



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.

022302

The AOAC Research Institute hereby certifies the method known as

InSiteTM Salmonella

manufactured by

Hygiena LLC

941 Avenida Acaso,

Camarillo, CA 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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METHOD NAME InSite™ <i>Salmonella</i>	CATALOG NUMBER ISO50
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INDEPENDENT LABORATORY Q.Laboratories 1930 Radcliff Drive Cincinnati, OH 45204
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APPLICABILITY OF METHOD Target organism – <i>Salmonella enterica</i> . Matrixes – stainless steel (12 in x 12 in, 4 in x 4 in), rubber (4 in x 4 in), plastic (4 in x 4 in), ceramic (4 in x 4 in), sealed concrete (4 in x 4 in) Performance claims – The study data were unable to find a statistically detectable difference from zero between Hygiena’s InSite <i>Salmonella</i> and ISO 6579-1:2017, <i>Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. reference method (2)</i> , with the exception of ceramic 4" x 4" high inoculum level where InSite <i>Salmonella</i> detected more positive results.	REFERENCE METHOD ISO 6579-1:2017, <i>Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. (2)</i>
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ORIGINAL CERTIFICATION DATE February 17, 2023	CERTIFICATION RENEWAL RECORD Renewed through December 2026.
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METHOD MODIFICATION RECORD 1. December 2024 Level 1	SUMMARY OF MODIFICATION 1. Editorial changes.
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Under this AOAC <i>Performance Tested Methods</i> SM License Number, 022302 this method is distributed by: NONE	Under this AOAC <i>Performance Tested Methods</i> SM License Number, 022302 this method is distributed as: NONE
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PRINCIPLE OF THE METHOD (1)
Hygiena’s InSite *Salmonella* device is a self-contained swab and screening test for the detection of *Salmonella enterica*. on selected food preparation and processing surfaces. The medium in the test apparatus contains a proprietary blend of selective agents and metabolic substrates that promotes the selective growth of *Salmonellae*, while inhibiting the growth of other Gram-negative and Gram-positive bacteria. As the population of *Salmonellae* increases in the medium, the pH of the medium lowers. This causes the pH indicators found in the medium to turn from purple to a bright yellow color. A visual color change from purple to bright yellow after 24–48 h at 37 ± 1°C indicates a presumptive positive test for *Salmonella spp.*.

DISCUSSION OF THE VALIDATION STUDY (1)

Hygiene's InSite *Salmonella* product is an easy-to-use screening test device for the detection of *Salmonella enterica* on environmental surfaces. The medium contains a proprietary blend of ingredients that selectively promote the growth of *Salmonella* while inhibiting growth of non-target organisms. If *Salmonella* are present in the medium their growth will decrease the pH of the medium which will cause the pH indicator in the medium to turn from purple to yellow. This presumptive positive result can be observed after 24–48 h at 37°C of incubation.

The inclusivity study showed that all *Salmonella enterica* isolates tested were detectable by InSite *Salmonella* within 48 h of incubation. The inoculum levels reported in Table 1 and 2, are cfu/mL; however, 100 µL was spiked onto the swab so the detection level is reduced tenfold. Taking that into account, the majority of strains were detected at 10–20 cfu/device. There were 18 strains detected below 10 cfu/device some as little as 5 cfu/device (*S. Typhi* ATCC 19430 and *S. Betioky* DD 1525). The InSite *Salmonella* device is capable of detection of *S. Typhi* ATCC 19430, *S. Paratyphi C* ATCC 13428, *S. Paratyphi B* R-SAL-41 and *S. Choleraesuis* ATCC 10458, dangerous human pathogens capable of causing severe disease (10).

In the exclusivity study two non-target strains produced presumptive positive results. Those two strains were *Citrobacter braakii* ATCC 6750 and *Raoultella planticola* ATCC 33531.

The InSite *Salmonella* device successfully detected *Salmonella enterica* from stainless steel matrixes when challenged with *C. freundii*, a Gram-negative bacterium, closely related competitor organism. The 12" x 12" surface area is the suggested size to test in the InSite *Salmonella* kit insert, as the larger test area is more applicable to testing conducted by customers, whereas the 4" x 4" size is dictated by the reference method ISO 6579-1:2017 guidelines.

The 12" x 12" stainless steel matrix sampled by InSite *Salmonella* was compared to the 4" x 4" stainless steel surface area as outlined in the reference method ISO 6579-1:2017. The POD analysis revealed no statistically significant difference in the results at any inoculum level. These results were also confirmed by the independent laboratory study findings. The test portions inoculated with 0 cfu/mL of target analyte but inoculated with 10⁴-60⁴ cfu/test area of competitor organism, were all negative using InSite *Salmonella*. The test portions inoculated with target analyte and co-inoculated with competitor organism at 10x higher level, were all recovered at the high inoculum level and had 15 of 20 positive results at the fractional recovery level.

The 4" x 4" stainless steel matrix study conducted by the method developer equally saw no statistically significant difference at any inoculum level. These results were also confirmed by the independent laboratory study findings. Each positive and negative InSite *Salmonella* result was confirmed using the ISO 6579-1:2017 reference method, nothing was recovered from the negative swabs, indicating that the results were negative due to no *Salmonella* being recovered by the swab. The robustness study demonstrated that InSite *Salmonella* will continue to produce accurate results even if the usage guidelines provided are not followed exactly. Although, it is still recommended to follow the kit insert guidelines closely (6 h pre-enrichment prior to activation, 3 s shake time and incubation at 37°C) to achieve the most accurate and reproducible results. The consistency and stability study has validated that the results from InSite *Salmonella* will remain accurate across different manufacturing batches across the shelf life (up to one year) of the product.

Table 1. Inclusivity results of the 100 strains tested for InSite *Salmonella*. (1)

No.	Serovar	Identifying Number	Sero-group	Source	Origin	cfu /mL	Result ^a
1	<i>Salmonella enterica</i> subsp. <i>arizonae</i>	ATCC 13314	O51	PHE ^b	Unknown	121	Positive
2	<i>Salmonella enterica</i> subsp. <i>arizonae</i>	ATCC 12324	R	ATCC ^c	Unknown	71	Positive
3	<i>Salmonella enterica</i> subsp. <i>arizonae</i> serotype IIIa 51:g,z51	SAFE-23	O51	HCC ^d	Unknown	161	Positive
4	<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	ATCC 43973	C1	ATCC	Unknown	97	Positive
5	<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	ATCC 12325	X	ATCC	Unknown	64	Positive
6	<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	ATCC BAA-216	O	ATCC	Human blood	129	Positive
7	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Abaetetuba	ATCC 35640	F	ATCC	Creek water	125	Positive
8	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Adelaide	NCTC 6386	E4	PHE	Human feces	163	Positive
9	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Agama	DD 3218	B	HCC	Cocoa Bean Environment	132	Positive
10	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Agona	DD 13743	B	GPLN ^e	Unknown	177	Positive
11	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Alabama	DD 13731	D1	GPLN	Unknown	132	Positive
12	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Alachua	DD 1556	O	HCC	Soil, abattoir	124	Positive
13	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Albany	DD 6735	C3	HCC	Sesame seeds	203	Positive
14	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Anatum	NCTC 3072	E1	PHE	Bird/Duckling	72	Positive
15	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Anfo	DD 1429	Q	HCC	African Meat Box (1967)	99	Positive
16	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Arkansas	DD 6177	E3	HCC	Chicken giblets	184	Positive
17	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Berkeley	DD 1523	U	HCC	Diseased Turkey	115	Positive
18	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Berta	DD 13730	D1	GPLN	Unknown	125	Positive
19	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Blegdam	DD 737	D1	HCC	Unknown	106	Positive
20	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Bovis-Morbificans	DD 1509	C2	HCC	Unknown	109	Positive
21	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Braenderup	DD 1337	C1	HCC	Chicken	126	Positive
22	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Brandenburg	DD 13746	B	GPLN	Unknown	133	Positive

23	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Bredeney	DD 964	B	HCC	Fresh Chicken	146	Positive
24	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Broughton	DD 3882	E4	HCC	Poultry feed	143	Positive
25	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Carmel	DD 1620	O:17	HCC	Unknown	118	Positive
26	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Cerro	ATCC 10723	K	ATCC	Unknown	101	Positive
27	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Champaign	DD 1623	Q	HCC	Liver of Hen	93	Positive
28	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Chester	DD 1625	B	HCC	Unknown	119	Positive
29	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Choleraesuis	ATCC 10708	C1	ATCC	Unknown	73	Positive
30	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Cubana	DD 13828	G	GPLN	Unknown	152	Positive
31	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Dublin	NCTC 12710	D1	PHE	Unknown	148	Positive
32	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Durban	DD 1641	D1	HCC	Feces	96	Positive
33	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Ealing	DD 1644	O	HCC	Dried Baby Milk (1985-1986)	121	Positive
34	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Enteritidis	DD 13759	D1	GPLN	Unknown	113	Positive
35	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Frintrop	DD 1428	D1	HCC	Animal Feed	111	Positive
36	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Give	DD 13813	E	GPLN	Unknown	131	Positive
37	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Haardt	DD 3915	C3	HCC	Broiler Breeders	135	Positive
38	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Hadar	NCTC 9877	C2	PHE	Unknown	105	Positive
39	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Hartford	DD 2290	C1	HCC	Cheesecake	94	Positive
40	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Heidelberg	NCTC 5717	B	PHE	Unknown	155	Positive
41	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Hvittingfoss	DD 3699	I	HCC	Herbs/Spices	205	Positive
42	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Indiana	DD 13915	B	HCC	Ground Turkey	121	Positive
43	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Infantis	ATCC BAA-1675	C1	ATCC	Unknown	131	Positive
44	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Javiana	ATCC BAA-1593	D1	ATCC	Tomato outbreak	142	Positive
45	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Kedougou	DD 1251	G2	HCC	Turkey	140	Positive
46	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Kentucky	NCTC 5799	C3	PHE	Unknown	122	Positive
47	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Kiambu	DD 13747	B	GPLN	Unknown	128	Positive
48	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Lexington	NCTC 6244	E1	PHE	Unknown	137	Positive
49	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Lille	DD 2263	C1	HCC	Pancake	109	Positive
50	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Liverpool	DD 13810	E	GPLN	Unknown	166	Positive
51	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Livingstone	DD 1650	C1	HCC	Feces	120	Positive
52	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Maastricht	ATCC 15789	F	ATCC	Fishmeal	156	Positive
53	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Madelia	DD 1698	H	HCC	Liver of Hen	136	Positive
54	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Manchester	DD 1424	C2	HCC	Autolysed Yeast	115	Positive
55	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Manhattan	DD 2673	C3	HCC	Avian	162	Positive
56	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Mbandaka	DD 13738	C1	GPLN	Unknown	143	Positive
57	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Miami	DD 13734	D1	GPLN	Unknown	131	Positive
58	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Mississippi	DD 1703	G	HCC	Feces from 1942	153	Positive
59	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Montevideo	NCTC 5747	C1	PHE	Unknown	160	Positive
60	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Muenster	DD 2748	E1	HCC	Chicken	187	Positive
61	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Newport	ATCC 6962	C2	ATCC	Food poisoning fatality	158	Positive

62	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Nottingham	NCTC 7832	I	PHE	Unknown	155	Positive
63	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Ohio	DD 2735	C1	HCC	Protein supplement for feed	190	Positive
64	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Ouakam	DD 13721	D2	GPLN	Unknown	134	Positive
65	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Panama	DD 1248	D1	HCC	Pork Sausages	128	Positive
66	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Paratyphi B	R-SAL-41	B	FDA DMS ^f 155/76	Human France	128	Positive
67	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Paratyphi C	ATCC 13428	C1	ATCC	Unknown	116	Positive
68	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Pomona	DD 1711	M	HCC	Turkey Intestine (1941)	156	Positive
69	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Poona	NCTC 4840	G1	PHE	Infant enteritis	170	Positive
70	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Pretoria	DD 1712	F	HCC	Pig	132	Positive
71	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Reading	DD 13694	B	USDA-ARS ^g	Unknown	133	Positive
72	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Rubislaw	ATCC 10717	F	ATCC	Unknown	152	Positive
73	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Ruiru	DD 13812	L	GPLN	Unknown	166	Positive
74	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Santiago	DD 6586	C2	HCC	Bourguignon powder	248	Positive
75	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Schleissheim	DD 8008	B	HCC	Cheese	126	Positive
76	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Schwarzengrund	DD 13741	B	GPLN	Unknown	126	Positive
77	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Sculcoates	DD 3184	I	HCC	Cocoa Bean Environment	184	Positive
78	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Senftenberg	DD 13356	E4	HCC	Unknown	117	Positive
79	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Sloterdijk	ATCC 15791	B	ATCC	Netherland outbreak	138	Positive
80	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Soerenga	DD 13814	N	GPLN	Unknown	127	Positive
81	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Stanley	DD 1333	B	HCC	Chicken	128	Positive
82	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Stanleyville	DD 2372	B	HCC	Coca Bean Environment	120	Positive
83	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Sya	DD 3186	X	HCC	Cocoa Bean Environment	147	Positive
84	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Tennessee	NCTC 6388	C1	PHE	Unknown	148	Positive
85	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Thompson	DD 1339	C1	HCC	Egg	71	Positive
86	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhi	ATCC 19430	D1	ATCC	Unknown	53	Positive
87	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	ATCC 14028	B	PHE	Heart and Liver of Chicks	120	Positive
88	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium 4,12:i	DD 13739	B	GPLN	Unknown	135	Positive
89	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Virchow	NCTC 5742	C1	PHE	Unknown	158	Positive
90	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Weltevreden	DD 13619	E1	HCC	Human stool 1977 Connecticut	110	Positive
91	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Widemarsh	DD 13809	O	GPLN	Unknown	186	Positive
92	<i>Salmonella enterica</i> subsp. <i>houtenae</i> serotype Houten	ATCC 43974	W	PHE	Unknown	167	Positive
93	<i>Salmonella enterica</i> subsp. <i>houtenae</i> serotype Volksdorf	DD 1614	U	HCC	Iguana Bladder	149	Positive
94	<i>Salmonella enterica</i> subsp. <i>houtenae</i> serotype Wassenaar	DD 1714	Z	HCC	Human	85	Positive
95	<i>Salmonella enterica</i> subsp. <i>indica</i>	ATCC 43976	H	PHE	Unknown	94	Positive
96	<i>Salmonella enterica</i> subsp. <i>indica</i>	SAFE-54	F	USDA-FSIS ^h 2229	Unknown	85	Positive
97	<i>Salmonella enterica</i> subsp. <i>indica</i> serotype Ferlac	NCTC 10458	B	PHE	Ceylonese desiccated coconut	130	Positive
98	<i>Salmonella enterica</i> subsp. <i>salamae</i> serotype	DD 1525	O:59	HCC	Unknown	51	Positive

	Betioky						
99	<i>Salmonella enterica</i> subsp. <i>salamae</i> serotype Dar-Es-Salaam	ATCC 43972	D1	PHE	Unknown	115	Positive
100	<i>Salmonella enterica</i> subsp. <i>salamae</i> serotype Westpark	DD 1560	E1	HCC	Tortoise Intestine	97	Positive

^a Presumptive InSite *Salmonella* result, recorded after 48 h of incubation at 37 °C.

^b Public Health England, Salisbury, UK.

^c American Type Culture Collection, Manassas, VA.

^d Hygiene Culture Collection, New Castle, DE.

^e Georgia Poultry Lab Network, Gainesville, GA.

^f United States Food and Drug Administration, College Park, MD.

^g United States Department of Agriculture-Agricultural Research Service, Wyndmoor, PA.

^h United States Department of Agriculture-Food Safety and Inspection Services, Athens, GA.

Table 2. Exclusivity results of the 30 non-target strains tested for InSite *Salmonella*. (1)

No.	Species	Identifying Number	Source	Origin	cfu/mL	Result ^a
1	<i>Citrobacter braakii</i>	ATCC 6750	PHE ^b	Human urine	78,000	Positive
2	<i>Citrobacter freundii</i>	ATCC 8090	PHE	Unknown	88,333	Negative
3	<i>Cronobacter sakazakii</i>	ATCC 29544	PHE	Human throat	81,333	Negative
4	<i>Edwardsiella tarda</i>	ATCC 15947	PHE	Human faeces	76,333	Negative
5	<i>Enterobacter aerogenes</i>	ATCC 13048	PHE	Sputum	115,000	Negative
6	<i>Enterobacter cloacae</i>	ATCC 13047	PHE	Human CSF	162,667	Negative
7	<i>Escherichia coli</i>	ATCC 11775	PHE	Human urine, cystitis	125,333	Negative
8	<i>Escherichia coli</i> O157:H7 Stx-/Eae-	NCTC 13127	PHE	Human faeces	71,333	Negative
9	<i>Escherichia coli</i>	ATCC 8739	PHE	Unknown	59,667	Negative
10	<i>Escherichia coli</i> O104:H4	NCTC 13562	PHE	2011 Germany Outbreak	139,333	Negative
11	<i>Escherichia fergusonii</i>	ATCC 35469	PHE	Human faeces	78,333	Negative
12	<i>Escherichia hermannii</i>	ATCC 33650	PHE	Infected toe	81,667	Negative
13	<i>Hafnia alvei</i>	ATCC 13337	PHE	Unknown	87,000	Negative
14	<i>Klebsiella oxytoca</i>	NCTC 8167	PHE	Unknown	82,000	Negative
15	<i>Klebsiella pneumoniae</i>	ATCC 13883	PHE	Unknown	79,333	Negative
16	<i>Kluyvera ascorbata</i>	NCTC 9737	PHE	Urine	79,333	Negative
17	<i>Morganella morganii</i>	ATCC 25830	PHE	Human faeces	76,000	Negative
18	<i>Proteus mirabilis</i>	ATCC 29906	PHE	Unknown	78,333	Negative
19	<i>Providencia rettgeri</i>	ATCC 29944	PHE	Unknown	91,667	Negative
20	<i>Raoultella planticola</i>	ATCC 33531	PHE	Unknown	99,000	Positive
21	<i>Serratia marcescens</i>	ATCC 13880	PHE	Pond water	47,000	Negative
22	<i>Shigella boydii</i>	ATCC 8700	PHE	Unknown	35,333	Negative
23	<i>Shigella flexneri</i>	NCTC 1	PHE	Dysentery case	31,333	Negative
24	<i>Shigella sonnei</i>	ATCC 29930	PHE	Unknown	37,333	Negative
25	<i>Shimwellia blattae</i>	ATCC 29907	PHE	Cockroach	100,000	Negative
26	<i>Yersinia enterocolitica</i> subsp. <i>enterocolitica</i>	ATCC 9610	PHE	Unknown	14,333	Negative
27	<i>Listeria innocua</i>	ATCC 33090	PHE	Cow brain	906,667	Negative
28	<i>Listeria monocytogenes</i>	ATCC 15313	PHE	Rabbit	60,667	Negative
29	<i>Pseudomonas aeruginosa</i>	ATCC 9027	PHE	Unknown	49,000	Negative
30	<i>Staphylococcus epidermidis</i>	ATCC 14990	PHE	Human nose	92,667	Negative

^a Presumptive InSite *Salmonella* result, recorded after 48 h of incubation at 37 °C.

^b Public Health England, Salisbury, UK.

Table 3. Matrix Study: InSite *Salmonella* Presumptive Results versus Confirmed Results (1)

Matrix	Strain	cfu ^a /test area	N ^b	InSite <i>Salmonella</i> Presumptive			InSite <i>Salmonella</i> Confirmed			dPOD _c ^f	95% CI ^g
				X ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Stainless steel (12" x 12")	<i>S. Typhimurium</i> (B) ATCC ^h 14028 / <i>C. freundii</i> ATCC 8090	1.90 x 10 ⁶ / 6.25 x 10 ⁷	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.47, 0.47)
		1.90 x 10 ⁴ / 6.25 x 10 ⁵	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.13, 0.13)
		0 / 6.25 x 10 ⁴	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
Stainless steel (4" x 4")	<i>S. Typhimurium</i> (B) ATCC 14028 / <i>C. freundii</i> ATCC 8090	3.29 x 10 ⁶ / 1.58 x 10 ⁷	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.47, 0.47)
		3.29 x 10 ⁴ / 1.58 x 10 ⁵	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.13, 0.13)
		0 / 1.58 x 10 ⁴	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
Stainless steel (12" x 12") _i	<i>S. Typhimurium</i> (B) ATCC 14028 / <i>C. freundii</i> ATCC 8090	122 / 1.50 x 10 ³	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.47, 0.47)
		55 / 480	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.13, 0.13)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
Stainless steel (4" x 4") _i	<i>S. Typhimurium</i> (B) ATCC 14028 / <i>C. freundii</i> ATCC 8090	117 / 1.30 x 10 ³	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.47, 0.47)
		48 / 620	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0.00	(-0.13, 0.13)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
Rubber (4" x 4")	<i>S. Montevideo</i> (C ₁) NCTC ^j 5747	3.02 x 10 ⁶	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.47, 0.47)
		3.02 x 10 ³	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.13, 0.13)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
Plastic (4" x 4")	<i>S. Newport</i> (C ₂) ATCC 6962	3.00 x 10 ⁵	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.47, 0.47)
		1.36 x 10 ⁴	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.13, 0.13)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
Ceramic (4" x 4")	<i>S. Salamae</i> (D ₁) ATCC 43972	3.52 x 10 ⁴	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.47, 0.47)
		3.52 x 10 ³	20	17	0.85	(0.64, 0.95)	17	0.85	(0.64, 0.95)	0.00	(-0.13, 0.13)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
Sealed concrete (4" x 4")	<i>S. Lexington</i> (E ₁) NCTC 6244	1.53 x 10 ⁵	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.47, 0.47)
		1.53 x 10 ³	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.13, 0.13)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)

^a cfu = colony forming units applied to each test area.

^b N = number of test portions.

^c X = number of positive test portions.

^d POD_{CP} = Candidate method presumptive results divided by the total number of trials.

^e POD_{CC} = Candidate method confirmed results divided by the total number of trials.

^f dPOD_c = Difference between the candidate method presumptive and confirmed POD values.

^g 95% CI = if the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^h ATCC = American Type Culture Collection, Manassas, VA.

ⁱ Matrix tested in the independent laboratory, Q-Laboratories, Cincinnati, OH.

^j NCTC = Public Health England, Salisbury, UK.

Table 4. Matrix Study: InSite *Salmonella* Confirmed Results versus Reference Method Results (1)

Matrix	Strain	cfu ^a /test area	N ^b	InSite <i>Salmonella</i> Presumptive			Reference Method ^e			dPOD _c ^g	95% CI ^h
				X ^c	POD _c ^d	95% CI	x	POD _R ^f	95% CI		
Stainless steel (12" x 12")	<i>S. Typhimurium</i> (B) ATCC ⁱ 14028 / <i>C. freundii</i> ATCC 8090	1.90 x 10 ⁶ / 6.25 x 10 ⁷	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
		1.90 x 10 ⁴ / 6.25 x 10 ⁵	20	15	0.75	(0.53, 0.89)	18	0.90	(0.70, 0.97)	-0.15	(-0.38, 0.09)
		0 / 6.25 x 10 ⁴	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
Stainless steel (4" x 4")	<i>S. Typhimurium</i> (B) ATCC 14028 / <i>C. freundii</i> ATCC 8090	3.29 x 10 ⁶ / 1.58 x 10 ⁷	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
		3.29 x 10 ⁴ / 1.58 x 10 ⁵	20	13	0.65	(0.43, 0.82)	18	0.90	(0.70, 0.97)	-0.25	(-0.48, 0.01)
		0 / 1.58 x 10 ⁴	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
Stainless steel (12" x 12") ^j	<i>S. Typhimurium</i> (B) ATCC 14028 / <i>C. freundii</i> ATCC 8090	122 / 1.50 x 10 ³	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
		55 / 480	20	6	0.30	(0.15, 0.52)	8	0.40	(0.22, 0.61)	-0.10	(-0.36, 0.18)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
Stainless steel (4" x 4") ^j	<i>S. Typhimurium</i> (B) ATCC 14028 / <i>C. freundii</i> ATCC 8090	117 / 1.30 x 10 ³	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
		48 / 620	20	5	0.25	(0.11, 0.47)	8	0.40	(0.22, 0.61)	-0.15	(-0.40, 0.13)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
Rubber (4" x 4")	<i>S. Montevideo</i> (C ₁) NCTC ^k 5747	3.02 x 10 ⁶	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
		3.02 x 10 ³	20	10	0.50	(0.30, 0.70)	7	0.35	(0.18, 0.57)	0.15	(-0.15, 0.41)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
Plastic (4" x 4")	<i>S. Newport</i> (C ₂) ATCC 6962	3.00 x 10 ⁵	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
		1.36 x 10 ⁴	20	13	0.65	(0.43, 0.82)	10	0.50	(0.30, 0.70)	0.15	(-0.15, 0.41)
		0	5	0	0.00	(0.00, 0.43)	1	0.20	(0.00, 0.62)	-0.20	(-0.62, 0.27)
Ceramic (4" x 4")	<i>S. Salamae</i> (D ₁) ATCC 43972	3.52 x 10 ⁴	5	5	1.00	(0.57, 1.00)	2	0.40	(0.12, 0.77)	0.60	(0.03, 0.88)
		3.52 x 10 ³	20	17	0.85	(0.64, 0.95)	13	0.65	(0.43, 0.82)	0.20	(-0.07, 0.44)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
Sealed concrete (4" x 4")	<i>S. Lexington</i> (E ₁) NCTC 6244	1.53 x 10 ⁵	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
		1.53 x 10 ³	20	9	0.45	(0.26, 0.66)	13	0.65	(0.43, 0.82)	-0.20	(-0.46, 0.10)
		0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)

^a cfu = colony forming units applied to each test area.

^b N = number of test portions.

^c X = number of positive test portions.

^d POD_c = Candidate method confirmed positive outcomes using the reference method procedure divided by the total number of trials.

^e Reference method = ISO 6579-1:2017 / Amd.1:2020 for *Salmonella*.

^f POD_R = Reference method confirmed positive outcomes divided by the total number of trials.

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

^h 95% CI = if the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

ⁱ ATCC = American Type Culture Collection, Manassas, VA.

^j Matrix tested in the independent laboratory, Q-Laboratories, Cincinnati, OH.

^k NCTC = Public Health England, Salisbury, UK.

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