CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

010803

The AOAC Research Institute hereby certifies the method known as:

iQ-Check *Salmonella* II 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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date December 19, 2023

Expiration Date December 31, 2024

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MODIFICATION MARCH 2021: Mike Clark
MODIFICATION DECEMBER 2022: Mike Clark
MODIFICATION JANUARY 2023: Mike Clark
MODIFICATION JULY 2023: Mike Clark and Leo Horine

SUBMITTING COMPANY
Bio-Rad Laboratories
2000 Alfred Nobel Drive
Hercules, CA 94547
USA

METHOD NAME
iQ-Check *Salmonella* II Real-Time PCR

CATALOG NUMBER
3578123

INDEPENDENT LABORATORY

Original
Silliker Food Science Center
160 Armory Drive
South Holland, IL, 60473, USA

June 2009
Chestnut Labs
3233 East Chestnut
expressway
Springfield, MO

November 2013
WBA Analytical Laboratories
Springdale, AR, USA

September 2016
Q Laboratories, Inc.
1400 Harrison Ave.
Cincinnati, OH 45214

December 2022; July 2023
TEQ Analytical Labs
6116 E Warren Ave
Denver, Colorado 80222

APPLICABILITY OF METHOD
Target organism – *Salmonella.*

Matrixes – Original Validation: FDA/BAM Ch. 5 – cantaloupe (25 g), eggs (25 g); USDA FSIS- raw chicken (25 g), raw beef (25 g)
MODIFICATION JUNE 2009 – FDA/BAM Ch. 5 – (25 g) - Peanut butter
MODIFICATION FEBRUARY 2010 – raw pork (25 g), fresh spinach (25 g)
MODIFICATION NOVEMBER 2013 – FDA/BAM Ch. 5 – ceramic (1 x 1 in), concrete (1 x 1 in), plastic (4 x 4 in), stainless steel (4 x 4 in), dry dog food (25 g) wet cat food (25 g)
MODIFICATION AUGUST 2014 – USDA MLG 4.07 - ready-to-eat deli ham (375 g), raw ground chicken (375 g)
FDA/BAM Ch. 5 – dry dog food (375 g)
MODIFICATION SEPTEMBER 2016 – FDA BAM Ch. 5 - (375 g) milk chocolate, raw milk cheese, Stainless steel (1 x 1 in, environmental swabs with HiCap Neutralizing Broth)
USDA/FSIS MLG 4.08 – chicken carcass rinse (30 mL)
MODIFICATION MARCH 2021 – USDA/FSIS MLG 4.10 – (375 g) - fresh raw ground beef, fresh raw beef trim
FDA BAM Ch. 5 – (375 g) fresh baby spinach, nonfat dry milk (NFDM), whey powder, white chocolate, chocolate liquor
SMPR 2020.002 – (10 g) – cannabis flower (>0.3% delta 9-tetrahydrocannabinol (THC))
MODIFICATION DECEMBER 2022 – FDA BAM Ch. 5 – Plant-based meat (375 g), All-purpose flour (375g)
SMPR 2020.002 – Dried hemp flower (<0.3% THC) (25 g)
MODIFICATION JULY 2023 – SMPR 2020.02 – cannabis infused gummies (25 g), cannabis infused chocolate (25 g), and cannabis derived concentrates (5 g)

Performance claims – The study data detected no statistical difference between the iQ-Check *Salmonella* II Real-Time PCR method and the reference methods.

REFERENCE METHODS
***Microbiology Laboratory Guidebook* (October 1, 2004) U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, XX, Chapter 4.03. (2)**

***Bacteriological Analytical Manual Online* (April 2003, updated September 2005, December 2005 and June 2006) 8th Ed., U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Chapter 5. (3)**

Microbiological Laboratory Guidebook Chapter 4.07 (effective 05/01/2013). United States Department of Agriculture, Food Safety and Inspection Service. 10)

United States Department of Agriculture Microbiological Laboratory Guidelines 4.08: *Isolation and Identification of Salmonella from Red Meat, Poultry, Pasteurized Egg, Catfish Products, and Environmental Sponges*. June 29th, 2014. (11)

USDA/FSIS MLG 4.10: *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges*. Updated: January 2019. (15)
AOAC International SMPR 2020.002, Standard Method Performance

Requirements for Detection of *Salmonella* species in Cannabis and Cannabis Products. (16)

ORIGINAL CERTIFICATION DATE	**CERTIFICATION RENEWAL RECORD**
January 20, 2008	**Renewed annually through December 2024.**

METHOD MODIFICATION RECORD	**SUMMARY OF MODIFICATION**
1. January 2009	**1. Modification of reagents B and C.**
2. June 2009 ERV Evaluation	**2. ERV Matrix extension to include peanut butter.**
3. February 2010	**3. Evaluation of new high Throughput Extraction Protocol.**
4. August 2010 Level 3	**4. Matrix extension to include ceramic, concrete, plastic, stainless steel, dry dog food, wet cat food.**
5. November 2013	**5. Shorten enrichment time in buffered peptone water supplemented with proprietary mix of selective agents.**
6. December 2013 Level 1	**6. Algorithm changes to software.**
7. August 2014 Level 2	**7. Matrix extension to include ready-to-eat deli ham, raw ground chicken.**
8. May 2015 Level 2	**8. Manufacturing location change from Steenvoorde, France to Hercules, CA.**
9. September 2016 Level 2	**9. Matrix extension to include milk chocolate, raw milk cheese, stainless steel, chicken carcass rinse and iQ-Check *Salmonella* II Free DNA Removal protocol evaluation.**
10. January 2020 Level 1	**10. Insert reformatted.**
11. January 2021 Level 1	**11. Editorial/clerical changes for clarity.**
12. March 2021 Level 2	**12. Evaluation of Fast Application Protocol File and matrix extension to include fresh raw ground beef, fresh raw beef trim, fresh baby spinach, nonfat dry milk powder, whey powder, white chocolate, chocolate liquor, cannabis flower.**
13. April 2021 Level 1	**13. Software was updated from Version 3 to Version 4 allowing compatibility with Windows 10.**
14. November 2021 Level 1	**14. Editorial changes and addition of user information in French, German, Spanish, Portuguese, and Italian.**
15. December 2022 Level 3	**15. Matrix extension to include plant-based meat, all-purpose flour, dried hemp flower (<0.3% THC).**
16. January 2023 Level 2	**16. Addition of CFX Opus Deepwell, with CFX Manager Software, Industrial Diagnostic Edition version 3.1 using Free DNA Removal Solution and Fast APF protocols.**
17. July 2023 Level 3	**17. Matrix extension to include cannabis infused gummies (25 g), cannabis infused chocolate (25 g), and cannabis derived concentrates (5 g).**

Under this AOAC *Performance Tested Methods*^{SM} License Number, 010803 this method is distributed by:	**Under this AOAC *Performance Tested Methods*^{SM} License Number, 010803 this method is distributed as:**
NONE	**NONE**

PRINCIPLE OF THE METHOD (1)

The iQ-Check *Salmonella* II kit is a test based on gene amplification and detection by real-time polymerase chain reaction, (RTi-PCR). Ready-to-use RTi-PCR reagents contain DNA primers and a DNA probe specific for *Salmonella*, as well as DNA polymerase and nucleotides. PCR is a technique used to generate many copies of target DNA. During the PCR reaction, several cycles of heating and cooling allow DNA denaturation, by heat, followed by primers binding to the target region. The DNA polymerase then uses these primers and deoxynucleotide triphosphates (dNTPs) to extend the DNA, creating copies of the target DNA. These copies are called amplicons. In real-time PCR, specific oligonucleotide probes called molecular beacons are used to detect the DNA during the amplification, by hybridizing to the amplicons. These probes are linked to a fluorophore which fluoresces only when hybridized to the target sequence. In the iQ-Check *Salmonella* II kits, carboxyfluorescein (FAM) is the fluorophore linked to the probe hybridizing to the *Salmonella* specific DNA sequence. In the absence of target DNA, no fluorescence will be detected, and the sample determined to be negative. As the amount of amplicons increases with each round of amplification, fluorescence intensity also increases. During each PCR cycle, at the annealing step, the real-time PCR system measures this fluorescence and the associated software plots the fluorescence intensity versus number of cycles. This method allows a simple determination of the presence of *Salmonella* in a sample. To monitor for a successful DNA amplification in each reaction tube, 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 *Salmonella* target DNA sequence, and detected by a second fluorophore.

DISCUSSION OF THE VALIDATION STUDY (1)

Previous studies have demonstrated the superior sensitivity of PCR methods compared to culture methods (9). The iQ-Check *Salmonella* II kit can be used after one single 21 h primary enrichment. The culture method requires a 48 h enrichment followed by 24 h on a selective agar plate. The culture method relies on the target bacteria's ability to grow on an agar plate, which can be suppressed by the cells being stressed or being out-competed for nutrients by background flora. The iQ-Check *Salmonella* II kit uses primers and molecular beacon probes targeting a specific sequence on the *Salmonella* genome. Detecting target DNA is possible even if the cells are stressed or in lower quantity than competitive organisms. The iQ-Check *Salmonella* II method utilizes Buffered Peptone Water as an enrichment broth. This creates a cost savings for the user since this media is inexpensive. BPW, a nonselective media, can be used with a PCR method because of the highly specific primers and probes used in the reaction. A selective enrichment step to inhibit competitor organism growth is not necessary since the iQ-Check real-time PCR system can distinguish between target DNA and non-target competitive DNA.

Table 1 - Inclusivity Results (1)

Serotype	Reference	Origin	Result
	Group A		
Salmonella Paratyphi A	ATCC 9150	IL Public Health Dept	+
Salmonella Paratyphi A	ATCC 11511	CDC	+
	Group B		
Salmonella Abony	CIP 8039	Pasteur Institute	+
Salmonella Agona	Ad 4869	Smoked sausage	+
Salmonella Agona	Ad 00V038	Swine feed	+
Salmonella Brandenburg	Ad 351	Seafood cocktail	+
Salmonella Brandenburg	Ad 499	Pork sausage	+
Salmonella Bredeney	Ad 141	Pork sausage	+
Salmonella Bredeney	Ad 464	Pork pâté	+
Salmonella Derby	Ad 374	Pork sausage	+
Salmonella Derby	Ad 17	Meat product	+
Salmonella Duisberg	Ad 42	Poultry environmental	+
Salmonella Essen	Ad 38	Poultry environmental	+
Salmonella Heidelberg	Ad 36	Food	+
Salmonella Heidelberg	Ad 285	Tomato + pork meat	+
Salmonella Heidelberg	Ad 24876	Poultry	+
Salmonella Indiana	Ad 2B	Brine	+
Salmonella Lagos	Ad 173	Chipolatas (sausage)	+
Salmonella Paratyphi B	Ad 301	Human	+
Salmonella Saintpaul	Ad 00C001	Pheasant	+
Salmonella Saintpaul	Ad 631	Poultry	+
Salmonella Saintpaul	Ad F31	Sardines	+
Salmonella Typhimurium	ATCC 13311	Human feces	+
Salmonella Typhimurium	ATCC 14028	Tissue	+
Salmonella Typhimurium	Ad ST 391	Swine abattoir	+
Salmonella Typhimurium	Ad 305	Paella (mixed rice dish)	+
Salmonella Typhimurium	Ad 528	Fish	+
Salmonella Typhimurium	Ad 633	Bread	+
Salmonella Typhimurium	Ad 702	Pork dry sausage	+
Salmonella Schwarzengrund	CMF 420	Pasteur Institute	+
	Group C1		
Salmonella Barielly	CMF 136	Pasteur Institute	+
Salmonella Braenderup	ATCC BNA 664	CDC	+
Salmonella Braenderup	Ad 111	Pork	+
Salmonella Diarizonae IIIb	ATCC 43973	Pasteur Institute	+
Salmonella Infantis	ATCC 51741	Pasta	+
Salmonella Infantis	Ad 14	Eggs	+
Salmonella Infantis	Ad 401B	Raw milk	+
Salmonella Infantis	Ad 128	Milk	+
Salmonella Lille	Ad 37	Poultry environmental	+
Salmonella Livingstone	Ad E1	White egg powder	+
Salmonella Lomita	CMF 125	Pasteur Institute	+
Salmonella Mbandaka	Ad 81	Eggs	+
Salmonella Montevideo	Ad 327	Intestine	+
Salmonella Montevideo	Ad 604	Raw milk	+
Salmonella Montevideo	Ad 510	Raw milk	+
Salmonella Oranienburg	CMF 360	Pasteur Institute	+
Salmonella Paratyphi C	ATCC 13428	MI Health Dept	+
Salmonella Potsdam	CMF 225	Pasteur Institute	+
Salmonella Rissen	Ad 59	Poultry environmental	+
Salmonella Singapore	CMF 427	Pasteur Institute	+
Salmonella Tennessee	Ad 00E006	Environmental	+
Salmonella Thompson	Ad ER 301	Poultry	+
Salmonella Virchow	Ad F276	Curry	+
Salmonella Virchow	CIP 105355	Human isolate	+
	Group C2		
Salmonella Bovismorbificans	Ad 728	Gelatin	+
Salmonella Bovismorbificans	Ad 132	Raw smoked pork breast	+
Salmonella Bovismorbificans	Ad 6629	Pork sausage	+
Salmonella Cremieu	Ad 230	Hare	+
Salmonella Glostrup	CMF 226	Pasteur Institute	+
Salmonella Hadar	Ad 35	Poultry	+

Salmonella Hadar	Ad 24871	Poultry	+
Salmonella Kottbus	Ad 1B	Poultry	+
Salmonella Manhattan	Ad 900	Dairy environmental (dust)	+
Salmonella Muenchen	CMF 337	Pasteur Institute	+
Salmonella Newport	Ad 972	Turkey	+
Salmonella Newport	Ad 540	Sausage	+
Salmonella Newport	Ad 586	Beef carcass	+
Salmonella Tallahassee	CMF 822	Pasteur Institute	+
Group C3			
Salmonella Albany	CMF 82	Pasteur Institute	+
Salmonella Bardo	Ad 569	Sausage meat	+
Salmonella Kentucky	CMF 264	Pasteur Institute	+
Group C4			
Samonella Nienstedten	CMF 352	Pasteur Institute	+
Group D1			
Salmonella Berta	CMF 141	Pasteur Institute	+
Salmonella Dublin	Ad 40	Poultry environmental	+
Salmonella Dublin	Ad 531	Unpasteurized cheese	+
Salmonella Dublin	Ad 528	Pancake mix	+
Salmonella Enteritidis	ATCC 13076	CDC	+
Salmonella Enteritidis	Ad 465	Eggs	+
Salmonella Enteritidis	Ad 657	Eggs	+
Salmonella Enteritidis	Ad 2532	Cooked ham	+
Salmonella Enteritidis	Ad 10	Egg white powder	+
Salmonella Gallinarum	Ad 1	Poultry	+
Salmonella Gallinarum	Ad 300	Poultry	+
Salmonella Miami	CMF 307	Pasteur Institute	+
Salmonella Panama	Ad 8	Ground beef	+
Salmonella Panama	Ad 882	Pork sausage with herbs	+
Salmonella Salamae II	ATCC 43972	Pasteur Institute	+
Salmonella Typhi	Ad 302	Human – Pasteur Institute	+
Group D2			
Salmonella Bambylor	CMF 135	Pasteur Institute	+
Salmonella Ouakam	CMF 364	Pasteur Institute	+
Group E1			
Salmonella Anatum	Ad 225	Food	+
Salmonella Anatum	Ad 298	Milk powder	+
Salmonella Falkensee	Ad 693	Sausage meat	+
Salmonella London	Ad 34	Food	+
Salmonella London	Ad 326	Beef	+
Salmonella Meleagridis	Ad 505	Raw milk	+
Group E2			
Salmonella Binza	Ad 27	Food	+
Salmonella Newbrunswick	Ad 436	Ground beef	+
Salmonella Newington	Ad 26	Dairy product	+
Group E3			
Salmonella Illinois	CMF 251	Pasteur Institute	+
Group E4			
Salmonella Regent	Ad 328	Duck	+
Salmonella Seftenberg	Ad 1A	Environmental	+
Salmonella Seftenberg	Ad 355	Sea food cocktail	+
Group F			
Salmonella Aberdeen	CMF 114	Pasteur Institute	+
Salmonella Rubislaw	CMF 414	Pasteur Institute	+
Group G1			
Salmonella Poona	CMF 689	Pasteur Institute	+
Group G2			
Salmonella Cubana	CMF 188	Pasteur Institute	+
Salmonella Grumpensis	CMF 142	Pasteur Institute	+
Salmonella Havana	CMF 237	Pasteur Institute	+
Salmonella Worthington	Ad 3506	Pâté	+
Group H			
Salmonella Carrau	CMF 142	Pasteur Institute	+
Salmonella Indica	Ad 600	Environmental	+
Salmonella Indica VI	ATCC 43976	Pasteur Institute	+
Salmonella Sundsvall	CMF 877	Pasteur Institute	+
Group I			
Salmonella Gaminara	CMF 221	Pasteur Institute	+
Salmonella Yoruba	CMF 3913	Pasteur Institute	+

	Group J		
Salmonella Kirkee	CMF 458	Pasteur Institute	+
	Group K		
Salmonella Cerro	CMF 166	Pasteur Institute	+
	Group L		
Salmonella Minnesota	CMF 146	Pasteur Institute	+
	Group N		
Salmonella Landau	CMF 277	Pasteur Institute	+
Salmonella Sternschauze	CMF 432	Pasteur Institute	+
Salmonella Urbana	CMF 438	Pasteur Institute	+
Salmonella Veneziana	Ad 233	Food	+
Salmonella Wayne	CMF499	Pasteur Institute	+
	Group O		
Salmonella Adelaide	CMF 482	Pasteur Institute	+
	Group P		
Salmonella Diarizonae	Ad 594	Frog legs	+
Salmonella Inverness	CMF 253	Pasteur Institute	+
Salmonella Sheffield	CMF 426	Pasteur Institute	+
	Group R		
Salmonella Johannesburg	CMF 256	Pasteur Institute	+
Salmonella Springs	CMF 431	Pasteur Institute	+
	Group T		
Salmonella Weslaco	CMF 688	Pasteur Institute	+
Salmonella Salamae	Ad 592	Kangaroo meat	+
Salmonella Salamae	Ad 593	Seed	+
	Group U		
Salmonella Houtenae	Ad 597	Cooked fish	+
	Group W		
Salmonella Houtenae IV	ATCC 43974	Pasteur Institute	+
	Group X		
Salmonella Phoenix	CMF 395	Pasteur Institute	+
	Group Y		
Salmonella bongori	Ad 598	Environmental sample	+
Salmonella Dalhem	CMF 924	Pasteur Institute	+
	Group Z		
Salmonella Arizonae	CIP 5526	Dried egg powder	+
Salmonella Houtenae	Ad 596	Milk product	+
	Group O51		
Salmonella Arizonae IIIa	ATCC 13314	Pasteur Institute	+
	Group O52		
Salmonella Utrecht	CMF 484	Pasteur Institute	+
	Group O61		
Salmonella Diarizonae	Ad 595	Cheese	+
	Group O66		
Salmonella Maregrosso	CMF 301	Pasteur Institute	+
Salmonella bongori	Ad 599	Breeding	+
Salmonella bongori V	ATCC 43975	Pasteur Institute	+
	Group O67		
Salmonella Crossness	CMF 165	Pasteur Institute	+

Ad = Culture collection ADRIA Developpement, Quimper, France
ATCC = American Type Culture Collection, USA
CIP = Collection Institut Pasteur, Paris, France
CMF = Culture Microbienne et Fongique (Microbiology and Fungus Culture Collection), France

Table 2 - Exclusivity Results (1)

Species	Reference	Origin	Result
Alcaligenes faecalis	ATCC 8750	Unknown	-
Bacillus cereus	ATCC 14579	Milk	-
Candida albicans	ATCC 10231	Clinical	-
Citrobacter diversus	Ad 140	Raw milk	-
Citrobacter freundii	ATCC 8090	Unknown	-
Citrobacter freundii	Ad 23	Sausage	-
Citrobacter freundii	Ad 59	Food	-
Citrobacter freundii	Ad 175	Duck	-
Citrobacter koseri	ATCC 27156	CDC	-
Edwarsiella tarda	ATCC 15947	Human feces	-
Enterobacter aerogenes	ATCC 13048	Food	-
Enterobacter agglomerans	Ad 11	Cheese	-
Enterobacter cloacae	Ad 128	Minced steak	-
Enterobacter cloacae	Ad 10	Raw milk	-
Enterobacter sakazakii	Ad 95	Cream cheese	-
Enterobacter sakazakii	Ad D7	Poultry	-
Escherichia blattae	ATCC 29907	Insect	-
Escherichia coli	ATCC 25922	Clinical isolate	-
Escherichia coli	Ad 19	Grated carrots	-
Escherichia coli	CIP 54117	Human feces	-
Escherichia coli	Ad 2B	Sausage	-
Escherichia coli	Ad 6	Sausage	-
Escherichia coli O157	Ad 525	Unknown	-
Escherichia fergusonii	ATCC 35469	Human feces	-
Escherichia hermanii	Ad 459	Unknown	-
Escherichia vulneris	Ad 134	Unknown	-
Hafnia alvei	Ad 168	Duck	-
Hafnia alvei	Ad 167	Sausage	-
Klebsiella oxytoca	Ad 57	Food	-
Klebsiella oxytoca	Ad 42	Food	-
Klebsiella pneumoniae	Ad 28	Food	-
Klebsiella pneumoniae	ATCC 13883	CDC	-
Lactococcus lactis	ATCC 11454	Unknown	-
Listeria monocytogenes	ATCC 19112	Clinical	-
Microbacterium arborescens	ATCC 4358	Unknown	-
Micrococcus luteus	ATCC 10240	Air	-
Morganella morganii	CIP A236	Pasteur Institute	-
Proteus mirabilis	Ad 54	Poultry	-
Proteus mirabilis	Ad 55	Food	-
Proteus vulgaris	Ad 56	Food	-
Pseudomonas aeruginosa	ATCC 27853	Blood culture	-
Serratia liquefaciens	Ad 5	Egg product	-
Serratia proteomaculans	Ad 00C056	Ham	-
Shigella flexneri	ATCC 29903	Unknown	-
Shigella sonnei	CIP 51.1	Pasteur Institute	-
Shigella sonnei	ATCC 29930	CDC	-
Staphylococcus aureus	ATCC 51740	Margarine	-
Streptococcus bovis	CIP 5623	Human isolate	-
Yersinia enterocolotica	Ad 32	Bacon fat	-
Yersinia enterocolotica	ATCC 9610	Human Tissue	-

Ad = Culture collection ADRIA Developpement, Quimper, France
ATCC = American Type Culture Collection, USA
CIP = Collection Institut Pasteur, Paris, France

Table 3 - Method Comparison Results (1)

Matrix	Level	MPN/25 g	# samples	iQ-Check positive	iQ-Check confirmed [a]	Reference positive	Method Agreement [b]	X^2 [c]	Sensitivity [d]	False Negative [e]	Specificity [f]	False Positive [g]
Raw chicken	Control	0	5	0	N/A	0	100%	-	-	-	100%	0%
(internal)	Low	0.5	20	14	N/A	14	100%	-	100%	0%	-	-
Raw beef	Control	0	5	0	N/A	0	100%	-	-	-	100%	0%
	Low	0.23	20	8	N/A	8	100%	-	100%	0%	-	-
Cantaloupe	Control	0	5	0	0	0	100%	-	-	-	100%	0%
	Low	2.325	20	15	14	15	95%	0.12	93%	7%	-	-
Eggs	Control	0	5	0	0	0	100%	-	-	-	100%	0%
	Low	0.38	20	13	13	8	75%	2.44	163%	0%	-	-
Raw chicken	Control	0	5	0	N/A	0	100%	-	-	-	100%	0%
(independent)	Low	2.3	20	14	N/A	12	90%	0.50	117%	0%	-	-

[a] N/A = Not applicable. The iQ-Check and reference method samples were paired and thus had the same confirmation procedure.

[b] Method agreement defined as number of spiked food samples identified compared to reference method identified samples, calculated by 1-(# iQ-Check *Salmonella* II positive - # reference method positive / Total # samples) x 100

[c] X^2 according to McNemar for raw chicken (internal and independent) and raw beef. X^2 according to Mantel-Haenszel for cantaloupe and eggs.

[d] Sensitivity rate defined as 100 times the total number of iQ-Check *Salmonella* II positive samples divided by the total number of samples positive by both methods.

[e] False negative rate is 100 – sensitivity rate.

[f] Specificity rate defined as 100 times the total number of iQ-Check *Salmonella* II negative samples divided by the total number of samples negative by both methods.

[g] False positive rate is 100 – specificity rate.

DISCUSSION OF MODIFICATION APPROVED JANUARY 2009 (5)

Due to a change in chemistry in iQ-Check real-time PCR test kits, AOAC-RI validation studies were repeated. Inclusivity, exclusivity, method comparison and lot-to-lot studies for iQ-Check *E. coli* O157:H7, *Salmonella* II, *Listeria monocytogenes* II and *Listeria* spp. showed no difference in the performance of the modified kits compared to the previously validated kits. A ruggedness study varying DNA extraction volumes was performed on iQ-Check *Salmonella* II and results were as expected. Data were analyzed manually and automatically with the new OM software version. Each analysis method yielded the same results. The data presented in this modification study support the renewal of Performance Tested Method status for all iQ-Check kits.

Table 2 – Inclusivity Results iQ-Check *Salmonella* II (5)

Serotype	Reference [a]	Origin	Result
Group A			
Salmonella Paratyphi A	ATCC 9150	IL Public Health Dept	+
Salmonella Paratyphi A	ATCC 11511	CDC	+
Group B			
Salmonella Abony	CIP 8039	Pasteur Institute	N/A [b]
Salmonella Agona	Ad 4869	Smoked sausage	+
Salmonella Agona	Ad 00V038	Swine feed	+
Salmonella Brandenburg	Ad 351	Seafood cocktail	+
Salmonella Brandenburg	Ad 499	Pork sausage	+
Salmonella Bredeney	Ad 141	Pork sausage	+
Salmonella Bredeney	Ad 464	Pork pâté	+
Salmonella Derby	Ad 374	Pork sausage	+
Salmonella Derby	Ad 17	Meat product	+
Salmonella Duisburg	Ad 42	Poultry environmental	+
Salmonella Essen	Ad 38	Poultry environmental	+
Salmonella Heidelberg	Ad 36	Food	+
Salmonella Heidelberg	Ad 285	Tomato + pork meat	+
Salmonella Heidelberg	Ad 24876	Poultry	+
Salmonella Indiana	Ad 2B	Feed	+
Salmonella Lagos	Ad 173	Chipolatas (sausage)	+
Salmonella Paratyphi B	Ad 301	Human	+
Salmonella Saintpaul	Ad 00C001	Pheasant	+
Salmonella Saintpaul	Ad 631	Poultry	+
Salmonella Saintpaul	Ad F31	Sardines	+
Salmonella Typhimurium	ATCC 13311	Human feces	+
Salmonella Typhimurium	ATCC 14028	Tissue	N/A [b]
Salmonella Typhimurium	Ad ST 391	Swine abattoir	+
Salmonella Typhimurium	Ad 305	Paella (mixed rice dish)	+
Salmonella Typhimurium	Ad 528	Fish	+
Salmonella Typhimurium	Ad 633	Bread	+
Salmonella Typhimurium	Ad 702	Pork dry sausage	+
Salmonella Schwarzengrund	CMF 420	Pasteur Institute	+
Group C1			
Salmonella Bareilly	CMF 136	Pasteur Institute	+
Salmonella Braenderup	ATCC BNA 664	CDC	+
Salmonella Braenderup	Ad 111	Pork	+
Salmonella Diarizonae IIIb	ATCC 43973	Pasteur Institute	+
Salmonella Infantis	ATCC 51741	Pasta	+
Salmonella Infantis	Ad 141	Eggs	+
Salmonella Infantis	Ad 401B	Raw milk	+
Salmonella Infantis	Ad 128	Milk	+
Salmonella Lille	Ad 37	Poultry environmental	+
Salmonella Livingstone	Ad E1	White egg powder	+
Salmonella Lomita	CMF 125	Pasteur Institute	+
Salmonella Mbandaka	Ad 81	Eggs	+
Salmonella Montevideo	Ad 327	Intestine	+
Salmonella Montevideo	Ad 604	Raw milk	+
Salmonella Montevideo	Ad 510	Raw milk	+
Salmonella Oranienburg	CMF 360	Pasteur Institute	+
Salmonella Paratyphi C	ATCC 13428	MI Health Dept	+
Salmonella Potsdam	CMF 225	Pasteur Institute	+
Salmonella Rissen	Ad 59	Poultry environmental	+
Salmonella Singapore	CMF 427	Pasteur Institute	+
Salmonella Tennessee	Ad 00E006	Environmental	+
Salmonella Thompson	Ad AER 301	Poultry	+

Salmonella Virchow	Ad F276	Curry	+
Salmonella Virchow	CIP 105355	Human isolate	+

Group O:8 (C2-C3)

Salmonella Albany	CMF 82	Pasteur Institute	+
Salmonella Bardo	Ad 569	Sausage meat	+
Salmonella Bovismorbificans	Ad 728	Gelatin	+
Salmonella Bovismorbificans	Ad 132	Raw smoked pork breast	+
Salmonella Bovismorbificans	Ad 6629	Pork sausage	+
Salmonella Cremieu	Ad 230	Hare	+
Salmonella Glostrup	CMF 226	Pasteur Institute	+
Salmonella Hadar	Ad 35	Poultry	+
Salmonella Hadar	Ad 24871	Poultry	+
Salmonella Kentucky	CMF 264	Pasteur Institute	+
Salmonella Kottbus	Ad 10	Poultry	+
Salmonella Manhattan	Ad 900	Dairy environmental (dust)	+
Salmonella Muenchen	CMF 337	Pasteur Institute	+
Salmonella Newport	Ad 972	Turkey	+
Salmonella Newport	Ad 540	Sausage	+
Salmonella Newport	Ad 586	Beef carcass	+
Salmonella Tallahassee	CMF 822	Pasteur Institute	+

Group C4

Samonella Ohio var 14+ (Nienstedten)	CMF 352	Pasteur Institute	+

Group D1

Salmonella Berta	CMF 141	Pasteur Institute	+
Salmonella Dublin	Ad 40	Poultry environmental	+
Salmonella Dublin	Ad 531	Unpasteurized cheese	+
Salmonella Dublin	Ad 528	Pancake mix	+
Salmonella Enteritidis	ATCC 13076	CDC	+
Salmonella Enteritidis	Ad 465	Eggs	+
Salmonella Enteritidis	Ad 657	Eggs	+
Salmonella Enteritidis	Ad 2532	Cooked ham	+
Salmonella Enteritidis	Ad 10b	Egg white powder	+
Salmonella Gallinarum	Ad 1b	Poultry	+
Salmonella Gallinarum	Ad 300	Poultry	+
Salmonella Miami	CMF 307	Pasteur Institute	+
Salmonella Panama	Ad 81	Ground beef	+
Salmonella Panama	Ad 882	Pork sausage with herbs	+
Salmonella Salamae II	ATCC 43972	Pasteur Institute	+
Salmonella Typhi	Ad 302	Human – Pasteur Institute	+

Group D2

Salmonella Bambylor	CMF 135	Pasteur Institute	+
Salmonella Ouakam	CMF 364	Pasteur Institute	+

Group E1

Salmonella Anatum	Ad 225	Food	+
Salmonella Anatum	Ad 298	Milk powder	+
Salmonella Anatum var 15+ (Newington)	Ad 26	Dairy product	+
Salmonella Falkensee	Ad 693	Sausage meat	+
Salmonella Give var 15+ (Newbrunswick)	Ad 436	Ground beef	+
Salmonella Lexington var 15+, 34+ (Illinois)	CMF 251	Pasteur Institute	+
Salmonella London	Ad 34	Food	+
Salmonella London	Ad 326	Beef	+
Salmonella Meleagridis	Ad 505	Raw milk	+
Salmonella Orion var 15+ (Binza)	Ad 27	Food	+
Salmonella Regent	Ad 328	Duck	+

Group E4

Salmonella Senftenberg	Ad 1A	Environmental	+
Salmonella Senftenberg	Ad 355	Sea food cocktail	+

Group F

Salmonella Aberdeen	CMF 114	Pasteur Institute	+
Salmonella Rubislaw	CMF 414	Pasteur Institute	+
Salmonella Veneziana	Ad 233	Food	+

Group O:13 (G)

Salmonella Cubana	CMF 188	Pasteur Institute	+
Salmonella Grumpensis	CMF 478	Pasteur Institute	+
Salmonella Havana	CMF 237	Pasteur Institute	+
Salmonella Poona	CMF 689	Pasteur Institute	+
Salmonella Worthington	Ad 3506	Pâté	+

Group H			
Salmonella Carrau	CMF 142	Pasteur Institute	+
Salmonella Indica VI	Ad 600	Environmental	+
Salmonella Indica VI	ATCC 43976	Pasteur Institute	+
Salmonella Sundsvall	CMF 877	Pasteur Institute	N/A [b]
Group I			
Salmonella Gaminara	CMF 221	Pasteur Institute	+
Salmonella Yoruba	CMF 3913	Pasteur Institute	+
Group J			
Salmonella Kirkee	CMF 458	Pasteur Institute	+
Group K			
Salmonella Cerro	CMF 166	Pasteur Institute	+
Group L			
Salmonella Minnesota	CMF 146	Pasteur Institute	+
Group N			
Salmonella Landau [c]	Ad 499 b	Pasteur Institute	+
Salmonella Landau	CMF 277	Pasteur Institute	N/A [b]
Salmonella Sternschanze [c]	Ad 500	Pasteur Institute	+
Salmonella Sternschanze	CMF 432	Pasteur Institute	N/A [b]
Salmonella Urbana [c]	Ad 501	Pasteur Institute	+
Salmonella Urbana	CMF 438	Pasteur Institute	N/A [b]
Salmonella Wayne [c]	Ad 502	Pasteur Institute	+
Salmonella Wayne	CMF499	Pasteur Institute	N/A [b]
Group O			
Salmonella Adelaide	CMF 482	Pasteur Institute	+
Group P			
Salmonella Diarizonae IIIb	Ad 594	Frog legs	+
Salmonella Inverness	CMF 253	Pasteur Institute	+
Salmonella Sheffield	CMF 426	Pasteur Institute	+
Group R			
Salmonella Johannesburg	CMF 256	Pasteur Institute	+
Salmonella Springs	CMF 431	Pasteur Institute	+
Group T			
Salmonella Weslaco	CMF 688	Pasteur Institute	+
Salmonella Salamae II	Ad 592	Kangaroo meat	+
Salmonella Salamae II	Ad 593	Seed	+
Group U			
Salmonella Houtenae IV	Ad 597	Cooked fish	+
Group W			
Salmonella Houtenae IV	ATCC 43974	Pasteur Institute	+
Group X			
Salmonella II 47:b:1,5 (Phoenix)	CMF 395	Pasteur Institute	+
Group Y			
Salmonella bongori V	Ad 598	Environmental sample	+
Salmonella Dalhem	CMF 924	Pasteur Institute	+
Group Z			
Salmonella Arizonae IIIa	CIP 55.26	Dried egg powder	+
Salmonella Houtenae IV	Ad 596	Milk product	+
Group O51			
Salmonella Arizonae IIIa	ATCC 13314	Pasteur Institute	+
Group O52			
Salmonella Utrecht	CMF 484	Pasteur Institute	+
Group O61			
Salmonella Diarizonae IIIb	Ad 595	Cheese	+
Group O66			
Salmonella Maregrosso	CMF 301	Pasteur Institute	+
Salmonella bongori V	Ad 599	Turkey	+
Salmonella bongori V	ATCC 43975	Pasteur Institute	+
Group O67			
Salmonella Crossness	CMF 165	Pasteur Institute	+
Salmonella Arizonae IIIa [c]	Ad 450	Ewe milk	+
Salmonella Arizonae IIIa [c]	Ad 478	Clams	+

[a] Ad = Culture collection ADRIA Developpement, Quimper, France
ATCC = American Type Culture Collection, USA
CIP = Collection Institut Pasteur, Paris, France
CMF = Culture Microbienne et Fongique (Microbiology and Fungus Culture Collection), France
[b] N/A = Not available for retest
[c] Strain added, not tested previously

Table 6 – Exclusivity Results iQ-Check *Salmonella* II (5)

Species	Reference [a]	Origin	Result
Alcaligenes faecalis	ATCC 8750	Unknown	-
Bacillus cereus	ATCC 14579	Milk	-
Candida albicans	ATCC 10231	Clinical	-
Citrobacter diversus	Ad 140	Raw milk	-
Citrobacter freundii	ATCC 8090	Unknown	-
Citrobacter freundii	Ad 23	Sausage	-
Citrobacter freundii	Ad 59	Food	-
Citrobacter freundii	Ad 175	Duck	-
Citrobacter koseri	ATCC 27156	CDC	-
Edwarsiella tarda	ATCC 15947	Human feces	-
Enterobacter aerogenes	ATCC 13048	Food	-
Enterobacter agglomerans	Ad 11	Cheese	-
Enterobacter cloacae	Ad 128	Minced steak	-
Enterobacter cloacae	Ad 10	Raw milk	-
Enterobacter sakazakii	Ad 95	Cream cheese	-
Enterobacter sakazakii	Ad D7	Poultry	-
Escherichia blattae	ATCC 29907	Insect	-
Escherichia coli	ATCC 25922	Clinical isolate	-
Escherichia coli	Ad 19	Grated carrots	-
Escherichia coli	CIP 54117	Human feces	-
Escherichia coli	Ad 2B	Sausage	-
Escherichia coli	Ad 6	Sausage	-
Escherichia coli O157	Ad 525	Unknown	-
Escherichia fergusonii	ATCC 35469	Human feces	-
Escherichia hermanii	Ad 459	Unknown	-
Escherichia vulneris	Ad 134	Unknown	-
Hafnia alvei	Ad 168	Duck	-
Hafnia alvei	Ad 167	Sausage	-
Klebsiella oxytoca	Ad 57	Food	-
Klebsiella oxytoca	Ad 42	Food	-
Klebsiella pneumoniae	Ad 28	Food	-
Klebsiella pneumoniae	ATCC 13883	CDC	-
Lactococcus lactis	ATCC 11454	Unknown	-
Listeria monocytogenes	ATCC 19112	Clinical	-
Microbacterium arborescens	ATCC 4358	Unknown	-
Micrococcus luteus	ATCC 10240	Air	-
Morganella morganii	CIP A236	Pasteur Institute	-
Proteus mirabilis	Ad 54	Poultry	-
Proteus mirabilis	Ad 55	Food	-
Proteus vulgaris	Ad 56	Food	-
Pseudomonas aeruginosa	ATCC 27853	Blood culture	-
Serratia liquefaciens	Ad 5	Egg product	-
Serratia proteomaculans	Ad 00C056	Ham	-
Shigella flexneri	ATCC 29903	Unknown	-
Shigella sonnei	CIP 51.1	Pasteur Institute	-
Shigella sonnei	ATCC 29930	CDC	-
Staphylococcus aureus	ATCC 51740	Margarine	-
Streptococcus bovis	CIP 5623	Human isolate	-
Yersinia enterocolotica	Ad 32	Bacon fat	-
Yersinia enterocolotica	ATCC 9610	Human Tissue	-

[a] Ad = Culture collection ADRIA Developpement, Quimper, France
ATCC = American Type Culture Collection, USA
CIP = Collection Institut Pasteur, Paris, France

Table 10 – Method Comparison Results iQ-Check *Salmonella* II (5)

Matrix	Level	MPN/25g	Samples	iQ-Check positive		iQ-Check culture confirmed	Reference positive	Method Agreement	X^2 [c]
				Previous Kit [a]	Modified Kit [b]				
Eggs	Control	< 0.075	5	0	0	0	0	100%	-
	Low	0.38	20	13	13	13	8	75%	2.44
Raw chicken	Control	< 0.075	5	0	0	0	0	100%	-
	Low	0.50	20	14	14	N/A	14	100%	-
Raw ground beef	Control	< 0.075	5	0	0	0	0	100%	-
	Low	5.75	20	N/A	15	N/A	15	100%	-

[a] Results included in the previous AOAC-RI validation report in 2007, except raw ground beef

[b] Results obtained by using saved DNA extracts from 2007 and PCR mix with the change of raw materials, except raw ground beef which was repeated in total

[c] X^2 according to Mantel-Haenszel for eggs and McNemar for raw chicken and raw ground beef

DISCUSSION OF MODIFICATION APPROVED JUNE 2009 (6)

iQ-Check *Salmonella* II allows for detection of *Salmonella* from food samples utilizing real-time PCR technology. When compared to the FDA BAM reference method for detection of *Salmonella* in peanut butter, iQ-Check *Salmonella* II was shown to be an effective and efficient alternative method for detection. There was no significant difference between the performances of the two methods. A shortened enrichment protocol (21 ± 1 h) was validated utilizing enrichment in BPW broth. Decreasing detection time by 2 days over the reference method will identify contamination sooner so appropriate action can be taken.

Summary Data Table – Peanut Butter (25 g) (6)

Round 1

Level	MPN per 25 g	Replicates per method	RM Pos.	CM Pres. Pos.	CM Conf. Pos.	CM Sens.	CM Spec.	RM Sens.	RM Spec.	χ^2
High	11.5	20	18	18	18	100%	-	100%	-	-
Low	1.08	20	4	3	3	75%	-	100%	-	0.08
Control	-	5	0	0	0	-	100%	-	100%	-

Round 2

Level	MPN per 25 g	Replicates per method	RM Pos.	CM Pres. Pos.	CM Conf. Pos.	CM Sens.	CM Spec.	RM Sens.	RM Spec.	χ^2
High	0.1	20	3	1	1	33%	-	100%	-	0.53
Low	0.5	20	6	4	4	66%	-	100%	-	0.25
Control	-	5	0	0	0	-	100%	-	100%	-

DISCUSSION OF MODIFICATION APPROVED FEBRUARY 2010 (7)

To meet the demands of high volume users, a high throughput DNA extraction procedure was validated with the iQ-Check real-time PCR test kits. A method comparison study was performed and no significant difference was observed between the iQ-Check and reference methods. The data presented in this modification study supports the renewal of Performance Tested Method[SM] status for all iQ-Check kits with the addition of the new DNA extraction protocol.

Modification Data Approved February 2010 (7)

Kit / Extraction	Matrix	Level	MPN/25g	Samples	iQ-Check Positive	iQ-Check Confirmed [a]	Reference Positive	Method Agreement	X^2
Salmonella	Raw chicken	Control	< 0.075	5	0	0	0	100%	-
Easy	breast	Low	1.14	20	9	N/A	9	100%	-
Salmonella	Raw chicken	Control	< 0.075	5	0	0	0	100%	-
Standard (8 hr)	breast	Low	1.14	20	8	N/A	9	95%	0.00
Salmonella	Raw ground	Control	< 0.075	5	0	0	0	100%	-
Standard (8 hr)	beef	Low	1.60	20	12	N/A	11	95%	0.00
Salmonella	Raw	Control	< 0.075	5	0	0	0	100%	-
Standard (8 hr)	pork	Low	1.10	20	6	N/A	7	95%	0.0
Salmonella	Fresh	Control	< 0.075	5	0	0	0	100%	-
Standard (8 hr)	spinach	Low	1.075	20	8	8	10	90%	0.39

DISCUSSION OF MODIFICATION APPROVED AUGUST 2010 (8)

To meet industry demands, four environmental surfaces and two pet foods were tested with iQ-Check *Salmonella* II. A method comparison study was performed and no significant difference was observed between the iQ-Check and reference methods. An independent study was also performed and no significant difference was observed. All samples that were identified as positive by iQ-Check *Salmonella* II were subsequently confirmed by reference method procedures. The data presented in this modification report supports a matrix extension claim for this kit under certificate # 010803.

Table 1 – Method Comparison Results (8)

Matrix	Level	MPN	Samples	iQ-Check Positive	iQ-Check Confirmed	Reference Positive	Method Agreement	X^2
Stainless steel	Control	0	5	0	0	0	100%	-
	Test	$3.3x10^3$/sq	20	15	15	14	95%	0.12
Plastic	Control	0	5	0	0	0	100%	-
	Test	$4.1x10^3$/sq	20	18	18	14	80%	2.44
Concrete	Control	0	5	0	0	0	100%	-
	Test	$3.0x10^3$/sq	20	10	10	12	90%	0.40
Ceramic	Control	0	5	0	0	0	100%	-
	Test	$5.7x10^3$/sq	20	14	14	15	95%	0.12
Dry dog food	Control	0	5	0	0	0	100%	-
	Test	1.60/25g	20	8	8	8	100%	-
Wet cat food	Control	0	5	0	0	0	100%	-
	Test	1.60/25g	20	14	14	11	85%	0.94
Stainless steel	Control	0	5	0	0	0	100%	-
(independent)	Test	$3.1x10^3$/sq	20	14	14	12	90%	0.40

DISCUSSION OF MODIFICATION APPROVED NOVEMBER 2013 (9)

This independent study was performed to extend the iQ-Check Salmonella II method validation to include a 375g sample size. iQ-Check sample results were compared to the USDA-MLG method for ground chicken and deli ham and to the FDA-BAM method for dry dog food. For all test products, no significant difference was observed between the presumptive and confirmed results or between the iQ-Check overall confirmed results and reference method results using the probability of detection (POD) statistical model. The data presented in this modification report supports an extension claim for this kit under certificate # 010803.

Table 1 – POD – iQ-Check Presumptive vs Confirmed (9)

Matrix	Strain	MPN[a]/test portion	N[c]	Bio-Rad iQ Check Presumptive				Bio-Rad iQ Check Confirmed				dPOD$_{cp}$[g]	95% CI[h]	
				x[d]	POD$_{cp}$[e]	95% CI		x	POD$_{cc}$[f]	95% CI				
Raw ground chicken	Naturally contaminated	0.39	20	7	0.35	0.1812	0.5671	7	0.35	0.1812	0.5671	0.00	-0.2750	0.2750
		0.1	20	1	0.05	0.0000	0.2361	1	0.05	0.0000	0.2361	0.00	-0.1927	0.1927
Deli Ham	Salmonella hadar	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.31	20	5	0.25	0.1119	0.4687	5	0.25	0.1119	0.4687	0.00	-0.2587	0.2587
		2.46	5	5	1.00	0.5655	1.0000	5	1.00	0.5655	1.0000	0.00	-0.4345	0.4345
Dry Dog Food 1:4	Salmonella infantis	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.99	20	17	0.85	0.6396	0.9476	17	0.85	0.6396	0.9476	0.00	-0.2320	0.2320
		1.2	5	4	0.80	0.3755	0.9638	5	1.00	0.5655	1.0000	-0.20	-0.6245	0.2643
Dry Dog Food 1:10	Salmonella infantis	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.43	20	13	0.65	0.4328	0.8188	13	0.65	0.4328	0.8188	0.00	-0.2750	0.2750
		0.99	5	4	0.80	0.3755	0.9638	4	0.80	0.3755	0.9638	0.00	-0.4550	0.4550

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator, with 95% confidence interval
[b]N/A = Not applicable
[c]N = Number of test portions
[d]x = Number of positive test portions
[e]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[f]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[g]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 2 – POS – Method Comparison (9)

Matrix	Strain	MPN[a]/test portion	N[c]	Bio-Rad iQ Check				Reference Method				dPOD$_C$[g]	95% CI[h]	
				x[d]	POD$_c$[e]	95% CI		x	POD$_R$[f]	95% CI				
Raw ground chicken	Naturally contaminated	0.39	20	7	0.35	0.1812	0.5671	8	0.40	0.2188	0.6134	-0.05	-0.3221	0.2328
		0.1	20	1	0.05	0.0000	0.2361	2	0.10	0.0279	0.3010	-0.05	-0.2572	0.1496
Deli Ham	Salmonella hadar	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.31	20	5	0.25	0.1119	0.4687	4	0.20	0.0807	0.4160	0.05	-0.2064	0.2991
		2.46	5	5	1.00	0.5655	1.0000	4	0.80	0.3755	0.9638	0.20	-0.2643	0.6245
Dry Dog Food 1:4	Salmonella infantis	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.99	20	17	0.85	0.6396	0.9476	12	0.60	0.3866	0.7812	0.25	-0.0277	0.4847
		1.2	5	4	0.80	0.3755	0.9638	2	0.40	0.1176	0.7693	0.40	-0.1626	0.7264
Dry Dog Food 1:10	Salmonella infantis	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.43	20	13	0.65	0.4328	0.8188	7	0.35	0.1812	0.5671	0.30	-0.0071	0.5387
		0.99	5	4	0.80	0.3755	0.9638	4	0.80	0.3755	0.9638	0.00	-0.4550	0.4550

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator, with 95% confidence interval

[b]N/A = Not applicable

[c]N = Number of test potions

[d]x = Number of positive test portions

[e]POD_C = Confirmed candidate method positive outcomes divided by the total number of trials

[f]POD_R = Confirmed reference method positive outcomes divided by the total number of trials

[g]$dPOD_C$ = Difference between the candidate method and reference method POD values

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

DISCUSSION OF MODIFICATION APPROVED AUGUST 2014 (10)

This independent study was performed to extend the iQ-Check Salmonella II method validation to include a 375g sample size. iQ-Check sample results were compared to the USDA-MLG method for ground chicken and deli ham and to the FDA-BAM method for dry dog food. For all test products, no significant difference was observed between the presumptive and confirmed results or between the iQ-Check overall confirmed results and reference method results using the probability of detection (POD) statistical model. The data presented in this modification report supports an extension claim for this kit under certificate # 010803.

Table 1 – POD – iQ-Check Presumptive vs Confirmed (10)

Matrix	Strain	MPN[a]/test portion	N[c]	Bio-Rad iQ Check Presumptive				Bio-Rad iQ Check Confirmed				dPOD$_{cp}$[g]	95% CI[h]	
				x[d]	POD$_{cp}$[e]	95% CI		x	POD$_{cc}$[f]	95% CI				
Raw ground chicken	Naturally contaminated	0.39	20	7	0.35	0.1812	0.5671	7	0.35	0.1812	0.5671	0.00	-0.2750	0.2750
		0.1	20	1	0.05	0.0000	0.2361	1	0.05	0.0000	0.2361	0.00	-0.1927	0.1927
Deli Ham	Salmonella hadar	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.31	20	5	0.25	0.1119	0.4687	5	0.25	0.1119	0.4687	0.00	-0.2587	0.2587
		2.46	5	5	1.00	0.5655	1.0000	5	1.00	0.5655	1.0000	0.00	-0.4345	0.4345
Dry Dog Food 1:4	Salmonella infantis	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.99	20	17	0.85	0.6396	0.9476	17	0.85	0.6396	0.9476	0.00	-0.2320	0.2320
		1.2	5	4	0.80	0.3755	0.9638	5	1.00	0.5655	1.0000	-0.20	-0.6245	0.2643
Dry Dog Food 1:10	Salmonella infantis	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.43	20	13	0.65	0.4328	0.8188	13	0.65	0.4328	0.8188	0.00	-0.2750	0.2750
		0.99	5	4	0.80	0.3755	0.9638	4	0.80	0.3755	0.9638	0.00	-0.4550	0.4550

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator, with 95% confidence interval

[b]N/A = Not applicable

[c]N = Number of test portions

[d]x = Number of positive test portions

[e]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

[f]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

[g]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values

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

Table 2 – POS – Method Comparison (10)

Matrix	Strain	MPN[a]/test portion	N[c]	Bio-Rad iQ Check				Reference Method				dPOD$_C$[g]	95% CI[h]	
				x[d]	POD$_c$[e]	95% CI		x	POD$_R$[f]	95% CI				
Raw ground chicken	Naturally contaminated	0.39	20	7	0.35	0.1812	0.5671	8	0.40	0.2188	0.6134	-0.05	-0.3221	0.2328
		0.1	20	1	0.05	0.0000	0.2361	2	0.10	0.0279	0.3010	-0.05	-0.2572	0.1496
Deli Ham	Salmonella hadar	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.31	20	5	0.25	0.1119	0.4687	4	0.20	0.0807	0.4160	0.05	-0.2064	0.2991
		2.46	5	5	1.00	0.5655	1.0000	4	0.80	0.3755	0.9638	0.20	-0.2643	0.6245
Dry Dog Food 1:4	Salmonella infantis	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.99	20	17	0.85	0.6396	0.9476	12	0.60	0.3866	0.7812	0.25	-0.0277	0.4847
		1.2	5	4	0.80	0.3755	0.9638	2	0.40	0.1176	0.7693	0.40	-0.1626	0.7264
Dry Dog Food 1:10	Salmonella infantis	0	5	0	0.00	0.0000	0.2000	0	0.00	0.0000	0.2000	0.00	-0.2000	0.2000
		0.43	20	13	0.65	0.4328	0.8188	7	0.35	0.1812	0.5671	0.30	-0.0071	0.5387
		0.99	5	4	0.80	0.3755	0.9638	4	0.80	0.3755	0.9638	0.00	-0.4550	0.4550

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator, with 95% confidence interval
[b]N/A = Not applicable
[c]N = Number of test potions
[d]x = Number of positive test portions
[e]POD$_C$ = Confirmed candidate method positive outcomes divided by the total number of trials
[f]POD$_R$ = Confirmed reference method positive outcomes divided by the total number of trials
[g]dPOD$_C$ = Difference between the candidate method and reference method POD values
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

DISCUSSION OF MODIFICATION APPROVED SEPTEMBER 2016 (11)

The iQ-Check *Salmonella* II Kit successfully detected *Salmonella* species from all three food matrixes and the environmental surfaces analyzed. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate method and the reference methods for both matrixes and both of the environmental surfaces tested.

The iQ-Check *Salmonella* II method is quick and simple to perform, providing results in about 2 hr post incubation of the selective enrichment for 30 sample replicates. The use of the iQ-Check Prep Automated System provides a hands free application that can reduce possible contamination caused by the analyst performing testing. iQ-Check Prep Automated System is able to perform DNA extraction and PCR preparation and provides added value to the lab, reducing the risk of cross contamination if the user is not proficient in DNA extraction or PCR preparation. The addition of the Free DNA Removal protocol gives the added benefit of removing free DNA that may be present in the sample and giving equivalent results as compared to the reference method. The CFX Manager IDE software is user friendly with the ability to track lot information and sample identification quickly and with ease. The software and instrument also offer the ability to utilize an open platform and set up unique runs before a run is completed. 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 D: Summary of Results (11)

Matrix	Milk Chocolate (375 g)						
Method	iQ-Check *Salmonella* II Kit						FDA/BAM Chapter 5
Result	Presumptive			Confirmed			
Uninoculated	0/5			0/5			0/5
Low	15/20			14/20			11/20
High	5/5			5/5			5/5
Matrix	Raw Milk Cheese (375 g)						
Method	iQ-Check *Salmonella* II Kit						FDA/BAM Chapter 5
Result	Presumptive			Confirmed			
	Easy I	FDRS	1:10	Easy I	FDRS	1:10	
Uninoculated	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Low	6/20	6/20	6/20	6/20	6/20	6/20	7/20
High	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Matrix	Chicken Carcass Rinse (30 mL)						
Method	iQ-Check *Salmonella* II Kit						USDA/FSIS MLG 4.08
Result	Presumptive			Confirmed			
Uninoculated	0/5			0/5			0/5
Low	9/20			9/20			6/20
High	5/5			5/5			5/5
Matrix	Stainless Steel (4" x 4" Test Area)						
Method	iQ-Check *Salmonella* II Kit						FDA/BAM Chapter 5
Result	Presumptive			Confirmed			
Uninoculated	0/5			0/5			0/5
Low	6/20			6/20			5/20
High	5/5			5/5			5/5

Table 9: iQ-Check *Salmonella* II Kit, Candidate vs. Reference – POD Results (11)

Matrix/Test Portion	Strain	Sample Treatment	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		
Milk Chocolate (375 g)	*Salmonella* Abony NCTC 6017 & *Citrobacter freundii* ATCC 8090	Easy I Protocol with FDRS	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.74 (0.43, 1.23)	20	14	0.70	0.48, 0.85	11	0.55	0.34, 0.74	0.15	-0.14, 0.41
			4.38 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Milk Cheese (375 g)	*Salmonella* Enteriditis ATCC 13076 & *Kluyvera intermedia* ATCC 33110	Easy I Protocol	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		Easy I Protocol with FDRS	3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		Easy I Protocol with 1:10 dilution										

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator version 1.6 provided by AOAC RI, with 95% confidence interval
[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 result and reference method confirmed result 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 10: iQ-Check *Salmonella* II Kit, Candidate vs. Reference – POD Results (11)

Matrix/Test Area	Strain	Sample Treatment	CFU[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		
Chicken Carcass Rinse (30 mL)	*Salmonella* Virchow ATCC 51955 & *Enterobacter aerogenes* ATCC 13048	Easy I Protocol with FDRS	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			20 & 400	20	9	0.45	0.11, 0.47	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
			230 & 4800	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel (4" x 4" Test Area)	*Salmonella* Derby NCTC 5721 & *Pantoea agglomerans* ATCC 19552	Easy I Protocol with FDRS	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			60 & 820	20	6	0.30	0.15, 0.52	5	0.25	0.11, 0.47	0.05	-0.22, 0.31
			330 & 6800	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[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 result and reference method confirmed result 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 11: iQ-Check *Salmonella* II Kit, Presumptive vs. Confirmed – POD Results (11)

Matrix/Test Portion	Strain	Sample Treatment	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		
Milk Chocolate (375 g)	*Salmonella* Abony NCTC 6017 & *Citrobacter freundii* ATCC 8090	Easy I Protocol with FDRS	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.74 (0.43, 1.23)	20	15	0.75	0.53, 0.89	14	0.70	0.48, 0.85	0.05	-0.22, 0.31
			4.38 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Milk Cheese (375 g)	*Salmonella* Enteriditis ATCC 13076 & *Kluyvera intermedia* ATCC 33110	Easy I Protocol	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
		Easy I Protocol with FDRS Easy I Protocol with 1:10 dilution	3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator version 1.6 provided by AOAC RI, with 95% confidence interval
[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 result and candidate method confirmed result 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 12: iQ-Check *Salmonella* II Kit, Presumptive vs. Confirmed – POD Results (11)

Matrix/Test Area	Strain	Sample Treatment	CFU[a]/ Test Area	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Chicken Carcass Rinse (30 mL)	*Salmonella* Virchow ATCC 51955 & *Enterobacter aerogenes* ATCC 13048	Easy I Protocol with FDRS	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			20 & 400	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			230 & 4800	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel (4" x 4" Test Area)	*Salmonella* Derby NCTC 5721 & *Pantoea agglomerans* ATCC 19552	Easy I Protocol with FDRS	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			60 & 820	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			330 & 6800	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[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 result and candidate method confirmed result 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

DISCUSSION OF MODIFICATION APPROVED MARCH 2021 (14)

The Bio-Rad iQ-Check *Salmonella* II Kit successfully detected *Salmonella* spp. in all matrixes tested and for all conditions analyzed. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods and the reference method for all samples tested. The data presented in the study demonstrates equivalent results between the classic APF and Fast APF on all matrixes with and without FDRS. The iQ-Check *Salmonella* II Kit successfully detected *Salmonella* spp. in fresh raw ground beef and fresh raw beef trim in 8 h of enrichment in BPW and in fresh baby spinach in 10 h of enrichment in BPW.

For the 10 g cannabis flower, one false negative result was observed when analyzed with FDRS treatment using the classic Salmonella APF, one false negative result when analyzed with Salmo Fast APF and 2 false negative results when analyzed with the Salmo Fast APF treated with FDRS from three samples. In all instances, there was no typical growth following secondary enrichments in RV and TT broths on the XLD plates and only one typical colony on each of the RAPID'*Salmonella* agar plates indicating a very low level of viable target *Salmonella* in the samples. This is further evidenced by the high Cq values observed from the PCR positive results from the other testing parameters.

The Bio-Rad iQ-Check *Salmonella* II Kit method is quick and simple to perform providing results in less than 75 min post enrichment and sample prep. The use of the iQ-Check Prep Automation system provides a hands-free application, that can reduce possible contamination caused by the analyst performing testing. The iQ-Check Prep is able to perform DNA extraction, PCR preparation, and automate the FDRS step while providing added value to the lab, reducing the risk of cross contamination, and improving traceability. 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 the iQ-Check *Salmonella* II Assay – BPW at 41.5±1°C for 8 h, Fast APF (14)

No.	Species and subspecies	Serovar	Source	Origin	Result
1	*S. enterica* subsp. *arizonae*	Not Available	ATCC[1] 13314	Not Available	+
2	*S. enterica* subsp. *arizonae*	Not Available	ATCC BAA-1577	Not Available	+
3	*S. enterica* subsp. *arizonae*	Not Available	QL[2]11007-4	Veterinary Isolate	+
4	*S. enterica* subsp. *diarizonae*	Not Available	ATCC BAA-1579	Not Available	+
5	*S. enterica* subsp. *diarizonae*	Not Available	ATCC BAA-216	Human blood	+
6	*S. enterica* subsp. *diarizonae*	Not Available	ATCC BAA-639	Human feces	+
7	*S. enterica* subsp. *houtenae*	Halmstad	QL024.1	Clinical isolate	+
8	*S. enterica* subsp. *houtenae*	Harmelen	ATCC 15783	Boa constrictor	+
9	*S. enterica* subsp. *houtenae*	Ochsenzoll	ATCC 29932	Not Available	+
10	*S. enterica* subsp. *indica*	Ferlac	ATCC 43976	Not Available	+
11	*S. enterica* subsp. *indica*	Ferlac	NCTC[3] 10458	Ceylonese dessicated coconut	+
12	*S. enterica* subsp. *indica*	Not Available	ATCC BAA-1578	India	+
13	*S. enterica* subsp. *salamae*	Artis	ATCC 700149	Not Available	+
14	*S. enterica* subsp. *salamae*	Basel	ATCC 700151	Not Available	+
15	*S. enterica* subsp. *salamae*	Not Available	QL02415	Pet food	+
16	*S. enterica* subsp. *enterica*	Abaetetuba	ATCC 35640	Creek water	+
17	*S. enterica* subsp. *enterica*	Abortusequi	FDA[4] 9842	Not Available	+
18	*S. enterica* subsp. *enterica*	Abortusovis	NCTC 10241	Not Available	+
19	*S. enterica* subsp. *enterica*	Abony	NCTC 6017	Not Available	+
20	*S. enterica* subsp. *enterica*	Adelaide	UPenn[5] STs 2	Not Available	+
21	*S. enterica* subsp. *enterica*	Anatum	ATCC 9270	Pork liver	+
22	*S. enterica* subsp. *enterica*	Arkansas	UPenn STs 11	Not Available	+
23	*S. enterica* subsp. *enterica*	Bareilly	FDA 1206H	Not Available	+
24	*S. enterica* subsp. *enterica*	Berta	UPenn STs 13	Not Available	+
25	*S. enterica* subsp. *enterica*	Binza	UPenn STs 14	Not Available	+
26	*S. enterica* subsp. *enterica*	Bovis-Morbificans	UPenn STs 16	Not Available	+
27	*S. enterica* subsp. *enterica*	Brandenburg	UPenn STs 18	Not Available	+
28	*S. enterica* subsp. *enterica*	Bredeney	NCTC 5731	Not Available	+
29	*S. enterica* subsp. *enterica*	California	NCTC 6018	Not Available	+
30	*S. enterica* subsp. *enterica*	Cerro	UPenn STs 22	Not Available	+
31	*S. enterica* subsp. *enterica*	Choleraesuis	ATCC 10708	Equine isolate	+
32	*S. enterica* subsp. *enterica*	Choleraesuis var Kunzendorf	ATCC 12011	Not Available	+
33	*S. enterica* subsp. *enterica*	Cubana	UPenn STs 24	Not Available	+
34	*S. enterica* subsp. *enterica*	Derby	NCTC 5721	Not Available	+
35	*S. enterica* subsp. *enterica*	Drypool	UPenn STs 26	Not Available	+
36	*S. enterica* subsp. *enterica*	Dublin	UPenn STs 27	Not Available	+
37	*S. enterica* subsp. *enterica*	Eastbourne	FDA 4017H	Not Available	+
38	*S. enterica* subsp. *enterica*	Enteritidis	ATCC 13076	Not Available	+
39	*S. enterica* subsp. *enterica*	Galiema	QL024.2	Environmental isolate	+
40	*S. enterica* subsp. *enterica*	Give	UPenn STs 42	Not Available	+
41	*S. enterica* subsp. *enterica*	Dublin	UPenn STs 27	Not Available	+
42	*S. enterica* subsp. *enterica*	Eastbourne	FDA 4017H	Not Available	+
43	*S. enterica* subsp. *enterica*	Enteritidis	ATCC 13076	Not Available	+
44	*S. enterica* subsp. *enterica*	Galiema	QL024.2	Environmental isolate	+
45	*S. enterica* subsp. *enterica*	Give	UPenn STs 42	Not Available	+
46	*S. enterica* subsp. *enterica*	Haardt	UPenn STs 44	Not Available	+
47	*S. enterica* subsp. *enterica*	Hadar	ATCC 51956	Not Available	+
48	*S. enterica* subsp. *enterica*	Havana	UPenn STs 47	Not Available	+
49	*S. enterica* subsp. *enterica*	Heidelberg	ATCC 8326	Not Available	+
50	*S. enterica* subsp. *enterica*	Illinois	ATCC 11646	Not Available	+
51	*S. enterica* subsp. *enterica*	Indiana	NCTC 11304	Turkey	+
52	*S. enterica* subsp. *enterica*	Infantis	ATCC 51741	Pasta	+
53	*S. enterica* subsp. *enterica*	Javiana	ATCC 10721	Not Available	+
54	*S. enterica* subsp. *enterica*	Jerusalem	QL024.12	Pet food	+
55	*S. enterica* subsp. *enterica*	Johannesburg	UPenn STs 56	Not Available	+
56	*S. enterica* subsp. *enterica*	Kahla	ATCC 17980	Not Available	+
57	*S. enterica* subsp. *enterica*	Kaitaan	QL024.7	Pet food	+
58	*S. enterica* subsp. *enterica*	Kentucky	ATCC 9263	Not Available	+
59	*S. enterica* subsp. *enterica*	Krefeld	UPenn STs 58	Not Available	+
60	*S. enterica* subsp. *enterica*	Lille	UPenn STs 59	Not Available	+
61	*S. enterica* subsp. *enterica*	Livingstone	UPenn STs 63	Not Available	+
62	*S. enterica* subsp. *enterica*	London	UPenn STs 64	Not Available	+
63	*S. enterica* subsp. *enterica*	Manhattan	UPenn STs 65	Not Available	+

64	*S. enterica* subsp. *enterica*	Mbankaka	FDA 37N	Low moisture ingredient	+
65	*S. enterica* subsp. *enterica*	Menden	ATCC 15992	Feces	+
66	*S. enterica* subsp. *enterica*	Meleagridis	QL12074-1	Environmental isolate	+
67	*S. enterica* subsp. *enterica*	Menhaden	QL024.20	Pet food	+
68	*S. enterica* subsp. *enterica*	Minnesota	UPenn STs 70	Not Available	+
69	*S. enterica* subsp. *enterica*	Montevideo	ATCC 8387	Not Available	+
70	*S. enterica* subsp. *enterica*	Muenchen	ATCC BAA-1594	Human stool	+
71	*S. enterica* subsp. *enterica*	Neasden	QL024.4	Raw material	+
72	*S. enterica* subsp. *enterica*	Newington	QL024.8	Fish oil	+
73	*S. enterica* subsp. *enterica*	Newport	ATCC 6962	Food poisoning	+
74	*S. enterica* subsp. *enterica*	Ohio	UPenn STs 81	Not Available	+
75	*S. enterica* subsp. *enterica*	Oranienburg	ATCC 9239	Not Available	+
76	*S. enterica* subsp. *enterica*	Orthmarshen	QL024.13	Pet kibble	+
77	*S. enterica* subsp. *enterica*	Paratyphi A	ATCC 9150	Not Available	+
78	*S. enterica* subsp. *enterica*	Paratyphi B	ATCC 10719	Not Available	+
79	*S. enterica* subsp. *enterica*	Paratyphi C	ATCC 13428	Not Available	+
80	*S. enterica* subsp. *enterica*	Pomona	ATCC 10729	Clinical isolate	+
81	*S. enterica* subsp. *enterica*	Poona	NCTC 4840	Infant enteritis	+
82	*S. enterica* subsp. *enterica*	Potsdam	QL15091-1A	Pet food	+
83	*S. enterica* subsp. *enterica*	Preston	QL024.16	Low moisture product	+
84	*S. enterica* subsp. *enterica*	Pullorum	ATCC 13036	Egg	+
85	*S. enterica* subsp. *enterica*	Rubislaw	UPenn STs 92	Not Available	+
86	*S. enterica* subsp. *enterica*	Saintpaul	ATCC 9712	Cystitis	+
87	*S. enterica* subsp. *enterica*	San-Diego	UPenn STs 94	Not Available	+
88	*S. enterica* subsp. *enterica*	Schalkwijk	QL024.10	Cat food	+
89	*S. enterica* subsp. *enterica*	Schwarzengrund	UPenn STs 95	Not Available	+
90	*S. enterica* subsp. *enterica*	Senftenberg	ATCC 43845	Not Available	+
91	*S. enterica* subsp. *enterica*	Stanley	ATCC 7308	Not Available	+
92	*S. enterica* subsp. *enterica*	Sylvania	QL091313.4	Raw dog food	+
93	*S. enterica* subsp. *enterica*	Tallahassee	ATCC 12002	Not Available	+
94	*S. enterica* subsp. *enterica*	Tennessee	QL024.6	Clinical isolate	+
95	*S. enterica* subsp. *enterica*	Thompson	FDA 2051H	Not Available	+
96	*S. enterica* subsp. *enterica*	Tranoroa	NCTC 10252	Not Available	+
97	*S. enterica* subsp. *enterica*	Typhi	ATCC 6539	Not Available	+
98	*S. enterica* subsp. *enterica*	Typhimurium	ATCC 14028	Animal tissue	+
99	*S. enterica* subsp. *enterica*	Utrect	NCTC 10077	Not Available	+
100	*S. enterica* subsp. *enterica*	Urbana	UPenn STs 110	Not Available	+
101	*S. enterica* subsp. *enterica*	Vellore	ATCC 15611	Rectal swab	+
102	*S. enterica* subsp. *enterica*	Virchow	ATCC 51955	Not Available	+
103	*S. enterica* subsp. *enterica*	Volta	QL024.9	Raw material	+
104	*S. enterica* subsp. *enterica*	Westhampton	QL024.14	Dog kibble	+
105	*S. enterica* subsp. *enterica*	Worthington	UPenn STs 114	Not Available	+
106	*Salmonella bongori*	Not Available	NCTC 10946	Amphibian; Frog	+
107	*Salmonella bongori*	Not Available	ATCC 43975	Not Available	+
108	*Salmonella bongori*	Not Available	NCTC 12419	Not Available	+

[1]ATCC = American Type Culture Collection, Manassas, USA
[2]QL = Q Laboratories Culture Collection, Cincinnati, USA
[3]NCTC = National Culture Type Collection, London, England
[4]FDA = US Food and Drug Administration Culture Collection, College Park, USA
[5]UPENN = University of Pennsylvania Culture Collection, Philadelphia, USA

Table 4. Inclusivity Results for the iQ-Check *Salmonella* II Assay – BPW at 37±1°C for 18 h, Fast APF (14)

No.	Species and subspecies	Serovar	Source	Origin	Result
1	*S. enterica* subsp. *enterica*	Aberdeen	CMF[1] 114	Pasteur Institute	+
2	*S. enterica* subsp. *enterica*	Adelaide	CMF 482	Pasteur Institute	+
3	*S. enterica* subsp. *enterica*	Agona	Ad[2] 4869	Smoked sausage	+
4	*S. enterica* subsp. *enterica*	Albany	CMF 82	Pasteur Institute	+
5	*S. enterica* subsp. *enterica*	Anatum	Ad 298	Milk powder	+
6	*S. enterica* subsp. *enterica*	Anatum var 15+ (Newington)	Ad 26	Dairy product	+
7	*S. enterica* subsp. *arizonae*	Not Available	CIP 55.26	Dried egg powder	+
8	*S. enterica* subsp. *arizonae*	Not Available	ATCC[3] 13314	Pasteur Institute	+
9	*S. enterica* subsp. *arizonae*	Not Available	Ad 450	Sheep milk	+
10	*S. enterica* subsp. *arizonae*	Not Available	Ad 478	Clams	+
11	*S. enterica* subsp. *enterica*	Bambylor	CMF 135	Pasteur Institute	+
12	*S. enterica* subsp. *enterica*	Bardo	Ad 569	Sausage meat	+
13	*S. enterica* subsp. *enterica*	Bareilly	CMF 136	Pasteur Institute	+
14	*S. enterica* subsp. *enterica*	Berta	CMF 141	Pasteur Institute	+
15	*S. enterica* subsp. *enterica*	Blockley	Ad 923	Poultry environmental	+
16	*Salmonella bongori*	Not Available	Ad 598	Environmental sample	+

17	*Salmonella bongori*	Not Available	Ad 599	Turkey	+
18	*Salmonella bongori*	Not Available	ATCC 43975	Pasteur Institute	+
19	*S. enterica* subsp. *enterica*	Bovismorbificans	Ad 728	Gelatin	+
20	*S. enterica* subsp. *enterica*	Braenderup	ATCC BNA 664	CDC	+
21	*S. enterica* subsp. *enterica*	Brandenburg	Ad 351	Seafood cocktail	+
22	*S. enterica* subsp. *enterica*	Bredeney	Ad 464	Pork pâté	+
23	*S. enterica* subsp. *enterica*	Carrau	CMF 142	Pasteur Institute	+
24	*S. enterica* subsp. *enterica*	Cerro	CMF 166	Pasteur Institute	+
25	*S. enterica* subsp. *enterica*	Cremieu	Ad 230	Hare	+
26	*S. enterica* subsp. *enterica*	Crossness	CMF 165	Pasteur Institute	+
27	*S. enterica* subsp. *enterica*	Cubana	CMF 188	Pasteur Institute	+
28	*S. enterica* subsp. *enterica*	Dalhem	CMF 924	Pasteur Institute	+
29	*S. enterica* subsp. *enterica*	Derby	Ad 374	Pork sausage	+
30	*S. enterica* subsp. *diarizonae*	Not Available	ATCC 43973	Pasteur Institute	+
31	*S. enterica* subsp. *diarizonae*	Not Available	Ad 594	Frog legs	+
32	*S. enterica* subsp. *diarizonae*	Not Available	Ad 595	Cheese	+
33	*S. enterica* subsp. *enterica*	Dublin	Ad 40	Poultry environmental	+
34	*S. enterica* subsp. *enterica*	Duisburg	Ad 42	Poultry environmental	+
35	*S. enterica* subsp. *enterica*	Enteritidis	ATCC 13076	CDC	+
36	*S. enterica* subsp. *enterica*	Essen	Ad 38	Poultry environmental	+
37	*S. enterica* subsp. *enterica*	Falkensee	Ad 693	Sausage meat	+
38	*S. enterica* subsp. *enterica*	Gallinarum	Ad 1	Poultry	+
39	*S. enterica* subsp. *enterica*	Gaminara	CMF 221	Pasteur Institute	+
40	*S. enterica* subsp. *enterica*	Give var 15+ (Newbrunswick)	Ad 436	Ground beef	+
41	*S. enterica* subsp. *enterica*	Glostrup	CMF 226	Pasteur Institute	+
42	*S. enterica* subsp. *enterica*	Grumpensis	CMF 478	Pasteur Institute	+
43	*S. enterica* subsp. *enterica*	Hadar	Ad 35	Poultry	+
44	*S. enterica* subsp. *enterica*	Havana	CMF 237	Pasteur Institute	+
45	*S. enterica* subsp. *enterica*	Heidelberg	Ad 24876	Poultry	+
46	*S. enterica* subsp. *houtenae*	Not Available	ATCC 43974	Pasteur Institute	+
47	*S. enterica* subsp. *enterica*	*Salmonella* II 47:b:1,5 (Phoenix)	CMF 395	Pasteur Institute	+
48	*S. enterica* subsp. *enterica*	Indiana	Ad 2B	Feed	+
49	*S. enterica* subsp. *indica*	Ferlac	ATCC 43976	Pasteur Institute	+
50	*S. enterica* subsp. *enterica*	Infantis	ATCC 51741	Pasta	+
51	*S. enterica* subsp. *enterica*	Inverness	CMF 253	Pasteur Institute	+
52	*S. enterica* subsp. *enterica*	Johannesburg	CMF 256	Pasteur Institute	+
53	*S. enterica* subsp. *enterica*	Kedougou	Ad 929	Bovine environmental	+
54	*S. enterica* subsp. *enterica*	Kentucky	CMF 264	Pasteur Institute	+
55	*S. enterica* subsp. *enterica*	Kirkee	CMF 458	Pasteur Institute	+
56	*S. enterica* subsp. *enterica*	Kottbus	Ad 1B	Poultry	+
57	*S. enterica* subsp. *enterica*	Lagos	Ad 173	Chipolatas (sausage)	+
58	*S. enterica* subsp. *enterica*	Landau	Ad 499 B	Pasteur Institute	+
59	*S. enterica* subsp. *enterica*	Lexington var 15+, 34+ (Illinois)	CMF 251	Pasteur Institute	+
60	*S. enterica* subsp. *enterica*	Lille	Ad 37	Poultry environmental	+
61	*S. enterica* subsp. *enterica*	Livingstone	Ad E1	White egg powder	+
62	*S. enterica* subsp. *enterica*	Lomita	CMF 125	Pasteur Institute	+
63	*S. enterica* subsp. *enterica*	London	Ad 34	Food	+
64	*S. enterica* subsp. *enterica*	Manhattan	Ad 900	Dairy environmental (dust)	+
65	*S. enterica* subsp. *enterica*	Maregrosso	CMF 301	Pasteur Institute	+
66	*S. enterica* subsp. *enterica*	Mbandaka	Ad 81	Eggs	+
67	*S. enterica* subsp. *enterica*	Meleagridis	Ad 505	Raw milk	+
68	*S. enterica* subsp. *enterica*	Miami	CMF 307	Pasteur Institute	+
69	*S. enterica* subsp. *enterica*	Minnesota	CMF 146	Pasteur Institute	+
70	*S. enterica* subsp. *enterica*	Montevideo	Ad 327	Intestine	+
71	*S. enterica* subsp. *enterica*	Muenchen	CMF 337	Pasteur Institute	+
72	*S. enterica* subsp. *enterica*	Newport	Ad 972	Turkey	+
73	*S. enterica* subsp. *enterica*	Oranienburg	CMF 360	Pasteur Institute	+
74	*S. enterica* subsp. *enterica*	Orion var 15+ (Binza)	Ad 27	Food	+
75	*S. enterica* subsp. *enterica*	Ouakam	CMF 364	Pasteur Institute	+
76	*S. enterica* subsp. *enterica*	Panama	Ad 81	Ground beef	+
77	*S. enterica* subsp. *enterica*	Paratyphi A	ATCC 9150	IL Public Health Dept	+
78	*S. enterica* subsp. *enterica*	Paratyphi B	Ad 301	Human	+
79	*S. enterica* subsp. *enterica*	Paratyphi C	ATCC 13428	MI Health Dept	+
80	*S. enterica* subsp. *enterica*	Poona	CMF 689	Pasteur Institute	+
81	*S. enterica* subsp. *enterica*	Potsdam	CMF 225	Pasteur Institute	+
82	*S. enterica* subsp. *enterica*	Regent	Ad 328	Duck	+
83	*S. enterica* subsp. *enterica*	Rissen	Ad 59	Poultry environmental	+
84	*S. enterica* subsp. *enterica*	Rubislaw	CMF 414	Pasteur Institute	+

85	*S. enterica* subsp. *enterica*	Saintpaul	Ad 00C001	Pheasant	+
86	*S. enterica* subsp. *salamae*	Not Available	ATCC 43972	Pasteur Institute	+
87	*S. enterica* subsp. *enterica*	Schwarzengrund	CMF 420	Pasteur Institute	+
88	*S. enterica* subsp. *enterica*	Senftenberg	Ad 355	Sea food cocktail	+
89	*S. enterica* subsp. *enterica*	Sheffield	CMF 426	Pasteur Institute	+
90	*S. enterica* subsp. *enterica*	Singapore	CMF 427	Pasteur Institute	+
91	*S. enterica* subsp. *enterica*	Springs	CMF 431	Pasteur Institute	+
92	*S. enterica* subsp. *enterica*	Sternschanze	CMF 432	Pasteur Institute	+
93	*S. enterica* subsp. *enterica*	Tallahassee	CMF 822	Pasteur Institute	+
94	*S. enterica* subsp. *enterica*	Tennessee	Ad 00E006	Environmental	+
95	*S. enterica* subsp. *enterica*	Thompson	Ad AER 301	Poultry	+
96	*S. enterica* subsp. *enterica*	Typhi	Ad 302	Pasteur Institute	+
97	*S. enterica* subsp. *enterica*	Typhimurium	ATCC 13311	Human feces	+
98	*S. enterica* subsp. *enterica*	Urbana	Ad 501	Pasteur Institute	+
99	*S. enterica* subsp. *enterica*	Utrecht	CMF 484	Pasteur Institute	+
100	*S. enterica* subsp. *enterica*	Veneziana	Ad 233	Food	+
101	*S. enterica* subsp. *enterica*	Virchow	CIP[4] 105355	Human isolate	+
102	*S. enterica* subsp. *enterica*	Wayne	Ad 502	Pasteur Institute	+
103	*S. enterica* subsp. *enterica*	Weslaco	CMF 688	Pasteur Institute	+
104	*S. enterica* subsp. *enterica*	Worthington	Ad 3506	Pâté	+
105	*S. enterica* subsp. *enterica*	Yoruba	CMF 3913	Pasteur Institute	+
106	*S. enterica* subsp. *enterica*	Ohio var 14+ (Nienstedten)	CMF 352	Pasteur Institute	+

[1]CMF = Culture Microbienne et Fongique (Microbiology and Fungus Culture Collection), France
[2]Ad = ADRIA Developpement Laboratory, Quimper, France
[3]ATCC = American Type Culture Collection, Manassas, USA
[4]CIP = Collection Institut Pasteur, Paris, France

Table 5. Inclusivity Results for the iQ-Check *Salmonella* II Assay – BPW + PIF Supplement at 37±1°C for 18 h, Fast APF (14)

No.	Species and subspecies	Serovar	Source	Origin	Result
1	*S. enterica* subsp. *enterica*	Abaetetuba	AD[1] Ad2318	Not Available	+
2	*S. enterica* subsp. *enterica*	Aberdeen	CIP[2] 105618	Not Available	+
3	*S. enterica* subsp. *enterica*	Abortusequi	AD Ad2321	Not Available	+
4	*S. enterica* subsp. *enterica*	Abortusovis	AD Ad2320	Ovine foetus	+
5	*S. enterica* subsp. *enterica*	Adelaïde	AD Ad2319	Turkey breeding environment	+
6	*S. enterica* subsp. *enterica*	Agona	AD A00V038	Feed for pork	+
7	*S. enterica* subsp. *enterica*	Anatum	AD A00E007	Dusts	+
8	*S. enterica* subsp. *arizonae*	51:z4,z24:-	CIP 55.23	Turkey meat	+
9	*S. enterica* subsp. *arizonae*	48:z4,z23:-	AD Ad1850	Poultry environmental sample	+
10	*S. enterica* subsp. *enterica*	Bardo	AD Adria 569	Meat for sausage	+
11	*S. enterica* subsp. *enterica*	Bareilly	AD Ad1687	Chocolate industry	+
12	*S. enterica* subsp. *enterica*	Blockley	AD Ad923	Poultry environment	+
13	*Salmonella bongori*	*66 :z35:-*	AD Ad598	Environmental samplE	+
14	*S. enterica* subsp. *enterica*	Bovismorbificans	AD Adria 6629	Sausage	+
15	*S. enterica* subsp. *enterica*	Braenderup	AD Adria 111	Pork meat	+
16	*S. enterica* subsp. *enterica*	Brandenburg	AD Ad351	Seafood cocktail	+
17	*S. enterica* subsp. *enterica*	Bredeney	AD Adria 396	Ground beef	+
18	*S. enterica* subsp. *enterica*	Caracas	AD Ad2322	Spice	+
19	*S. enterica* subsp. *enterica*	Cerro	AD Ad689	Dehydrated poultry protein	+
20	*S. enterica* subsp. *enterica*	Chester	CIP 103543	Not Available	+
21	*S. enterica* subsp. *enterica*	Cubana	AD Ad2323	Dust feed environment	+
22	*S. enterica* subsp. *enterica*	Derby	AD Ad1093	Fish fillet	+
23	*S. enterica* subsp. *diarizonae*	38:lv:z53	AD Ad451	Ewe milk cheese	+
24	*S. enterica* subsp. *diarizonae*	61:k:1,5,7	AD Ad1300	Raw ewe milk	+
25	*S. enterica* subsp. *enterica*	Dublin	AD Ad529	Beef meat	+
26	*S. enterica* subsp. *enterica*	Emek	AD Ad333	Not Available	+
27	*S. enterica* subsp. *enterica*	Enteritidis	AD Ad477	Hen meat	+
28	*S. enterica* subsp. *enterica*	Gallinarum biovar pullorum	AD Ad300	Poultry environment	+
29	*S. enterica* subsp. *enterica*	Gaminara	AD Ad2324	Boar meat	+
30	*S. enterica* subsp. *enterica*	Give	AD 436	Ground beef	+
31	*S. enterica* subsp. *enterica*	Guinea	AD 29	Not Available	+
32	*S. enterica* subsp. *enterica*	Hadar	AD 24871	Chicken meat	+
33	*S. enterica* subsp. *enterica*	Havana	AD Ad 930	Poultry environment	+
34	*S. enterica* subsp. *enterica*	Heidelberg	AD A00E005	Dusts from dairy industry	+
35	*S. enterica* subsp. *houtenae*	1,40:z4:z23:-	AD Ad2682	Primary production sample (poultry)	+
36	*S. enterica* subsp. *enterica*	Hvittingfoss	AD Ad2325	Raw stuff	+
37	*S. enterica* subsp. *enterica*	Indiana	AD Ad174	White cheese	+
38	*S. enterica* subsp. *indica*	1,6,14,25:a:enx	AD Ad 600	Environmental sample	+

39	*S. enterica* subsp. *indica*	11:b:e,n,x	AD Ad2337	Chicken breeding environment	+
40	*S. enterica* subsp. *enterica*	Infantis	AD F401B	Cheese	+
41	*S. enterica* subsp. *enterica*	Javiana	AD Ad2326	Turkey meat	+
42	*S. enterica* subsp. *enterica*	Kedougou	AD Ad929	Bovine environmental sample	+
43	*S. enterica* subsp. *enterica*	Kentucky	AD Ad1756	Poultry environmental sample	+
44	*S. enterica* subsp. *enterica*	Kottbus	AD Adria 1	Poultry environmental sample	+
45	*S. enterica* subsp. *enterica*	Landau	AD Ad499	Not Available	+
46	*S. enterica* subsp. *enterica*	Lille	AD Adria 37	Food product	+
47	*S. enterica* subsp. *enterica*	Livingstone	AD Ad1107	Dusts	+
48	*S. enterica* subsp. *enterica*	London	AD Adria 326	Cooked meat sample	+
49	*S. enterica* subsp. *enterica*	Luciana	CIP 105626	Not Available	+
50	*S. enterica* subsp. *enterica*	Manhattan	AD Adria 900	Dusts from dairy industry	+
51	*S. enterica* subsp. *enterica*	Maracaibo	CIP 54143	Not Available	+
52	*S. enterica* subsp. *enterica*	Marseille	CIP105627	Not Available	+
53	*S. enterica* subsp. *enterica*	Mbandaka	AD Ad 914	Mayonnaise	+
54	*S. enterica* subsp. *enterica*	Meleagridis	AD 505	Raw milk	+
55	*S. enterica* subsp. *enterica*	Michigan	AD Ad2327	Low moisture sausage	+
56	*S. enterica* subsp. *enterica*	Mikawasima	AD Ad1811	Raw ewe milk	+
57	*S. enterica* subsp. *enterica*	Minnesota	AD Ad2328	Feed	+
58	*S. enterica* subsp. *enterica*	Missisipi	AD Ad2329	Parakeet	+
59	*S. enterica* subsp. *enterica*	Montevideo	AD Ad912	Raw milk	+
60	*S. enterica* subsp. *enterica*	Muenchen	CIP 106178	Not Available	+
61	*S. enterica* subsp. *enterica*	Napoli	AD Ad928	Clinical	+
62	*S. enterica* subsp. *enterica*	Newport	AD Adria 586	Sausage	+
63	*S. enterica* subsp. *enterica*	Norwich	AD Ad1172	Not Available	+
64	*S. enterica* subsp. *enterica*	Ohio	AD Ad1482	Raw cow milk	+
65	*S. enterica* subsp. *enterica*	Orion	AD 27	Not Available	+
66	*S. enterica* subsp. *enterica*	Oranienburg	AD Ad1724	Cereals	+
67	*S. enterica* subsp. *enterica*	Ouakam	AD Ad1647	Compost	+
68	*S. enterica* subsp. *enterica*	Panama	AD Adria 8	Ground beef	+
69	*S. enterica* subsp. *enterica*	Paratyphi A	ATCC[3] 9150	Not Available	+
70	*S. enterica* subsp. *enterica*	Paratyphi B	AD Ad301	Clinical	+
71	*S. enterica* subsp. *enterica*	Paratyphi C	ATCC 13428	Not Available	+
72	*S. enterica* subsp. *enterica*	Pomona	CIP105630	Not Available	+
73	*S. enterica* subsp. *enterica*	Poona	AD Ad2330	Poultry feed	+
74	*S. enterica* subsp. *enterica*	Putten	AD Ad2331	Feed for chicken	+
75	*S. enterica* subsp. *enterica*	Regent	AD Adria 328	Duck	+
76	*S. enterica* subsp. *enterica*	Rissen	AD Adria 39	Food product	+
77	*S. enterica* subsp. *enterica*	Rubislaw	AD Ad2332	Shark cartilage	+
78	*S. enterica* subsp. *enterica*	Saintpaul	AD Adria F31	Pilchard fillets	+
79	*S. enterica* subsp. *salamae*	42,b:e,n,x,z15	AD Ad 593	Cereals	+
80	*S. enterica* subsp. *enterica*	Schwarzengrund	AD Ad2333	Egg products environment	+
81	*S. enterica* subsp. *enterica*	Senftenberg	AD Ad 355	Seafood cocktail	+
82	*S. enterica* subsp. *enterica*	Stanley	AD Ad1688	Chocolate industry	+
83	*S. enterica* subsp. *enterica*	Stourbridge	AD Ad2297	Raw milk cheese	+
84	*S. enterica* subsp. *enterica*	Strasbourg	CIP105632	Not Available	+
85	*S. enterica* subsp. *enterica*	Tananarive	CIP54142	Not Available	+
86	*S. enterica* subsp. *enterica*	Tennessee	AD A00E006	Dusts from dairy industry	+
87	*S. enterica* subsp. *enterica*	Thompson	AD AER301	Poultry	+
88	*S. enterica* subsp. *enterica*	Typhi	AD Ad302	Clinical	+
89	*S. enterica* subsp. *enterica*	Typhimurium	AD Ad1070	Pork meat	+
90	*S. enterica* subsp. *enterica*	Typhimurium 1,4 [5], I2:-:-	AD Ad1333	Tiramisu	+
91	*S. enterica* subsp. *enterica*	Typhimurium 1,4 [5], I2:-:1,2	AD Ad1335	Poultry environmental sample	+
92	*S. enterica* subsp. *enterica*	Typhimurium 1,4 [5], II2:i:-	AD Ad1334	Ready to cook pork	+
93	*S. enterica* subsp. *enterica*	Urbana	AD Ad2334	Shrimps	+
94	*S. enterica* subsp. *enterica*	Veneziana	AD Adria 233	Food product	+
95	*S. enterica* subsp. *enterica*	Virchow	AD Adria F276	Curry	+
96	*S. enterica* subsp. *enterica*	Wandsworth	AD Ad2335	Fillet of mullet	+
97	*S. enterica* subsp. *enterica*	Waycross	CIP105634	Not Available	+
98	*S. enterica* subsp. *enterica*	Wayne	AD Ad502	Not Available	+
99	*S. enterica* subsp. *enterica*	Weltevreden	AD Ad2336	Treated water	+
100	*S. enterica* subsp. *enterica*	Worthington	AD Adria 3506	Pâté	+

[1]AD = ADRIA Developpement Laboratory, Quimper, France

[2]CIP = Collection Institut Pasteur, Paris, France

[3]ATCC = American Type Culture Collection, Manassas, USA

Table 6. Exclusivity Results for the iQ-Check *Salmonella* II Assay - Fast APF (14)

(No.)	Organism	Source	Origin	Result
1	*Acinetobacter baumanii*	ATCC[1] 19606	Urine	-
2	*Alcaligenes faecalis* subsp. *faecalis*	ATCC 8750	Not Available	-
3	*Aeromonoas hydrophila*	ATCC 49140	Clinical isolate	-
4	*Citrobacter braakii*	ATCC 43162	Clinical isolate	-
5	*Citrobacter farmeri*	ATCC 51633	Human feces	-
6	*Citrobacter freundii*	QL[2] 11007-10	Clinical isolate	-
7	*Cronobacter sakazakii*	ATCC 29544	Infant formula	-
8	*Edwardsiella tarda*	ATCC 15947	Human feces	-
9	*Enterobacter aerogenes*	ATCC 35029	Not Available	-
10	*Enterobacter cloacae*	ATCC 13047	Spinal fluid	-
11	*Escherichia coli*	ATCC 8739	Feces	-
12	*Escherichia coli* O157	ATCC 43895	Raw hamburger	-
13	*Escherichia fergusonii*	ATCC 35469	Human feces	-
14	*Escherichia hermanii*	ATCC 33650	Mouse brain	-
15	*Escherichia vulneris*	ATCC 29943	Human wound	-
16	*Hafnia alvei*	ATCC 51815	Milk	-
17	*Haemophilus influenzae*	ATCC 19418	Not Available	-
18	*Klebsiella oxytoca*	ATCC 43165	Clinical isolate	-
19	*Klebsiella pneumoniae* subsp. *pneumonia*	ATCC 4352	Cow's milk	-
20	*Morganella morganii*	ATCC 25829	Human	-
21	*Mycobacterium smegmatis*	ATCC 19420	Not Available	-
22	*Pantoea agglomerans*	ATCC 19552	Sewage	-
23	*Proteus mirabilis*	ATCC 7002	Urine	-
24	*Providencia rettgeri*	ATCC 14505	Not Available	-
25	*Pseudomonas aeruginosa*	ATCC 9027	Outer ear infection	-
26	*Rahnella aquatilis*	ATCC 55046	Soil	-
27	*Salmonella bongori*	ATCC 43975	Not Available	-
28	*Serratia marcescens*	ATCC 13880	Human	-
29	*Shigella boydii*	ATCC 9207	Feces	-
30	*Shimwellia blattae*	ATCC 29907	Clinical isolate	-
31	*Vibrio vunificus*	QL 02111-1A	Shellfish	-
32	*Bacillus cereus*	ATCC 14579	Not Available	-
33	*Bacillus subtilis*	ATCC 6051	Not Available	-

[1]ATCC = American Type Culture Collection, Manassas, USA

[2]QL = Q Laboratories Culture Collection, Cincinnati, USA

Table 8. Bio-Rad iQ-Check *Salmonella* II Kit, Candidate vs. Reference – POD Results (14)

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Candidate[1,2,3,4]			Reference			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Fresh Raw Ground Beef (375 g)	*Salmonella* Anatum ATCC 9270	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.57 (0.31, 0.97)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh Raw Beef Trim (375 g)	*Salmonella* Mbandaka ATCC 51958	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.69 (0.40, 1.14)	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh Baby Spinach (375 g)	*Salmonella* Kentucky ATCC 9263	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.20 (0.74, 2.01)	20	14	0.70	0.48, 0.85	12	0.60	0.39, 0.78	0.10	-0.18, 0.36
		2.58 (1.15, 5.78	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Nonfat Dry Milk (375 g)	*Salmonella* Senftenberg ATCC 43845	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.44 (0.21, 0.76)	20	8	0.40	0.22, 0.61	6	0.30	0.15, 0.52	0.10	-0.18, 0.36
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Whey Powder (375 g)	*Salmonella* Cerro ATCC 10723	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.34 (0.14, 0.61)	20	6	0.30	0.15, 0.52	5	0.25	0.11, 0.47	0.05	-0.22, 0.31
		1.56 (0.73, 3.35)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
White Chocolate (375 g)	*Salmonella* Abony NCTC 6017	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.48 (0.22, 0.78)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Chocolate Liquor (375 g)	*Salmonella* Montevideo ATCC 8387	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.61 (0.33, 1.01)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[1]Identical results were produced at 8 & 22 h for fresh raw ground beef and fresh raw beef trim and at 10 & 22 h for fresh baby spinach

[2]Results were identical for the white chocolate and chocolate liquor samples analyzed with the purification step using iQ-Check Purification Reagent and without the purification step.

[3]Identical results were produced using both *Salmonella* APF files (Salmo Fast and *Salmonella* Classic)

[4]Identical results were produced with and without Free DNA Removal Solution

[a]MPN = Most Probable Number was calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval

[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 result and reference method confirmed result 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 9. Bio-Rad iQ-Check *Salmonella* II Kit, Presumptive vs. Confirmed–POD Results (14)

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Presumptive[1,2,3,4]			Confirmed[5,6]			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{R}$[e]	95% CI		
Fresh Raw Ground Beef (375 g)	*Salmonella* Anatum ATCC 9270	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.57 (0.31, 0.97)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	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
Fresh Raw Beef Trim (375 g)	*Salmonella* Mbandaka ATCC 51958	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.69 (0.40, 1.14)	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
Fresh Baby Spinach (375 g)	*Salmonella* Kentucky ATCC 9263	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.20 (0.74, 2.01)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	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
Nonfat Dry Milk (375 g)	*Salmonella* Senftenberg ATCC 43845	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.44 (0.21, 0.76)	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
Whey Powder (375 g)	*Salmonella* Cerro ATCC 10723	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.34 (0.14, 0.61)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		1.56 (0.73, 3.35)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
White Chocolate (375 g)	*Salmonella* Abony NCTC 6017	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.48 (0.22, 0.78)	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
Chocolate Liquor (375 g)	*Salmonella* Montevideo ATCC 8387	-	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, 1.01)	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

[1]Identical results were produced at 8 & 22 h for fresh raw ground beef and fresh raw beef trim and at 10 & 22 h for fresh baby spinach.
[2]Results were identical for the white chocolate and chocolate liquor samples analyzed with the purification step using iQ-Check Purification Reagent and without the purification step.
[3]Identical results were produced using both *Salmonella* APF files (Salmo Fast and *Salmonella* Classic)
[4]Identical results were produced with and without Free DNA Removal Solution
[5]Results obtained following the alternative confirmation were identical to results obtain from confirmation by reference methods.
[6]Fresh raw ground beef, fresh raw beef trim, and fresh baby spinach were confirmed at 22 h only
[a]MPN = Most Probable Number was calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval
[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 result and candidate method confirmed result 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 10. Bio-Rad iQ-Check *Salmonella* II Kit, Presumptive vs. Confirmed–POD Results (14)

Matrix	Test Parameters	Strain	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{R}$[e]	95% CI		
Cannabis Flower (10 g)	Classic APF	*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
			0.59 (0.31, 1.01)	20	8	0.4	0.22, 0.61	8	0.4	0.22, 0.61	0.00	-0.13, 0.13
			3.26 (1.33, 7.99)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Cannabis Flower (10 g)	Classic APF with FDRS	*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
			0.59 (0.31, 1.01)	20	7	0.35	0.39, 0.78	8	0.4	0.22, 0.61	-0.05	-0.21, 0.11
			3.26 (1.33, 7.99)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Cannabis Flower (10 g)	Salmo Fast APF	*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
			0.59 (0.31, 1.01)	20	7	0.35	0.39, 0.78	8	0.4	0.22, 0.61	-0.05	-0.21, 0.11
			3.26 (1.33, 7.99)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Cannabis Flower (10 g)	Salmo Fast APF	*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
			0.59 (0.31, 1.01)	20	6	0.30	0.15, 0.52	8	0.4	0.22, 0.61	-0.10	-0.28, 0.08
			3.26 (1.33, 7.99)	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

[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

DISCUSSION OF MODIFICATION APPROVED DECEMBER 2022 (17)

The Bio-Rad *Salmonella* II kit successfully detected *Salmonella* in plant-based meat (375 g), all-purpose flour (375 g), and dried hemp flower (25 g). Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods and the reference method for all samples tested.

The evaluation of the alternative confirmation protocol showed identical results for the alternative confirmation agar plate for the high-level test portions and the low level all-purpose flour test portions. With the 8 discordant results seen with hemp flower, an additional step was taken to streak directly from TT and RV to RAPID'*Salmonella* agar plates which allowed for the easier isolation of *Salmonella* colonies versus XLD agar plates. The target organism present in the hemp flower test portions was difficult to isolate and detect on the agar plates due to the high level of background contamination present in the samples.

The Bio-Rad *Salmonella* II kit is robust, quick, and simple to perform, providing results in around 80 minutes post enrichment. The CFX Manager Software, IDE 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 can 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.

The addition of PIF Supplement in flour matrices improved the recovery of *Salmonella* species when co-inoculated with Pathogenic *E. coli*. This recovery is observed with RAPID'*Salmonella* chromogenic agar for confirmation following both the reference method and the alternative confirmation method.

In the independent laboratory testing, the Bio-Rad iQ-Check *Salmonella* II targets *Salmonella* in plant-based meat at a 375 g test portion size. POD analysis showed no statistically significant difference in the number of presumptive positive samples versus confirmed positives. Also, there was no statistically significant difference between the candidate and reference method. RAPID'*Salmonella* chromogenic agar is not recommended for use with hemp flower.

Processing samples using these test kits was very user friendly. The DNA extraction procedure included the use of deep well plates, a single lysis reagent, and a heated lysis step. The use of deep well plates optimizes the overall workflow and sample throughput versus using individual tubes. The addition of the FDRS treatment only added a few extra steps and minimal hands-on time. Preparation of the PCR master mix was easily performed by combining two refrigerated reagents and aliquoting into appropriate wells.

Table 3. Bio-Rad iQ-Check *Salmonella* II Kit, Presumptive vs. Confirmed (Paired)–POD Results (17)

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
				X[b]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Plant-Based Meat (375 g) With FDRS[h]	*Salmonella* Abony CIP 80.39	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.50 (0.27, 0.87)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		3.62 (1.75, 7.57)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
All-Purpose Flour (375 g) With FDRS[i]	*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
		0.71 (0.40, 1.18)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		2.37 (1.26, 4.45)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Dried Hemp Flower (25 g) With FDRS[h]	*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)
		1.45 (0.88, 2.60)	20	14	0.70	0.48, 0.86	14	0.70	0.48, 0.86	0.00	(-0.13, 0.13)
		4.65 (1.80, 12.0)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Plant-Based Meat (375 g) With FDRS Independent Laboratory	*Salmonella* Abony NCTC 6017	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.27, 0.86)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		4.45 (2.37, 9.26)	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 ver. 2.0 provided by AOAC RI, with 95% confidence interval
[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 result and candidate method confirmed result 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]Identical results with and without FDRS
[i]Test portions analyzed with 1:3 and 1:9 enrichment ratios produced identical result.

Table 4. Bio-Rad iQ-Check *Salmonella* II, Candidate vs. Reference (Unpaired) – POD Results (17)

Matrix	Strain	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		
Plant-Based Meat (375 g) With FDRS[h]	*Salmonella* Abony CIP 80.39	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.50 (0.27, 0.87)	20	10	0.50	0.30, 0.70	9	0.50	0.30, 0.70	0.00	-0.28, 0.28
		3.62 (1.75, 7.57)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
All-Purpose Flour (375 g) With FDRS[i]	*Salmonella* Typhimurium ATCC 14028	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.71 (0.40, 1.18)	20	10	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		2.37 (1.26, 4.45)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plant-Based Meat (375 g) With FDRS Independent Laboratory	*Salmonella* Abony NCTC 6017	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.51 (0.27, 0.86)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		4.45 (2.37, 9.26)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval
[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 result and reference method confirmed result 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]Identical results with and without FDRS
[i]Test portions analyzed with 1:3 and 1:9 enrichment ratios produced identical results

Table 5. Alternative Confirmation Results for the iQ-Check *Salmonella* II Kit for All-Purpose Flour (17)

All-Purpose Flour (375 g) *Salmonella* Typhimurium ATCC 14028					
Low Level 0.71 (0.40, 1.18)					
Sample #	iQ-Check *Salmonella* II Kit	Confirmed (Direct streak from enrichment)		FDA BAM Chapter 5 (From RV &TT broths)	
		RAPID *Salmonella* Agar	FDA BAM Chapter 5	RAPID *Salmonella* Agar	Traditional Confirmation
1	-	-	-	+	+
2	+	+	+	-	-
3	-	-	-	+	+
4	+	+	+	+	+
5	+	+	+	-	-
6	-	-	-	-	-
7	+	+	+	+	+
8	-	-	-	-	-
9	+	+	+	-	-
10	-	-	-	+	+
11	+	+	+	-	-
12	-	-	-	+	+
13	+	+	+	+	+
14	-	-	-	-	-
15	-	-	-	+	+
16	-	-	-	-	-
17	+	+	+	+	+
18	+	+	+	+	+
19	-	-	-	+	+
20	+	+	+	-	-
Total	10/20	10/20	10/20	11/20	11/20
High Level 2.37 (1.26, 4.45)					
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
Total	5/5	5/5	5/5	5/5	5/5
Uninoculated					
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
Total	0/5	0/5	0/5	0/5	0/5

Table 6. Alternative Confirmation Results for the iQ-Check *Salmonella* II Kit for Dried Hemp Flower (17)

Dried Hemp Flower (25 g) *Salmonella* Typhimurium ATCC 14028				
Low Level 1.45 (0.88, 2.60)				
Sample #	iQ-Check *Salmonella* II Kit	Confirmed		
		RAPID *Salmonella* Agar (Direct streak from enrichment)	AOAC SMPR 2020.002 (From RV &TT broths)	
			RAPID *Salmonella* Agar	XLD Agar
1	+	-	+	+
2	-	-	-	-
3	+	-	+	+
4	+	+	+	+
5	-	-	-	-
6	+	-	+	+
7	+	-	+	+
8	+	+	+	+
9	+	-	+	+
10	-	-	-	-
11	+	+	+	+
12	+	+	+	+
13	-	-	-	-
14	+	-	+	+
15	+	-	+	+
16	-	-	-	-
17	+	+	+	+
18	-	-	-	-
19	+	+	+	+
20	+	-	+	+
Total	14/20	6/20	14/20	14/20
High Level 4.65 (1.80, 12.0)				
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
Total	5/5	5/5	5/5	5/5
Uninoculated				
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	0/5	0/5	0/5	0/5

Table 7. Bio-Rad iQ-Check *Salmonella* II - Plant Based Meat 375 g - Confirmation Results, Method Developer (17)

Inoculation Level	RSA[a] Direct Streak	XLD[b] (RV[c])	XLD (TT[d])	HE[e] (RV)	HE (TT)	BS[f] (RV)	BS (TT)	RSA (TT)	RSA (RV)	LIA[g]	TSI[h]
0 CFU	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	N/A	N/A
0 CFU	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	N/A	N/A
0 CFU	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	N/A	N/A
0 CFU	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	N/A	N/A
0 CFU	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	N/A	N/A
Low	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Low	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
Low	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Low	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Low	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Low	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Low	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
Low	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Low	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
Low	**POS**	**POS**	**POS**	**POS**	**POS**	NEG	NEG	**POS**	**POS**	**POS**	**POS**
Low	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
Low	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Low	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Low	**POS**	**POS**	**POS**	**POS**	**POS**	NEG	NEG	**POS**	**POS**	**POS**	**POS**
Low	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
Low	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Low	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
Low	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
Low	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
Low	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
High	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
High	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
High	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	NEG
High	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**
High	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**

[a]RSA = RAPID'*Salmonella* Agar
[b]XLD = Xylose Lysine Deoxycholate agar
[c]RV = Rappaport – Vassiliadis Broth
[d]TT = Tetrathionate Broth
[e]HE = Hektoen Enteric agar
[f]BS = Bismuth Sulfite agar
[g]LIA = Lysine Iron Agar slant
[h]TSI = Triple Sugar Iron slant
[i]N/A = Not applicable

DISCUSSION OF MODIFICATION APPROVED JANUARY 2023 (18)

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. The iQ-Check *Salmonella* II kit showed a negative PCR result for the CFX Opus Deepwell compared to the CFX96 Touch Deep Well instrument for the low inoculation level. This was likely due to the low level of target *Salmonella* (0.48 MPN/25 g) in the 375 g test portions and the normal distribution of the target DNA and sampling of the test portions. No other discrepancies were observed. Any differences observed between the candidate and reference methods are likely due to tests being conducted under unpaired testing conditions or possibly homogeneity issues with the sample preparation. An additional set of data was analyzed to determine if the differences were likely due to laboratory error. These data show fewer differences between the candidate and reference method indicating no issues with the method performance. In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified, and all exclusivity organisms were correctly excluded.

Table 13. Inclusivity Results, iQ-Check *Salmonella* II Kit (14)

No.	Genus	Species	Subspecies/Serovar	Source	Origin	CFX96 Touch Deep Well Result[a]	CFX Opus Deepwell Result[a]
1	*Salmonella*	*bongori*	66:z41:-	NCTC[b] 10946	Frog	+	+
2	*Salmonella*	*bongori*	48:i:-	FDA[c] 94-0708	Unknown	+	+
3	*Salmonella*	*bongori*	40:z35:-	FDA 95-0123	Unknown	+	+
4	*Salmonella*	*bongori*	*66 :z35:-*	Ad[d] 598	Unknown	+	+
5	*Salmonella*	*enterica*	Abaetetuba	Ad 2318	Unknown	+	+
6	*Salmonella*	*enterica*	Aberdeen	CIP[e] 105618	Human	+	+
7	*Salmonella*	*enterica*	Abortusequi	Ad 2321	Unknown	+	+
8	*Salmonella*	*enterica*	Abortusovis	Ad 2320	Unknown	+	+
9	*Salmonella*	*enterica*	Adelaïde	Ad 2319	Unknown	+	+
10	*Salmonella*	*enterica*	Agona	A00V038[d]	Unknown	+	+

11	*Salmonella*	*enterica*	Anatum	A00E007	Unknown	+	+
12	*Salmonella*	*enterica*	*arizonae* 51:z4,z24:-	CIP 55.23	Turkey	+	+
13	*Salmonella*	*enterica*	*arizonae* 48:z4,z23:-	Ad 1850	Unknown	+	+
14	*Salmonella*	*enterica*	arizonae	ATCC[f] 13314	Unknown	+	+
15	*Salmonella*	*enterica*	Bardo	Adria[d] 569	Unknown	+	+
16	*Salmonella*	*enterica*	Bareilly	Ad 1687	Unknown	+	+
17	*Salmonella*	*enterica*	Blockley	Ad 923	Unknown	+	+
18	*Salmonella*	*enterica*	Bovismorbificans	Adria 6629	Unknown	+	+
19	*Salmonella*	*enterica*	Braenderup	Adria 111	Unknown	+	+
20	*Salmonella*	*enterica*	Brandenburg	Ad 351	Unknown	+	+
21	*Salmonella*	*enterica*	Bredeney	Adria 396	Unknown	+	+
22	*Salmonella*	*enterica*	Caracas	Ad2322	Unknown	+	+
23	*Salmonella*	*enterica*	Cerro	Ad 689	Unknown	+	+
24	*Salmonella*	*enterica*	Cubana	Ad 2323	Unknown	+	+
25	*Salmonella*	*enterica*	Derby	Ad 1093	Unknown	+	+
26	*Salmonella*	*enterica*	diarizonae	ATCC BAA-1579	Unknown	+	+
27	*Salmonella*	*enterica*	diarizonae	ATCC BAA-216	Human Blood	+	+
28	*Salmonella*	*enterica*	diarizonae	ATCC BAA-639	Human Feces	+	+
29	*Salmonella*	*enterica*	*diarizonae* 38:lv:z53	Ad 451	Unknown	+	+
30	*Salmonella*	*enterica*	*diarizonae* 61:k:1,5,7	Ad 1300	Unknown	+	+
31	*Salmonella*	*enterica*	Dublin	Ad 529	Unknown	+	+
32	*Salmonella*	*enterica*	Emek	Ad 333	Unknown	+	+
33	*Salmonella*	*enterica*	Enteritidis	Ad 477	Unknown	+	+
34	*Salmonella*	*enterica*	Gallinarum biovar pullorum	Ad 300	Unknown	+	+
35	*Salmonella*	*enterica*	Gaminara	Ad 2324	Unknown	+	+
36	*Salmonella*	*enterica*	Give	Ad 436	Unknown	+	+
37	*Salmonella*	*enterica*	Guinea	Ad 29	Unknown	+	+
38	*Salmonella*	*enterica*	Hadar	Ad 24871	Unknown	+	+
39	*Salmonella*	*enterica*	Havana	Ad 930	Unknown	+	+
40	*Salmonella*	*enterica*	Heidelberg	A00E005	Unknown	+	+
41	*Salmonella*	*enterica*	*houtenae 1,40:z4:z23:-*	Ad 2682	Unknown	+	+
42	*Salmonella*	*enterica*	houtenae	CPS[g] FSL R9-0517	Unknown	+	+
43	*Salmonella*	*enterica*	houtenae	ATCC 43974	Unknown	+	+
44	*Salmonella*	*enterica*	houtenae	ATCC 15783	Boa Constrictor	+	+
45	*Salmonella*	*enterica*	Hvittingfoss	Ad 2325	Unknown	+	+
46	*Salmonella*	*enterica*	Indiana	Ad 174	Unknown	+	+
47	*Salmonella*	*enterica*	indica	ATCC 43976	India	+	+
48	*Salmonella*	*enterica*	indica	NCTC 10458	Unknown	+	+
49	*Salmonella*	*enterica*	indica	CPS FSL R9-5884	Unknown	+	+
50	*Salmonella*	*enterica*	*indica 1*,6,14,25:a:enx	Ad 600	Unknown	+	+
51	*Salmonella*	*enterica*	*indica*11:b:e,n,x	Ad 2337	Unknown	+	+
52	*Salmonella*	*enterica*	Infantis	Ad F401B	Unknown	+	+
53	*Salmonella*	*enterica*	Javiana	Ad2326	Unknown	+	+
54	*Salmonella*	*enterica*	Kedougou	Ad 929	Unknown	+	+
55	*Salmonella*	*enterica*	Kentucky	Ad 1756	Unknown	+	+
56	*Salmonella*	*enterica*	Kottbus	Adria 1	Unknown	+	+
57	*Salmonella*	*enterica*	Landau	Ad 499	Unknown	+	+
58	*Salmonella*	*enterica*	Lille	Adria 37	Unknown	+	+
59	*Salmonella*	*enterica*	Livingstone	Ad 1107	Unknown	+	+
60	*Salmonella*	*enterica*	London	Adria 326	Unknown	+	+
61	*Salmonella*	*enterica*	Luciana	CIP 105626	Human	+	+
62	*Salmonella*	*enterica*	Manhattan	Adria 900	Unknown	+	+
63	*Salmonella*	*enterica*	Maracaibo	CIP 54143	Unknown	+	+
64	*Salmonella*	*enterica*	Marseille	CIP105627	Human	+	+
65	*Salmonella*	*enterica*	Mbandaka	Ad 914	Unknown	+	+
66	*Salmonella*	*enterica*	Meleagridis	Ad 505	Unknown	+	+
67	*Salmonella*	*enterica*	Michigan	Ad 2327	Unknown	+	+
68	*Salmonella*	*enterica*	Mikawasima	Ad 1811	Unknown	+	+
69	*Salmonella*	*enterica*	Minnesota	Ad 2328	Unknown	+	+
70	*Salmonella*	*enterica*	Mississipi	Ad 2329	Unknown	+	+
71	*Salmonella*	*enterica*	Montevideo	Ad 912	Unknown	+	+
72	*Salmonella*	*enterica*	Muenchen	CIP 106178	Unknown	+	+
73	*Salmonella*	*enterica*	Napoli	Ad 928	Unknown	+	+
74	*Salmonella*	*enterica*	Newport	Adria 586	Unknown	+	+
75	*Salmonella*	*enterica*	Norwich	Ad 1172	Unknown	+	+
76	*Salmonella*	*enterica*	Ohio	Ad 1482	Unknown	+	+
77	*Salmonella*	*enterica*	Orion	Ad 27	Unknown	+	+
78	*Salmonella*	*enterica*	Oranienburg	Ad 1724	Unknown	+	+

79	*Salmonella*	*enterica*	Ouakam	Ad 1647	Unknown	+	+
80	*Salmonella*	*enterica*	Panama	Adria 8	Unknown	+	+
81	*Salmonella*	*enterica*	Paratyphi A	ATCC 9150	Unknown	+	+
82	*Salmonella*	*enterica*	Paratyphi B	Ad 301	Unknown	+	+
83	*Salmonella*	*enterica*	Paratyphi C	ATCC 13428	Unknown	+	+
84	*Salmonella*	*enterica*	Pomona	CIP105630	Cock	+	+
85	*Salmonella*	*enterica*	Poona	Ad 2330	Unknown	+	+
86	*Salmonella*	*enterica*	Putten	Ad 2331	Unknown	+	+
87	*Salmonella*	*enterica*	Regent	Adria 328	Unknown	+	+
88	*Salmonella*	*enterica*	Rissen	Adria 39	Unknown	+	+
89	*Salmonella*	*enterica*	Rubislaw	Ad 2332	Unknown	+	+
90	*Salmonella*	*enterica*	Saintpaul	Adria F31	Unknown	+	+
91	*Salmonella*	*enterica*	*salamae* 42,b:e,n,x,z15	Ad 593	Unknown	+	+
92	*Salmonella*	*enterica*	salamae	QL[h] 024.15	Pet Food	+	+
93	*Salmonella*	*enterica*	salamae	ATCC 700149	Unknown	+	+
94	*Salmonella*	*enterica*	salamae	ATCC 700151	Unknown	+	+
95	*Salmonella*	*enterica*	Schwarzengrund	Ad 2333	Unknown	+	+
96	*Salmonella*	*enterica*	Senftenberg	Ad 355	Unknown	+	+
97	*Salmonella*	*enterica*	Stanley	Ad 1688	Unknown	+	+
98	*Salmonella*	*enterica*	Stourbridge	Ad 2297	Unknown	+	+
99	*Salmonella*	*enterica*	Strasbourg	CIP105632	Human	+	+
100	*Salmonella*	*enterica*	Tananarive	CIP54.142	Pig	+	+
101	*Salmonella*	*enterica*	Tennessee	A00E006	Unknown	+	+
102	*Salmonella*	*enterica*	Thompson	Ad AER301	Unknown	+	+
103	*Salmonella*	*enterica*	Typhi	Ad 302	Unknown	+	+
104	*Salmonella*	*enterica*	Typhimurium	Ad 1070	Unknown	+	+
105	*Salmonella*	*enterica*	Typhimurium 1,4 [5], I2:-:-	Ad 1333	Unknown	+	+
106	*Salmonella*	*enterica*	Typhimurium 1,4 [5], I2:-:1,2	Ad 1335	Unknown	+	+
107	*Salmonella*	*enterica*	Typhimurium 1,4 [5], II2:i:-	Ad 1334	Unknown	+	+
108	*Salmonella*	*enterica*	Urbana	Ad 2334	Unknown	+	+
109	*Salmonella*	*enterica*	Veneziana	Adria 233	Unknown	+	+
110	*Salmonella*	*enterica*	Virchow	Adria F276	Unknown	+	+
111	*Salmonella*	*enterica*	Wandsworth	Ad 2335	Unknown	+	+
112	*Salmonella*	*enterica*	Waycross	CIP105634	Human	+	+
113	*Salmonella*	*enterica*	Wayne	Ad 502	Unknown	+	+
114	*Salmonella*	*enterica*	Weltevreden	Ad 2336	Unknown	+	+
115	*Salmonella*	*enterica*	Worthington	Adria 3506	Unknown	+	+

[a] "+" indicates the target analyte was detected.
[b] NCTC = National Collection of Type Collection, Salisbury, UK
[c] FDA = U.S. Food and Drug Administration Culture Collection, Silver Spring, MD
[d] Ad, Adria, A00 = ADRIA Développement culture collection, Quimper, France
[e] CIP = Collection de l'institut Pasteur, Paris, France
[f] ATCC = American Type Culture Collection, Manassas, VA, United States
[g] CSP = Cornell University CPS Strain Collection, Ithaca, NY, United States
[h] QL = Q Laboratories, Inc., Culture Collection, Cincinnati, OH, United States

Table 18. Exclusivity Results, iQ-Check *Salmonella* II Kit (14)

No.	Organism	Source	Origin	CFX96 Touch Deep Well Result[a]	CFX Opus Deepwell Result[a]
1	*Acinetobacter baumanii*	ATCC[b] 19606	Urine	-	-
2	*Alcaligenes faecalis* subsp. *faecalis*	ATCC 8750	Unknown	-	-
3	*Aeromonoas hydrophila*	ATCC 49140	Clinical isolate	-	-
4	*Citrobacter braakii*	ATCC 43162	Clinical isolate	-	-
5	*Citrobacter farmeri*	ATCC 51633	Human feces	-	-
6	*Citrobacter freundii*	QL[c] 11007-10	Clinical isolate	-	-
7	*Cronobacter sakazakii*	ATCC 29544	Infant formula	-	-
8	*Edwardsiella tarda*	ATCC 15947	Human feces	-	-
9	*Enterobacter aerogenes*	ATCC 35029	Unknown	-	-
10	*Enterobacter cloacae*	ATCC 13047	Spinal fluid	-	-
11	*Escherichia coli*	ATCC 8739	Feces	-	-
12	*Escherichia coli* O157	ATCC 43895	Raw hamburger	-	-
13	*Escherichia fergusonii*	ATCC 35469	Human feces	-	-
14	*Escherichia hermanii*	ATCC 33650	Mouse brain	-	-

15	*Escherichia vulneris*	ATCC 29943	Human wound	-	-
16	*Hafnia alvei*	ATCC 51815	Milk	-	-
17	*Haemophilus influenzae*	ATCC 19418	Unknown	-	-
18	*Klebsiella oxytoca*	ATCC 43165	Clinical isolate	-	-
19	*Klebsiella pneumoniae* subsp. *pneumonia*	ATCC 4352	Cow's milk	-	-
20	*Morganella morganii*	ATCC 25829	Human	-	-
21	*Mycobacterium smegmatis*	ATCC 19420	Unknown	-	-
22	*Pantoea agglomerans*	ATCC 19552	Sewage	-	-
23	*Proteus mirabilis*	ATCC 7002	Urine	-	-
24	*Providencia rettgeri*	ATCC 14505	Unknown	-	-
25	*Pseudomonas aeruginosa*	ATCC 9027	Outer ear infection	-	-
26	*Rahnella aquatilis*	ATCC 55046	Soil	-	-
27	*Salmonella bongori*	ATCC 43975	Unknown	-	-
28	*Serratia marcescens*	ATCC 13880	Human	-	-
29	*Shigella boydii*	ATCC 9207	Feces	-	-
30	*Shimwellia blattae*	ATCC 29907	Clinical isolate	-	-
31	*Vibrio vunificus*	QL 02111-1A	Shellfish	-	-
32	*Bacillus cereus*	ATCC 14579	Unknown	-	-
33	*Bacillus subtilis*	ATCC 6051	Unknown	-	-

[a] "-" indicates the target analyte was not detected
[b] ATCC = American Type Culture Collection, Manassas, VA, United States
[c] QL = Q Laboratories, Inc., Culture Collection, Cincinnati, OH, United States

Table 23. Bio-Rad iQ-Check *Salmonella* II Kit, Presumptive vs. Confirmed–POD Results (14)

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
				X[b]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Fresh ground beef, 85% lean (375 g) CFX96 Touch Deep Well	*Salmonella* Newport Ad 2730	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.48 (0.27, 0.82)	20	6	0.30	0.15, 0.52	5	0.25	0.11, 0.47	0.05	-0.11, 0.21
		1.54 (0.70, 3.34)	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Fresh ground beef, 85% lean (375 g) CFX Opus Deepwell	*Salmonella* Newport Ad 2730	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.48 (0.27, 0.82)	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.13, 0.13
		1.54 (0.70, 3.34)	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval
[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 result and candidate method confirmed result 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 24. Bio-Rad iQ-Check *Salmonella* II, Candidate vs. Reference (Unpaired) – POD Results (14)

Matrix	Strain	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		
Fresh ground beef, 85% lean (375 g) CFX96 Touch Deep Well	*Salmonella* Newport Ad 2730	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.48 (0.27, 0.82)	20	5	0.30	0.15, 0.52	10	0.50	0.30, 0.70	-0.20	-0.45, 0.10
		1.54 (0.70, 3.34)	5	4	0.80	0.38, 1.00	5	1.00	0.57, 1.00	-0.20	-0.62, 0.28
Fresh ground beef, 85% lean (375 g) CFX Opus Deepwell	*Salmonella* Newport Ad 2730	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.48 (0.27, 0.82)	20	5	0.50	0.30, 0.70	10	0.50	0.30, 0.70	-0.25	-0.50, 0.05
		1.54 (0.70, 3.34)	5	4	0.80	0.38, 1.00	5	1.00	0.57, 1.00	-0.20	-0.62, 0.28

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval
[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 result and reference method confirmed result 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 25. Bio-Rad iQ-Check *Salmonella* II Kit, CFX Opus Deepwell vs. CFX96 Touch Deep Well–POD Results (14)

Matrix	Strain	MPN[a]/ Test Portion	N[b]	CFX Opus Deepwell			CFX96 Touch Deep Well			dPOD$_{OT}$[f]	95% CI[g]
				X[c]	POD$_{OC}$[d]	95% CI	X	POD$_{TC}$[e]	95% CI		
Fresh ground beef, 85% lean (375 g)	*Salmonella* Newport Ad 2730	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.48 (0.27, 0.82)	20	5	0.25	0.11, 0.47	6	0.30	0.15, 0.52	-0.05	-0.21, 0.11
		1.54 (0.70, 3.34)	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_{OC}$ = CFX Opus Deepwell confirmed positive outcomes divided by the total number of trials
[e]POD$_{TC}$ = CFX96 Touch Deep Well confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{OT}$= Difference between the CFX Opus Deepwell confirmed result and CFX96 Touch Deep Well confirmed result 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

Supplemental Data. Bio-Rad iQ-Check *Salmonella* II Kit, Presumptive vs. Confirmed–POD Results (18)

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
				X[b]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Fresh ground beef, 85% lean (375 g) CFX96 Touch Deep Well	*Salmonella* Anatum ATCC 9270	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.57 (0.31, 0.97)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	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
Fresh ground beef, 85% lean (375 g) CFX Opus Deepwell	*Salmonella* Anatum ATCC 9270	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.57 (0.31, 0.97)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	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
Fresh beef trim (375 g) CFX96 Touch Deep Well	*Salmonella* Mbandaka ATCC 51958	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.69 (0.40, 1.14)	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
Fresh beef trim (375 g) CFX Opus Deepwell	*Salmonella* Mbandaka ATCC 51958	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.69 (0.40, 1.14)	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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval
[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 result and candidate method confirmed result 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

Supplemental Data. Bio-Rad iQ-Check *Salmonella* II, Candidate vs. Reference (Unpaired) – POD Results (18)

Matrix	Strain	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		
Fresh ground beef, 85% lean (375 g) CFX96 Touch Deep Well	*Salmonella* Anatum ATCC 9270	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.57 (0.31, 0.97)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh ground beef, 85% lean (375 g) CFX Opus Deepwell	*Salmonella* Anatum ATCC 9270	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.57 (0.31, 0.97)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh beef trim (375 g) CFX96 Touch Deep Well	*Salmonella* Mbandaka ATCC 51958	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.69 (0.40, 1.14)	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh beef trim (375 g) CFX Opus Deepwell	*Salmonella* Mbandaka ATCC 51958	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.69 (0.40, 1.14)	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval
[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 result and reference method confirmed result 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

Supplemental Data. Bio-Rad iQ-Check *Salmonella* II Kit, CFX Opus Deepwell vs. CFX96 Touch Deep Well–POD Results (18)

Matrix	Strain	MPN[a]/ Test Portion	N[b]	CFX Opus Deepwell			CFX96 Touch Deep Well			$dPOD_{OT}$[f]	95% CI[g]
				X[c]	POD_{OC}[d]	95% CI	X	POD_{TC}[e]	95% CI		
Fresh ground beef, 85% lean (375 g)	*Salmonella* Anatum ATCC 9270	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.57 (0.31, 0.97)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	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	-0.43, 0.43
Fresh beef trim (357 g)	*Salmonella* Mbandaka ATCC 51958	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.69 (0.40, 1.14)	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	-0.43, 0.43

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{OC} = CFX Opus Deepwell confirmed positive outcomes divided by the total number of trials
[e]POD_{TC} = CFX96 Touch Deep Well confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{OT}$= Difference between the CFX Opus Deepwell confirmed result and CFX96 Touch Deep Well confirmed result 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

DISCUSSION OF MODIFICATION APPROVED JULY 2023 (19)

The iQ-Check *Salmonella* II method successfully detected target *Salmonella* species in cannabis infused gummies (25 g), cannabis infused chocolate (25 g), and cannabis derived concentrate (5 g). POD analysis proved that the study data were unable to find a statistically detectable difference from zero between the candidate method presumptive and reference method confirmed results. Results in this study also provided evidence that the alternative plate confirmation using RAPID'*Salmonella* agar can be used in lieu of standard confirmation procedures outlined in SMPR 2020.002.

Table 1: Bio-Rad iQ-Check *Salmonella* II Presumptive vs. Confirmed Results (Paired) – POD Results (19)

Matrix and Inoculum	MPN_a / Test Portion	N[b]	x[c]	Presumptive		x	Confirmed		$dPOD_{cp}$[f]	95% CI[g]
				POD_{cp}[d]	95% CI		POD_{cc}[e]	95% CI		
Cannabis infused chocolate, 25 g (*Salmonella Typhimurium ATCC 14028 and E. coli O157:H7 ATCC 43895*)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
	0.65 (0.32, 1.15)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	(-0.13, 0.13)
	4.07 (2.15, 234)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Cannabis infused gummies, 25 g (*Salmonella* Newport ATCC 6962 *and E. coli* O111 CDC 2010C 3114)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
	0.83 (0.44, 1.44)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
	4.65 (3.37, 234)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Cannabis derived concentrate, 5 g (*Salmonella* Heidelberg ATCC 8326 *and E. coli* O45 CDC 00-3039)	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
	0.80 (0.42, 1.35)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	(-0.13, 0.13)
	6.74 (3.18, 187)	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 based on the POD of cultural confirmation of test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval. [b]N = Number of test potions; [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 result and candidate method confirmed result 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]NA – Not Applicable.

Table 2: Cannabis infused chocolate (25 g) Alternative Plating Confirmation (RAPID'*Salmonella*) vs. SMPR 2020.002 Confirmation (19)

Inoculum Level	Sample Number	Direct Streak RAPID'Sal	SMPR 2020.002 Confirmation									
			TT		RV		TSI	LIA	Poly O	Poly H	API20E ID	Reported Result
			XLD	RAPID'Sal	XLD	RAPID'Sal						
Blank	2	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	9	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	23	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	25	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	28	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
Low	4	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	5	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	6	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	7	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	8	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	10	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	11	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	12	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-

	13	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	14	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	15	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	17	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	18	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	19	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	20	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	21	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	22	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	24	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	27	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	29	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
High	1	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	3	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	16	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	26	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	30	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+

Table 3: Cannabis infused gummies (25 g) Alternative Plating Confirmation (RAPID'*Salmonella*) vs. SMPR 2020.002 Confirmation (19)

Inoculum Level	Sample Number	Direct Streak RAPID'Sal	SMPR 2020.002 Confirmation									
			TT		RV		TSI	LIA	Poly O	Poly H	API20E ID	Reported Result
			XLD	RAPID'Sal	XLD	RAPID'Sal						
Blank	1	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	4	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	17	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	18	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	21	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
Low	2	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	3	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	5	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	6	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	7	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	8	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	9	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	10	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	11	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	12	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	13	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	14	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	15	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	16	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	20	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	22	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	25	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	27	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	28	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	29	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
High	19	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	23	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	24	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	26	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+
	30	+	+	+	+	+	+	+	+	+	*Salmonella* spp.	+

Table 4: Cannabis derived concentrate (5 g) Alternative Plating Confirmation (RAPID'*Salmonella*) vs SMPR 2020.002 Confirmation (19)

Inoculum Level	Sample Number	Direct Streak RAPID'Sal	SMPR 2020.002 Confirmation									
			TT		RV		TSI	LIA	Poly O	Poly H	API20E ID	Reported Result
			XLD	RAPID'Sal	XLD	RAPID'Sal						
Blank	10	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	11	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	19	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	22	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	28	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
Low	1	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	2	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	4	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	6	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	7	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	8	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	9	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	13	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	14	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+

	15	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	16	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	17	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	18	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	20	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	21	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	23	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	24	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	25	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	27	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	-
	29	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
High	3	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	5	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	12	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	26	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+
	30	+	+	+	+	+	+	+	+	+	*Salmonella spp.*	+

REFERENCES CITED

1. Lauer, W., Tourniaire, J.P., Sidi, C., Patel, A.., Evaluation of the iQ-Check™ *Salmonella* II Real-Time PCR Test Kit in Select Foods, AOAC *Performance Tested Methods*℠ certification number 010803.
2. *Microbiology Laboratory Guidebook* (October 1, 2004) U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, XX, Chapter 4.03. Online at http://www.fsis.usda.gov/PDF/MLG_4_03.pdf, Accessed May 2, 2007.
3. *Bacteriological Analytical Manual Online* (April 2003, updated September 2005, December 2005 and June 2006) 8th Ed., U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Chapter 5. Online at http://www.cfsan.fda.gov/~ebam/bam-5.html, Accessed May 2, 2007.
4. Seigel, S. (1956) *Nonparametric Statistics for the Behavioral Sciences*, McGraw Hill Book Co. New York, NY.
5. Garrity, G.M. (2005) *Bergey's Manual of Systematic Bacteriology*, The Williams and Wilkins Co. Baltimore, MD. Bio-Rad Laboratories. Comparative Evaluation of a Modification of Raw Materials for iQ-Check™ Real-Time PCR Test Kit. AOAC *Performance Tested Methods*℠ certification number 010803. Modification approved January 2009.
6. Lauer, W.F. iQ-Check™ *Salmonella* II: AOAC® *Performance Tested*℠ certification number 010803 Salmonella in Peanut Butter Emergency Response Validation. Approved June 2009.
7. Bio-Rad Laboratories. Validation of a New High Throughput Extraction Protocol for iQ-Check™ Salmonella II Real-Time PCR Test Kit. AOAC *Performance Tested Methods*℠ certification number 010803. Modification approved February 2010.
8. Bio-Rad Laboratories. Evaluation of Matrix Extension Modification Report for iQ-Check™ Salmonella II Real-Time PCR Test Kit. AOAC *Performance Tested Methods*℠ certification number 010803. Modification approved August 2010.
9. Bio-Rad Laboratories. Matrix Extension Modification Report for iQ-Check™ Salmonella II Real-Time PCR Test Kit. AOAC *Performance Tested Methods*℠ certification number 010803. Modification approved November 2013.
10. Bio-Rad Laboratories, Matrix Extension Modification Report for iQ-Check *Salmonella* II Real-Time PCR Test Kit, AOAC *Performance Tested Methods*℠ certification number 010803. Modification approved August 2014.
11. Clark, M., Bastin, B., Bird, P., Benzinger, Jr., M.J., Crowley, E., Agin, J., and Goins, D., Independent Evaluation of the Bio-Rad iQ,Check Salmonella II Kit for the Detection of *Salmonella* Species in Select Foods and Environmental Surfaces Modification/Matrix Extension, AOAC *Performance Tested Methods*℠ certification number 010803. Modification approved September 2016.
12. Microbiological Laboratory Guidebook Chapter 4.07 (effective 05/01/2013). United States Department of Agriculture, Food Safety and Inspection Service. Online at http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERESUnited States Department of Agriculture Microbiological Laboratory Guidelines 4.08: *Isolation and Identification of Salmonella from Red Meat, Poultry, Pasteurized Egg, Catfish Products, and Environmental Sponges*. June 29th, 2014. http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG.4.pdf?MOD=AJPERES
13. United States Department of Agriculture Microbiological Laboratory Guidelines 4.08: *Isolation and Identification of Salmonella from Red Meat, Poultry, Pasteurized Egg, Catfish Products, and Environmental Sponges*. June 29th, 2014. http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG.4.pdf?MOD=AJPERES
14. Clark, M., Method Modification of the Bio-Rad iQ-Check *Salmonella* II for the Detection of *Salmonella* Species in Select matrixes, AOAC *Performance Tested Methods*℠ certification number 010803. Modification approved March 2021.
15. USDA/FSIS MLG 4.10: *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges*. Updated: January 2019. (Accessed December 2020) https://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/mlg-4.pdf?MOD=AJPERES
16. AOAC International SMPR 2020.002, Standard Method Performance Requirements for Detection of *Salmonella* species in Cannabis and Cannabis Products. https://www.aoac.org/wp-content/uploads/2020/07/SMPR-2020_002.pdf (accessed January 2021)
17. Clark, M., Matrix Extension Validation Study of the iQ-Check *Salmonella* II Real-Time PCR Method for Plant-Based Meat, All Purpose Flour and Dried Hemp Flower: Level 3 Modification, AOAC *Performance Tested Methods*℠ certification number 010803. Modification approved December 16, 2022.
18. Clark, M., Validation of the Group Modification for the Addition of the CFX Opus Deepwell Real-Time PCR Instrument and CFX Manager Software, IDE v3.1, AOAC *Performance Tested Methods*℠ certification number 010803. Modification approved January 2023.
19. Clark, M. and Horine, L., Matrix Extension Study for the iQ-Check *Salmonella* II Real-Time PCR Method for Cannabis Infused Gummies, Cannabis Infused Chocolate, and Cannabis Derived Concentrates, AOAC *Performance Tested Methods*℠ certification number 010803. Modification Approved July 2023.