrete, ceramic tile, and plastic) raw meat (20% fat ground beef), dairy (2% milk), chicken carcasses and in RTE (hot dogs) foods. POD statistical analysis of all eight matrices tested indicate that there is no significant difference in performance between the methods at specific time points as assayed in this study, and importantly, no false positive or false negative results were observed in the entirety of the study. The successful validation of the assay is further supported by the results of the inclusivity and exclusivity testing, indicating that the Veriflow® SS assay was able to accurately detect over 104 *Salmonella* isolates while correctly excluding all non-specific bacteria tested.

The Veriflow® SS assay provides flexibility and ease of use for the end user by providing accurate results across multiple surfaces with sampling by either swabs or sponges, and across multiple food matrices, without complex sample preparation after enrichment. The Veriflow® system also offers significant savings in time compared to the reference methods used in this study, by producing accurate presumptive results after an enrichment time of only 18 hours, as compared to the reference methods that require 3-4 days to reach presumptive results. 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. Thus the results of this study demonstrated that the easy to follow Veriflow® SS protocol provides for a sensitive, reliable and simple to use rapid detection method for *Salmonella* species.

Table 3. Veriflow® SS Inclusivity Evaluation (1)

No.	Organism	Reference Number	Serogroup	Source	Veriflow® SS Results	Reference method confirmation
1	<i>Salmonella bongori</i>	NCTC ¹ 12419	Not Available	Not Available	+	+
2	<i>Salmonella Bongori</i> , Serotype Brookfield	ATCC ² 43975	Not Available	Not Available	+	+
3	<i>Salmonella bongori</i> CIP 82.33	NCTC 10946	Not Available	Frog	+	+
4	<i>Salmonella enterica</i> subsp. <i>salamae</i> serovar Artis	ATCC 700149	56	Not Available	+	+
5	<i>Salmonella enterica</i> subsp. Arizonae	ATCC 13314	Not Available	Not Available	+	+
6	<i>Salmonella enterica</i> subsp. Arizonae	ATCC BAA-1557	Not Available	Not Available	+	+
7	<i>Salmonella enterica</i> subsp. Arizonae	QL ³ 11007-4	Not Available	Veterinary Isolate	+	+
8	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abaetetuba	ATCC 35640	F	Creek Water, Argentina	+	+
9	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony	NCTC 6017	B	Not Available	+	+
10	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abortusequi	FDA ⁴ 9842	B	Not Available	+	+
11	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abortusovis	NCTC 10241	B	Not Available	+	+
12	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Adelaide	STS ⁵ 2	O	Not Available	+	+
13	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Agama	STS 3	B	Not Available	+	+

14	Salmonella enterica subsp. enterica serovar Agona	ATCC 51957	B	Not Available	+	+
15	Salmonella enterica subsp. enterica serovar Agoueve	STS 5	G	Not Available	+	+
16	Salmonella enterica subsp. enterica serovar Alachua	STS 6	O	Not Available	+	+
17	Salmonella enterica subsp. enterica serovar Albany	STS 7	C2	Not Available	+	+
18	Salmonella enterica subsp. enterica serovar Anatum	ATCC 9270	E1	Pork Liver	+	+
19	Salmonella enterica subsp. enterica serovar Arkansas	STS 11	E3	Not Available	+	+
20	Salmonella enterica subsp. enterica serovar Bareilly	FDA1206H	C1	Not Available	+	+
21	Salmonella enterica subsp. Salamae serovar Basel	ATCC 700151	58	Not Available	+	+
22	Salmonella enterica subsp. enterica serovar Berta	STS 13	D1	Not Available	+	+
23	Salmonella enterica subsp. enterica serovar Binza	STS 14	E2	Not Available	+	+
24	Salmonella enterica subsp. enterica serovar Bovis-morbificans	STS 16	C2	Not Available	+	+
25	Salmonella enterica subsp. enterica serovar Brandenburg	STS 18	B	Not Available	+	+
26	Salmonella enterica subsp. enterica serovar Bredeney	NCTC 5731	B	Not Available	+	+
27	Salmonella enterica subsp. enterica serovar California	NCTC 6018	B	Not Available	+	+
28	Salmonella enterica subsp. enterica serovar Cerro	STS 27	K	Not Available	+	+
29	Salmonella enterica subsp. enterica serovar Choleraesuis	ATCC 10708	C1	Not Available	+	+
30	Salmonella enterica subsp. enterica serovar Choleraesuis var Kunzendorf	ATCC 12011	C1	Not Available	+	+

31	Salmonella enterica subsp. enterica serovar Cubana	STS 24	G	Not Available	+	+
32	Salmonella enterica subsp. enterica serovar Derby	NCTC 5721	B	Not Available	+	+
33	Salmonella enterica subsp. Diarizonae	ATCC BAA-1579	Not Available	Not Available	+	+
34	Salmonella enterica subsp. Diarizonae	BAA-216	Not Available	Blood, Human	+	+
35	Salmonella enterica subsp. Diarizonae	BEI NR-516	Not Available	Not Available	+	+
36	Salmonella enterica subsp. enterica serovar Drypool	STS 26	E2	Not Available	+	+
37	Salmonella enterica subsp. enterica serovar Dublin	STS 27	D1	Not Available	+	+
38	Salmonella enterica subsp. enterica serovar Eastbourne	FDA 4017H	D1	Not Available	+	+
39	Salmonella enterica subsp. enterica serovar Enteritidis	ATCC 13076	D1	Not Available	+	+
40	Salmonella enterica subsp. Enterica serovar Galiema	QL024.2	C1	Not Available	+	+
41	Salmonella enterica subsp. Enterica serovar Give	STS 42	E1	Not Available	+	+
42	Salmonella enterica subsp. Enterica serovar Haardt	STS 44	C2	Not Available	+	+
43	Salmonella enterica subsp. Enterica serovar Hadar	ATCC 51956	C2	Not Available	+	+
44	Salmonella enterica subsp. Enterica serovar Havana	STS 47	G	Not Available	+	+
45	Salmonella enterica subsp. enterica serovar Heidelberg	ATCC 8326	B	Not Available	+	+
46	Salmonella enterica subsp. enterica serovar Illinois	ATCC 11646	E3	Not Available	+	+
47	Salmonella enterica subsp. enterica serovar Indiana	NCTC 11304	B	Bird, Turkey	+	+

48	Salmonella enterica subsp. Indica	ATCC BAA-1578	Not Available	India	+	+
49	Salmonella enterica subsp. Indica serovar Ferlac	NCTC 10458	H	Desiccated Coconut	+	+
50	Salmonella enterica subsp. Indica serovar Ferlac	ATCC 43976		Not Available	+	+
51	Salmonella enterica subsp. Enterica serovar Infantis	ATCC 51741	C1	Pasta	+	+
52	Salmonella enterica subsp. Enterica serovar Javiana	ATCC 10721	D1	Not Available	+	+
53	Salmonella enterica subsp. Enterica serovar Jerusalem	QL024.12	C1	Not Available	+	+
54	Salmonella enterica subsp. Enterica serovar Johannesburg	STS 56	R	Not Available	+	+
55	Salmonella enterica subsp. Enterica serovar Kahla	ATCC 17980	T	Feces	+	+
56	Salmonella enterica subsp. Enterica serovar Kaitaan	QL024.7	H	Not Available	+	+
57	Salmonella enterica subsp. Enterica serovar Kentucky	ATCC 9263	C2	Not Available	+	+
58	Salmonella enterica subsp. Enterica serovar Krefeld	STS 58	E4	Not Available	+	+
59	Salmonella enterica subsp. Enterica serovar Lille	STS 59	C1	Not Available	+	+
60	Salmonella enterica subsp. Enterica serovar Livingstone	STS 63	C1	Not Available	+	+
61	Salmonella enterica subsp. Enterica serovar London	STS 64	E1	Not Available	+	+
62	Salmonella enterica subsp. Enterica serovar Manhattan	STS 65	C2	Not Available	+	+
63	Salmonella enterica subsp. enterica serovar Mbankaka	37N	C1	Not Available	+	+
64	Salmonella enterica subsp. enterica serovar Menden	ATCC 15992	C1	Feces	+	+
65	Salmonella enterica subsp. enterica serovar Meleagridis	QL12074-1	E1	Not Available	+	+

66	Salmonella enterica subsp. enterica serovar Menhaden	QL024.20	E3	Not Available	+	+
67	Salmonella enterica subsp. enterica serovar Montevideo	ATCC 8387	C1	Not Available	+	+
68	Salmonella enterica subsp. enterica serovar Muenchen	ATCC BAA-1594	C2	Feces	+	+
69	Salmonella enterica subsp. enterica serovar Neasden	QL024.4	D1	Not Available	+	+
70	Salmonella enterica subsp. enterica serovar Newington	QL024.8	E2	Not Available	+	+
71	Salmonella enterica subsp. enterica serovar Newport	ATCC 6962	C2	Food Poisoning Patient	+	+
72	Salmonella enterica subsp. enterica serovar Ohio	STS 81	C1	Not Available	+	+
73	Salmonella enterica subsp. enterica serovar Oranienburg	ATCC 9239	C1	Not Available	+	+
74	Salmonella enterica subsp. enterica serovar Orthmarshen	QL024.13	C1	Not Available	+	+
75	Salmonella enterica subsp. enterica serovar Paratyphi A	ATCC 9150	A	Not Available	+	+
76	Salmonella enterica subsp. enterica serovar Paratyphi B	ATCC 10719	B	Not Available	+	+
77	Salmonella enterica subsp. enterica serovar Paratyphi C	ATCC 13428	C1	Not Available	+	+
78	Salmonella enterica subsp. enterica serovar Pomona	ATCC 10729	M	Not Available	+	+
79	Salmonella enterica subsp. Enterica serovar Poona	NCTC 4840	G	Infant Enteritis	+	+
80	Salmonella enterica subsp. enterica serovar Preston	QL024.16	B	Not Available	+	+
81	Salmonella enterica subsp. Enterica serovar Pullorum	ATCC 13036	D1	Egg	+	+

82	Salmonella enterica subsp. Enterica serovar Rubislaw	STS 92	F	Not Available	+	+
83	Salmonella enterica subsp. enterica serovar Saintpaul	ATCC 9712	B	Cystitis	+	+
84	Salmonella enterica subsp. Salamae	QL02415	C1	Not Available	+	+
85	Salmonella enterica subsp. Enterica serovar San-Diego	STS 94	B	Not Available	+	+
86	Salmonella enterica subsp. enterica serovar Schalkwijk	QL024.10	H	Not Available	+	+
87	Salmonella enterica subsp. enterica serovar Schwarzengrund	STS 95	B	Not Available	+	+
88	Salmonella enterica subsp. enterica serovar Senftenberg	ATCC 43845	E4	Not Available	+	+
89	Salmonella enterica subsp. enterica serovar Stanley	ATCC 7308	B	Not Available	+	+
90	Salmonella enterica subsp. enterica serovar Tallahassee	ATCC 12002	C2	Not Available	+	+
91	Salmonella enterica subsp. enterica serovar Tennessee	QL024.6	C1	Not Available	+	+
92	Salmonella enterica subsp. enterica serovar Thompson	2051H	C1	Not Available	+	+
93	Salmonella enterica subsp. enterica serovar Typhi	ATCC 6539	D1	Not Available	+	+
94	Salmonella enterica subsp. enterica serovar Typhimurium	ATCC 14028	B	Tissue, animal	+	+
95	Salmonella enterica subsp. enterica Utrecht	NCTC 10077	52	Not Available	+	+
96	Salmonella enterica subsp. enterica Vellore	ATCC 15611	B	Rectal Swab	+	+
97	Salmonella enterica subsp. enterica Virchow	ATCC 51955	C1	Not Available	+	+
98	Salmonella enterica subsp. enterica Volta	QL024.9	F	Not Available	+	+
99	Salmonella enterica subsp. enterica Westhampton	QL024.14	E1	Not Available	+	+
100	Salmonella enterica subsp. enterica Worthington	STS 114	G	Not Available	+	+

101	Salmonella enterica subsp. Houtenae serovar Halmstad	QL024.1	E2	Not Available	+	+
102	Salmonella enterica subsp. Houtenae serovar Harmelen	ATCC 15783	51	Boa Constrictor	+	+
103	Salmonella enterica subsp. Houtenae serovar Ochsenzoll	ATCC 29932	I	Not Available	+	+
104	Salmonella enterica subsp. enterica Urbana	STS 110	N	Not Available	+	+

¹ NCTC – National Collection of Type Cultures, UK

² ATCC – American Type Culture Collection

³ STS – University of Pennsylvania

⁴ FDA – Food and Drug Administration Isolate

⁵ QL – Q Laboratories Isolate

Table 4. Veriflow® SS Exclusivity Evaluation (1)

No.	Organism	Reference Number	Source	Veriflow® SS Result	Reference Method Result
1	<i>Alcaligenes faecalis</i> ¹	ATCC 8750	Not Available	-	-
2	<i>Bacillus cereus</i> ¹	ATCC 14579	Not Available	-	-
3	<i>Bacillus subtilis</i> ¹	ATCC 6051	Not Available	-	-
4	<i>Campylobacter lari</i> ¹	ATCC BAA-1060	Feces, Human	-	-
5	<i>Campylobacter jejuni</i> ¹	ATCC 33560	Feces,Bovine	-	-
6	<i>Candida albicans</i> ¹	ATCC 24433	Nail Infection	-	-
7	<i>Carnobacterium maltaromaticum</i> ¹	ATCC 43224	Vacuum-packed beef	-	-
8	<i>Citrobacter farmeri</i> ¹	ATCC 51112	Feces, Human	-	-
9	<i>Citrobacter freundii</i> ¹	ATCC 8090	Not Available	-	-
10	<i>Edwardsiella tarda</i> ¹	ATCC 15947	Feces, Human	-	-
11	<i>Enterobacter aerogenes</i> ¹	ATCC 13048	Sputum	-	-
12	<i>Enterobacter cloacea</i> ¹	ATCC 23355	Not Available	-	-
13	<i>Enterococcus faecium</i> ¹	ATCC 19434	Not Available	-	-
14	<i>Enterococcus faecalis</i> ¹	ATCC 29212	Urine	-	-
15	<i>Escherichia coli</i> ¹	ATCC 25922	Clinical Isolate	-	-
16	<i>Escherichia coli</i> ²	BEI NR 4356	Not Available	-	-
17	<i>Escherichia coli</i> ²	BEI NR-12	Not Available	-	-
18	<i>Hafnia alvei</i> ¹	ATCC 51813	Milk, Minnesota	-	-
19	<i>Lactobacillus acidophilus</i> ¹	ATCC 314	Not Available	-	-
20	<i>Lactobacillus kefir</i> ¹	ATCC 35411	Kefir	-	-
21	<i>Lactobacillus lactis</i> ¹	ATCC 4794	Not Available	-	-
22	<i>Listeria monocytogenes</i> ¹	ATCC 7644	Human Isolate	-	-
23	<i>Listeria innocua</i> ¹	ATCC 33090	Cow Brain	-	-
24	<i>Klebsiella pneumonia</i> ¹	ATCC 13883	Not Available	-	-
25	<i>Kocuria rhizophila</i> ¹	ATCC 9341	Not Available	-	-
26	<i>Morganella morganii</i> ¹	ATCC 25829	Urine	-	-
27	<i>Proteus mirabilis</i> ¹	ATCC 7002	Clinical Isolate	-	-
28	<i>Proteus vulgaris</i> ¹	ATCC 6380	Not Available	-	-
29	<i>Pseudomonas aeruginosa</i> ¹	ATCC 27853	Not Available	-	-
30	<i>Shigella sonnei</i> ¹	ATCC 29930	Milk, Minnesota	-	-
31	<i>Staphylococcus aureus</i> ¹	ATCC 10832	Not Available	-	-
32	<i>Staphylococcus epidermidis</i> ¹	ATCC 12228	Kefir	-	-
33	<i>Staphylococcus haemolyticus</i> ¹	ATCC 29970	Not Available	-	-
34	<i>Staphylococcus hominis</i> ¹	ATCC 27844	Human Isolate	-	-
35	<i>Streptococcus pneumoniae</i> ¹	ATCC 6302	Cow Brain	-	-

¹ATCC: American Type Culture Collection²Isolates obtained from IS culture collection

Table 5. Veriflow[®] SS Presumptive vs. Confirmed Results for Environmental Surfaces – POD Results (1)

Matrix	Strain	CFU/Test Area ^a	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Stainless Steel	<i>Salmonella</i> Typhimurium/ <i>Citrobacter freundii</i> ATCC 14028/ ATCC 8090	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		9	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		90	5	3	0.60	0.23, .88	3	0.60	0.23, .88	0.00	-0.43, 0.43
Sealed Concrete	<i>Salmonella</i> Enteritidis ATCC BAA-1045	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		9	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.28, 0.28
		456	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic Tile	<i>Salmonella</i> Choleraesuis ATCC 10708	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		975	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	-0.05	-0.26, 0.26
		9750	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic	<i>Salmonella</i> Arizonae ATCC 13314	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		14	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		140	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^bN = Number of test portions

^cx = Number of positive test portions

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

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

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

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 6: Veriflow® SS Environmental Surfaces, Candidate vs. Reference – POD Results (1)

Matrix	Strain	CFU/Test Area ^a	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
				x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Stainless Steel	<i>Salmonella</i> Typhimurium/ <i>Citrobacter freundii</i> ATCC 14028/ ATCC 8090	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		9	20	10	0.50	0.30, 0.70	10	0.50	0.3, 0.7	0.00	-0.28, 0.28
		90	5	3	0.60	0.23, .88	3	0.60	0.23, .88	0.00	-0.46, 0.46
Sealed Concrete	<i>Salmonella</i> Enteritidis ATCC BAA-1045	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		9	20	14	0.70	0.48, 0.85	11	0.55	0.34, 0.74	0.15	-0.14, 0.41
		456	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic Tile	<i>Salmonella</i> Choleraesuis ATCC 10708	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		975	20	12	0.60	0.39, 0.78	9	0.45	0.26, 0.66	0.15	-0.15, 0.41
		9750	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic	<i>Salmonella</i> Arizonae ATCC 13314	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		14	20	10	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		140	5	5	1.00	0.57, 1.00	4	0.80	0.57, 1.00	0.20	-0.26, 0.62

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^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 results and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 7. Veriflow SS Presumptive vs. Confirmed for Raw Meat, RTE Food, Dairy and Chicken Carcasses Matrices – POD Results (1)

Matrix	Strain	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		
Ground Beef	<i>Salmonella</i> Typhimurium ATCC 14028	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		.11	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		0.34	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Hot Dogs	<i>Salmonella</i> Infantis ATCC 51741	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.58	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
2% Milk	<i>Salmonella</i> Choleraesuis ATCC 10708	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		.45	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
		2.46	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Chicken Carcasses	Naturally Occurring	Lot 1	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0.00	-0.25, 0.25
		Lot 2	20	7	.35	0.18, 0.57	7	.35	0.18, 0.57	0.00	-0.28, 0.28

^aMPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, 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 candidate method confirmed results and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 8: Veriflow® SS Raw Meat, RTE Food, Dairy and Chicken Carcass Matrices Candidate vs. Reference – POD Results (1)

Matrix	Strain	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		
Ground Beef	<i>Salmonella</i> Typhimurium ATCC 14028	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		.11	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		0.34	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Hot Dogs	<i>Salmonella</i> Infantis ATCC 51741	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.58	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
2% Milk	<i>Salmonella</i> Choleraesuis ATCC 10708	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		.45	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-.05	-0.32, 0.23
		2.46	5	5	1.00	0.57, 1.00	4	0.80	0.57, 1.00	0.20	-0.26, 0.62
Chicken Carcasses	Naturally Occurring	Lot 1	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
		Lot 2	20	7	.35	0.18, 0.57	10	.50	0.30, 0.70	-0.15	-0.41, 0.15

^aMPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, 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 candidate method confirmed results 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

DISCUSSION OF THE JULY 2015 MODIFICATION (8)

The results of this study demonstrated the specificity, accuracy and reliability of the modified Veriflow® SS assay as compared to the traditional FDA BAM Chapter 5 and USDA/FSISI MLG chapter 4.06 culture based reference methods (3,4) for the detection of *Salmonella* species on environmental surfaces, raw meat, dairy (2% milk), and in RTE (hot dogs) foods. POD statistical analysis of the matrices tested indicate that there is no significant difference in performance between the methods at specific time points as assayed in this study, and importantly, no false positive or false negative results were observed in the entirety of the study.

Table 1. Veriflow® SS Presumptive vs. Confirmed Results for Environmental Surfaces – POD Results (8)

Matrix	Strain	CFU/Test Area ^a	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Stainless Steel	<i>Salmonella</i> Kentucky/ <i>Citrobacter freundii</i> ATCC 9263/ ATCC 8090	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		55	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		520	5	5	0.60	0.23, .88	5	0.60	0.23, .88	0.00	-0.43, 0.43

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^bN = Number of test portions

^cx = Number of positive test portions

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

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

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

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 2: Veriflow® SS Environmental Surfaces, Candidate vs. Reference – POD Results (8)

Matrix	Strain	CFU/Test Area ^a	N ^b	Candidate			Reference			dPOD _C ^f	95% CI ^g
				x ^c	POD _C ^d	95% CI	X	POD _R ^e	95% CI		
Stainless Steel	<i>Salmonella</i> Kentucky/ <i>Citrobacter freundii</i> ATCC 9263/ ATCC 8090	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		55	20	8	0.40	0.22, 0.61	10	0.50	0.30,0.70	-0.10.	-0.36, 0.19
		550	5	5	1.0	0.57, 1.00	5	1.0	0.57, 1.00	0.00	-0.43, 0.43

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^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 results and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 3. Veriflow® SS Presumptive vs. Confirmed for Raw Meat, RTE Food, Dairy and Chicken Carcasses Matrices – POD Results (8)

Matrix	Strain	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		
Hot Dogs	<i>Salmonella</i> Typhimurium ATCC 14028	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45, 0.45
		.49	20	10	0.50	0.30,0.70	10	0.50	0.30,0.70	0.00	-0.14, 0.14
		3.30	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.45, 0.45
2% Milk	<i>Salmonella</i> Typhimurium ATCC 14028	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45, 0.45
		0.85	20	12	0.60	0.39, 0.75	12	0.60	0.39, 0.75	0.00	-0.14, 0.14
		2.20	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.45, 0.45
Ground Turkey	<i>Salmonella</i> Typhimurium ATCC 14028	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45, 0.45
		.53	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.14, 0.14
		3.00	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.45, 0.45

^aMPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, 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 candidate method confirmed results and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 4: Veriflow® SS Raw Meat, RTE Food, Dairy and Chicken Carcass Matrices Candidate vs. Reference – POD Results (8)

Matrix	Strain	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		
Hot Dogs	<i>Salmonella</i> Typhimurium ATCC 14028	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45, 0.45
		.49	20	10	0.50	0.30,0.70	10	0.50	0.30,0.70	0.00	-0.14, 0.14
		3.30	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.45, 0.45
2% Milk	<i>Salmonella</i> Typhimurium ATCC 14028	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45, 0.45
		0.85	20	12	0.60	0.39, 0.75	12	0.60	0.39, 0.75	0.00	-0.14, 0.14
		2.20	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.45, 0.45
Ground Turkey	<i>Salmonella</i> Typhimurium ATCC 14028	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45, 0.45
		.53	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.14, 0.14
		3.00	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.45, 0.45

^aMPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, 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 candidate method confirmed results 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

DISCUSSION OF MATRIX EXTENSION VALIDATION STUDY AUGUST 2015 (9)

Analysis of the paired enrichment peanut butter RTE meat samples was performed on three inoculation levels of *Salmonella enterica* serotype Typhimurium (ATCC 14028): 0, .35 and 3.74 CFU/25g for the Veriflow® SS assay and the FDA BAM chapter 5 reference method. For the low level of contamination, there were 8 presumptive positive results and 9 confirmed positives (via the FDA BAM chapter 5 method) for the Veriflow® SS samples after 18 hours of enrichment. All un-inoculated control test portions were negative for both methods and all high level test portions were positive for both methods. There were no significant differences between the Veriflow® SS assay results and the USDA/FSIS reference method results based on the POD analysis.

Table 1. Veriflow® SS Presumptive vs. Confirmed for Raw Meat, RTE Food, Dairy and Chicken Carcasses Matrices – POD Result (9)

Matrix	Strain	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		
Peanut Butter	<i>Salmonella</i> Typhimurium ATCC 14028	-	5	0	0.00	0.00,0.43	0	0.00	0.00,0.43	0.00	-0.45,0.45
		.34	20	8	0.40	0.22,0.61	9	0.45	0.26,0.66	-.05	-0.11,0.31
		3.74	5	5	1.00	0.57,1.00	5	1.00	0.57,1.00	0.00	-0.45,0.45

^aMPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, 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 candidate method confirmed results and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 2: Veriflow® SS Raw Meat, RTE Food, Dairy and Chicken Carcass Matrices Candidate vs. Reference – POD Results (9)

Matrix	Strain	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		
Peanut Butter	<i>Salmonella</i> Typhimurium ATCC 14028	-	5	0	0.00	0.00,0.43	0	0.00	0.00,0.43	0.00	-0.45,0.45
		0.34	20	8	0.40	0.22,0.61	9	0.45	0.26,0.66	-.05	-0.11,0.35
		3.74	5	5	1.00	0.57,1.00	5	1.00	0.57,1.00	0.00	-0.45,0.45

^aMPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, 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 candidate method confirmed results 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

DISCUSSION OF MATRIX EXTENSION VALIDATION STUDY JANUARY 2016 Black Pepper (10)

The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow® SS assay as compared to the traditional FDA BAM Chapter 5 culture based reference method (2) for the detection of *Salmonella* in ground black pepper. POD statistical analysis indicate that there is no significant difference in performance between the method at specific time points as assayed in this study.

The Veriflow® SS assay provides flexibility and ease of use for the end user by providing accurate results across multiple surfaces with sampling by either swabs or sponges, and across multiple food matrices, without complex sample preparation after enrichment. The Veriflow® system also offers significant savings in time compared to the reference method used in this study for the analysis of spice, by producing accurate presumptive results after an enrichment time of 24 hours, as compared to the reference methods that require 3-4 days to reach presumptive results. Thus the results of this study demonstrated that the easy to follow Veriflow® SS protocol provides for a sensitive, reliable and simple to use rapid detection method for *Salmonella* species in ground black pepper.

Table 1. Veriflow® SS Presumptive vs. Confirmed for Black Ground Pepper – POD Result (10)

Matrix	Strain	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		
Ground Black Pepper	<i>Salmonella</i> Abeatetuba ATCC 35640	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.451,0.451
		.63	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.139,0.139
		2.73	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.451,0.451

^aMPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, 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 candidate method confirmed results and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 2: Veriflow® SS Ground Black Pepper Matrix Candidate vs. Reference – POD Results (10)

Matrix	Strain	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		
Ground Black Pepper	<i>Salmonella</i> Abeatetuba ATCC 35640	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.45,0.45
		0.63	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.11,0.35
		2.73	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.45,0.45

^aMPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, 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 candidate method confirmed results 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

DISCUSSION OF MATRIX EXTENSION VALIDATION STUDY JANUARY 2016 Whey Protein (11)

The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow® O157:H7 assay as compared to the traditional FDA BAM Chapter 4A and culture based reference method for the detection of *E. coli* O157:H7 in whey protein isolate. POD statistical analysis indicate that there is no significant difference in performance between the methods at specific time points as assayed in this study, and importantly, no false positive or false negative results were observed in the study.

The Veriflow® O157:H7 assay provides flexibility and ease of use for the end user by providing accurate results across multiple food matrixes, without complex sample preparation after enrichment. The Veriflow® system also offers significant savings in time compared to the reference methods used in this study, by producing accurate presumptive results after an enrichment time of only 18-20 hours, as compared to the reference methods that require 3-4 days to reach presumptive results. 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. Thus the results of this study demonstrated that the easy to follow Veriflow® O157:H7 protocol provides for a sensitive, reliable and simple to use rapid detection method for *E. coli* O157:H7.

Table 1: Veriflow® O157:H7 Assay, Candidate vs. Reference – POD Results (11)

Matrix	Analysis Time Point	Strain	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		
Whey protein isolate powder	20 h	<i>E. coli</i> O157:H7 ATCC ^h 35150	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.97	20	14	0.70	0.48, 0.85	12	0.6	0.42, 0.75	0.10	-0.17, 0.33
			2.85	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 reference method test portions using the LCF MPN calculator version 1.6, 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.

^hAmerican Type Culture Collection, Manassas, VI.

Table 2: Veriflow® O157:H7 Assay, Presumptive vs. Confirmed – POD Results (11)

Matrix	Analysis Time Point	Strain	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		
Whey protein isolate powder	20 h	<i>E. coli</i> O157:H7 ATCC ^h 35150	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.97	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.28, 0.28
			2.85	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 reference method test portions using the LCF MPN calculator version 1.6, 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.

^hAmerican Type Culture Collection, Manassas, VI.

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