CERTIFICATION

AOAC® *Performance Tested*℠

Certificate No.

120802

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

Salmonella Rapid Culture Method Using ONE Broth Salmonella and Brilliance™ Salmonella

manufactured by

Oxoid Ltd. part of Thermo Fisher Scientific
Wade Rd
Basingstoke, Hampshire
RG24 8PW, UK

This method has been evaluated in the AOAC® *Performance Tested Methods*℠ 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*℠ 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 26, 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 26, 2019
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KIT NAME(S)
Salmonella Rapid Culture Method using ONE Broth Salmonella and Brilliance™ Salmonella

CATALOG NUMBERS
CM1091B, CM1092B, SR0194E, SRO242B

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APPLICABILITY OF METHOD
Target organism – *Salmonella* spp.

Matrices – ground beef, ground chicken, lettuce, shrimp, & shell eggs

Performance claims - The Salmonella Rapid Culture Method using ONE Broth Salmonella + Brilliance™ Salmonella was not significantly different from the USDA-FSIS and FDA-BAM) reference methods, while being superior to the reference method in both reduced incubation time and ease of identifying typical colonies.

REFERENCE METHODS
Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg Products. USDA Microbiology Laboratory Guidebook 4.03. USDA/FSIS, Laboratory QA/QC Division, Athens, GA. (http://www.fsis.usda.gov/PDF/MLG_4_04.pdf) (3)

Andrews, W.H,, & Hammack, T.S.Wallace H. Andrews and Thomas S. Hammack. (1998) in *US FDA/CFSAN Bacteriological Analytical Manual (FDA-BAM)*, 8th edition, Chapter 5: Salmonella, U.S. Food and Drug Administration, Washington, DC. (online)(http://www.cfsan.fda.gov/~ebam/bam-5.html) (4)

ORIGINAL CERTIFICATION DATE
December 24, 2008

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2020

METHOD MODIFICATION RECORD
1. **December 2017 Level 1**
2. **December 2018 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial changes in insert and labels**
2. **Format of insert updated and edited for clarity.**

Under this AOAC® *Performance Tested*SM License Number, 120802 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*SM License Number, 120802 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
The Salmonella Rapid Culture method involves a single enrichment in ONE Broth Salmonella, followed by plating on Brilliance™ Salmonella chromogenic medium, taking a total of two days to generate an initial result.
ONE Broth-Salmonella is a highly nutritious enrichment broth containing a specific growth promoter to ensure excellent recovery of stressed and damaged *Salmonella* cells, while inhibiting the growth of competing micro-organisms. This highly effective medium allows enrichment to be performed in a single 18-24 hour incubation, eliminating the need for a secondary enrichment.
Following enrichment, the sample is plated onto Brilliance™ Salmonella, the first in a new class of novel chromogenic culture media to utilize Inhibigen™ technology. This new technology improves the recovery and differentiation of *Salmonella* by selectively reducing background flora, allowing clearer visualization of target colonies in mixed cultures.
Chromogens within the medium enable differentiation of *Salmonella* colonies (bright purple) from any remaining organisms that are able to grow, such as *Klebsiella* (blue colonies) and *Enterobacter* (clear colonies to no growth), thus reducing the number of false-positives requiring confirmation.
An Inhibigen™ compound is comprised of two components combined together by a bond that can only be cleaved by specific enzymes. When bound, the inhibitor component is not toxic and therefore can exist in a medium without harming micro-organisms. Once inside the cell, the bond will be cleaved if the target enzyme is present. When the bond is cleaved, the inhibitor molecule is released, disrupting cell replication, resulting in targeted inhibition. As cells die and lyse, free inhibitor is released but cannot be taken up by other cells. The Inhibigen™ in Brilliance™ Salmonella is used to specifically inhibit growth of *Escherichia coli*.
Differentiation of *Salmonella* from any other organism that is able to grow on Brilliance™ Salmonella is achieved through the inclusion of two chromogens in the medium: magenta-caprylate and X-ß-glucopyranoside. Caprylate esterase is an enzyme present in all Salmonella and some species of *Klebsiella, Enterobacter,* and *Proteus*. Organisms possessing caprylate esterase cleave the chromogen to release an insoluble purple chromophore. As the cells grow, the chromophore builds up and produces a purple-colored colony. However, some Enterobacteriaceae, including *Klebsiella* and *Enterobacter*, but not *Salmonella*, possess ß-glucosidase, which means that they are capable of also taking up and cleaving X-ßglucopyranoside. Therefore, if these organisms grow, they will form blue/dark blue colonies, which make them easy to

DISCUSSION OF THE VALIDATION STUDY (1)

differentiate from purple *Salmonella* colonies even if they are caprylate esterase positive. Novobiocin and cefsulodin inhibit the growth of other competing flora such as *Proteus* spp. and pseudomonads.The internal and independent method comparison evaluations of the Salmonella Rapid Culture Method (ONE Broth Salmonella/Brilliance™ Salmonella) clearly demonstrated that this method is equivalent to the USDA/FSIS reference method for the detection and presumptive identification of *Salmonella* spp. at spiked levels from low (1 colony forming unit (CFU)/25g) to high (1.1 CFU/g) in the following selected foods: ground beef, ground chicken, lettuce, shrimp, and shell eggs. In addition, the Salmonella Rapid Culture Method was found to be superior to the reference method in incubation time and ease of identifying typical colonies. The inclusivity data demonstrated that the Salmonella Rapid Culture Method (ONE Broth Salmonella/Brilliance™ Salmonella) detected essentially all species and serovars of Salmonella tested. The exclusivity data confirmed that the Salmonella Rapid Culture Method was able to discriminate *Salmonella* spp. from non-Salmonella microorganisms. Lot-to-lot comparability and stability data along with the ruggedness data verified that the Salmonella Rapid Culture Method was robust and can provide reproducible results over a range of culture conditions (time and temperature).

Based on these results, the Salmonella Rapid Culture Method (ONE Broth Salmonella enrichment followed by Brilliance™ Salmonella plating) should be recommended as the first line culture methodology for rapid detection of *Salmonella* spp. contamination in food products, saving time and making identification easier than the reference method.

Table 3a. Inclusivity Results (1)

Sample	Genus	Serovar	ID or ATCC #	Source	Origin	Result	Property
1	*Salmonella*	4,27:undetermined	238	Clinical	Unknown	Typical Growth	
2	*Salmonella*	4,5,12: i:-	268	Clinical	Unknown	Typical Growth	
3	*Salmonella*	4,5,12:b:- variant L(+)	197	Clinical	Unknown	Typical Growth	
4	*Salmonella*	4,5,12:i:-	275	Clinical	Unknown	Typical Growth	
5	*Salmonella*	6,7:k:-	237	Clinical	Unknown	Typical Growth	
6	*Salmonella*	abaetetuba	35460	ATCC	Creek water, Argentina	Typical Growth	
7	*Salmonella*	Abony	6017	NTCC	Unknown	Typical Growth	
8	*Salmonella*	abortusovis	31685	ATCC	CIP 1-097	No growth*	
9	*Salmonella*	adelaide	10718	ATCC	Walter Reed	Typical Growth	
10	*Salmonella*	Agona	131	USDA	Unknown	Typical Growth	
11	*Salmonella*	Albany	51960	ATCC	Unknown	Typical Growth	
12	*Salmonella*	Anatum	211	Clinical	Unknown	Typical Growth	
13	*Salmonella*	Arizonae	13314	ATCC	NCTC	Typical Growth	Lactose +
14	*Salmonella*	bareilly	9115	ATCC	Unknown	Typical Growth	
15	*Salmonella*	berta	8392	ATCC	Int. Salmonella Ctr.	Typical Growth	
16	*Salmonella*	blockley	51961	ATCC	Beth Isr. Hosp.	Typical Growth	
17	*Salmonella*	bongori	43975	ATCC	Unknown	Typical Growth	
18	*Salmonella*	Braenderup	216	Clinical	Unknown	Typical Growth	
19	*Salmonella*	Brandenburg	711	Turkey	Unknown	Typical Growth	
20	*Salmonella*	Bredeney	10728	ATCC	Walter Reed	Typical Growth	
21	*Salmonella*	Breukelen	15782	ATCC	Unknown	Typical Growth	
22	*Salmonella*	california	23201	ATCC	Vet Lab (U of Cal.)	Typical Growth	
23	*Salmonella*	Cerro	10723	ATCC	Walter Reed	Typical Growth	
24	*Salmonella*	chaco	49214	ATCC	NCTC	Typical Growth	
25	*Salmonella*	Chailey	227	Clinical	Unknown	Typical Growth	
26	*Salmonella*	Chester	11997	ATCC	CDC	Typical Growth	
27	*Salmonella*	Cholerasuis	10708	ATCC	Walter Reed	Typical Growth	
28	*Salmonella*	Cubana	697	Turkey	Unknown	Typical Growth	
29	*Salmonella*	Derby	123	USDA	Unknown	Typical Growth	
30	*Salmonella*	Diarizonae Lactose (+) H_2S (-)	29934	ATCC	Unknown	Typical Growth	Lactose +
31	*Salmonella*	Dublin	126	USDA	Unknown	Typical Growth	
32	*Salmonella*	Enteritidis	13076	ATCC	CDC	Typical Growth	
33	*Salmonella*	essen	6961	ATCC	NCTC	Typical Growth	
34	*Salmonella*	Etterbeek	19128	ATCC	Unknown	Typical Growth	
35	*Salmonella*	Ferlac	43976	ATCC	CIP	Typical Growth	
36	*Salmonella*	Florida	10727	ATCC	Walter Reed	Typical Growth	
37	*Salmonella*	gallinarum	9184	ATCC	Unknown	Typical Growth	

38	*Salmonella*	give	9268	ATCC	Rat	Typical Growth	
39	*Salmonella*	Hadar	689	Turkey	Unknown	Typical Growth	
40	*Salmonella*	Harmelen	15783	ATCC	Unknown	Typical Growth	
41	*Salmonella*	Heerlen	15792	ATCC	feces, Netherlands	Typical Growth	
42	*Salmonella*	Heidelberg	127	USDA	Unknown	Typical Growth	
43	*Salmonella*	Hilversum	15784	ATCC	snake, Mapolon	Typical Growth	
44	*Salmonella*	Hooggraven	15786	ATCC	Lizard, Cordylus	Typical Growth	
45	*Salmonella*	houten	29834	ATCC	NCTC, Frog, Gonyocephalus	Typical Growth	
46	*Salmonella*	Illinois	11646	ATCC	CDC	Typical Growth	
47	*Salmonella*	indiana	51959	ATCC	Unknown	Typical Growth	
48	*Salmonella*	indica	BAA-1576	ATCC	Sidney Kimmel Cancer Ctr	Typical Growth	
49	*Salmonella*	Infantis	51741	ATCC	pasta	Typical Growth	Sucrose +
50	*Salmonella*	Inverness	10720	ATCC	Walter Reed	Typical Growth	
51	*Salmonella*	Javiana	243	Clinical	unknown	Typical Growth	
52	*Salmonella*	jena	49221	ATCC	NCTC	Typical Growth	
53	*Salmonella*	Kahla	17980	ATCC	Unknown	Typical Growth	
54	*Salmonella*	Kentucky	9263	ATCC	Unknown	Typical Growth	
55	*Salmonella*	Kitenge	19126	ATCC	feces	Typical Growth	
56	*Salmonella*	Kunzendorf	12011	ATCC	CDC	Typical Growth	
57	*Salmonella*	Litchfield	125	USDA	Human	Typical Growth	
58	*Salmonella*	London	8389	ATCC	Unknown	Typical Growth	
59	*Salmonella*	Maarssen	15793	ATCC	Lizard, Zonosaurus	Typical Growth	
60	*Salmonella*	Maartensdijk	15790	ATCC	Calf lymph nodes	Typical Growth	
61	*Salmonella*	Maastricht	15789	ATCC	Fishmeal, Netherlands	Typical Growth	
62	*Salmonella*	Mbandaka	51958	in-house	Unknown	Typical Growth	Sucrose +
63	*Salmonella*	Meleagridis	256	Clinical	Unknown	Typical Growth	
64	*Salmonella*	Menden	15992	ATCC	feces	Typical Growth	
65	*Salmonella*	michigan	BAA-709	ATCC	cantalope/FDA	Typical Growth	
66	*Salmonella*	Minnesota	9700	ATCC	Michigan Dept. Hlth.	Typical Growth	
67	*Salmonella*	Montevideo	133	USDA	Unknown	Typical Growth	
68	*Salmonella*	Muenchen	132	USDA	Unknown	Typical Growth	
69	*Salmonella*	muenster	BAA-1575	ATCC	Stool, female patient	Typical Growth	
70	*Salmonella*	Newington	29628	ATCC	ducklings, CDC	No growth*	
71	*Salmonella*	Newport	218	Clinical	Unknown	Typical Growth	
72	*Salmonella*	Ngili	19127	ATCC	feces	Typical Growth	
73	*Salmonella*	Ochsenzoll	29932	ATCC	CIP	Typical Growth	
74	*Salmonella*	Ohio	129	USDA	Unknown	Typical Growth	
75	*Salmonella*	oranienberg	9239	ATCC	Unknown, food poisoning	Typical Growth	
76	*Salmonella*	panama	7378	ATCC	baby, New York	Typical Growth	
77	*Salmonella*	Paratyphi A	9150	ATCC	Unknown	Typical Growth	
78	*Salmonella*	Paratyphi B var L(+) tartrate +	173	Clinical	Unknown	Typical Growth	
79	*Salmonella*	paratyphi C	9068	ATCC	Unknown, East Africa	No growth*	
80	*Salmonella*	Parera	15788	ATCC	Water, BonAire	Typical Growth	
81	*Salmonella*	Phoenix	29931	ATCC	Unknown	Typical Growth	

82	*Salmonella*	Pomona	10729	ATCC	Walter Reed	Typical Growth	
83	*Salmonella*	Poona	304	Clinical	Unknown	Typical Growth	
84	*Salmonella*	pullorum	9120	ATCC	feces (diarrhea patient)	Typical Growth	
85	*Salmonella*	Putten	15787	ATCC	Lizard, Lacerta (Netherlands)	Typical Growth	
86	*Salmonella*	Reading	630	Turkey	Unknown	Typical Growth	
87	*Salmonella*	San Diego	23199	ATCC	Urinary bladder, turtle	Typical Growth	
88	*Salmonella*	Senftenberg	710	Turkey	Unknown	Typical Growth	
89	*Salmonella*	simsbury	12004	ATCC	CDC	Typical Growth	
90	*Salmonella*	Sloterdijk	15791	ATCC	Family illness, Netherlands	Typical Growth	
91	*Salmonella*	St. Paul	687	Turkey	Unknown	Typical Growth	
92	*Salmonella*	stanley	7308	ATCC	Unknown	Typical Growth	
93	*Salmonella*	tallahassee	12002	ATCC	CDC	Typical Growth	
94	*Salmonella*	Tennessee	10722	ATCC	Walter Reed	Typical Growth	
95	*Salmonella*	typhi	6539	ATCC	Walter Reed	No growth*	
96	*Salmonella*	Typhimurium	13311	ATCC	NCTC	Typical Growth	
97	*Salmonella*	Typhimurium Var Copenhagen	233	Clinical	Unknown	Typical Growth	
98	*Salmonella*	Typhimurium Var O:5-	337	Clinical	Unknown	Typical Growth	
99	*Salmonella*	typhisuis	8321	ATCC	Unknown	Typical Growth	
100	*Salmonella*	Urbana	9261	ATCC	Unknown	Typical Growth	
101	*Salmonella*	Vellore	15611	ATCC	Rectal	Typical Growth	
102	*Salmonella*	Worthington	9607	ATCC	Unknown	Typical Growth	

* S. Newington, typhi (6439) and paratyphi C showed typical growth on Brilliance™ Salmonella agar when plated directly from blood agar (no growth in ONE Broth directly). S. abortusovis appeared to grow in ONE Broth but did not grow on Brilliance™ Salmonella agar when plated.

Table 3b. Inclusivity Results (1)

Sample	Genus	Serovar	ID #	Source	Origin	Result[a]	Direct Plating onto Chromogenic Agar[b]
1	*Salmonella*	*paratyphi C*	3006	Univ. Calgary	unknown	No Growth	Typical Growth
2	*Salmonella*	*paratyphi C*	2288	Univ. Calgary	unknown	Typical Growth	Typical Growth
3	*Salmonella*	*paratyphi C*	2419	Univ. Calgary	unknown	Typical Growth	Typical Growth
4	*Salmonella*	*paratyphi C*	2505	Univ. Calgary	unknown	No Growth	Typical Growth
5	*Salmonella*	*paratyphi C*	2681	Univ. Calgary	unknown	No Growth	No Growth
6	*Salmonella*	*paratyphi C*	2719	Univ. Calgary	unknown	Typical Growth	Typical Growth
7	*Salmonella*	*paratyphi C*	2729	Univ. Calgary	unknown	Typical Growth	Typical Growth
8	*Salmonella*	*paratyphi C*	3000	Univ. Calgary	unknown	No Growth	Typical Growth
9	*Salmonella*	*paratyphi C*	3592	Univ. Calgary	unknown	No Growth	Typical Growth
10	*Salmonella*	*paratyphi C*	3596	Univ. Calgary	unknown	No Growth	Typical Growth
11	*Salmonella*	*typhi*	2682	Univ. Calgary	unknown	No Growth	Typical Growth
12	*Salmonella*	*typhi*	2692	Univ. Calgary	unknown	No Growth	Typical Growth
13	*Salmonella*	*typhi*	2728	Univ. Calgary	unknown	No Growth	Typical Growth
14	*Salmonella*	*typhi*	2780	Univ. Calgary	Malaysia	No Growth	No Growth
15	*Salmonella*	*typhi*	2990	Univ. Calgary	Canada	No Growth	Typical Growth
16	*Salmonella*	*typhi*	3124	Univ. Calgary	Canada	Typical Growth	Typical Growth
17	*Salmonella*	*typhi*	3158	Univ. Calgary	Canada	No Growth	Typical Growth
18	*Salmonella*	*typhi*	3220	Univ. Calgary	US	No Growth	Typical Growth
19	*Salmonella*	*typhi*	2698	Univ. Calgary	Canada	No Growth	Typical Growth
20	*Salmonella*	*typhi*	3489	Univ. Calgary	Chile	No Growth	Typical Growth

[a] Typical growth indicates purple colonies on chromogenic media after incubation in Salmonella ONE broth.

[b] Cultures were grown in BHI and plated directly onto Brilliance™ Salmonella media.

Table 2. Exclusivity Results (1)

Organism	Source	Origin	Result
Rhodococcus equi	ATCC 6939	foal lung abscess	No Growth
Alcaligenes faecalis	ATCC 35655	unknown	No Growth
Pseudomonas aeriginosa	ATCC 27853	unknown	Atypical Growth
Shigella sonnei	ATCC 9290	Walter Reed	Atypical Growth
Morganella morganii	ATCC 25829	stool, infant	Atypical Growth
Shigella flexneri	ATCC 9199	Walter Reed	Atypical Growth
Klebsiella pneumoniae	ATCC 13883	NCTC	Atypical Growth
Streptococcus pneumoniae	ATCC 6303	unknown	No Growth
Candida albicans	ATCC 10231	human	No Growth
Lactobacillus fermentum	ATCC 9338	unknown	No Growth
Proteus hauseri	ATCC 13315	NCTC	No Growth
Enterobacter aerogenes	ATCC 13048	CDC	Atypical Growth
Edwardsiella tarda	ATCC 15947	human feces	No Growth
Enterobacter sakazakii	ATCC 51329	unknown	Typical Growth[1]
Citrobacter farmeri	Clinical	unknown	Atypical Growth
Citrobacter braakii	Clinical	unknown	Atypical Growth
Citrobacter diversus	Clinical	unknown	Atypical Growth
Citrobacter freundii	Clinical	unknown	Atypical Growth
Staphylococcus aureus	ATCC 6538	human lesion	No Growth
Pantoea agglomerans	Clinical	unknown	No Growth
Bacillus cereus	ATCC 13061	FDA	No Growth
Enterobacter cloacae	ATCC 700323	unknown	Atypical Growth
Proteus vulgaris	ATCC 33420	clinical isolate	Atypical Growth
Listeria monocytogenes	ATCC 19115	human	Atypical Growth
Micrococcus luteus	ATCC 10240	air isolate	No Growth
Microbacterium testaceum	ATCC 15829	paddy	No Growth
E. coli O157:H7	Food	beef	Atypical Growth
E. coli 055:H7	USDA	unknown	Atypical Growth
E. coli (Generic)	51813	unknown	Atypical Growth
E. coli O145:NM	USDA	unknown	Atypical Growth

[1] Isolate confirmed as 99% *E. sakazakii* by Vitek ID. Note: No growth observed when cultured in ONE Broth Salmonella and plated onto Brilliance™ Salmonella.

Table 5. Method Comparison Results (1)

					Reference Method	Test Kit			Test Kit Performance			
Matrix	**Inoculating organism**	**Level**	**MPN/25g**	**No. test portions**	**Positive**	**Presump. Positive**	**Confirmed Positive**	**Chi square[a]**	**Sensitivity[b] %**	**False neg. %**	**Specificity[c] %**	**False pos. %**
Ground Beef	*Salmonella* Muenchen	Low	0.9	20	10	8	8	0.394	80	20	-	-
		Control	0	5	0	0	0	-	-	-	100	0
Ground Chicken	*Salmonella* Montevideo	Low	2.3	20	17	15	15	0.609	88	12	-	-
		Control	0	5	0	0	0	-	-	-	100	0
Lettuce	*Salmonella* Heidelberg	Low	5.75	20	15	14	14	0.122	93	7	-	-
		Control	0	5	0	0	0	-	-	-	100	0
Shrimp	*Salmonella* Cholersuis	Low	<0.75	20	9	10	10	0.098	111	0	-	-
		Control	0	5	0	0	0	-	-	-	100	0
Shell Eggs	*Salmonella* Typhimurium	Low	0.9	20	8	11	11	0.880	138	0	-	-
		Control	0	5	0	0	0	-	-	-	100	0

a Chi-square values greater than 3.84 indicate the two methods differ significantly ($P \leq 0.05$). (See appendix for complete Chi-square calculations)
b Sensitivity (%) = confirmed positive inoculated sample (test method)/confirmed positive inoculated sample (reference method) X 100
c Specificity (%) = confirmed positive controls (test method)/total number of controls

REFERENCES CITED

1. Hilber, Christine and McCallie, James., Evaluation of the Salmonella Rapid Culture Method using ONE Broth Salmonella and Brilliance™ Salmonella, AOAC® *Performance Tested℠* certification number 120802.
2. AOAC Research Institute Validation Outline for Salmonella Rapid Culture Method using ONE Broth Salmonella and Brilliance™ Salmonella , Approved – December 2008.
3. Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg Products. USDA Microbiology Laboratory Guidebook 4.03. USDA/FSIS, Laboratory QA/QC Division, Athens, GA. (http://www.fsis.usda.gov/PDF/MLG_4_04.pdf)
4. Andrews, W.H,, & Hammack, T.S.Wallace H. Andrews and Thomas S. Hammack. (1998) in *US FDA/CFSAN Bacteriological Analytical Manual (FDA-BAM)*, 8th edition, Chapter 5: Salmonella, U.S. Food and Drug Administration, Washington, DC. (online)(http://www.cfsan.fda.gov/~ebam/bam-5.html)