CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

081904

The AOAC Research Institute hereby certifies the method known as

iQ-Check *S.* Typhimurium 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

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Bradley A. Stawick, AOAC Research Institute Senior Director

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METHOD NAME
iQ-Check *S.* Typhimurium Real-Time PCR

CATALOG NUMBER
12004306

INDEPENDENT LABORATORY
Q Laboratories
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Cincinnati, OH USA 45214

APPLICABILITY OF METHOD
Target Analyte – *Salmonella* Typhimurium.

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.* Typhimurium method and the reference methods.

REFERENCE METHODS

U.S. Department of Agriculture Food Safety and Inspection Service (2018) Microbiology Laboratory Guidebook, Revision 4.09 *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges* (2)

FDA, *Environmental Sampling and Detection of Salmonella in Poultry Houses.* (3)

ORIGINAL CERTIFICATION DATE
August 21, 2019

CERTIFICATION RENEWAL RECORD
Renewed through December 2026.

METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. January 2020 Level 1	1. Insert reformatted.
2. January 2021 Level 1	2. Editorial changes.
3. April 2021 Level 1	3. Software was updated from Version 3 to Version 4 allowing compatibility with Windows 10.
4. October 2021 Level 1	4. Editorial changes and addition of user information in French, German, Spanish, Portuguese, and Italian.
5. January 2023 Level 2	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. October 2023 Level 1	6. Editorial changes.
7. November 2024 Level 1	7. Editorial changes including software update to CFX Maestro Software, IDE v4.0.

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PRINCIPLE OF THE METHOD (1)
The iQ-Check *S.* Typhimurium 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.* Typhimurium (ST) 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 ST present in a sample. Finally, the kits can be used to identify isolated suspected ST colonies directly from selective agar plates such as RAPID'*Salmonella* chromogenic agar.
The iQ-Check *S.* Typhimurium 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.* Typhimurium, 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.* Typhimurium Assays successfully detected ST from all six matrixes analyzed. The ability to screen for ST 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 two of the most prevalent serotypes in the poultry industry with two assays. 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 kits allow 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 on the kit as part of the overall confirmation protocol for ST. In the method comparison study, the iQ-Check test kit demonstrated no statistically significant difference 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 kits 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 *S.* Typhimurium assay.

The iQ-Check *S.* Typhimurium 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 3. Inclusivity Results for iQ-Check *S.* Typhimurium Assay (1)

No.	Species	Subspecies	Serovar	Antigenic Formula	Source	Origin	BPW	BPW + Suppl.	TCS	RSA
1	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	[1]ATCC 14028	Chicken heart and liver tissue	+	+	+	+
2	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	[2]n° Anses: 38.09	Brine	+	+	+	+
3	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 442.09	Beef meat	+	+	+	+
4	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 447.09	Pork (crépine)	+	+	+	+
5	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 591.09	Lamb with sauce	+	+	+	+
6	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 695.09	Stuffed quail	+	+	+	+
7	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 839.09	Culture from veal feces	+	+	+	+
8	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 838.09	Environment (Duck)	+	+	+	+
9	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 553.11	Culture from horse feces	+	+	+	+
10	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 564.11	White pepper	+	+	+	+
11	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 708.11	Hoki filet with cream	+	+	+	+
12	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 781.11	Pigeon viscera	+	+	+	+
13	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 792.11	Urban compost	+	+	+	+
14	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 835.11	Env. (Chicken)	+	+	+	+
15	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 840.11	Streaky ham	+	+	+	+
16	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 845.11	Whole quail	+	+	+	+
17	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 880.11	Spareribs	+	+	+	+
18	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 886.11	Culture from swine feces	+	+	+	+
19	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 907.11	Raw milk (cow)	+	+	+	+
20	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 976.11	Sausage	+	+	+	+
21	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 977.11	Tomato filling	+	+	+	+
22	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 979.11	Stuffed potatoes	+	+	+	+
23	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 985.11	Fish meal	+	+	+	+
24	*Salmonella*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses:	Pet food	+	+	+	+

	enterica				1175.11					
25	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	n° Anses: 119.11	Foie gras (liver)	+	+	+	+
26	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	[3]ADRIA n°4	Milk powder	+	+	+	+
27	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°13	Pasteurized liquid egg	+	+	+	+
28	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°206	Pasteurized liquid egg	+	+	+	+
29	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°472	Egg yolk	+	+	+	+
30	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°776	Pasteurized liquid egg	+	+	+	+
31	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	[4]CIP 58.58	Ready-to-eat	+	+	+	+
32	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°19	Foie (Liver)	+	+	+	+
33	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°22	Raw ground meat	+	+	+	+
34	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°167	Ready-to-eat	+	+	+	+
35	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°193	Chipolatas sausages	+	+	+	+
36	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°830	Chipolatas sausages	+	+	+	+
37	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°911	Merguez sausages	+	+	+	+
38	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°987	Chipolatas sausages	+	+	+	+
39	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°4874	Meat (pâté)	+	+	+	+
40	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°A00C003	Frozen meat	+	+	+	+
41	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°A00C059	Frozen beef trim	+	+	+	+
42	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°A00C060	ground beef	+	+	+	+
43	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n°Ad1070	Pork	+	+	+	+
44	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n° ST325	Pork	+	+	+	+
45	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n° ST1	Pork	+	+	+	+
46	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n° ST394	Pork	+	+	+	+
47	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n° ST719	Pork	+	+	+	+
48	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n° ST11	Pork	+	+	+	+
49	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n° JES411	Liquid egg	+	+	+	+
50	*Salmonella enterica*	*enterica*	Typhimurium	1,4,[5],12:i:1,2	ADRIA n° Ad913	Beef trim	+	+	+	+

[1]American Type Culture Collection, Manassas, VA
[2]French Agency for Food, Environmental and Occupational Health & Safety, Maisons-Alfort, France
[3]Culture Collection ADRIA Developpement, Quimper, France
[4]Collection Institute Pasteur, Paris, France

Table 4. Exclusivity Results for iQ-Check *S.* Typhimurium Assay (1)

No.	Species	Subspecies	Serovar	Antigenic Formula	Source	Origin	BPW	BPW + Suppl.	TCS	RSA
1	*Salmonella bongori*			V 66:z41:–	[1]CIP 82.33T	Unknown	-	-	-	-
2	*Salmonella bongori*			V 48:z35:–	[2]RDCM 001	Unknown	-	-	-	-
3	*Salmonella enterica*	*arizonae*		51 : z4,z23	CIP 82.30T	Unknown	-	-	-	-
4	*Salmonella enterica*	*arizonae*			[3]ADRIA/AFSSA n° Ad 478	Shell fish	-	-	-	-
5	*Salmonella enterica*	*arizonae*			[4]RDC 36	Unknown	-	-	-	-
6	*Salmonella enterica*	*arizonae*			ADRIA/AFSSA n° Ad 450	Sheep milk	-	-	-	-
7	*Salmonella enterica*	*arizonae*			ADRIA/AFSSA n° Ad 451	Unknown	-	-	-	-
8	*Salmonella enterica*	*arizonae*			ADRIA/AFSSA n° Ad 452	Unknown	-	-	-	-
9	*Salmonella enterica*	*arizonae*			ADRIA/AFSSA n° Ad 453	Unknown	-	-	-	-
10	*Salmonella enterica*	*arizonae*			RDM 1 Hollande	Unknown	-	-	-	-
11	*Salmonella enterica*	*diarizonae*		IIIb 6,7 : l,v:z53	CIP 82.31T	Unknown	-	-	-	-
12	*Salmonella enterica*	*diarizonae*		IIIb 38 : l,v:z35	ADRIA/AFSSA n°Ad 594	Frog legs	-	-	-	-
13	*Salmonella enterica*	*diarizonae*		IIIb 61 : i:z53	ADRIA/AFSSA n° Ad 595	Cheese	-	-	-	-
14	*Salmonella enterica*	*enterica*	Abaetetuba	11 : k:1,5	[5]ATCC 35640	Unknown	-	-	-	-
15	*Salmonella enterica*	*enterica*	Aberdeen	11 : i:1,2	[6]CMF 114.1	Unknown	-	-	-	-
16	*Salmonella enterica*	*enterica*	Abony	1,4,12,27:b:e,n,x	[7]NCTC 6017	Unknown	-	-	-	-
17	*Salmonella enterica*	*enterica*	Adelaïde	35 : f,g: –	CMF 482.2	Unknown	-	-	-	-
18	*Salmonella enterica*	*enterica*	Agama	4,12 : i:1,6	RDCM 205 Hey's food (NL)	Unknown	-	-	-	-
19	*Salmonella enterica*	*enterica*	Agona	1,4,[5],12 : f,g,s:[1,2]	RDCM 057	Unknown	-	-	-	-
20	*Salmonella enterica*	*enterica*	Albany	8,20 : z4,z24:–	CMF 82.2	Unknown	-	-	-	-
21	*Salmonella enterica*	*enterica*	Anatum	3,{10}{15}{15,34} : e,h:1,6	CMF 814.4	Unknown	-	-	-	-
22	*Salmonella enterica*	*enterica*	Bambylor	9,46 : z:e,n,z15	CMF 135.1	Unknown	-	-	-	-
23	*Salmonella enterica*	*enterica*	Bareilly	6,7,14 : y:1,5	CMF 136.1	Unknown	-	-	-	-
24	*Salmonella enterica*	*enterica*	Berta	1,9,12 : [f],g,[t]:–	CMF 141.2	Unknown	-	-	-	-
25	*Salmonella enterica*	*enterica*	Blegdam	9,12 : g,m,q:–	NCTC 5769	Unknown	-	-	-	-
26	*Salmonella enterica*	*enterica*	Blockley	6,8 : k:1,5	RDCM 127 ADRIA	Unknown	-	-	-	-
27	*Salmonella enterica*	*enterica*	Braenderup	6,7,14 : e,h:e,n,z15	CMF 151	Unknown	-	-	-	-
28	*Salmonella enterica*	*enterica*	Brandenburg	4,[5],12 : l,v:e,n,z15	RDC 106	Unknown	-	-	-	-
29	*Salmonella enterica*	*enterica*	Bredeney	1,4,12,27 : l,v:1,7	CMF 817.1	Unknown	-	-	-	-
30	*Salmonella enterica*	*enterica*	California	4,12 : g,m,t:[z67]	RDCM 051	Unknown	-	-	-	-
31	*Salmonella enterica*	*enterica*	Cerro	6,14,18 : z4,z23:[1,5]	CMF 166.2	Unknown	-	-	-	-
32	*Salmonella enterica*	*enterica*	Carrau	6,14,[24] : y:1,7	CMF 142.2	Unknown	-	-	-	-
33	*Salmonella enterica*	*enterica*	Crossness	67 : r:1,2	CMF 165.1	Unknown	-	-	-	-
34	*Salmonella enterica*	*enterica*	Cubana	1,13,23 : z29:–	CMF 188	Unknown	-	-	-	-

35	*Salmonella enterica*	*enterica*	Choleraesuis	6,7 : c:1,5	CMF 181.1	Unknown	-	-	-	-
36	*Salmonella enterica*	*enterica*	Dahlem	48 : k:e,n,z15	CMF 924.1	Unknown	-	-	-	-
37	*Salmonella enterica*	*enterica*	Derby	1,4,[5],12 : f,g:[1,2]	RDC 91	Unknown	-	-	-	-
38	*Salmonella enterica*	*enterica*	Dublin	1,9,12[Vi] : g,p:–	RDC 31	Unknown	-	-	-	-
39	*Salmonella enterica*	*enterica*	Duisburg	1,4,12,[27] : d:e,n,z15	RDCM 199 Hey's food (NL)	Unknown	-	-	-	-
40	*Salmonella enterica*	*enterica*	Emek	8,20 : g,m,s:–	ADRIA n° Ad333	Unknown	-	-	-	-
41	*Salmonella enterica*	*enterica*	Enteritidis	1,9,12 : g,m:–	RDC 338	Unknown	-	-	-	-
42	*Salmonella enterica*	*enterica*	Fischerkietz	1,6,14,25 : y:e,n,x	RDCM 202 Hey's food (NL)	Unknown	-	-	-	-
43	*Salmonella enterica*	*enterica*	Ferruch	8 : e,h:1,5	RDCM 207 Hey's food (NL)	Unknown	-	-	-	-
44	*Salmonella enterica*	*enterica*	Gallinarum	9,12:-:-	[8]DSM 13674	Unknown	-	-	-	-
45	*Salmonella enterica*	*enterica*	Gaminara	16 : d:1,7	CMF 221.1	Unknown	-	-	-	-
46	*Salmonella enterica*	*enterica*	Give	3,{10}{15}{15,34} : l,v:1,7	RDM 2 Hollande	Unknown	-	-	-	-
47	*Salmonella enterica*	*enterica*	Glostrup	6,8 : z10:e,n,z15	CMF 226.3	Unknown	-	-	-	-
48	*Salmonella enterica*	*enterica*	Goldcoast	6,8 : r:l,w	RDCM 210 Hey's food (NL)	Unknown	-	-	-	-
49	*Salmonella enterica*	*enterica*	Grumpensis	1,13,23 : d:1,7	CMF 478.2	Unknown	-	-	-	-
50	*Salmonella enterica*	*enterica*	Guinea	1,44 : z10:1,7	RDCM 062	Unknown	-	-	-	-
51	*Salmonella enterica*	*enterica*	Hadar	6,8 : z10:e,n,x	CMF 234.1	Unknown	-	-	-	-
52	*Salmonella enterica*	*enterica*	Havana	1,13,23 : f,g,[s]:–	CMF 237.1	Unknown	-	-	-	-
53	*Salmonella enterica*	*enterica*	Heidelberg	1,4,[5],12 : r:1,2	RDC 93	Unknown	-	-	-	-
54	*Salmonella enterica*	*enterica*	Illinois	3,{10}{15}{15,34} : z10:1,5	CMF 251.2	Unknown	-	-	-	-
55	*Salmonella enterica*	*enterica*	Indiana	1,4,12 : z:1,7	RDCM 128 ADRIA	Unknown	-	-	-	-
56	*Salmonella enterica*	*enterica*	Infantis	6,7,14 : r:1,5	CIP 82.97	Unknown	-	-	-	-
57	*Salmonella enterica*	*enterica*	Inverness	38 : k:1,6	CMF 253.1	Unknown	-	-	-	-
58	*Salmonella enterica*	*enterica*	Johannesburg	1,40 : b:e,n,x	CMF 256.1	Unknown	-	-	-	-
59	*Salmonella enterica*	*enterica*	Kedougou	1,13,23 : i:l,w	RDCM 212 Hey's food (NL)	Unknown	-	-	-	-
60	*Salmonella enterica*	*enterica*	Kentucky	8,20 : i:z6	CMF 264.2	Unknown	-	-	-	-
61	*Salmonella enterica*	*enterica*	Kottbus	6,8 : e,h:1,5	RDCM 198 Hey's food (NL)	Unknown	-	-	-	-
62	*Salmonella enterica*	*enterica*	Livingstone	6,7,14 : d:l,w	RDCM 155 Friesland Campina	16S ID	-	-	-	-
63	*Salmonella enterica*	*enterica*	Livingstone	6,7,14 : d:l,w	[9]LAV n°1	Env.	-	-	-	-
64	*Salmonella enterica*	*enterica*	Lomita	6,7 : e,h:1,5	CMF 125.3	Unknown	-	-	-	-
65	*Salmonella enterica*	*enterica*	London	3,{10}{15} : l,v:1,6	RDCM 129 ADRIA	Unknown	-	-	-	-
66	*Salmonella enterica*	*enterica*	Mbandaka	6,7,14 : z10:e,n,z15	RDCM 130 ADRIA	Unknown	-	-	-	-
67	*Salmonella enterica*	*enterica*	Miami	1,9,12 : a:1,5	CMF 307	Unknown	-	-	-	-
68	*Salmonella enterica*	*enterica*	Minnesota	21 : b:e,n,x	CMF 146.3	Unknown	-	-	-	-
69	*Salmonella enterica*	*enterica*	Montevideo	6,7,14,[54] : g,m,[p],s:[1,2,7]	RDCM 45	Unknown	-	-	-	-

70	*Salmonella enterica*	*enterica*	Moscow	1,9,12 : g,q:–	RDCM 053	Unknown	-	-	-	-
71	*Salmonella enterica*	*enterica*	Muenchen	6,8 : d:1,2	CMF 337	Unknown	-	-	-	-
72	*Salmonella enterica*	*enterica*	Newport	6,8,20 : e,h:1,2	RDCM 208 Hey's food (NL)	Unknown	-	-	-	-
73	*Salmonella enterica*	*enterica*	Nienstedten	6,7,14 : b:l,w	CMF 352	Unknown	-	-	-	-
74	*Salmonella enterica*	*enterica*	Nottingham	16 : d:e,n,z15	NCTC 7832T	Unknown	-	-	-	-
75	*Salmonella enterica*	*enterica*	Ohio	6,7,14 : b:l,w	RDCM 132 ADRIA	Unknown	-	-	-	-
76	*Salmonella enterica*	*enterica*	Oranienburg	6,7,14 : m,t: [z57]	CMF 360	Unknown	-	-	-	-
77	*Salmonella enterica*	*enterica*	Ouakam	9,46 : z29:–	CMF 364	Unknown	-	-	-	-
78	*Salmonella enterica*	*enterica*	Paratyphi B Java	1,4,[5],12 : b:1,2	RDCM 192 Hey's food (NL)	Unknown	-	-	-	-
79	*Salmonella enterica*	*enterica*	Potsdam	6,7,14 : l,v:e,n,z15	CMF 225.2	Unknown	-	-	-	-
80	*Salmonella enterica*	*enterica*	Putten	13,23 : d:l,w	RDCM 214	Unknown	-	-	-	-
81	*Salmonella enterica*	*enterica*	Rostock	1,9,12:g,p,u:-	RDCM 55	Unknown	-	-	-	-
82	*Salmonella enterica*	*enterica*	Rubislaw	11 : r:e,n,x	CMF 414.2	Unknown	-	-	-	-
83	*Salmonella enterica*	*enterica*	Saintpaul	1,4,[5],12 : e,h:1,2	RDCM 101 ADRIA	Ad203	-	-	-	-
84	*Salmonella enterica*	*enterica*	Senftenberg	1,3,19 : g,[s],t:–	RDCM 209 Hey's food (NL)	Unknown	-	-	-	-
85	*Salmonella enterica*	*enterica*	Sundsvall	[1],6,14,[25] : z:e,n,x	CMF 877.2	Unknown	-	-	-	-
86	*Salmonella enterica*	*enterica*	Taksony	1,3,19 : i:z6	RDCM 203 Hey's food (NL)	Unknown	-	-	-	-
87	*Salmonella enterica*	*enterica*	Tallahassee	6,8 : z4,z32:–	CMF 822.1	Unknown	-	-	-	-
88	*Salmonella enterica*	*enterica*	Tennessee	6,7,14 : z29:[1,2,7]	RDCM Nestlé 599	Env.	-	-	-	-
89	*Salmonella enterica*	*enterica*	Thompson	6,7,14 : k:1,5	RDCM 056	Unknown	-	-	-	-
90	*Salmonella enterica*	*enterica*	Tournai	3,{10}{15} : y:z6	CMF 448.1	Unknown	-	-	-	-
91	*Salmonella enterica*	*enterica*	Virchow	6,7,14 : r:1,2	CMF 805.2	Unknown	-	-	-	-
92	*Salmonella enterica*	*enterica*	Wayne	30:g,z51:-	RDCM 54	Unknown	-	-	-	-
93	*Salmonella enterica*	*enterica*	Worthington	1,13,23 : z :l,w	RDCM 157 Friesland Campina	16S ID	-	-	-	-
94	*Salmonella enterica*	*enterica*	Yoruba	16 : c:l,w	CMF 3913/83	Unknown	-	-	-	-
95	*Salmonella enterica*	*houtenae*		IV 45 : g,z51:–	CIP 82.32T	Unknown	-	-	-	-
96	*Salmonella enterica*	*houtenae*		IV 50 : g,z51:–	ADRIA/AFSSA n° Ad 596	Milk product	-	-	-	-
97	*Salmonella enterica*	*houtenae*		IV 43 : z4,z32:–	ADRIA/AFSSA n° Ad 597	Cooked fish	-	-	-	-
98	*Salmonella enterica*	*indica*		VI [1],6,14,[25] : a:e,n,x	CIP 102501T	Unknown	-	-	-	-
99	*Salmonella enterica*	*salamae*	Betioky	II 59 : k:z65	CMF 141.1	Unknown	-	-	-	-
100	*Salmonella enterica*	*salamae*	Grabouw	II 11 : g,m,s,t:z39	RDCM 047	Unknown	-	-	-	-
101	*Salmonella enterica*	*salamae*	Manica	II 1,9,12 : g,m,s,t:(z42)	RDCM 048	Unknown	-	-	-	-
102	*Salmonella enterica*	*salamae*	Daressalaam	II 1,9,12 : l,w:e,n,x	CIP 82.29T	Unknown	-	-	-	-
103	*Salmonella enterica*	*salamae*		II 42 : g,t:–	ADRIA/AFSSA n° Ad 592	Kangaroo meat	-	-	-	-
104	*Salmonella enterica*	*salamae*		II 42 : b:e,n,x,z15	ADRIA/AFSSA n° Ad 593	Seed	-	-	-	-

105	*Salmonella enterica*	*salamae*	Tranoroa	II 55 : k:z39	CMF 463.1	Unknown	-	-	-	-
106	*Salmonella enterica*	*salamae*	Zuerich	II 1,9,12,46,27 : c:z39	CMF 510.1	Unknown	-	-	-	-
107	*Acinetobacter baumanii*				RDC 146	Unknown	-		-	
108	*Aeromonas hydrophila*				[10]LMG 2844T	Frog red-leg	-		-	
109	*Aeromonas hydrophila/ caviae*				RDCM 152 Friesland Campina	16S ID	-		-	
110	*Bacillus licheniformis*				RDC 88	Unknown	-		-	
111	*Bacillus cereus*				RDCM 218 Friesland Campina	16S ID	-		-	
112	*Campylobacter jejuni*				ATCC 29428	Unknown	-		-	
113	*Campylobacter coli*				ATCC 43489	Unknown	-		-	
114	*Campylobacter lari*				ATCC 35221	Unknown	-		-	
115	*Campylobacter upsaliensis*				RDC 68	Unknown	-		-	
116	*Citrobacter freundii*				RDCM 154 Friesland Campina	16S ID	-		-	
117	*Citrobacter freundii*				ATCC 8090	Unknown	-		-	
118	*Cronobacter sakazakii*				RDCM 210 Friesland Campina	16S ID	-		-	
119	*Cronobacter sakazakii*				RDCM 213 Friesland Campina	16S ID	-		-	
120	*Enterobacter aerogenes*				ATCC 13048	Unknown	-		-	
121	*Enterobacter amnigenus*				RDCM 204 Friesland Campina	16S ID	-		-	
122	*Enterobacter amnigenus*				RDCM 144 Friesland Campina	16S ID	-		-	
123	*Enterobacter asburiae*				RDCM 142 Friesland Campina	16S ID	-		-	
124	*Enterobacter cloacae*				RDCM 151 Friesland Campina	16S ID	-		-	
125	*Enterobacter cloacae*				LMG 2783	Unknown	-		-	
126	*Enterobacter cowanii*				RDCM 202 Friesland Campina	16S ID	-		-	
127	*Enterobacter pyrinus*				RDCM 211 Friesland Campina	16S ID	-		-	
128	*Enterobacter sakazakii*				RDC 236	Milk product	-		-	
129	*Enterobacter sakazakii*				RDCM 150 Friesland Campina	16S ID	-		-	
130	*Enterococcus faecium*				Bio-Rad RIVH WR63	Unknown	-		-	
131	*Escherichia coli*			O157H7 VT-	ATCC 700728	Unknown	-		-	
132	*Escherichia coli*				RDCM 203 Friesland Campina	16S ID	-		-	
133	*Escherichia hermanii*				RDC 72 ADRIA ADRIA	White egg	-		-	
134	*Hafnia alvei*				CIP 57.31T	Unknown	-		-	

135	*Hafnia alvei*				RDCM 145 Friesland Campina	16S ID	-		-	
136	*Hafnia alvei*				RDCM 205 Friesland Campina	16S ID	-		-	
137	*Klebsiella oxytoca*				RDC 30	Unknown	-		-	
138	*Klebsiella oxytoca*				RDCM 208 Friesland Campina	16S ID	-		-	
139	*Klebsiella oxytoca*				RDCM 148 Friesland Campina	16S ID	-		-	
140	*Klebsiella pneumoniae*				RDCM 209 Friesland Campina	16S ID	-		-	
141	*Klebsiella pneumoniae*	*pneumoniae*			RDCM 149 Friesland Campina	16S ID	-		-	
142	*Listeria monocytogenes*			1/2b	[11]CNRL 87373	Unknown	-		-	
143	*Listeria monocytogenes*			1/2c	CNRL 103573	Unknown	-		-	
144	*Listeria monocytogenes*			1/2a	CIP 78.31	mesenteric lymph node, guinea pig	-		-	
145	*Micrococcus luteus*				RDC 70	soil	-		-	
146	*Pantoea agglomerans*				RDCM 141 Friesland Campina	16S ID	-		-	
147	*Pantoea agglomerans*				RDCM 201 Friesland Campina	16S ID	-		-	
148	*Proteus mirabilis*				ATCC 29906	Unknown	-		-	
149	*Proteus mirabilis*				ATCC 25933	Unknown	-		-	
150	*Proteus mirabilis*				RDCM T04.37.17	Roquefort	-		-	
151	*Pseudomonas aeruginosa*				RDCM 153 Friesland Campina	16S ID	-		-	
152	*Pseudomonas aeruginosa*				RDCM 212 Friesland Campina	16S ID	-		-	
153	*Raoultella terrigena*				RDCM 146 Friesland Campina	16S ID	-		-	
154	*Raoultella terrigena*				RDCM 206 Friesland Campina	16S ID	-		-	
155	*Serratia marcescens*				ATCC 8100	Unknown	-		-	
156	*Serratia marsescens*				RDCM 147 Friesland Campina	16S ID	-		-	
157	*Serratia marcescens*				RDCM 207 Friesland Campina	16S ID	-		-	
158	*Shigella flexneri*				ATCC 12022	Unknown	-		-	
159	*Shigella sonnei*				ATCC 25931	Human feces	-		-	
160	*Staphylococcus aureus*				ATCC 25923	Unknown	-		-	
161	*Staphylococcus aureus*				RDCM 217 Friesland Campina	16S ID	-		-	
162	*Staphylococcus epidermidis*				RDCM 216 Friesland Campina	16S ID	-		-	
163	*Staphylococcus intermedius*				RDCM 61.221	Unknown	-		-	

164	*Staphylococcus xylosus*				RDC 85	Unknown	-		-	
165	*Yersinia enterocolitica*				CIP 80.27T	Unknown	-		-	

[1]Collection Institute Pasteur, Paris, France
[2]Bio-Rad R&D Collection Marnes, Marnes, France
[3]Culture Collection ADRIA Developpement, Quimper, France
[4]Bio-Rad R&D Collection, Marnes, France
[5]American Type Culture Collection, Manassas, VA
[6]Bio-Rad Internal Collection, Steenvoorde, France
[7]National Collection of Type Cultures, Salisbury, England
[8]The Leibniz Institute DSMZ, Brunswick, Germany
[9]Lavetan Reference Laboratory, Turnhout, Belgium
[10]Belgian Coordinated Collections of Microorganisms, Ghent, Belgium
[11]Centre National de référence des Legionelles, Lyon**, France**

Table 6: Summary of *Salmonella* Typhimurium Results (1)

	iQ-Check *S.* Typhimurium				
	Presumptive		Confirmed		
Level	With FDRS[1]	Without FDRS	Alternative	Traditional	Reference Method
Raw chicken breast without skin					
Noninoculated	0/5	0/5	0/5	0/5	0/5
Low	8/20	8/20	8/20	8/20	8/20
High	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
Low	9/20	9/20	9/20	9/20	9/20
High	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
Low	8/20	8/20	8/20	8/20	8/20
High	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
Low	10/20	10/20	10/20	10/20	10/20
High	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
Low	7/20	7/20	7/20	7/20	7/20
High	5/5	5/5	5/5	5/5	5/5
Boot/Drag swabs					
Noninoculated	0/5	0/5	0/5	0/5	0/5
Low	8/20	8/20	8/20	8/20	7/20
High	5/5	5/5	5/5	5/5	5/5

[1]FDRS = Free DNA Removal Solution

Table 7. iQ-Check *S.* Typhimurium 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) *Salmonella* Typhimurium (ATCC BAA-215)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.55 (0.29, 0.93)	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 breast with skin (25 g) *Salmonella* Typhimurium (ATCC 13311)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.61 (0.33, 1.04)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	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 without skin + 2% w/w salt (25 g) *Salmonella* Typhimurium (QL 11007-2)	-	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
	3.70 (1.52, 9.02)	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) *Salmonella* Typhimurium (ATCC 19585)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.70 (0.40, 1.14)	20	10	0.50	0.25, 0.75	10	0.50	0.25, 0.75	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 thigh with skin (25 g) *Salmonella* Typhimurium (ATCC 14028)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
	3.70 (1.52, 9.01)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Boot Swabs *Salmonella* Typhimurium (QL 011414.2)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	67	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
	180	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]MPN = 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
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method and reference method 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

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

Matrix and Inoculum	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			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) *Salmonella* Typhimurium (ATCC BAA-215)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.55 (0.29, 0.93)	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 breast with skin (25 g) *Salmonella* Typhimurium (ATCC 13311)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.61 (0.33, 1.04)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	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 without skin + 2% w/w salt (25 g) *Salmonella* Typhimurium (QL 11007-2)	-	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
	3.70 (1.52, 9.02)	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) *Salmonella* Typhimurium (ATCC 19585)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.70 (0.40, 1.14)	20	10	0.50	0.25, 0.75	10	0.50	0.25, 0.75	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 thigh with skin (25 g) *Salmonella* Typhimurium (ATCC 14028)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
	3.70 (1.52, 9.01)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Boot Swabs *Salmonella* Typhimurium (QL 011414.2)	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	67	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
	180	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]MPN = 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
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= 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]Results 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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