



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
081903

The AOAC Research Institute hereby certifies the method known as:

iQ-Check S. Enteritidis Real-Time PCR

Corporate Location
Bio-Rad Laboratories
2000 Alfred Nobel Drive
Hercules, CA 94547 USA

Manufacturing Location **Bio-Rad**
Laboratories
925 Alfred Nobel Drive
Hercules, CA 94547 USA

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. 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 that reads 'Scott Coates'.

Scott Coates, Senior Director
Signature for AOAC Research Institute

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METHOD NAME iQ-Check S. Enteritidis	CATALOG NUMBER 3578142
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INDEPENDENT LABORATORY Q Laboratories 1930 Radcliffe Dr. Cincinnati, OH USA 45214

APPLICABILITY OF METHOD Target Analyte – <i>Salmonella</i> Enteritidis. Matrixes – (25 g) – raw chicken breast with skin, raw chicken breast without skin, raw chicken breast without skin containing 2% w/w salt, raw chicken thigh with skin, raw chicken thigh without skin. Environmental boot swabs, drag swabs. Performance claims – The study data detected no statistical difference between the iQ-Check S. Enteritidis method and the reference methods.	REFERENCE METHODS U.S. Department of Agriculture Food Safety and Inspection Service (2018) Microbiology Laboratory Guidebook, Revision 4.09 <i>Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges (2)</i> FDA, <i>Environmental Sampling and Detection of Salmonella in Poultry Houses.</i> (3)
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ORIGINAL CERTIFICATION DATE August 21, 2019	CERTIFICATION RENEWAL RECORD Renewed annually through December 2024.
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METHOD MODIFICATION RECORD 1. January 2020 Level 1 2. January 2021 Level 1 3. April 2021 Level 1 4. October 2021 Level 1 5. January 2023 Level 2 6. October 2023 Level 1	SUMMARY OF MODIFICATION 1. Insert formatting change. 2. Editorial/clerical changes. 3. Software was updated from Version 3 to Version 4 allowing compatibility with Windows 10. 4. Editorial changes and addition of user information in French, German, Spanish, Portuguese, and Italian. 5. Addition of CFX Opus Deepwell, with CFX Manager Software, Industrial Diagnostic Edition version 3.1 using Free DNA Removal Solution and Fast APF protocols. 6. Editorial/clerical changes.
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PRINCIPLE OF THE METHOD (1)

The iQ-Check S. Enteritidis real-time PCR assay has the flexibility to be used in multiple ways. The kit can be used as a primary screening tool for the direct detection of S. Enteritidis (SE) in select foods and primary production samples such as boot or drag swabs. The kit can also be used as a secondary screening tool after first screening for presumptive positive samples with the iQ-Check *Salmonella* spp. kit using the same extracted DNA enabling quicker confirmation of SE present in a sample. Finally, the kit can be used to identify isolated suspected SE colonies directly from selective agar plates such as RAPID[®] *Salmonella* chromogenic agar.

The iQ-Check S. Enteritidis real-time PCR assay is based on gene amplification and detection by real-time polymerase chain reaction (PCR) technology. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific for S. Enteritidis as well as DNA polymerase and nucleotides. Detection and data analysis are optimized for use with Bio-Rad real-time PCR instruments, such as the CFX 96 Touch Deep Well system. The iQ-Check Prep is a robotic liquid handling platform that performs DNA extraction and PCR plate set-up. It is designed for all the iQ-Check Food Pathogen Detection Kits and the CFX96 Touch Deep Well real-time PCR System. It is a completely integrated automated solution for food pathogen testing. The iQ-Check Free DNA Removal Solution is also provided in a separate kit to safely reduce free DNA present in the matrixes.

PCR is a powerful technique used to generate many copies of target DNA. During the PCR reaction, several cycles of heating and cooling facilitate DNA denaturation, primer binding to the target region, and DNA polymerase extension of the DNA, creating copies of the target region. These copies are called amplicons.

A synthetic DNA “internal control” is included in the reaction mix. This control is amplified with a specific probe at the same time as the target analytes. It allows for the validation of any negative result (4).

DISCUSSION OF THE VALIDATION STUDY (1)

The iQ-Check S. Enteritidis Assay successfully detected SE from all six matrixes analyzed. The ability to screen for SE allows the end user to follow the current guidelines outlined by the USDA/FSIS MLG 4.09 and FDA *Env Sampling & Det of Salm in Poultry Houses* and identify the most prevalent serotypes in the poultry industry. The alternative confirmation procedure allows the end user to confirm samples at the same degree of sensitivity as the USDA/FSIS MLG 4.09 and FDA *Env Sampling & Det of Salm in Poultry Houses*, but one to two days sooner than the traditional confirmation method. With the addition of the Free DNA Removal Solution, the test kit allows the user to safely reduce free DNA present in the matrixes.

In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified, and all exclusivity organisms were correctly excluded. The inclusivity data also demonstrated that positive isolated colonies from the RAPID[®] *Salmonella* could be tested as part of the overall confirmation protocol for SE. In the method comparison study, the iQ-Check test kit demonstrated no statistically significant differences between candidate and reference method results (dPOD_C), or between presumptive and confirmed results (dPOD_{CP}) for all target pathogens.

The lot-to-lot consistency and stability study show no significant differences observed across the shelf life of the kit for three different lots of kits at each time point tested. The robustness study shows no discrepant results observed for all 8 treatment combinations for the iQ-Check S. Enteritidis assay.

The iQ-Check S. Enteritidis Assay is quick and simple to perform, providing results in approximately 4 hours post incubation of the enrichment for up to 94 sample replicates. The use of the iQ-Check Prep Instrument provides a hands-free application that can reduce possible contamination caused by the analyst performing testing. The iQ-Check Prep Instrument can perform DNA extraction and PCR preparation and provides added value of traceability to the lab. The CFX Manager IDE software is user friendly with the ability to track lot information and sample identification quickly and with ease. Because results are displayed in real-time, the user is able to quickly and accurately determine if results will be valid before the end of the run. The software also provides the user the option to analyze each individual Cq curves to help aid in problem solving any issues within an individual reaction.

Table 1. Inclusivity Results for the iQ-Check S. Enteritidis Assay (1)

No.	Species	Subspecies	Serovar	Antigenic Formula	Source	Origin	BPW	BPW + Suppl.	TCS	RSA
1	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	¹ RDC 338	Unknown	+	+	+	+
2	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	² ATCC 13076	Unknown	+	+	+	+
3	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	³ n° Anses: 4253.03	Oysters	+	+	+	+
4	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 3982.08	Turkey meat	+	+	+	+
5	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 6484.08	Culture from cow feces	+	+	+	+
6	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 1005.09	Chicken neck skin	+	+	+	+
7	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 8939.10	Env. chicken farm	+	+	+	+
8	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 318.11	Egg white	+	+	+	+
9	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 320.11	Salted egg yolk	+	+	+	+
10	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 322.11	Fresh eggshell	+	+	+	+
11	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 499.11	Toulouse sausage	+	+	+	+
12	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 639.11	Ground beef	+	+	+	+
13	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 778.11	Chicken thigh	+	+	+	+
14	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	⁴ RDCM 037 Labovet	Pork (carcass)	+	+	+	+
15	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 969.11	Layer hen env.	+	+	+	+
16	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 971.11	Pork meat	+	+	+	+
17	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 972.11	Chicken breast	+	+	+	+
18	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 987.11	Mango Bavarian cake	+	+	+	+
19	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	n° Anses: 1137.11	Flesh chicken	+	+	+	+
20	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	⁵ ADRIA n°23	Raw liquid egg	+	+	+	+
21	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°11195.90	Unknown	+	+	+	+
22	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°5	Unknown	+	+	+	+
23	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°0011	Unknown	+	+	+	+

24	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°0115	Unknown	+	+	+	+
25	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°0211	Unknown	+	+	+	+
26	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°0212	Unknown	+	+	+	+
27	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°0214	Unknown	+	+	+	+
28	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°0216	Unknown	+	+	+	+
29	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°0217	Unknown	+	+	+	+
30	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°20268	Unknown	+	+	+	+
31	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	⁶ Ad211	Unknown	+	+	+	+
32	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	Ad213	Unknown	+	+	+	+
33	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	RDCM 076 ADRIA	Raw liquid egg	+	+	+	+
34	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	Ad638	Mayonnaise	+	+	+	+
35	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	Ad926	Veal meat (paupiette)	+	+	+	+
36	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA	NCTC 6676	+	+	+	+
37	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ADRIA n°Ad477	Hen breast	+	+	+	+
38	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	RDCM 188 Hey's Food (NL)	Unknown	+	+	+	+
39	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	⁷ NCCB 100284	Milk	+	+	+	+
40	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	⁸ DSM 26655	Unknown	+	+	+	+
41	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ATCC 4931	human gastro-enteritis	+	+	+	+
42	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ATCC 31194	Unknown	+	+	+	+
43	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	9,12:g,m	ATCC 49220	Unknown	+	+	+	+
44	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	⁹ CIP 105150	Unknown	+	+	+	+
45	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	CIP 106158	Unknown	+	+	+	+
46	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	CIP 57.29	Unknown	+	+	+	+
47	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	9,12:g,m:-	CIP 56.29	Unknown	+	+	+	+
48	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	9,12:g,m:-	CIP 81.3	Unknown	+	+	+	+
49	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ATCC 49221	Unknown	+	+	+	+
50	<i>Salmonella enterica</i>	<i>enterica</i>	Enteritidis	1,9,12:g,m:-	ATCC 49219	Unknown	+	+	+	+

¹Bio-Rad R&D Collection, Marnes, France²American Type Culture Collection, Manassas, VA³French Agency for Food, Environmental and Occupational Health & Safety, Maisons-Alfort, France⁴Bio-Rad R&D Collection Marnes, Marnes, France^{5,6}Culture Collection ADRIA Developpement, Quimper, France⁷Netherlands Culture Collection of Bacteria, Utrecht, Netherlands⁸The Leibniz Institute DSMZ, Brunswick, Germany⁹Collection Institute Pasteur, Paris, France

Table 2. Exclusivity Results for iQ-Check S. Enteritidis Assay (1)										
No.	Species	Subspecies	Serovar	Antigenic Formula	Source	Origin	BPW	BPW + Suppl.	TCS	RSA
1	<i>Salmonella bongori</i>			V 66 : z41:-	¹ CIP 82.33T	Unknown	-	-	-	-
2	<i>Salmonella bongori</i>		Maregrosso	V 66 : z35:-	² CMF 301.1	Unknown	-	-	-	-
3	<i>Salmonella enterica</i>	<i>arizonae</i>			³ RDC 36	Unknown	-	-	-	-
4	<i>Salmonella enterica</i>	<i>arizonae</i>		51 : z4,z23	CIP 82.30T	Unknown	-	-	-	-
5	<i>Salmonella enterica</i>	<i>diarizonae</i>		IIIb 61 : i:z53	⁴ ADRIA/AFSSA AD 595	Cheese	-	-	-	-
6	<i>Salmonella enterica</i>	<i>diarizonae</i>		IIIb 38 : l,v:z35	ADRIA/AFSSA AD 594	Frog legs	-	-	-	-
7	<i>Salmonella enterica</i>	<i>diarizonae</i>		IIIb 6,7 : l,v:z53	CIP 82.31T	Unknown	-	-	-	-
8	<i>Salmonella enterica</i>	<i>enterica</i>	Abaetetuba	11 : k:1,5	⁵ ATCC 35640	Unknown	-	-	-	-
9	<i>Salmonella enterica</i>	<i>enterica</i>	Aberdeen	11 : i:1,2	CMF 114.1	Unknown	-	-	-	-
10	<i>Salmonella enterica</i>	<i>enterica</i>	Abony	1,4,12,27:b:e,n,x	⁶ NCTC 6017	Unknown	-	-	-	-
11	<i>Salmonella enterica</i>	<i>enterica</i>	Adelaide	35 : f,g: -	CMF 482.2	Unknown	-	-	-	-
12	<i>Salmonella enterica</i>	<i>enterica</i>	Agama	4,12 : i:1,6	⁷ RDCM 205 Hey's food (NL)	Unknown	-	-	-	-
13	<i>Salmonella enterica</i>	<i>enterica</i>	Agona	1,4,[5],12 : f,g,s:[1,2]	RDCM 57	Unknown	-	-	-	-
14	<i>Salmonella enterica</i>	<i>enterica</i>	Albany	8,2 : z4,z24:-	CMF 82.2	Unknown	-	-	-	-
15	<i>Salmonella enterica</i>	<i>enterica</i>	Anatum	3,{10}{15}{15,34} : e,h:1,6	CMF 814.4	Unknown	-	-	-	-
16	<i>Salmonella enterica</i>	<i>enterica</i>	Bambylor	9,46 : z:e,n,z15	CMF 135.1	Unknown	-	-	-	-
17	<i>Salmonella enterica</i>	<i>enterica</i>	Bareilly	6,7,14 : y:1,5	CMF 136.1	Unknown	-	-	-	-
18	<i>Salmonella enterica</i>	<i>enterica</i>	Blockley	6,8 : k:1,5	RDCM 127 ADRIA	Hen	-	-	-	-
19	<i>Salmonella enterica</i>	<i>enterica</i>	Braenderup	6,7,14 : e,h:e,n,z15	CMF 151	Unknown	-	-	-	-
20	<i>Salmonella enterica</i>	<i>enterica</i>	Brandenburg	4,[5],12 : l,v:e,n,z15	RDC 106	Unknown	-	-	-	-
21	<i>Salmonella enterica</i>	<i>enterica</i>	California	4,12 : g,m,t:[z67]	RDCM 051	Unknown	-	-	-	-
22	<i>Salmonella enterica</i>	<i>enterica</i>	Canoga (Westhampton)	3,15,34:g,s,t:-	RDCM 46	Unknown	-	-	-	-
23	<i>Salmonella enterica</i>	<i>enterica</i>	Cerro	6,14,18 : z4,z23:[1,5]	CMF 166.2	Unknown	-	-	-	-
24	<i>Salmonella enterica</i>	<i>enterica</i>	Crossness	67 : r:1,2	CMF 165.1	Unknown	-	-	-	-
25	<i>Salmonella enterica</i>	<i>enterica</i>	Cubana	1,13,23 : z29:-	CMF 188	Unknown	-	-	-	-
26	<i>Salmonella enterica</i>	<i>enterica</i>	Dahlem	48 : k:e,n,z15	CMF 924.1	Unknown	-	-	-	-
27	<i>Salmonella enterica</i>	<i>enterica</i>	Derby	1,4,[5],12 : f,g:[1,2]	RDC 91	Unknown	-	-	-	-
28	<i>Salmonella enterica</i>	<i>enterica</i>	Duisburg	1,4,12,[27] : d:e,n,z15	RDCM 199 Hey's food (NL)	Unknown	-	-	-	-
29	<i>Salmonella enterica</i>	<i>enterica</i>	Emek	8,20 : g,m,s:-	RDCM 75 ADRIA	Unknown	-	-	-	-
30	<i>Salmonella enterica</i>	<i>enterica</i>	Fischerkietz	1,6,14,25 : y:e,n,x	RDCM 202 Hey's food (NL)	Unknown	-	-	-	-
31	<i>Salmonella enterica</i>	<i>enterica</i>	Ferruch	8 : e,h:1,5	RDCM 207 Hey's food (NL)	Unknown	-	-	-	-
32	<i>Salmonella enterica</i>	<i>enterica</i>	Give	3,{10}{15}{15,34} : l,v:1,7	RDM 2	Hollande	-	-	-	-
33	<i>Salmonella enterica</i>	<i>enterica</i>	Glostrup	6,8 : z10:e,n,z15	CMF 226.3	Unknown	-	-	-	-
34	<i>Salmonella enterica</i>	<i>enterica</i>	Goldcoast	6,8 : r:l,w	RDCM 210 Hey's food (NL)	Unknown	-	-	-	-

35	<i>Salmonella enterica</i>	<i>enterica</i>	Grumpensis	1,13,23 : d:1,7	CMF 478.2	Unknown	-	-	-	-
36	<i>Salmonella enterica</i>	<i>enterica</i>	Guinea	1,44 : z10:1,7	RDCM 62 ADRIA	Unknown	-	-	-	-
37	<i>Salmonella enterica</i>	<i>enterica</i>	Hadar	6,8 : z10:e,n,x	CMF 234.1	Unknown	-	-	-	-
38	<i>Salmonella enterica</i>	<i>enterica</i>	Havana	1,13,23 : f,g,[s]:-	CMF 237.1	Unknown	-	-	-	-
39	<i>Salmonella enterica</i>	<i>enterica</i>	Heidelberg	<u>1</u> ,4,[5],12 : r:1,2	RDC 93	Unknown	-	-	-	-
40	<i>Salmonella enterica</i>	<i>enterica</i>	Illinois	3,{10}{15}{15,34} : z10:1,5	CMF 251.2	Unknown	-	-	-	-
41	<i>Salmonella enterica</i>	<i>enterica</i>	Indiana	1,4,12 : z:1,7	RDCM 128 ADRIA	Fishmeal	-	-	-	-
42	<i>Salmonella enterica</i>	<i>enterica</i>	Infantis	6,7,14 : r:1,5	RDCM 71 ADRIA	CIP 82.97	-	-	-	-
43	<i>Salmonella enterica</i>	<i>enterica</i>	Inverness	38 : k:1,6	CMF 253.1	Unknown	-	-	-	-
44	<i>Salmonella enterica</i>	<i>enterica</i>	Johannesburg	1,40 : b:e,n,x	CMF 256.1	Unknown	-	-	-	-
45	<i>Salmonella enterica</i>	<i>enterica</i>	Kedougou	1,13,23 : i:l,w	RDCM 212 Hey's food (NL)	Unknown	-	-	-	-
46	<i>Salmonella enterica</i>	<i>enterica</i>	Kentucky	8,20 : i:z6	CMF 264.2	Unknown	-	-	-	-
47	<i>Salmonella enterica</i>	<i>enterica</i>	Kirkee	17 : b:1,2	CMF 458.2	Unknown	-	-	-	-
48	<i>Salmonella enterica</i>	<i>enterica</i>	Livingstone	6,7,14 : d:l,w	⁸ LAV 1	Env.	-	-	-	-
49	<i>Salmonella enterica</i>	<i>enterica</i>	Lomita	6,7 : e,h:1,5	CMF 125.3	Unknown	-	-	-	-
50	<i>Salmonella enterica</i>	<i>enterica</i>	London	3,{10}{15} : l,v:1,6	RDCM 129 ADRIA	Cooked pork shoulder	-	-	-	-
51	<i>Salmonella enterica</i>	<i>enterica</i>	Mbandaka	6,7,14 : z10:e,n,z15	RDCM 130 ADRIA	Mayonnaise	-	-	-	-
52	<i>Salmonella enterica</i>	<i>enterica</i>	Miami	1,9,12 : a:1,5	CMF 307	Unknown	-	-	-	-
53	<i>Salmonella enterica</i>	<i>enterica</i>	Minnesota	21 : b:e,n,x	CMF 146.3	Unknown	-	-	-	-
54	<i>Salmonella enterica</i>	<i>enterica</i>	Montevideo	6,7,14,[54] : g,m,[p],s:[1,2,7]	RDCM 45	Unknown	-	-	-	-
55	<i>Salmonella enterica</i>	<i>enterica</i>	Muenchen	6,8 : d:1,2	CMF 337	Unknown	-	-	-	-
56	<i>Salmonella enterica</i>	<i>enterica</i>	Newport	6,8,20 : e,h:1,2	RDCM 208 Hey's food (NL)	Unknown	-	-	-	-
57	<i>Salmonella enterica</i>	<i>enterica</i>	Nienstedten	6,7,14 : b:l,w	CMF 352	Unknown	-	-	-	-
58	<i>Salmonella enterica</i>	<i>enterica</i>	Nottingham	16 : d:e,n,z15	NCTC 7832T	Unknown	-	-	-	-
59	<i>Salmonella enterica</i>	<i>enterica</i>	Ohio	6,7,14 : b:l,w	RDCM 132 ADRIA	Raw cow milk	-	-	-	-
60	<i>Salmonella enterica</i>	<i>enterica</i>	Oranienburg	6,7,14 : m,t:[z57]	CMF 360	Unknown	-	-	-	-
61	<i>Salmonella enterica</i>	<i>enterica</i>	Ouakam	9,46 : z29:-	CMF 364	Unknown	-	-	-	-
62	<i>Salmonella enterica</i>	<i>enterica</i>	Paratyphi B	1,4,[5],12 : b:1,2	RDCM 131 ADRIA	Unknown	-	-	-	-
63	<i>Salmonella enterica</i>	<i>enterica</i>	Paratyphi B Java	<u>1</u> ,4,[5],12 : b:1,2	RDCM 192	Unknown	-	-	-	-
64	<i>Salmonella enterica</i>	<i>enterica</i>	Paratyphi B	1,4,[5],12 : b:1,2	RDCM 131	Unknown	-	-	-	-
65	<i>Salmonella enterica</i>	<i>enterica</i>	Poona	1,13,22 : z:1,6	CMF 689.2	Unknown	-	-	-	-
66	<i>Salmonella enterica</i>	<i>enterica</i>	Potsdam	6,7,14 : l,v:e,n,z15	CMF 225.2	Unknown	-	-	-	-
67	<i>Salmonella enterica</i>	<i>enterica</i>	Putten	13,23 : d:l,w	RDCM 214	Unknown	-	-	-	-
68	<i>Salmonella enterica</i>	<i>enterica</i>	Rubislaw	11 : r:e,n,x	CMF 414.2	Unknown	-	-	-	-
69	<i>Salmonella enterica</i>	<i>enterica</i>	Saint Paul	1,4,[5],12 : e,h:1,2	RDCM 101 ADRIA	Unknown	-	-	-	-
70	<i>Salmonella</i>	<i>enterica</i>	Senftenberg	1,3,19 : g,[s],t:-	RDCM 209	Unknown	-	-	-	-

	<i>enterica</i>				Hey's food (NL)					
71	<i>Salmonella enterica</i>	<i>enterica</i>	Strasbourg	9,46 : d:1,7	CMF 457.2	Unknown	-	-	-	-
72	<i>Salmonella enterica</i>	<i>enterica</i>	Sundsvall	[1],6,14,[25] : z:e,n,x	CMF 877.2	Unknown	-	-	-	-
73	<i>Salmonella enterica</i>	<i>enterica</i>	Taksony	1,3,19 : i:z6	RDCM 203 Hey's food (NL)	Unknown	-	-	-	-
74	<i>Salmonella enterica</i>	<i>enterica</i>	Tallahassee	6,8 : z4,z32:-	CMF 822.1	Unknown	-	-	-	-
75	<i>Salmonella enterica</i>	<i>enterica</i>	Tennessee	6,7,14 : z29:-	RDCM Nestlé 599	Env.	-	-	-	-
76	<i>Salmonella enterica</i>	<i>enterica</i>	Thompson	6,7,14 : k:1,5	RDCM 056	Unknown	-	-	-	-
77	<i>Salmonella enterica</i>	<i>enterica</i>	Tournai	3,{10}{15} : y:z6	CMF 448.1	Unknown	-	-	-	-
78	<i>Salmonella enterica</i>	<i>enterica</i>	Typhimurium	1,4,[5],12:i:1,2	ATCC 14028	Chicken heart and liver tissue	-	-	-	-
79	<i>Salmonella enterica</i>	<i>enterica</i>	Utrecht	52 : d:1,5	CMF 484.1	Unknown	-	-	-	-
80	<i>Salmonella enterica</i>	<i>enterica</i>	Virchow	6,7,14 : r:1,2	CMF 805.2	Unknown	-	-	-	-
81	<i>Salmonella enterica</i>	<i>enterica</i>	Wayne	30:g,z51:-	RDCM 54	Unknown	-	-	-	-
82	<i>Salmonella enterica</i>	<i>enterica</i>	Weslaco	42 : z36:-	CMF 688.1	Unknown	-	-	-	-
83	<i>Salmonella enterica</i>	<i>enterica</i>	Worthington	1,13,23 : z :l,w	RDCM 157 Friesland Campina	16S ID	-	-	-	-
84	<i>Salmonella enterica</i>	<i>enterica</i>	Yoruba	16 : c:l,w	RDCM 3913/83	Unknown	-	-	-	-
85	<i>Salmonella enterica</i>	<i>houtanae</i>		IV 45 : g,z51:-	CIP 82.32T	Unknown	-	-	-	-
86	<i>Salmonella enterica</i>	<i>houtanae</i>		IV 43 : z4,z32:-	ADRIA/AFSSA AD 597	Cooked fish	-	-	-	-
87	<i>Salmonella enterica</i>	<i>houtanae</i>		IV 50 : g,z51:-	ADRIA/AFSSA AD 596	Milk product	-	-	-	-
88	<i>Salmonella enterica</i>	<i>indica</i>		VI [1],6,14,[25] : a:e,n,x	CIP 102501T	Unknown	-	-	-	-
89	<i>Salmonella enterica</i>	<i>salamae</i>	Betioky	II 59 : k:z65	CMF 141.1	Unknown	-	-	-	-
90	<i>Salmonella enterica</i>	<i>salamae</i>	Daressalaam	II 1,9,12 : l,w:e,n,x	CIP 82.29T	Unknown	-	-	-	-
91	<i>Salmonella enterica</i>	<i>salamae</i>	Grabouw	II 11 : g,m,s,t:z39	RDCM 047	Unknown	-	-	-	-
92	<i>Salmonella enterica</i>	<i>salamae</i>		II 42 : b:e,n,x,z15	ADRIA/AFSSA AD 593	Seed	-	-	-	-
93	<i>Salmonella enterica</i>	<i>salamae</i>		II 42 : g,t:-	ADRIA/AFSSA AD 592	Kangaroo meat	-	-	-	-
94	<i>Salmonella enterica</i>	<i>salamae</i>	Manica	II 1,9,12 : g,m,s,t:(z42)	RDCM 048	Unknown	-	-	-	-
95	<i>Salmonella enterica</i>	<i>salamae</i>	Phoenix	II 47 : b:1,5	CMF 395.1	Unknown	-	-	-	-
96	<i>Salmonella enterica</i>	<i>salamae</i>	Tranoroa	II 55 : k:z39	CMF 463.1	Unknown	-	-	-	-
97	<i>Salmonella enterica</i>	<i>salamae</i>	Zuerich	II 1,9,12,46,27 : c:z39	CMF 510.1	Unknown	-	-	-	-
98	<i>Acinetobacter baumannii</i>				RDC 146	Unknown	-		-	
99	<i>Aeromonas hydrophila</i>				⁹ LMG 2844T	Frog red-leg	-		-	
100	<i>Bacillus licheniformis</i>				RDC 88	Unknown	-		-	
101	<i>Campylobacter coli</i>				CIP 70.77 c1	Unknown	-		-	
102	<i>Campylobacter coli</i>				CIP 70.80T a	Unknown	-		-	
103	<i>Campylobacter coli</i>				CIP 70.54 a	Unknown	-		-	
104	<i>Campylobacter coli</i>				CIP 103753	Unknown	-		-	

105	<i>Campylobacter coli</i>				CIP 70.54 c	Unknown	-		-	
106	<i>Campylobacter coli</i>				CIP 70.77 c2	Unknown	-		-	
107	<i>Campylobacter concisus</i>				CIP 103757T b	Unknown	-		-	
108	<i>Campylobacter curvus</i>				CIP 103747T b	Unknown	-		-	
109	<i>Campylobacter fetus</i>	fetus			CIP 53.96T a	Unknown	-		-	
110	<i>Campylobacter fetus</i>	fetus			CIP 53.96T c	Unknown	-		-	
111	<i>Campylobacter fetus</i>	vener			CIP 68.29T c	Unknown	-		-	
112	<i>Campylobacter jejuni</i>				CIP 70.2T a	Unknown	-		-	
113	<i>Campylobacter jejuni</i>				CIP 70.86 T a	Unknown	-		-	
114	<i>Campylobacter jejuni</i>				CIP 103778 a	Unknown	-		-	
115	<i>Campylobacter jejuni</i>				CIP 103726 b	Unknown	-		-	
116	<i>Campylobacter jejuni</i>				CIP 70.2T b	Unknown	-		-	
117	<i>Campylobacter jejuni</i>				CIP 70.2 T b	Unknown	-		-	
118	<i>Campylobacter jejuni</i>				CIP 103778	Unknown	-		-	
119	<i>Campylobacter jejuni</i>	doylei			CIP 103751T a	Unknown	-		-	
120	<i>Campylobacter lari</i>				CIP 102722T a	Unknown	-		-	
121	<i>Campylobacter lari</i>				CIP 102722T c	Unknown	-		-	
122	<i>Campylobacter mucosalis</i>				CIP 103750T a	Unknown	-		-	
123	<i>Campylobacter mucosalis</i>				CIP 103750T c	Unknown	-		-	
124	<i>Campylobacter showae</i>				CIP 103970T a	Unknown	-		-	
125	<i>Campylobacter showae</i>				CIP 103970T b	Unknown	-		-	
126	<i>Campylobacter sputorum</i>				CIP 53.103T	Unknown	-		-	
127	<i>Campylobacter sputorum</i>				CIP 103749T c2	Unknown	-		-	
128	<i>Campylobacter sputorum</i>				CIP 103749T a	Unknown	-		-	
129	<i>Campylobacter sputorum</i>				CIP 103749T c1	Unknown	-		-	
130	<i>Campylobacter upsaliensis</i>				CIP 103681T a	Unknown	-		-	
131	<i>Citrobacter freundii</i>				ATCC 8090	Unknown	-		-	
132	<i>Enterobacter aerogenes</i>				ATCC 13048	Unknown	-		-	
133	<i>Enterobacter cloacae</i>				LMG 2783	Unknown	-		-	
134	<i>Enterobacter sakazakii</i>				RDC 236	Milk product	-		-	
135	<i>Escherichia coli</i>			O157H7 VT-	ATCC 700728	Unknown	-		-	
136	<i>Escherichia hermanii</i>				RDC 72	White egg	-		-	
137	<i>Hafnia alvei</i>				CIP 57.31T	Unknown	-		-	
138	<i>Klebsiella oxytoca</i>				RDC 30	Unknown	-		-	
139	<i>Listeria monocytogenes</i>			1/2b	¹⁰ CNRL 87373	Unknown	-		-	
140	<i>Listeria monocytogenes</i>			1/2c	CNRL 103573	Unknown	-		-	
141	<i>Listeria</i>			1/2a	CIP 78.31	mesenteric	-		-	

	<i>monocytogenes</i>					lymph node, guinea pig				
142	<i>Micrococcus luteus</i>				RDC 70	soil	-		-	
143	<i>Proteus mirabilis</i>				ATCC 29906	Unknown	-		-	
144	<i>Pseudomonas aeromonas</i>				ATCC 27853	Unknown	-		-	
145	<i>Pseudomonas aeruginosa</i>				CIP 76.110	Unknown	-		-	
146	<i>Serratia marcescens</i>				RDC 33	Unknown	-		-	
147	<i>Shigella flexneri</i>				ATCC 12022	Unknown	-		-	
148	<i>Shigella sonnei</i>				ATCC 25931	Human feces	-		-	
149	<i>Staphylococcus aureus</i>				ATCC 25923	Unknown	-		-	
150	<i>Staphylococcus epidermidis</i>				ATCC 14990	nasal swab	-		-	
151	<i>Staphylococcus intermedius</i>				RDCM SDP 1.14.1	Unknown	-		-	
152	<i>Staphylococcus xylosus</i>				RDC 85	Unknown	-		-	
153	<i>Streptococcus B agalactiae</i>				ATCC 13813	Unknown	-		-	
154	<i>Yersinia enterocolitica</i>				CIP 80.27T	Face, glanders like infection	-		-	

¹Collection Institute Pasteur, Paris, France

²Bio-Rad Internal Collection, Steenvoorde, France

³Bio-Rad R&D Collection, Marnes, France

⁴Culture Collection ADRIA Developpement, Quimper, France

⁵American Type Culture Collection, Manassas, VA

⁶National Collection of Type Cultures, Salisbury, England

⁷Bio-Rad R&D Collection Marnes, Marnes, France

⁸Lavetan Reference Laboratory, Turnhout, Belgium

⁹Belgian Coordinated Collections of Microorganisms, Ghent, Belgium

¹⁰Centre National de référence des Legionelles, Lyon, France

Table 5. Summary of Salmonella Enteritidis Results (1)

Level	iQ-Check S. Enteritidis					Reference Method
	Presumptive		Confirmed			
	With FDRS ¹	Without FDRS	Alternative	Traditional		
Raw chicken breast without skin						
Noninoculated	0/5	0/5	0/5	0/5	0/5	0/5
Low	8/20	8/20	8/20	8/20	8/20	8/20
High	5/5	5/5	5/5	5/5	5/5	5/5
Raw chicken breast with skin						
Noninoculated	0/5	0/5	0/5	0/5	0/5	0/5
Low	9/20	9/20	9/20	9/20	9/20	9/20
High	5/5	5/5	5/5	5/5	5/5	5/5
Raw chicken breast without skin containing 2% w/w salt						
Noninoculated	0/5	0/5	0/5	0/5	0/5	0/5
Low	11/20	11/20	11/20	11/20	11/20	11/20
High	5/5	5/5	5/5	5/5	5/5	5/5
Raw chicken thigh without skin						
Noninoculated	0/5	0/5	0/5	0/5	0/5	0/5
Low	8/20	8/20	8/20	8/20	8/20	8/20
High	5/5	5/5	5/5	5/5	5/5	5/5
Raw chicken thigh with skin						
Noninoculated	0/5	0/5	0/5	0/5	0/5	0/5
Low	10/20	10/20	10/20	10/20	10/20	10/20
High	5/5	5/5	5/5	5/5	5/5	5/5
Boot/Drag swabs						
Noninoculated	0/5	0/5	0/5	0/5	0/5	0/5
Low	11/20	11/20	11/20	11/20	11/20	10/20
High	5/5	5/5	5/5	5/5	5/5	5/5

¹FDRS = Free DNA Removal Solution

Table 7. iQ-Check S. Enteritidis Results – Candidate vs. Reference (1)

Matrix and Inoculum	MPN ^a / Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
			x ^c	POD _c ^d	95% CI	x	POD _R ^e	95% CI		
Raw chicken breast without skin (25 g) <i>Salmonella</i> Enteritidis (CCUG 21288)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.50 (0.25, 0.86)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
	1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw chicken breast with skin (25 g) <i>Salmonella</i> Enteritidis (ATCC 13076)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.49 (0.25, 0.84)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw chicken breast without skin + 2% w/w salt (25 g) <i>Salmonella</i> Enteritidis (CCUG 26522)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.84 (0.49, 1.40)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw chicken thigh without skin (25 g) <i>Salmonella</i> Enteritidis (CCUG 27004)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.50 (0.25, 0.86)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw chicken thigh with skin (25 g) <i>Salmonella</i> Enteritidis (ATCC 4931)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.76 (0.45, 1.27)	20	10	0.50	0.25, 0.75	10	0.50	0.25, 0.75	0.00	-0.13, 0.13
	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Boot Swabs <i>Salmonella</i> Enteritidis (CCUG 27021)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	74	20	11	0.55	0.34, 0.74	10	0.50	0.25, 0.75	0.05	-0.24, 0.33
	220	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval; Test portions for boot swabs was determined by plating the inoculum

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method and reference method confirmed POD values

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

Table 8. iQ-Check S. Enteritidis Results – Presumptive vs. Confirmed (1)

Matrix and Inoculum	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
			x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Raw chicken breast without skin (25 g) <i>Salmonella</i> Enteritidis (CCUG 21288)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00 ^h	-0.47, 0.47
	0.50 (0.25, 0.86)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
	1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw chicken breast with skin (25 g) <i>Salmonella</i> Enteritidis (ATCC 13076)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.49 (0.25, 0.84)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw chicken breast without skin + 2% w/w salt (25 g) <i>Salmonella</i> Enteritidis (CCUG 26522)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.84 (0.49, 1.40)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw chicken thigh without skin (25 g) <i>Salmonella</i> Enteritidis (CCUG 27004)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.50 (0.25, 0.86)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw chicken thigh with skin (25 g) <i>Salmonella</i> Enteritidis (ATCC 4931)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.76 (0.45, 1.27)	20	10	0.50	0.25, 0.75	10	0.50	0.25, 0.75	0.00	-0.13, 0.13
	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Boot Swabs <i>Salmonella</i> Enteritidis (CCUG 27021)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	74	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
	220	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval; Test portions for boot swabs was determined by plating the inoculum

^bN = Number of test portions

^cx = Number of positive test portions

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

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

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

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

^hResults between traditional and alternative confirmation were identical

DISCUSSION OF THE MODIFICATION STUDY APPROVED JANUARY 2023 (5)

The new CFX Opus Deepwell instrument delivers the same performance as the current CFX96 Touch Deep Well instrument but with a more modern design and cloud capabilities. The improved stability of the thermal block ensures a more uniform thermal protocol. The CFX Manager Software, IDE v 3.1 brings the same performance, algorithm, and interpretation as the current CFX Manager Software, IDE v 3.0 with the only change being compatibility to both CFX96 Touch Deep Well and CFX Opus Deepwell instruments.

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