



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**042002**

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

**Molecular Environmental Monitoring Program (MEMP) *Salmonella* Assay**

manufactured by

**Applied Food Diagnostics, Inc.**

**387 Hazle Street**

**Nuremberg, PA**

**USA**

This method has been evaluated in the AOAC<sup>®</sup> *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*<sup>SM</sup> 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 (April 14, 2020 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

*Scott Coates*

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

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April 24, 2020  
Date

<b>METHOD AUTHORS</b> Thomas Lonczynski and Laura Cowin	<b>SUBMITTING COMPANY</b> Applied Food Diagnostics, Inc. 387 Hazle Street Nuremberg, PA USA
<b>KIT NAME(S)</b> Molecular Environmental Monitoring Program (MEMP) <i>Salmonella</i> Assay	<b>CATALOG NUMBERS</b> MEMP-SAL-032- MEMP <i>Salmonella</i> Assay Kit MEMP-SWB-032 – AFD MEMP Swab Kit
<b>INDEPENDENT LABORATORY</b> Q Laboratories 1930 Radcliff Drive Cincinnati, OH 45204 USA	<b>AOAC EXPERTS AND PEER REVIEWERS</b> Thomas Hammack <sup>1</sup> , Michael Brodsky <sup>2</sup> , Wayne Ziemer <sup>3</sup> <sup>1</sup> US FDA CFSAN, College Park, MD, USA <sup>2</sup> Brodsky Consultants, Ontario, CANADA <sup>3</sup> Independent Consultant, Loganville, MD, USA
<b>APPLICABILITY OF METHOD</b> Analyte – <i>Salmonella</i> species  Matrices – Stainless steel (1”x 1”), plastic (1”x 1”), rubber (1”x 1”), ceramic tile (1”x 1”) and sealed concrete (1”x 1”)  Performance claims - Performance equivalent to that of the U.S. Food and Drug Administration (FDA) <i>Bacteriological Analytical Manual</i> (BAM) Chapter 5 (2) for environmental surface swabs.	<b>REFERENCE METHOD</b> Food and Drug Administration <i>Bacteriological Analytical Manual, Salmonella</i> , Chapter 5 (2018) <a href="https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm">https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm</a> (2)
<b>ORIGINAL CERTIFICATION DATE</b> April 14, 2020	<b>CERTIFICATION RENEWAL RECORD</b> New Approval
<b>METHOD MODIFICATION RECORD</b> NONE	<b>SUMMARY OF MODIFICATION</b> NONE
Under this AOAC® <i>Performance Tested</i> <sup>SM</sup> License Number, 042002 this method is distributed by: <b>NONE</b>	Under this AOAC® <i>Performance Tested</i> <sup>SM</sup> License Number, 042002 this method is distributed as: <b>NONE</b>

**PRINCIPLE OF THE METHOD (1)**

For qPCR amplification and detection, forward and reverse primers hybridize to a unique sequence *Salmonella* genomic DNA. A fluorogenic probe consisting of a DNA probe labeled with a 5'-dye and a 3'-quencher is included in the same reaction mixture. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on the real-time PCR instrument. Unique primer and probe mixtures specific for *Salmonella* are present in this assay.

## DISCUSSION OF THE VALIDATION STUDY (1)

### *Inclusivity and Exclusivity*

Of the 100 inclusivity strains analyzed by the MEMP *Salmonella* Assay, all 100 inclusivity strains were correctly detected by the appropriate PCR target within the *Salmonella* assay kit. Of the 30 exclusivity strains, none were detected by the *Salmonella* MEMP method. See Tables 1 and 2.

### *Matrix Study*

Results from both the method developer and independent studies of the MEMP *Salmonella* Assay for the environmental samples are outlined in Tables 3 and 4. Throughout the study, the method developer had difficulty achieving fractional results, and repeat attempts had to be made to spike at the correct inoculation levels. Environmental surfaces proved challenging to recover cells off the surface at low levels due to the humidity in the environment. Multiple trials were run for some of the surfaces to achieve fractional levels.

For stainless steel, a 1" x 1" area was tested using a swab for the candidate method. For the high inoculation level, the swab detected all five high positive samples, which all confirmed. For the low inoculation level, the swab detected 13 presumptive results in the 1" x 1" area, 10 of which confirmed. The MEMP swabs are able to collect all cell types, including live and dead cells. For the presumptive positive test portions that did not confirm, it's likely that dead cells were present, leading to a positive PCR reaction, but these cells were not enriched. The FDA BAM Chapter 5 method had 10 confirmed results, which showed no statistically significant difference when analyzed using POD analysis.

The plastic was swabbed in a 1" x 1" area for the candidate method. Five samples had presumptive calls, and all presumptive swabs confirmed. The FDA BAM Chapter 5 method had five samples confirm positive, leading to no statistically significant differences between the two methods.

The rubber was also swabbed in a 1" x 1" area for the candidate method. For the low-level inoculation, nine samples were presumptive on the PCR, all nine of which confirmed positive. For the FDA BAM Chapter 5 method, 9 samples confirmed. When performing the POD analysis on the two methods, no statistically significant differences were observed.

For ceramic, a 1" x 1" area was swabbed for the candidate method. For the low inoculation level, the candidate had fifteen presumptive calls, and fourteen of those presumptive results confirmed. The FDA BAM Chapter 5 method had eleven confirmed positives. For ceramic, the candidate method had more confirmed positives than the reference method, and POD analysis showed no statistical difference.

For concrete, 1" x 1" surface was also swabbed for the candidate method. For the low inoculation set, the candidate method had nine presumptive calls, seven of which confirmed. Again, it is likely that MEMP assay is detecting dead cells from the surface. The FDA BAM Chapter 5 reference method had five confirmed calls. POD analysis did not show any statistically significant differences in the two methods.

### *Independent Laboratory Study*

In the independent study, there were 10 presumptive positive results at the low level, and 10 confirmed results, indicating no difference between the confirmed and presumptive results. For the reference method, there were 8 positive results at the low level. The POD analysis between the MEMP Assay and the reference method indicated that there was no significant difference, with 95% confidence. A summary of POD analyses is presented in Tables 3 and 4.

## Data from validation study

Table 1. Inclusivity List – *Salmonella* Strains (1)

No.	Genus	Species	Serovar	Source	Origin	MEMP Result
1	<i>Salmonella</i>	<i>enterica</i> subsp. <i>houtenae</i>	1, 40:g, z51:-	SGSC <sup>c</sup> 3120	Unknown, Tonga	+
2	<i>Salmonella</i>	<i>enterica</i> subsp. <i>indica</i>	11:b:e, n, x	SGSC 3118	Unknown	+
3	<i>Salmonella</i>	<i>enterica</i> subsp. <i>houtenae</i>	16:z4,z32:-	SGSC 3086	Human, Illinois	+
4	<i>Salmonella</i>	<i>enterica</i> subsp. <i>diarizonae</i>	38[k]:z35:-	SGSC 3069	Human, California	+
5	<i>Salmonella</i>	<i>enterica</i> subsp. VII	40:z4, z24:-	SGSC 3121	Human, Florida	+
6	<i>Salmonella</i>	<i>enterica</i> subsp. <i>salamae</i>	42:f:g,t:-	SGSC 3047	Unknown	+
7	<i>Salmonella</i>	<i>enterica</i> subsp. <i>indica</i>	45:a:e, n, x	SGSC 3116	Unknown, India	+
8	<i>Salmonella</i>	<i>enterica</i> subsp. IV	45a,b:g,z32:-	SGSC 3074	Animal, Canal Zone	+
9	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Agona	SGSC 2458	Unknown, Peru	+
10	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Anatum	SGSC 2459	Human, Washington	+
11	<i>Salmonella</i>	<i>enterica</i> subsp. <i>arizonae</i>	62:z4,z23: - -	SGSC 3061	Corn snake, Oregon	+
12	<i>Salmonella</i>	<i>enterica</i> subsp. <i>arizonae</i>	62:z36: - -	SGSC 3063	Human, California	+
13	<i>Salmonella</i>	<i>enterica</i> subsp. <i>arizonae</i>	51:z(4),z(23):(-)	SGSC 2426	Unknown	+
14	<i>Salmonella</i>	<i>enterica</i> subsp. <i>salamae</i>	Bleadon	SGSC 2415	Unknown	+
15	<i>Salmonella</i>	<i>enterica</i> subsp. <i>salamae</i>	Bloemfontein	SGSC 2414	Unknown	+
16	<i>Salmonella</i>	<i>bongori</i>	48:z35:-	SGSC 2556	Unknown	+
17	<i>Salmonella</i>	<i>bongori</i>	66:z41: - -	SGSC 3100	Frog	+
18	<i>Salmonella</i>	<i>bongori</i>	48:z41: - -	SGSC 3103	Parakeet, USA	+
19	<i>Salmonella</i>	<i>bongori</i>	66:z(41):(-)	SGSC 2429	Unknown	+
20	<i>Salmonella</i>	<i>bongori</i>	66:z41:-	SGSC 2557	Unknown	+
21	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Choleraesuis	SGSC 2461	Swine, Minnesota	+
22	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Decatur	SGSC 2465	Unknown, France	+
23	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Derby	SGSC 2466	Avian, Oklahoma	+
24	<i>Salmonella</i>	<i>enterica</i> subsp. <i>diarizonae</i>	6,7:1,v:z(53)	SGSC 2427	Unknown	+
25	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Dublin	SGSC 3611	Unknown	+

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26	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Bredeney	SGSC 4931	Unknown	+
27	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Duisberg	SGSC 2472	Unknown, Scotland	+
28	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Emek	SGSC 2477	Unknown, Israel	+
29	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Newport	ATCC <sup>b</sup> 6962	Food poisoning fatality	+
30	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Enteritidis	ATCC 13076	Unknown	+
31	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Montevideo	ATCC 8387	Unknown	+
32	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Schwarzengrund	BEI <sup>c</sup> NR-28796	Human, Oregon	+
33	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Typhi	BEI NR-513	USA	+
34	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Typhimurium	USDA ERRC <sup>d</sup> B-4211	Ames, B. N. (U California, Davis, CA)	+
35	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Poona	SGSC 4934	Unknown	+
36	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Blockley	SGSC 4935	Unknown	+
37	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Livingstone	SGSC 4937	Unknown	+
38	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Hvittingfoss	SGSC 4947	Unknown	+
39	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Bareilly	SGSC 4949	Unknown	+
40	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Mbandaka	SGSC 2958	Unknown	+
41	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Setubal	SGSC 2567	Unknown, Mississippi	+
42	<i>Salmonella</i>	<i>enterica</i> subsp. <i>indica</i>	Ferlac	SGSC 2581	Unknown	+
43	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Flint	SGSC 2554	Unknown	+
44	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Gallinarum	SGSC 2478	Human, Connecticut	+
45	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Haifa	SGSC 2479	Unknown, Scotland	+
46	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Heidelberg	SGSC 2480	Chicken, Pennsylvania	+
47	<i>Salmonella</i>	<i>enterica</i> subsp. <i>houtenae</i>	45:g,z(51):-	SGSC 2428	Unknown	+
48	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Indiana	SGSC 2482	Unknown, Scotland	+
49	<i>Salmonella</i>	<i>enterica</i> subsp. <i>indica</i>	1,6,14,25:a:e,n,x	SGSC 2430	Unknown	+
50	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Infantis	SGSC 2483	Human, North Carolina	+
51	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Argentina	SGSC 2555	Unknown	+
52	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Paratyphi B var. Java	SGSC 4951	Unknown	+
53	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Krefeld	SGSC 4945	Unknown, France	+

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54	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Malawi	SGSC 2577	Unknown	+
55	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Maregresso	SGSC 2578	Unknown	+
56	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Miami	SGSC 2486	Human, French Guiana	+
57	<i>Salmonella</i>	<i>enterica</i> subsp. <i>salamae</i>	58:d:z	SGSC 3039	Human, Massachusetts	+
58	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Muncheon	SGSC 2490	Human, France	+
59	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Naestved	SGSC 3612	Unknown	+
60	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Newport	SGSC 2493	Human, North Carolina	+
61	<i>Salmonella</i>	<i>enterica</i> subsp. <i>diarizonae</i>	50:k:z	SGSC 3068	Human, Oregon	+
62	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Ohio	SGSC 4943	Unknown	+
63	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Panama	SGSC 3583	Unknown	+
64	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Paratyphi A	SGSC 4568	Unknown	+
65	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Paratyphi B	SGSC 4567	Unknown	+
66	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Paratyphi C	SGSC 2290	Unknown	+
67	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Oranienburg	BEI NR-171	Human, Illinois	+
68	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Javiana	BEI NR-4296	Human, Pennsylvania	+
69	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Pullorum	SGSC 2508	Unknown, Germany	+
70	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Reading	SGSC 2510	Unknown, Scotland	+
71	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Rissen	SGSC 4941	Unknown	+
72	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Rubinslaw	SGSC 2511	Unknown, Laboratory Strain	+
73	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	S.4, 5, 12:i:-	SGSC 4956	Unknown	+
74	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Saintpaul	SGSC 2512	Human, Texas	+
75	<i>Salmonella</i>	<i>enterica</i> subsp. <i>salamae</i>	1,9,12:1,w:e,n,x	SGSC 2425	Unknown	+
76	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Abortusoris	BEI NR-13556	Unknown, France	+
77	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Sendai	SGSC 2515	Human, California	+
78	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Senftenberg	SGSC 2516	Chicken, Maryland	+
79	<i>Salmonella</i>	spp.	44:z39: - -	SGSC 3105	Food, Ghana	+
80	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Stanley	SGSC 2517	Unknown, Scotland	+
81	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Stanleyville	SGSC 2518	Unknown, Scotland	+

82	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Tennessee	SGSC 4939	Unknown	+
83	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Thompson	SGSC 2519	Human, Florida	+
84	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Hadar	BEI NR-28799	Human, Massachusetts	+
85	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Virchow	BEI NR-28801	Human, Connecticut	+
86	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Kentucky	BEI NR-28795	Human, Wisconsin	+
87	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Typhisuis	SGSC 2526	Swine, California	+
88	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Vrindaban	SGSC 2582	Unknown	+
89	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Wassenaar	SGSC 2576	Unknown	+
90	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Weltevreden	SGSC 4929	Unknown	+
91	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Wien	SGSC 2529	Human, France	+
92	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i>	Brandenberg	SGSC 2460	Unknown, Scotland	+
93	<i>Salmonella</i>	<i>enterica</i> subsp. <i>indica</i>	45:a:e,n,x	ATCC BAA-1578	India	+
94	<i>Salmonella</i>	<i>enterica</i> subsp. <i>diarizonae</i>	6,7:l,v:z53	ATCC 43973	Unknown	+
95	<i>Salmonella</i>	<i>enterica</i> subsp. <i>dublin</i>	1,9,12,[Vi]:g,p: - -	SGSC 2471	Unknown, Thailand	+
96	<i>Salmonella</i>	<i>enterica</i> subsp. <i>houtenae</i>	11:z4,z23:	ATCC 15788	Water, Bonaire	+
97	<i>Salmonella</i>	<i>enterica</i> subsp. <i>salamae</i>	9,46:z4z24:z39:z42	ATCC 15793	Lizard, Netherlands	+
98	<i>Salmonella</i>	<i>enterica</i> subsp. <i>salamae</i>	55:k:z39	ATCC 700148	Unknown	+
99	<i>Salmonella</i>	<i>enterica</i> subsp. <i>wien</i>	1,4,12,27:b:l,w	SGSC 2528	Human, France	+
100	<i>Salmonella</i>	<i>enterica</i> subsp. <i>diarizonae</i>	IIIb 47:i:z53:z57	ATCC 12325	CDC	+

<sup>a</sup>Genetic Stock Centre, University of Calgary, Canada.

<sup>b</sup>American Type Culture Collection, Manassas, VA.

<sup>c</sup>BEI Resources, Manassas, VA.

<sup>d</sup>United States Department of Agriculture Eastern Regional Research Center, Wyndmoor, PA.

**Table 2. Exclusivity List – Non-Salmonella Strains (2)**

No.	Genus	Species	Source	Origin	MEMP Result
1	<i>Bacillus</i>	<i>cereus</i>	BEI <sup>a</sup> NR-2488	Blood	-
2	<i>Bacillus</i>	<i>subtilis</i>	BEI NR-607	Unknown	-
3	<i>Bacillus</i>	<i>pumilus</i>	BEI NR-605	Unknown	-
4	<i>Carnobacterium</i>	<i>divergens</i>	ATCC <sup>b</sup> 35677	Vacuum-packed minced beef	-
5	<i>Citrobacter</i>	<i>freundii</i>	ATCC 43864	Unknown	-
6	<i>Citrobacter</i>	<i>koseri</i>	SGSC <sup>c</sup> 5610	Clinical	-
7	<i>Ewingella</i>	<i>americana</i>	SGSC 5640	Human feces	-
8	<i>Enterobacter</i>	<i>cloacae</i>	SGSC 5330	Unknown	-
9	<i>Enterobacter</i>	<i>aerogenes</i>	SGSC 5347	Unknown	-
10	<i>Enterobacter</i>	<i>taylorae</i>	SGSC 5283	Unknown	-
11	<i>Escherichia</i>	<i>coli</i> O157:H7	SGSC 5982	Unknown	-
12	<i>Escherichia</i>	<i>coli</i> O1:K1:H(-)	SGSC 2811	Unknown	-
13	<i>Escherichia</i>	<i>fergusoni</i>	SGSC 5718	Human feces	-
14	<i>Klebsiella</i>	<i>oxytoca</i>	SGSC 5366	Unknown	-
15	<i>Klebsiella</i>	<i>pneumoniae</i> subsp. <i>pneumoniae</i>	SGSC 5926	Unknown	-
16	<i>Klebsiella</i>	<i>planticola</i>	SGSC 5929	Radish root	-
17	<i>Pseudomonas</i>	<i>aeruginosa</i>	BEI NR-48982	Human	-
18	<i>Proteus</i>	<i>mirabilis</i>	SGSC 5445	Unknown	-
19	<i>Providencia</i>	<i>stuartii</i>	SGSC 5639	Clinical	-
20	<i>Serratia</i>	<i>marcescens</i>	SGSC 5354	Unknown	-
21	<i>Serratia</i>	<i>odorifera</i>	SGSC 5720	Sputum	-
22	<i>Shigella</i>	<i>flexneri</i>	SGSC 5577	Unknown	-
23	<i>Streptococcus</i>	<i>pyogenes</i>	BEI NR-51272	Human	-
24	<i>Staphylococcus</i>	<i>aureus</i>	BEI NR-46412	Urine	-
25	<i>Streptococcus</i>	<i>bovis</i>	USDA <sup>d</sup> ERRC B-23788	Diseased bovine tissue	-
26	<i>Yersinia</i>	<i>enterocolitica</i>	USDA ERRC B-41479	Ground beef	-
27	<i>Listeria</i>	<i>monocytogenes</i> 4c	BEI NR-111	Chicken	-
28	<i>Listeria</i>	<i>marthii</i>	BEI NR-9582	River water	-
29	<i>Escherichia</i>	<i>coli</i> O121:H19	BEI NR-17630	Human	-
30	<i>Escherichia</i>	<i>hermanii</i>	SGSC 5715	Human toe	-

<sup>a</sup>BEI Resources, Manassas, VA.

<sup>b</sup>American Type Culture Collection, Manassas, VA.

<sup>c</sup>*Salmonella* Genetic Stock Centre, University of Calgary, Canada.

<sup>d</sup>United States Department of Agriculture Eastern Regional Research Center, Windsor, PA.



**Table 3. MEMP *Salmonella* Assay Presumptive vs. Confirmed Results (1)**

Matrix	Strain	CFU <sup>a</sup> /test area	MEMP <i>Salmonella</i> presumptive results				MEMP <i>Salmonella</i> confirmed results				
			N <sup>b</sup>	x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI	dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
Stainless steel (1" x 1")	<i>S. Heidelberg</i> , B (SGSC <sup>h</sup> 2480)/10X C. <i>freundii</i> <sup>i</sup> (ATCC 43864)	N/A <sup>j</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		125 & 1500	20	13	0.65	0.43, 0.82	10	0.50	0.30, 0.70	0.15	-0.15, 0.41
		500 & 10000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel <sup>k</sup> (1" x 1")	<i>S. Heidelberg</i> , B (SGSC 2480)/10X C. <i>freundii</i> (ATCC 43864)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		66 & 780	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		170 & 2800	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic (1" x 1")	<i>S. Montevideo</i> , C <sub>1</sub> (ATCC 8387)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		75	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
		500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Rubber (1" x 1")	<i>S. Panama</i> , D <sub>1</sub> (SGSC 3583)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		125	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic tile (1" x 1")	<i>S. Poona</i> , G <sub>1</sub> (SGSC 3583)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		115	20	15	0.75	0.53, 0.89	14	0.70	0.48, 0.85	0.05	-0.22, 0.31
		500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sealed concrete (1" x 1")	<i>S. Anatum</i> , E <sub>1</sub> (SGSC 2459)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		125	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		400	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU = Colony forming units per test 1" x 1" test area

<sup>b</sup>N = Number of test portions.

<sup>c</sup>x = Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>*Salmonella* Genetic Stock Centre, University of Calgary, Canada.

<sup>i</sup>ATCC = American Type Culture Collection, Manassas. VA.

<sup>j</sup>N/A = Not applicable.

<sup>k</sup>Matrix tested in the independent laboratory.

Table 4. MEMP *Salmonella* Assay vs. Reference Method BAM Ch. 5 Results (1)

Matrix	Strain	CFU <sup>a</sup> /test area	N <sup>b</sup>	MEMP <i>Salmonella</i> results			Reference method results			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
				x <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	x	POD <sub>R</sub> <sup>e</sup>	95% CI		
Stainless steel (1" x 1")	<i>S. Heidelberg</i> , B (SGSC <sup>h</sup> 2480)/10X C. <i>freundii</i> (ATCC <sup>i</sup> 43864)	N/A <sup>j</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		125 & 1500	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		500 & 10000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel <sup>k</sup> (1" x 1")	<i>S. Heidelberg</i> , B (SGSC 2480)/10X C. <i>freundii</i> (ATCC 43864)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		66 & 780	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
		170 & 2800	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic (1" x 1")	<i>S. Montevideo</i> , C <sub>1</sub> (ATCC 8387)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		75	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
		500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Rubber (1" x 1")	<i>S. Panama</i> , D <sub>1</sub> (SGSC 3583)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		125	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic tile (1" x 1")	<i>S. Poona</i> , G <sub>1</sub> (SGSC 3583)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		115	20	14	0.70	0.48, 0.85	11	0.55	0.34, 0.74	0.15	-0.14, 0.41
		500	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
Sealed concrete (1" x 1")	<i>S. Anatum</i> , E <sub>1</sub> (SGSC 2459)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		125	20	7	0.35	0.18, 0.57	5	0.25	0.11, 0.47	0.10	-0.18, 0.36
		400	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU = Colony forming units per test 1" x 1" test area.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>x = Number of positive test portions.

<sup>d</sup>POD<sub>c</sub> = Candidate method presumptive positive outcomes that confirmed positive divided by the total number of trials.

<sup>e</sup>POD<sub>R</sub> = Reference method presumptive positive outcomes divided by the total number of trials.

<sup>f</sup>dPOD<sub>c</sub> = Difference between the candidate method result and reference method result POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>*Salmonella* Genetic Stock Centre, University of Calgary, Canada.

<sup>i</sup>ATCC = American Type Culture Collection, Manassas, VA.

<sup>j</sup>N/A = Not applicable.

<sup>k</sup>Matrix tested in the independent laboratory.

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