CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

111903

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

BAC*Spec Salmonella* 2

manufactured by

Eurofins GeneScan Technologies GmbH
Engesserstraße 4
D-79108 Freiburg im Breisgau
Germany

This method has been evaluated in the AOAC® *Performance Tested Methods*SM 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 Tested*SM 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 (November 22, 2019 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

November 22, 2019
Date

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KIT NAME(S)
BAC*Spec Salmonella* 2

CATALOG NUMBERS
4323410301, 4323410305

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APPLICABILITY OF METHOD
Target Organism – *Salmonella* spp.

Matrices – (25 g): Mayonnaise-based vegetable salad, ground beef (fat content approx. 20%), raw milk (fat content approx. 4%), fresh spinach, pasteurized liquid egg, dry pet food
stainless steel (304L) surface (1" x 1")
sealed ceramic tile surface (4' x 4')

Performance claims - Performance of the BAC*Spec Salmonella* 2 is equivalent to the reference culture method ISO 6579-1: Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: detection of *Salmonella* spp

REFERENCE METHOD
ISO 6579-1:2017, *Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp.* (https://www.iso.org/standard/56712.html) (2)

ORIGINAL CERTIFICATION DATE
November 22, 2019

CERTIFICATION RENEWAL RECORD
New Approval

METHOD MODIFICATION RECORD
None

SUMMARY OF MODIFICATION
None

Under this AOAC® *Performance Tested*SM License Number, 111903 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*SM License Number, 111903 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)

The BAC*Spec Salmonella* 2 method is a qualitative ELISA for the detection of the H and O antigens of *Salmonella* spp. in selected foodstuffs, feed products and environmental samples. Utilizing antigen-antibody binding and enzyme catalyzed color reactions, the assay ensures the potential for high specificity and sensitivity in detection of *Salmonella spp*. Test portions are first enriched in Buffered Peptone Water (BPW). This enrichment step is identical with the first enrichment step defined in the reference method ISO 6579-1 [1]. After the primary enrichment a secondary enrichment in Rappaport Vassiliadis Soy broth (RVS) follows to suppress growth of non-*Salmonella* strains and support the subsequent growth of *Salmonella* spp. After completion of the enrichment steps, an aliquot is removed and heat-inactivated. These sample preparations are then analyzed by ELISA. There are two types of ELISA: Sandwich and Competitive. For detection of large molecules like the *Salmonella*-antigens, the principle of sandwich ELISA is applied. The method BAC*Spec Salmonella* 2 is carried out on a 96-well microtiter plate (MTP). In each well of the MTP, antibodies against *Salmonella* antigens are immobilized. If samples containing *Salmonella* antigens are added into the wells, the antibodies recognize and capture the antigens. After a washing step an antibody-peroxidase conjugate is added, which binds to the immobilized antigens. After a second washing step, a substrate (TMB) solution for peroxidase is added. The peroxidase then catalyzes a reaction, which generates a blue color in the substrate solution. After incubation, the reaction is stopped with diluted sulfuric acid and the color turns yellow. The optical density (OD) of the yellow-colored solution is measured with a microplate reader (Fig 1). The OD is proportional to the concentration of *Salmonella* antigens in the sample. A cut-off is defined based on the OD value of the positive control. Samples with OD signals above the cut-off are designated as positive.

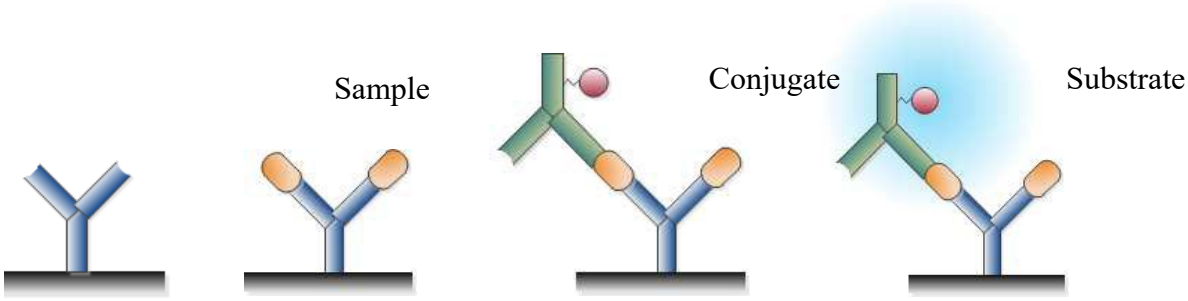

Figure 1: Principle of sandwich ELISA for BAC*Spec Salmonella* 2

The BAC*Spec Salmonella* 2 can be conducted either manually or using automated ELISA analysers. All automated analysers, which can fulfill the test conditions defined in the kit manual, can be used for BAC*Spec Salmonella* 2 ELISA.

DISCUSSION OF THE VALIDATION STUDY (1)

In the inclusivity study all 106 *Salmonella* strains were tested positive. Among the strains, all *Salmonella* species and subspecies were present (at least 3 of each). Therefore, BAC*Spec Salmonella* 2 kit is suitable to detect *Salmonella* spp.

In the exclusivity study, 9 strains (*Citrobacter braakii, Citrobacter diversus, Citrobacter freundii, Citrobacter koseri, Enterobacter agglomerans, Escherichia hermanii, Escherichia coli, Enterobacter amnigenus, Hafnia alvei*) tested positive when the strains were enriched in BPW. However, after the full protocol including the secondary enrichment in the selective media (RVS), all strains tested negative. These results show that the RVS enrichment successfully suppresses non-Salmonella strain growth. The enrichment steps in the BAC*Spec Salmonella* 2 method ensure the additional selectivity of the method.

In the matrix study, the results of BAC*Spec Salmonella* 2 method and the confirmation results are identical for all tested matrixes. The confirmed results of the BAC*Spec Salmonella* 2 method and the ISO 6579-1 method are identical for all tested matrixes as well. Therefore, the BAC*Spec Salmonella* 2 method and the ISO 6579-1 method are not significantly different. The results of manual ELISA and automated ELISA did not show any difference according to POD analysis for all three tested matrixes. Hence, there is no statistically significant difference between manual and automated ELISA.

In the robustness study, the data show that variations (±10% to reference condition) of sample volume or reagents volume or changes of the incubation temperature (±2 °C to reference condition) will not influence the test performance. No deviation of qualitative results was observed. According to the ANOVA analysis, all tested groups do not have significantly different test results.

In the lot-to-lot comparison study, the data and statistical analysis show that the performance of BAC*Spec Salmonella* 2 ELISA is consistent using different lots produced at Eurofins GeneScan Technologies. This data demonstrates constant quality of the product.

The initial results of the long-term stability study showed, that BAC*Spec Salmonella* 2 kit is stable under the storage condition defined in the kit manual for at least 8 months. The complete results will be presented in 2020.

Table 1. Inclusivity study results for BAC*Spec Salmonella* 2 (1)

				ELISA Result			Confirmation		
No.	Strain	Source[a]	Origin	OD	Ratio to cut-off[b]	Result	XLD	ASAP	Latex
1	*S.* Abaetetuba	Ad2318	/[c]	2.642	7.2	+	+	+	+
2	*S.* Aberdeen	CIP 105618	Human	3.112	8.5	+	+	+	+
3	*S.* Abortusequi	Ad2321	/	2.665	7.2	+	+(H2S-)[d]	+	+
4	*S.* Abortusovis	Ad2320	Ovine foetus	0.758	2.1	+	+(H2S-)	+(white colonies)	+
5	*S.* Adelaïde	Ad2319	Turkey breeding environment	1.377	3.7	+	+(H2S-)	+	+

6	*S*. Agona	A00V038	Feed for pork	2.869	7.8	+	+	+	+
7	*S*. Anatum	A00E007	Dusts	3.201	8.7	+	+	+	+
8	*S*. arizonae 18:z4,z32:-	CIP55.28	/	0.606	1.7	+	+	+ (light colonies)	+
9	*S*. arizonae 51:z4,z23	CIP 5523	Poultry environmental sample	0.732	2.1	+	+ (yellow colonies)	+(light colonies)	+
10	*S*. arizonae 48:z4,z23:-	Ad1850	Primary production sample (poultry)	0.716	1.9	+	+	+(light colonies)	+
11	*S*. arizonae 50:z4,z23	CIP5526	Egg powder	1.193	3.2	+	+ (white colonies)	+ (white colonies)	+
12	*S*. Bardo	Adria 569	Meat for sausage	2.534	6.9	+	+	+	+
13	*S*. Bareilly	Ad 1687	Chocolate industry	3.139	8.5	+	+	+	+
14	*S*. Berta	CIP105682	/	2.884	7.7	+	+	+	+
15	*S*. Blockley	Ad 923	Poultry environment	2.322	6.3	+	+	+	+
16	*S*. bongori 1,40 :z81 :-	Ad2683	Ready to reheat meal	1.065	2.9	+	+	+ (white colonies)	+
17	*S*. bongori 48 :z35	Ad 598	Environmental sample	1.210	3.4	+	+	+ (light colonies)	+
18	*S*. bongori 66 :z35:-	Ad 599	Primary production sample	0.580	1.6	+	+	+(white colonies)	+
19	*S*. Bovismorbificans	Adria 6629	Sausage	2.200	6.0	+	+	+	+
20	*S*. Braenderup	Adria 111	Pork meat	3.286	8.9	+	+	+	+
21	*S*. Brandenburg	Ad 351	Seafood cocktail	3.640	9.9	+	+	+	+
22	*S*. Bredeney	Adria 396	Ground beef	3.086	8.4	+	+	+	+
23	*S*. Caracas	Ad2322	Spice	1.760	4.8	+	+	+	+
24	*S*. Cerro	Ad 689	Dehydrated poultry protein	1.818	4.9	+	+	+	+
25	*S*. Chester	CIP 103543	/	2.681	7.3	+	+	+	+
26	*S*. Cremieu	230	Hare meat	2.111	4.8	+	+ (Yellow colonies)	+	+
27	*S*. Cubana	Ad2323	Dust feed environment	1.304	3.5	+	+	+	+
28	*S*. Derby	Ad 1093	Fish fillet	2.699	7.3	+	+	+	+
29	*S*. diarizonae 38:lv:z53	Ad 451	Ewe milk cheese	0.829	2.3	+	+	+	+
30	*S*. diarizonae 61:k:1,5,7	Ad 1300	Raw ewe milk	2.944	8.0	+	+	+	+
31	*S*. diarizonae 61:-:1,5,7	Ad1280	Raw ewe milk	1.084	3.0	+	+ (yellow colonies)	+	+
32	*S*. Dublin	Ad 529	Beef meat	2.494	6.8	+	+	+(white colonies)	+
33	*S*. Duisburg	Ad1812	/	2.746	6.2	+	+	+	+
34	*S*. Emek	Ad 333	/	1.560	4.2	+	+	+	+
35	*S*. Enteritidis	Ad 477	Hen meat	2.671	7.3	+	+	+	+
36	*S*. Essen	38	/	2.122	5.6	+	+	+	+
37	*S*. Falkensee	693	Pork meat	3.726	8.5	+	+	+	+
38	*S*. Gallinarum	Ad1840	Primary production sample (Poultry)	1.880	5.0	+	+ (white colonies)	+	+
39	*S*. Gaminara	Ad2324	Boar meat	1.799	4.9	+	+	+	+
40	*S*. Garoli	CIP54139		3.769	8.6	+	+	+	+
41	*S*. Give	436	Ground beef	3.328	9.1	+	+	+	+
42	*S*. Guinea	29	/	1.258	3.4	+	+(H2S-)	+	+
43	*S*. Hadar	24871	Chicken meat	2.823	7.7	+	+	+	+
44	*S*. Haifa	Ad1727	/	2.900	6.6	+	+	+	+
45	*S*. Havana	Ad 930	Poultry environment	2.394	6.5	+	+	+	+
46	*S*. Heidelberg	A00E005	Dusts from dairy industry	3.400	9.2	+	+(H2S-)	+	+
47	*S*.	Ad597	Cooked fish	1.713	4.7	+	+	+	+

	houtenae 43 :z4,z32								
48	*S*. houtenae 1,40:z4,z23:-	Ad2682	Primary production sample (Poultry)	1.153	3.2	+	+	+	+
49	*S*. houtenae 50:g,z51	Ad 596	Dairy product	0.453	1.2	+	+	+	+
50	*S*. Hvittingfoss	Ad2325	Raw stuff	1.323	3.6	+	+	+	+
51	*S*. Idikan	Ad2567	Feed product	1.120	3.0	+	+	+	+
52	*S*. Indiana	Ad 174	White cheese	3.392	9.2	+	+	+	+
53	*S*. indica 45:a,enx	ATCC BAA-1578	/	1.197	2.7	+	+	+ (grey colonies)	+
54	*S*. indica 1,6,14,25:a:enx	Ad 600	Environmental sample	2.124	5.8	+	+(H2S-; yellow colonies)	+(light colonies)	+
55	*S*. indica11:b:enx	Ad2337	Chicken breeding environment	2.164	5.9	+	+(H2S-)	+(light colonies)	+
56	*S*. Infantis	F401B	Cheese	3.169	8.6	+	+	+	+
57	*S*. Javiana	Ad2326	Turkey meat	2.906	7.9	+	+	+	+
58	*S*. Kedougou	Ad 929	Bovine environmental sample	2.731	7.4	+	+	+	+
59	*S*. Kentucky	Ad1756	Poultry environmental sample	3.367	9.2	+	+	+	+
60	*S*. Kottbus	Adria 1	Poultry environmental sample	2.861	7.8	+	+	+	+
61	*S*. Landau	Ad 499	/	0.828	2.3	+	+(H2S-)	+	+
62	*S*. Lille	Adria 37	Food product	0.965	2.6	+	+	+	+
63	*S*. Livingstone	Ad 1107	Dusts	1.466	4.0	+	+	+	+
64	*S*. London	Adria 326	Cooked meat sample	3.830	10.4	+	+	+	+
65	*S*. Manhattan	Adria 900	Dusts from dairy industry	1.901	5.2	+	+	+	+
66	*S*. Mbandaka	Ad 914	Mayonnaise	2.739	7.7	+	+	+	+
67	*S*. Meleagridis	505	Raw milk	0.698	2.0	+	+	+	+
68	*S*. Michigan	Ad2327	Low moisture sausage	1.189	3.4	+	+	+	+
69	*S*. Mikawasima	Ad1811	Raw ewe milk	2.055	5.8	+	+	+	+
70	*S*. Minnesota	Ad2328	Feed	0.903	2.5	+	+	+	+
71	*S*. Missisipi	Ad2329	Parakeet	1.210	3.4	+	+	+	+
72	*S*. Montevideo	Ad912	Raw milk	2.308	6.5	+	+	+	+
73	*S*. Muenchen	CIP 106178	/	1.053	3.0	+	+	+	+
74	*S*. Napoli	Ad 928	Clinical	2.445	6.9	+	+	+	+
75	*S*. Newport	Adria 586	Sausage	1.775	5.0	+	+	+	+
76	*S*. Ohio	Ad1482	Raw cow milk	3.366	9.5	+	+	+	+
77	*S*. Oranienburg	Ad1724	Cereals	2.122	6.0	+	+	+	+
78	*S*. Orion	27	/	2.645	7.5	+	+	+	+
79	*S*. Ouakam	Ad1647	Compost	1.037	2.9	+	+	+	+
80	*S*. Panama	Adria 8	Ground beef	2.954	8.3	+	+	+	+
81	*S*. Paratyphi B	Ad 301	Clinical	1.880	5.2	+	+	+	+
82	*S*. Paratyphi C	ATCC 13428	/	0.655	1.8	+	+	+(light colonies)	+
83	*S*. Pomona	CIP105630	/	1.813	5.1	+	+	+	+
84	*S*. Poona	Ad2330	Poultry feed	1.631	4.6	+	+	+	+
85	*S*. Putten	Ad2331	Feed for chicken	1.570	4.4	+	+	+	+
86	*S*. Regent	Adria 328	Duck	2.057	5.7	+	+	+	+
87	*S*. Rissen	Adria 39	Food product	1.162	3.3	+	+	+	+

88	*S.* Rubislaw	Ad2332	Shark cartilage	2.261	6.4	+	+	+	+
89	*S.* Saintpaul	Adria F31	Pilchard fillets	3.169	8.9	+	+	+	+
90	*S.* Salamae 42,b:e,n,x,z15	Ad593	Cereals	1.555	4.4	+	+	+	+
91	*S.* Salamae 9:gmt	Ad212	/	3.285	7.9	+	+	+	+
92	*S.* Salamae 42:gt:-	Ad592	Kangaroo meat	0.837	1.6	+	+	+	+
93	*S.* Schwarzengrund	Ad2333	Egg products environment	1.952	5.5	+	+	+	+
94	*S.* Senftenberg	Ad355	Seafood cocktail	2.684	7.4	+	+	+	+
95	*S.* Stanley	Ad1688	Chocolate industry	1.726	4.8	+	+	+	+
96	*S.* Stourbridge	Ad2297	Raw milk cheese	3.298	9.1	+	+	+	+
97	*S.* Tananarive	CIP54142	/	2.316	6.4	+	+	+	+
98	*S.* Tennessee	A00E006	Dusts from dairy industry	1.681	4.7	+	+	+	+
99	*S.* Thompson	AER301	Poultry	2.358	6.5	+	+	+	+
100	*S.* Typhi	Ad302	Clinical	1.284	3.6	+	+ (H2S-)	+	+
101	*S.* Typhimurium	Ad1070	Pork meat	2.711	7.5	+	+	+	+
102	*S.* Urbana	Ad2334	Shrimps	0.701	1.9	+	+	+	+
103	*S.* Veneziana	Adria 233	Food product	2.545	7.0	+	+	+	+
104	*S.* Virchow	Adria F276	Curry	2.297	6.4	+	+	+	+
105	*S.* Weltevreden	Ad2336	Treated water	3.030	8.4	+	+	+	+
106	*S.* Wien	CIP8122	/	1.138	3.0	+	+	+	+

[a] Source: Adria, Ad, A000. AER000 and 000: collection of Adria Développement, France. CIP: Collection of Institute Pasteur, France. ATCC: American Type Culture Collection, USA. [b] Ratio to cut-off = OD / cut-off, when the value ≥ 1, the sample is evaluated as positive; [c] / = origin of the strain is unknown. [d] +(H2S-) = positive, but without H2S reaction.

Table 2. Exclusivity study results for BAC*Spec Salmonella* 2 (1)

				ELISA Result BPW			ELISA Result RVS			Confirmation	
No.	**Strain**	**Source[a]**	**Origin**	**OD**	**Ratio to cut-off[b]**	**Result**	**OD**	**Ratio to cut-off**	**Result**	**XLD**	**ASAP**
1	*Citrobacter braakii*	Ad833	Raw beef meat	2.443	6.9	+	0.258	0.9	-	-	-
2	*Citrobacter diversus*	adria 140	Raw milk	2.202	6.2	+	0.248	0.9	-	-	-
3	*Citrobacter freundii*	adria 23	Raw pork sausage	1.570	4.4	+	0.188	0.7	-	-	-
4	*Citrobacter koseri*	adria 71	Frozen vegetables	1.727	4.9	+	0.109	0.4	-	-	-
5	*Enterobacter agglomerans*	adria 11	Cheese	1.089	3.1	+	0.184	0.7	-	-	-
6	*Enterobacter amnigenus*	A00C068	Raw poultry meat	0.356	1.0	+	0.103	0.4	-	-	-
7	*Enterobacter cloacae*	adria 10	Raw milk	0.094	0.3	-	N/A[c]	N/A	N/A	N/A	N/A
8	*Enterobacter intermedius*	adria 60	Bean	0.088	0.2	-	N/A	N/A	N/A	N/A	N/A
9	*Enterobacter kobei*	Ad342	Ham	0.173	0.5	-	N/A	N/A	N/A	N/A	N/A
10	*Enterobacter sakazakii*	adria 95	Fermented milk	0.285	0.8	-	N/A	N/A	N/A	N/A	N/A
11	*Erwinia carotovora*	CIP 8283	Potatoes	0.227	0.6	-	N/A	N/A	N/A	N/A	N/A
12	*Escherichia coli*	adria 19	Grated carrots	0.373	1.1	+	0.083	0.3	-	-	-
13	*Escherichia coli O157 :H7*	Ad485	Ground beef	0.135	0.4	-	N/A	N/A	N/A	N/A	N/A
14	*Escherichia hermanii*	Ad 461	Dessert	1.040	2.9	+	0.165	0.6	-	-	-
15	*Escherichia vulneris*	adria 132	Veal liver	0.217	0.6	-	N/A	N/A	N/A	N/A	N/A
16	*Hafnia alvei*	adria 167	Raw pork sausage	0.766	2.2	+	0.130	0.5	-	-	-
17	*Klebsiella oxytoca*	57	Food product	0.071	0.2	-	N/A	N/A	N/A	N/A	N/A

18	*Klebsiella pneumoniae*	47	Raw turkey meat	0.077	0.2	-	N/A	N/A	N/A	N/A	N/A
19	*Kluyvera spp*	adria 41	Raw milk	0.263	0.7	-	N/A	N/A	N/A	N/A	N/A
20	*Morganella morganii*	CIP A236	/[d]	0.077	0.2	-	N/A	N/A	N/A	N/A	N/A
21	*Pantoea agglomerans*	adria 62	Frozen vegetables	0.170	0.5	-	N/A	N/A	N/A	N/A	N/A
22	*Proteus mirabilis*	Ad639	Mayonnaise	0.122	0.3	-	N/A	N/A	N/A	N/A	N/A
23	*Proteus vulgaris*	adria 43	Sliced ham	0.073	0.2	-	N/A	N/A	N/A	N/A	N/A
24	*Providencia rettgeri*	adria 112	White liquid egg	0.113	0.3	-	N/A	N/A	N/A	N/A	N/A
25	*Rhanella aquatilis*	adria 69	Molluscs	0.101	0.3	-	N/A	N/A	N/A	N/A	N/A
26	*Serratia liquefaciens*	26	Egg product	0.301	0.8	-	N/A	N/A	N/A	N/A	N/A
27	*Serratia marcescen*	Ad2604	Dairy product	0.065	0.2	-	N/A	N/A	N/A	N/A	N/A
28	*Shigella flexneri*	CIP 8248	/	0.100	0.3	-	N/A	N/A	N/A	N/A	N/A
29	*Shigella sonnei*	CIP 8249T (ATCC 29930)	/	0.087	0.2	-	N/A	N/A	N/A	N/A	N/A
30	*Yersinia enterocolotica*	adria 32	Bacon	0.084	0.2	-	N/A	N/A	N/A	N/A	N/A

[a] Source: Adria, Ad, A000. AER000 and 000: collection of Adria Développement, France. CIP: Collection of Institute Pasteur, France. ATCC: American Type Culture Collection, USA. [b] Ratio to cut-off = OD / cut-off, when the value ≥ 1, the sample is evaluated as positive. [c] If the result of BPW culture is negative, no selective enrichment is performed. [d] / = origin of the strain is unknown.

Table 3. POD statistics of candidate presumptive vs. candidate confirmed method results of BAC*Spec Salmonella* 2 (1)

					Presumptive Candidate Result (after RVS enrichment)			Confirmation Result				
Matrix	Strain(s)	Inoculation Level	N^a	MPN[b] / test portion	x^c	POD_{CP}[d]	95% CI	x_{CC}	POD_{CC}[e]	95% CI	$dPOD_{CP,CC}$[f]	95% CI[g]
Mayonnaise based vegetable-salad	*S*.Mbandaka	uninoculated	5	N/A[h]	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	0.4 (0.18, 0.69)	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		high level	5	1.59 (0.62, 4.15)	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Ground beef	*S*. Typhimurium	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	0.84 (0.49, 1.4)	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.28, 0.28)
		high level	5	2.39 (1.47, 4.71)	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw milk	*S*. Ohio	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	0.51 (0.26, 0.84)	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		high level	5	1.93 (0.77, 5.26)	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Fresh spinach	*S*. Virchow	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	1.42 (0.88, 2.59)	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.26, 0.26)
		high level	5	3.65 (1.87, 0.72)	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized liquid egg	*S*. Havana	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	0.74 (0.48, 1.12)	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
		high level	5	1.56 (0.57, 3.8)	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Dry pet food	*S*. Derby	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	0.35 (0.17, 0.63)	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		high level	5	1.93 (0.77, 4.96)	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Stainless steel	*S*. Livingstone & *C. freundii*	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	7.4[i] & 99.2[j]	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		high level	5	13.8[i] & 137.8[j]	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Ceramic tile surface	*S*. cerro	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	40.5[k]	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		high level	5	81.0[k]	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

[a] N = Number of test potions; [b] MPN = Most Probable Number; [c] x = Number of positive test portions; [d] POD_{CP} = Probability of Detection of presumptive candidate method - positive outcomes divided by the total number of trials; [e] POD_{CC} = probability of detection for the confirmation method according to ISO 6579-1 - positive outcomes divided by the total number of trials; [f] $dPOD_{CP,CC}$ = Difference between POD values of presumptive candidate method (POD_{CP}) and confirmation (POD_{CC}) results; [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] N/A = not applicable; [i] Instead MPN, CFU/test in the inoculum were determined for *S.* Livingstone; [j] Instead MPN, CFU/test in the inoculum were determined for *C. freundii*; [k] Instead MPN, CFU/test in the inoculum were determined for *S.* Cerro

Table 4. POD statistics of candidate confirmed results versus reference method results of BAC*Spec Salmonella* 2 (1)

					Confirmed Candidate Result			Reference method Result				
Matrix	Strain(s)	Inoculation Level	N^a	MPN / test portion[b]	x^c	$POD_C{}^d$	95% CI	x_R	$POD_R{}^e$	95% CI	$dPOD_{C,R}{}^f$	95% CI[g]
Mayonnaise based vegetable-salad	*S*.Mbandaka	uninoculated	5	N/A[h]	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	0.4 (0.18, 0.69)	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		high level	5	1.59 (0.62, 4.15)	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Ground beef	*S*. Typhimurium	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	0.84 (0.49, 1.4)	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.28, 0.28)
		high level	5	2.39 (1.47, 4.71)	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw milk	*S*. Ohio	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	0.51 (0.26, 0.84)	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		high level	5	1.93 (0.77, 5.26)	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Fresh spinach	*S*. Virchow	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	1.42 (0.88, 2.59)	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.26, 0.26)
		high level	5	3.65 (1.87, 0.72)	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized liquid egg	*S*. Havana	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	0.74 (0.48, 1.12)	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
		high level	5	1.56 (0.57, 3.8)	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Dry pet food	*S*. Derby	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	0.35 (0.17, 0.63)	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		high level	5	1.93 (0.77, 4.96)	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Stainless steel	*S*. Livingstone & *C. freundii*	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	7.4[i] & 99.2[j]	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		high level	5	13.8[i] & 137.8[j]	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Ceramic tile surface	*S*. cerro	uninoculated	5	N/A	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		low-level	20	40.5[k]	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		high level	5	81.0[k]	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

[a] N = Number of test potions; [b] MPN = Most Probable Number; [c] x = Number of positive test portions; [d] POD_C = Probability of Detection of confirmed candidate method - positive outcomes divided by the total number of trials; [e] POD_R = probability of detection for the referance method ISO 6579-1 - positive outcomes divided by the total number of trials; [f] $dPOD_{C,R}$ = Difference between POD values of confirmed candidate method (POD_C) and reference method (POD_R) results; [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] N/A = not applicable; [i] Instead MPN, CFU/test in the inoculum were determined for *S.* Livingstone; [j] Instead MPN, CFU/test in the inoculum were determined for *C. freundii*; [k] Instead MPN, CFU/test in the inoculum were determined for *S.* Cerro

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