

# Thermo Scientific RapidFinder Salmonella species, Typhimurium, and Enteritidis PCR Kit

## AOAC-RI PTM Validation: Method Comparison Study

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### Summary

As part of the validation studies for AOAC Research Institute (RI) Performance Tested Method<sup>SM</sup> certification, method comparison and independent laboratory studies were conducted to validate the performance of the Thermo Scientific<sup>™</sup> RapidFinder<sup>™</sup> *Salmonella* species, Typhimurium, and Enteritidis PCR Kit (candidate method) for use with the Applied Biosystems<sup>™</sup> 7500 Fast Food Safety Real-Time PCR Instrument and associated Applied Biosystems<sup>™</sup> RapidFinder<sup>™</sup> Express Software (version 2.0 or greater) and the Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 5 Food Safety Real-Time PCR Instrument and associated Thermo Scientific RapidFinder<sup>™</sup> Analysis Software (version 1.0 or greater) for the detection and differentiation of *Salmonella* species, *Salmonella* Typhimurium and *Salmonella* Enteritidis from poultry and pork meat and production environmental samples. The following is a summary of the method comparison study.

### Methodology

The performance of the candidate method was assessed as an unpaired study in comparison to the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook (MLG) 4.09 for raw chicken thighs with skin, raw chicken wings with skin, chicken nuggets and raw pork sausage. The performance of the candidate method was also evaluated in comparison to the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) Chapter 5 for stainless steel environmental surface sponges.

Method developer studies were conducted by Thermo Fisher Scientific on raw chicken thighs with skin, raw chicken wings with skin, chicken nuggets, raw pork sausage and stainless steel surface sponges. The independent laboratory study was conducted by Q Laboratories, Inc., Ohio, USA for one claimed food matrix

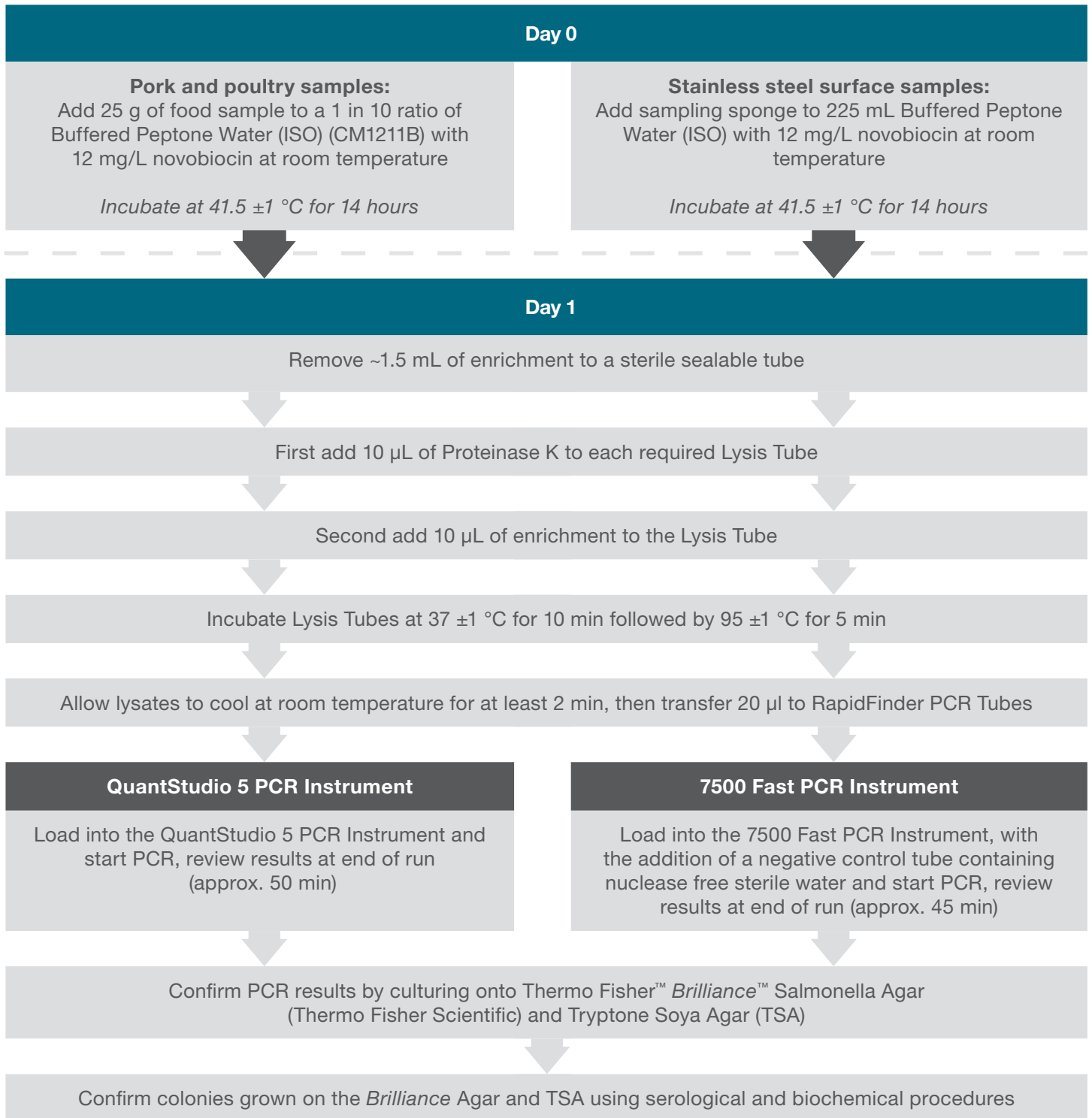
(chicken thighs with skin) and one environmental surface (stainless steel surface sponges).

#### Sample preparation

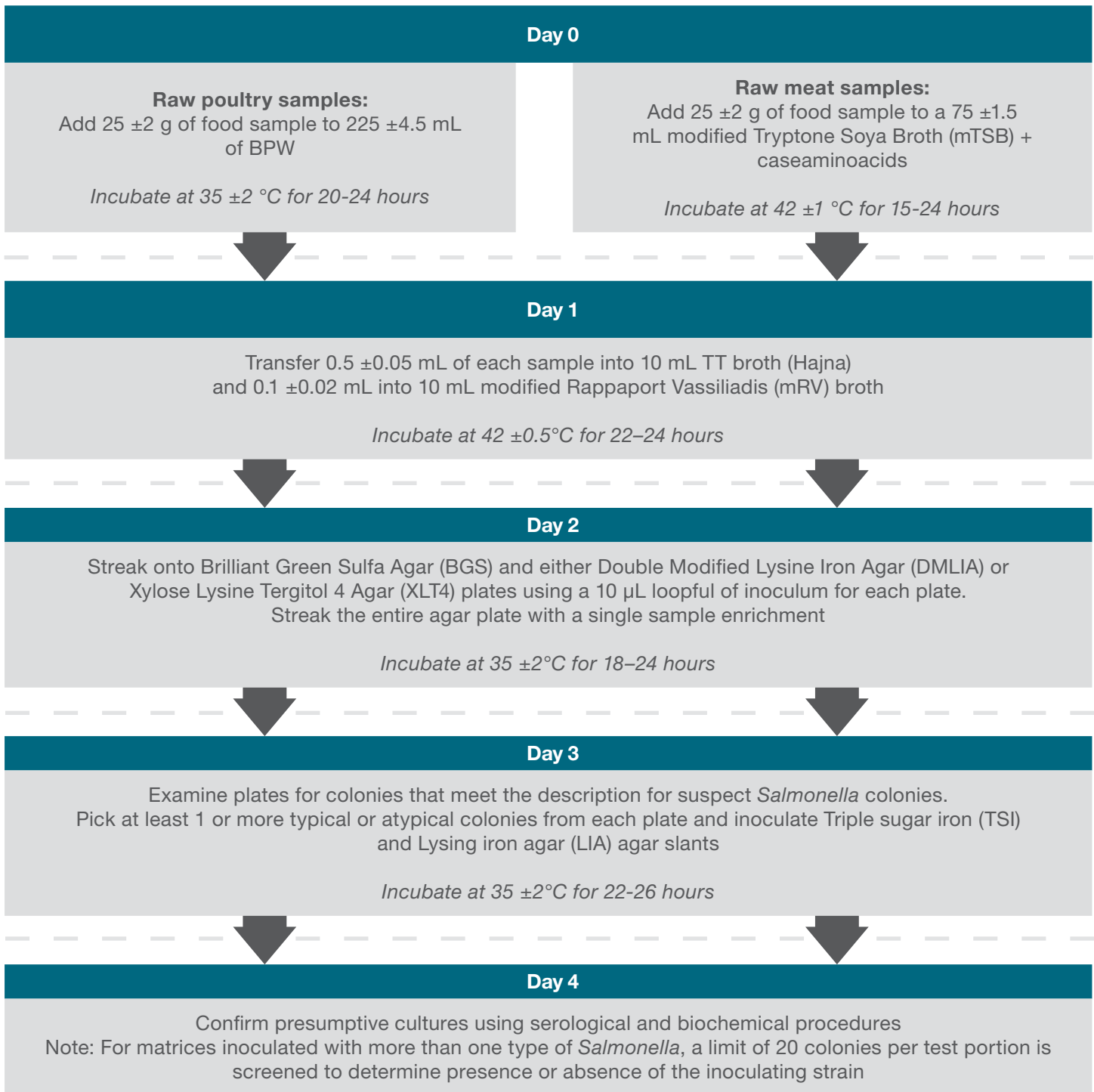
For each food matrix, samples were portioned into homogenizer bags and analyzed using the candidate method and USDA FSIS MLG 4.09 reference method protocols. After addition of enrichment broth, samples were homogenized for 30-60 seconds at 230 rpm and incubated.

Each stainless steel testing area was sampled with horizontal and vertical motions using a sponge pre-moistened with Dey-Engley broth. Each sample sponge was held at 4 °C for 2 hours prior to analysis. The sample sponges were then analysed using the candidate method and FDA BAM Ch. 5 reference method protocols.

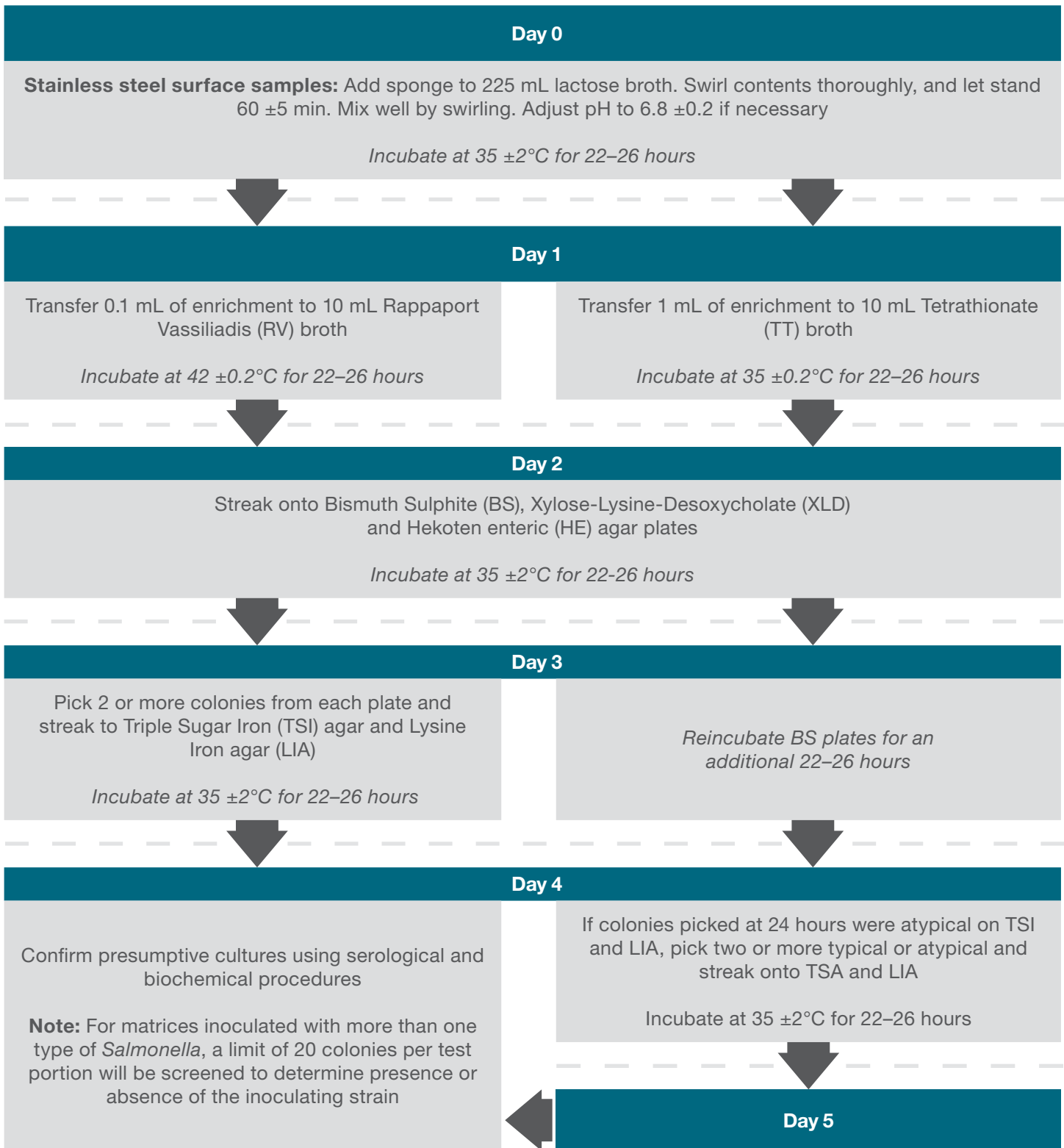
**Table A: RapidFinder Salmonella species, Typhimurium, and Enteritidis PCR Kit method for pork, poultry and stainless steel surface samples using the QuantStudio 5 PCR Instrument or the 7500 Fast PCR Instrument**



**Table B: USDA FSIS MLG 4.09 Reference method protocol for poultry and pork samples**



**Table C: FDA BAM Chapter 5 Reference method protocol for stainless steel surface samples**



## Results

All test results were analyzed by probability of detection (POD) statistical analysis to 95% confidence intervals. POD analysis of the study data were conducted as detailed in the AOAC INTERNATIONAL validation guidelines. All data from the POD analysis is detailed in Appendix 1.

For all matrices tested, when using both the 7500 Fast PCR Instrument with RapidFinder Express software and the QuantStudio 5 PCR Instrument with RapidFinder Analysis software, there was no statistical difference by POD analysis between the candidate method presumptive PCR results compared to the candidate method confirmed PCR results using both candidate and reference method confirmation protocols (detailed in Appendix 1, Tables 1, 2, 6 and 7). There was no POD statistical difference between the candidate method confirmation protocol and reference method confirmation protocols (detailed in Appendix 1, Tables 3 and 8).

For the majority of matrices tested, there was no statistical difference by POD analysis between the candidate method confirmed PCR results and the reference method results (detailed in Appendix 1, Tables 4, 5, 9 and 10). Where there were statistically significant differences, the candidate method returned a greater number of confirmed positive results than the USDA FSIS MLG 4.09 reference method. Significantly greater numbers of *Salmonella* Enteritidis positive results from the candidate method were observed on both the raw chicken thighs with skin (co-inoculated with *Salmonella* Enteritidis and *Salmonella* Kentucky)

and raw pork sausage co-inoculated with *Salmonella* Ohio, *Salmonella* Typhimurium and *Salmonella* Enteritidis) compared to the USDA FSIS MLG 4.09 reference method, demonstrating the candidate method was better at detecting *Salmonella* Enteritidis from these samples than the USDA FSIS MLG 4.09 reference method when using both the 7500 Fast PCR Instrument with RapidFinder Express software and the QuantStudio 5 PCR Instrument with RapidFinder Analysis software. The USDA FSIS MLG 4.09 reference method was unable to detect *Salmonella* Enteritidis in many of the samples tested where the candidate method did detect *Salmonella* Enteritidis.

## Conclusion

The data collected demonstrates that the candidate method showed equivalent or improved sensitivity performance to the USDA FSIS MLG 4.09 reference method for the detection of *Salmonella* spp., *Salmonella* Enteritidis, and *Salmonella* Typhimurium from raw chicken thighs with skin, raw chicken wings with skin, chicken nuggets, raw pork sausage, and the FDA BAM Ch. 5 reference method for stainless steel environmental surface sponges. POD analysis conducted during the validation study demonstrated there were no statistical differences between the candidate method and the reference method. Where there were statistically significant differences, these were in favor of the candidate method. The AOAC-RI PTM validation certificate (License number: 081707) is available from either [www.thermofisher.com](http://www.thermofisher.com) or the AOAC Research Institute at [www.aoac.org](http://www.aoac.org).

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# Appendix 1. RapidFinder Salmonella species, Typhimurium, and Enteritidis PCR method developer study results

**Table 1. RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit Results using the 7500 Fast PCR Instrument: POD summary of Candidate method presumptive PCR result vs. Candidate method confirmed result (using candidate method confirmation protocol)**

Matrix	Inoculating strain(s)	MPN <sup>a</sup> /test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw chicken thighs with skin	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A <sup>i</sup>	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		1.84	20	19	0.95	0.76, 1.00	19	0.95	0.76, 1.00	0	-0.19, 0.19
		1.77	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		0.34	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		0.2	5	4	0.8	0.38, 1.00	4	0.8	0.38, 1.00	0	-0.47, 0.47
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.28	NR	NR
		1.01	20	NR	NR	NR	11	0.55	0.34, 0.74	NR	NR
		1.51	5	NR	NR	NR	2	0.4	0.12, 0.77	NR	NR
Raw chicken thighs with skin (independent lab) <sup>k</sup>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		1.87	20	16	0.8	0.58, 0.92	16	0.8	0.58, 0.92	0	-0.25, 0.25
		3.63	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		0.89	20	12	0.6	0.39, 0.78	12	0.6	0.37, 0.78	0	-0.28, 0.28
		2.28	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.43	NR	NR
		0.81	20	NR	NR	NR	10	0.5	0.30, 0.70	NR	NR
		2.37	5	NR	NR	NR	5	1	0.57, 1.00	NR	NR
Raw chicken wings with skin	<i>Salmonella</i> Typhimurium B	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		0.99	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		3.14	5	4	0.8	0.38, 1.00	4	0.8	0.38, 1.00	0	-0.47, 0.47
Chicken nuggets	<i>Salmonella</i> Montevideo C <sub>1</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.43	NR	NR
		0.52	20	NR	NR	NR	11	0.55	0.34, 0.74	NR	NR
		1.32	5	NR	NR	NR	4	0.8	0.38, 1.00	NR	NR
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		1.5	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		3.01	5	3	0.6	0.23, 0.88	3	0.6	0.23, 0.88	0	-0.46, 0.46

Table continued on the next page ►

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw pork sausage	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Ohio C1, <i>Salmonella</i> Typhimurium B and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		3.8	20	20	1	0.84, 1.00	20	1	0.84, 1.00	0	-0.16, 0.16
		>4.38	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		0.63	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0	-0.28, 0.28
		0.8	5	4	0.8	0.38, 1.00	4	0.8	0.38, 1.00	0	-0.47, 0.47
	<i>Salmonella</i> Enteritidis D1	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		0.36	20	15	0.75	0.53, 0.89	14	0.7	0.48, 0.85	0.05	-0.22, 0.31
		0.27	5	5	1	0.57, 1.00	3	0.6	0.23, 0.88	0.4	-0.12, 0.77
	<i>Salmonella</i> Ohio C <sub>1</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.43	NR	NR
		1.25	20	NR	NR	NR	12	0.6	0.39, 0.78	NR	NR
		4.38	5	NR	NR	NR	5	1	0.57, 1.00	NR	NR
Stainless steel	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		N/A	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0	-0.23, 0.23
		N/A	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		N/A	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.23, 0.23
		N/A	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.43	NR	NR
		N/A	20	NR	NR	NR	10	0.5	0.30, 0.70	NR	NR
		N/A	5	NR	NR	NR	4	0.8	0.38, 1.00	NR	NR
Stainless steel (independent lab) <sup>k</sup>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		N/A	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		N/A	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		N/A	20	10	0.5	0.30, 0.70	10	0.5	0.30, 0.70	0	-0.28, 0.28
		N/A	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.43	NR	NR
		N/A	20	NR	NR	NR	9	0.45	0.26, 0.66	NR	NR
		N/A	5	NR	NR	NR	5	1	0.57, 1.00	NR	NR

a MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

b N = Number of test portions.

c x = Number of positive test portions.

d POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

e All strains were confirmed by serotyping.

f POD<sub>CC</sub> = Candidate method confirmed (via *Brilliance* Salmonella method) positive outcomes divided by the total number of trials.

g dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed (via *Brilliance* Salmonella method) 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.

i RapidFinder presumptive PCR result for *Salmonella* species reflects all species present and therefore does not discriminate between the species.

j N/A = Not applicable

k Matrix tested in the independent laboratory.

l NR = Not reported. *Salmonella* strains that are not a specific PCR target cannot have presumptive PCR results for the specific strain. Therefore POD and dPOD cannot be calculated.



**Table 2. RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit Results using the 7500 Fast PCR Instrument: POD summary of Candidate method presumptive PCR result vs. Candidate method confirmed result (using reference method confirmation protocol)**

Matrix	Inoculating strain(s)	MPN <sup>a</sup> /test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw chicken thighs with skin	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		1.84	20	19	0.95	0.76, 1.00	19	0.95	0.76, 1.00	0	-0.19, 0.19
		1.77	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		0.34	20	15	0.75	0.53, 0.89	15'	0.75	0.53, 0.89	0	-0.26, 0.26
		0.2	5	4	0.8	0.38, 1.00	4	0.8	0.38, 1.00	0	-0.47, 0.47
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	NRn	NR	NR	0	0	0.00, 0.28	NR	NR
		1.01	20	NR	NR	NR	12	0.6	0.39, 0.78	NR	NR
		1.51	5	NR	NR	NR	2	0.4	0.12, 0.77	NR	NR
Raw chicken thighs with skin (independent lab)*	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		1.87	20	16	0.8	0.58, 0.92	16	0.8	0.58, 0.92	0	-0.25, 0.25
		3.63	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		0.89	20	12	0.6	0.39, 0.78	12	0.6	0.39, 0.78	0	-0.25, 0.25
		2.28	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.43	NR	NR
		0.81	20	NR	NR	NR	10	0.5	0.30, 0.70	NR	NR
		2.37	5	NR	NR	NR	5	1	0.57, 1.00	NR	NR
Raw chicken wings with skin	<i>Salmonella</i> Typhimurium B	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		0.99	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		3.14	5	4	0.8	0.38, 1.00	4	0.8	0.38, 1.00	0	-0.47, 0.47
Chicken nuggets	<i>Salmonella</i> Montevideo C <sub>1</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.43	NR	NR
		0.52	20	NR	NR	NR	11	0.55	0.34, 0.74	NR	NR
		1.32	5	NR	NR	NR	4	0.8	0.38, 1.00	NR	NR
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		1.5	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		3.01	5	3	0.6	0.23, 0.88	3	0.6	0.23, 0.88	0	-0.46, 0.46

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Matrix	Inoculating strain(s)	MPN <sup>a</sup> /test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw pork sausage	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Ohio C <sub>1</sub> , <i>Salmonella</i> Typhimurium B and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		3.8	20	20	1	0.84, 1.00	20	1	0.84, 1.00	0	-0.16, 0.16
		>4.38	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		0.63	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0	-0.28, 0.28
		0.8	5	4	0.8	0.38, 1.00	3	0.6	0.23, 0.88	0.2	-0.31, 0.62
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		0.36	20	15	0.75	0.53, 0.89	12 <sup>m</sup>	0.6	0.39, 0.78	0.15	-0.13, 0.40
		0.27	5	5	1	0.57, 1.00	3	0.6	0.23, 0.88	0.4	-0.12, 0.77
	<i>Salmonella</i> Ohio C <sub>1</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.43	NR	NR
		1.25	20	NR	NR	NR	12	0.6	0.39, 0.78	NR	NR
		4.38	5	NR	NR	NR	5	1	0.57, 1.00	NR	NR
Stainless Steel environmental surface sponges	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		N/A	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0	-0.23, 0.23
		N/A	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		N/A	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.23, 0.23
		N/A	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.43	NR	NR
		N/A	20	NR	NR	NR	10	0.5	0.30, 0.70	NR	NR
		N/A	5	NR	NR	NR	4	0.8	0.38, 1.00	NR	NR
Stainless Steel environmental surface sponges (independent lab <sup>k</sup> )	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		N/A	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		N/A	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		N/A	20	10	0.5	0.30, 0.70	10	0.5	0.30, 0.70	0	-0.28, 0.28
		N/A	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	NR	NR	NR	0	0	0.00, 0.43	NR	NR
		N/A	20	NR	NR	NR	9	0.45	0.26, 0.66	NR	NR
		N/A	5	NR	NR	NR	5	1	0.57, 1.00	NR	NR

a MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

b N = Number of test portions.

c x = Number of positive test portions.

d POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

e All strains were confirmed by serotyping.

f POD<sub>CC</sub> = Candidate method confirmed (via *Brilliance* Salmonella method) positive outcomes divided by the total number of trials.

g dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed (via *Brilliance* Salmonella method) 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.

i RapidFinder presumptive PCR result for *Salmonella* species reflects all species present and therefore does not discriminate between the species.

j N/A = Not applicable.

k Matrix tested in the independent laboratory.

l 14 of the 15 positives confirmed using standard confirmation protocol. Additional 1 positive confirmed using extended confirmation protocol.

m 11 of the 12 positives confirmed using standard protocol. An additional 1 positive confirmed by testing more than the recommended 20 colonies.

n NR = Not reported. *Salmonella* strains that are not a specific PCR target cannot have presumptive PCR results for the specific strain, therefore POD and dPOD cannot be calculated.

**Table 3. RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit Results with 7500 Fast PCR Instrument: POD summary of Candidate method confirmed result (using candidate confirmation protocol) vs. Candidate method confirmed result (using reference method confirmation protocol)**

Matrix	Inoculating strain(s)	MPN <sup>a</sup> /test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw chicken thighs with skin	Salmonella spp. totali (Salmonella Kentucky C <sub>3</sub> and Salmonella Enteritidis D <sub>1</sub> )	N/A <sup>k</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.84	20	19	0.95	0.76, 1.00	19	0.95	0.76, 1.00	0.00	-0.19, 0.19
		1.77	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	Salmonella Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.34	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		0.20	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	Salmonella Kentucky C <sub>3</sub>	N/A	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		1.01	20	11	0.55	0.34, 0.74	12	0.60	0.39, 0.78	-0.05	-0.33, 0.24
		1.51	5	2	0.44	0.12, 0.77	2	0.40	0.12, 0.77	0.00	-0.46, 0.46
Raw chicken thighs with skin (independent lab) <sup>j</sup>	Salmonella spp. totali (Salmonella Kentucky C <sub>3</sub> and Salmonella Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.87	20	16	0.80	0.58, 0.92	16	0.80	0.80, 0.92	0.00	-0.25, 0.25
		3.63	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43
	Salmonella Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.89	20	12	0.60	0.39, 0.78	12	0.60	0.50, 0.70	0.00	-0.28, 0.25
		2.28	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43
	Salmonella Kentucky C <sub>3</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.81	20	10	0.50	0.30, 0.70	10	0.50	0.60, 0.78	0.00	-0.28, 0.28
		2.37	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43
Raw chicken wings with skin	Salmonella Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		3.14	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Chicken nuggets	Salmonella Montevideo C <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.52	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		1.32	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	Salmonella Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.50	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		3.01	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46

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Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw pork sausage	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Ohio C <sub>1</sub> , <i>Salmonella</i> Typhimurium B and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		3.80	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
		>4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.63	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		0.8	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.16, 0.75
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.36	20	14	0.70	0.48, 0.85	12 <sup>m</sup>	0.60	0.39, 0.78	0.10	-0.18, 0.36
		0.27	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46
	<i>Salmonella</i> Ohio C <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.25	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
		4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel environmental surface sponges	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel environmental surface sponges (independent lab) <sup>l</sup>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	15	0.75	0.53, 0.89	15	0.75	0.75, 0.89	0.00	-0.26, 0.26
		N/A	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.26, 0.66	10	0.50	0.45, 0.66	0.00	-0.28, 0.28
		N/A	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	9	0.45	0.30, 0.70	9	0.45	0.50, 0.70	0.00	-0.28, 0.28
		N/A	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43

a MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

b N = Number of test portions.

c x = Number of positive test portions.

d POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

e Candidate method confirmed using Brilliance Salmonella Agar method.

f Candidate method confirmed using reference method. All strains were confirmed by serotyping.

g PODCC2 = Candidate method confirmed positive outcomes divided by the total number of trials.

h dPODCC = Difference between the candidate method confirmed result (using Brilliance Salmonella Agar method) and candidate method confirmed result (using reference method) POD values.

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

j RapidFinder presumptive PCR result for *Salmonella* species reflects all species present and therefore does not discriminate between the species.

k N/A = Not applicable.

l Matrix tested in the independent laboratory.

m 11 of the 12 positives confirmed using standard protocol. An additional 1 positive confirmed by testing more than the recommended 20 colonies.

**Table 4. RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit Results using the 7500 Fast PCR Instrument: POD summary of Candidate method confirmed result (using candidate method confirmation protocol) vs. USDA FSIS MLG 4.09/FDA BAM Ch.5 reference method result**

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw chicken thighs with skin	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.84	20	19	0.95	0.76, 1.00	18	0.90	0.70, 0.97	0.05	-0.15, 0.26
		1.77	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		0.34	20	15	0.75	0.53, 0.89	8	0.40	0.22, 0.61	0.35	0.04, 0.58
		0.20	5	4	0.80	0.38, 1.00	1	0.20	0.0, 0.62	0.60	0.00, 0.88
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		1.01	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		1.51	5	2	0.40	0.12, 0.77	3	0.60	0.23, 0.88	-0.20	-0.60, 0.32
Raw chicken thighs with skin (independent lab) <sup>k</sup>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.87	20	16	0.80	0.58, 0.92	17	0.85	0.64, 0.95	-0.05	-0.29, 0.19
		3.63	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.89	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.37, 0.19
		2.28	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.81	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.32, 0.23
		2.37	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw chicken wings with skin	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
		3.14	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Chicken nuggets	<i>Salmonella</i> Montevideo C <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.52	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	-0.15, 0.41
		1.32	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.50	20	15	0.75	0.53, 0.89	16	0.80	0.58, 0.92	-0.05	-0.30, 0.21
		3.01	5	3	0.60	0.23, 0.88	5	1.00	0.57, 1.00	-0.40	-0.77, 0.12

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Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw pork sausage	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Ohio C <sub>1</sub> , <i>Salmonella</i> Typhimurium B and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		3.80	20	20	1.00	0.84, 1.00	19	0.95	0.76, 1.00	0.05	-0.12, 0.24
		>4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.63	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		0.8	5	4	0.80	0.38, 1.00	2	0.40	0.12, 0.77	0.40	-0.16, 0.75
	<i>Salmonella</i> Enteritidis D1	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.36	20	14	0.70	0.48, 0.85	5	0.25	0.11, 0.47	0.45	0.14, 0.66
		0.27	5	3	0.60	0.23, 0.88	1	0.20	0.00, 0.62	0.40	-0.16, 0.75
	<i>Salmonella</i> Ohio C1	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.25	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.32, 0.23
		4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel environmental surface sponges	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	13	0.65	0.43, 0.82	15	0.75	0.53, 0.89	-0.10	-0.36, 0.18
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
		N/A	5	4	0.80	0.38, 1.00	2	0.40	0.12, 0.77	0.40	-0.16, 0.75
Stainless Steel environmental surface sponges (independent lab) <sup>k</sup>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.00	-0.19, 0.37
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.10	-0.28, 0.28
		N/A	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 based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

b N = Number of test portions.

c x = Number of positive test portions.

d POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

e Reference methods = MLG 4.09 for poultry and meat products; BAM Ch. 5 for environmental surface sponges.

f PODR = Reference method confirmed positive outcomes divided by the total number of trials.

g dPODC = Difference between the candidate method confirmed (using Brilliance Salmonella Agar) 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.

i RapidFinder presumptive PCR result for *Salmonella* species reflects all species present and therefore does not discriminate between the species. All strains were confirmed by serotyping.

j N/A = Not applicable.

k Matrix tested in the independent laboratory.

l NR = Not reported. *Salmonella* strains that are not a specific PCR target cannot have presumptive PCR results for the specific strain, therefore POD and dPOD cannot be calculated

**Table 5. RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit Results with the 7500 Fast PCR Instrument: POD summary of Candidate method confirmed result (using reference method confirmation protocol) vs. USDA FSIS MLG 4.09/FDA BAM Ch.5 reference method result**

Matrix	Inoculating strain(s)	MPN <sup>a</sup> /test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw chicken thighs with skin	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.84	20	19	0.95	0.76, 1.00	18	0.90	0.70, 0.97	0.05	-0.15, 0.26
		1.77	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		0.34	20	15	0.75	0.53, 0.89	8	0.40	0.22, 0.61	0.35	0.04, 0.58
		0.20	5	4	0.80	0.38, 1.00	1	0.20	0.00, 0.62	0.60	0.00, 0.88
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		1.01	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.32, 0.23
		1.51	5	2	0.40	0.12, 0.77	3	0.60	0.23, 0.88	-0.20	-0.60, 0.32
Raw chicken thighs with skin (independent lab) <sub>k</sub>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.87	20	16	0.80	0.58, 0.92	17	0.85	0.64, 0.95	-0.05	-0.29, 0.19
		3.63	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.89	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.32, 0.23
		2.28	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.81	20	10	0.50	0.39, 0.78	12	0.60	0.43, 0.82	-0.10	-0.37, 0.19
		2.37	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw chicken wings with skin	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
		3.14	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Chicken nuggets	<i>Salmonella</i> Montevideo C <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.52	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	-0.15, 0.41
		1.32	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.50	20	15	0.75	0.53, 0.89	16	0.80	0.58, 0.92	-0.05	-0.30, 0.21
		3.01	5	3	0.60	0.23, 0.88	5	1.00	0.57, 1.00	-0.40	-0.77, 0.12

Table continued on the next page ►

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw pork sausage	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Ohio C <sub>1</sub> , <i>Salmonella</i> Typhimurium B and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		3.80	20	20	1.00	0.84, 1.00	19	0.95	0.76, 1.00	0.05	-0.12, 0.24
		>4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.63	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		0.8	5	3	0.60	0.23, 0.88	2	0.40	0.12, 0.77	0.20	-0.32, 0.60
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.36	20	12 <sup>i</sup>	0.60	0.39, 0.78	5	0.25	0.11, 0.47	0.35	0.04, 0.58
		0.27	5	3	0.60	0.23, 0.88	1	0.20	0.00, 0.62	0.40	-0.16, 0.75
	<i>Salmonella</i> Ohio C <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.25	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.32, 0.23
		4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel environmental surface sponges	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	13	0.65	0.43, 0.82	15	0.75	0.53, 0.89	-0.10	-0.36, 0.18
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
		N/A	5	4	0.80	0.38, 1.00	2	0.40	0.12, 0.77	0.40	-0.16, 0.75
Stainless Steel environmental surface sponges (independent lab) <sub>k</sub>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.19, 0.37
		N/A	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.43, 0.43
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		N/A	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 based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

b N = Number of test portions.

c x = Number of positive test portions.

d POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

e Reference methods = MLG 4.09 for poultry and meat products; BAM Ch. 5 for environmental surface sponges.

f POD<sub>CC</sub> = Reference method confirmed positive outcomes divided by the total number of trials.

g dPOD<sub>C</sub> = Difference between the candidate method confirmed (using reference 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.

i RapidFinder presumptive PCR result for *Salmonella* species reflects all species present and therefore does not discriminate between the species. All strains were confirmed by serotyping.

j N/A = Not applicable.

k Matrix tested in the independent laboratory.

l 11 of the 12 positives confirmed using standard protocol. An additional 1 positive confirmed by testing more than the recommended 20 colonies.



**Table 6. RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit Results using the QuantStudio 5 PCR Instrument: POD summary of Candidate method presumptive PCR result vs. Candidate method confirmed result (using candidate method confirmation protocol)**

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw chicken thighs with skin	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A <sup>i</sup>	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		1.84	20	19	0.95	0.76, 1.00	19	0.95	0.76, 1.00	0.00	-0.19, 0.19
		1.77	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.34	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		0.20	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.28	NR	NR
		1.01	20	NR	NR	NR	11	0.55	0.34, 0.74	NR	NR
		1.51	5	NR	NR	NR	2	0.40	0.12, 0.77	NR	NR
Raw chicken thighs with skin (independent lab) <sub>k</sub>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.87	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0.00	-0.25, 0.25
		3.63	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.89	20	12	0.60	0.39, 0.78	12	0.60	0.30, 0.70	0.00	-0.28, 0.28
		2.28	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.43	NR	NR
		0.81	20	NR	NR	NR	10	0.50	0.39, 0.78	NR	NR
		2.37	5	NR	NR	NR	5	1.00	0.57, 1.00	NR	NR
Raw chicken wings with skin	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		3.14	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Chicken nuggets	<i>Salmonella</i> Montevideo C <sub>1</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.43	NR	NR
		0.52	20	NR	NR	NR	11	0.55	0.34, 0.74	NR	NR
		1.32	5	NR	NR	NR	4	0.80	0.38, 1.00	NR	NR
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.50	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		3.01	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46

Table continued on the next page ►

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw pork sausage	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Ohio C <sub>1</sub> , <i>Salmonella</i> Typhimurium B and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		3.80	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
		>4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.63	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		0.8	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.36	20	15	0.75	0.53, 0.89	14	0.70	0.48, 0.85	0.05	-0.22, 0.31
		0.27	5	5	1.00	0.57, 1.00	3	0.60	0.23, 0.88	0.40	-0.12, 0.77
	<i>Salmonella</i> Ohio C <sub>1</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.43	NR	NR
		1.25	20	NR	NR	NR	12	0.60	0.39, 0.78	NR	NR
		4.38	5	NR	NR	NR	5	1.00	0.57, 1.00	NR	NR
Stainless Steel environmental surface sponges	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.43	NR	NR
		N/A	20	NR	NR	NR	10	0.50	0.30, 0.70	NR	NR
		N/A	5	NR	NR	NR	4	0.80	0.38, 1.00	NR	NR
Stainless Steel environmental surface sponges (independent lab) <sub>k</sub>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.43	NR	NR
		N/A	20	NR	NR	NR	9	0.45	0.26, 0.66	NR	NR
		N/A	5	NR	NR	NR	5	1.00	0.57, 1.00	NR	NR

a MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

b N = Number of test portions.

c x = Number of positive test portions.

d POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

e All strains were confirmed by serotyping.

f POD<sub>CC</sub> = Candidate method confirmed (via *Brilliance* Salmonella method) positive outcomes divided by the total number of trials

g dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed (via *Brilliance* Salmonella method) 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.

i RapidFinder presumptive PCR result for Salmonella species reflects all species present and therefore does not discriminate between the species.

j N/A = Not applicable.

k Matrix tested in the independent laboratory.

l NR = Not reported. Salmonella strains that are not a specific PCR target cannot have presumptive PCR results for the specific strain, therefore POD and dPOD cannot be calculated.

**Table 7. RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit Results using the QuantStudio 5 PCR Instrument: POD summary of Candidate method presumptive PCR result vs. Candidate method confirmed result (using reference method confirmation protocol)**

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw chicken thighs with skin	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		1.84	20	19	0.95	0.76, 1.00	19	0.95	0.76, 1.00	0.00	-0.19, 0.19
		1.77	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.34	20	15	0.75	0.53, 0.89	15 <sup>j</sup>	0.75	0.53, 0.89	0.00	-0.26, 0.26
		0.20	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	NRn	NR	NR	0	0.00	0.00, 0.28	NR	NR
		1.01	20	NR	NR	NR	12	0.60	0.39, 0.78	NR	NR
		1.51	5	NR	NR	NR	2	0.40	0.12, 0.77	NR	NR
Raw chicken thighs with skin (independent lab) <sup>k</sup>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.87	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0.00	-0.25, 0.25
		3.63	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.89	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
		2.28	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.43	NR	NR
		0.81	20	NR	NR	NR	10	0.50	0.30, 0.70	NR	NR
		2.37	5	NR	NR	NR	5	1.00	0.57, 1.00	NR	NR
Raw chicken wings with skin	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		3.14	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Chicken nuggets	<i>Salmonella</i> Montevideo C <sub>1</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.43	NR	NR
		0.52	20	NR	NR	NR	11	0.55	0.34, 0.74	NR	NR
		1.32	5	NR	NR	NR	4	0.80	0.38, 1.00	NR	NR
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.50	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		3.01	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46

Table continued on the next page ►

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw pork sausage	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Ohio C <sub>1</sub> , <i>Salmonella</i> Typhimurium B and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		3.80	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
		>4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.63	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		0.8	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	-0.20	-0.31, 0.62
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.36	20	15	0.75	0.53, 0.89	12 <sup>m</sup>	0.60	0.39, 0.78	-0.15	-0.13, 0.40
		0.27	5	5	1.00	0.57, 1.00	3	0.60	0.23, 0.88	-0.40	-0.12, 0.77
	<i>Salmonella</i> Ohio C <sub>1</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.43	NR	NR
		1.25	20	NR	NR	NR	12	0.60	0.39, 0.78	NR	NR
		4.38	5	NR	NR	NR	5	1.00	0.57, 1.00	NR	NR
Stainless Steel environmental surface sponges	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.43	NR	NR
		N/A	20	NR	NR	NR	10	0.50	0.30, 0.70	NR	NR
		N/A	5	NR	NR	NR	4	0.80	0.38, 1.00	NR	NR
Stainless Steel environmental surface sponges (independent lab) <sub>k</sub>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	15	0.75	0.58, 0.92	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	NR	NR	NR	0	0.00	0.00, 0.43	NR	NR
		N/A	20	NR	NR	NR	9	0.45	0.26, 0.66	NR	NR
		N/A	5	NR	NR	NR	5	1.00	0.57, 1.00	NR	NR

a MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

b N = Number of test portions.

c x = Number of positive test portions.

d POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

e All strains were confirmed by serotyping.

f POD<sub>CC</sub> = Candidate method confirmed (via Reference confirmation method) positive outcomes divided by the total number of trials.

g dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed (via Reference confirmation method) 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.

i RapidFinder presumptive PCR result for *Salmonella* species reflects all species present and therefore does not discriminate between the species.

j N/A = Not applicable.

k Matrix tested in the independent laboratory.

l 14 of the 15 positives confirmed using standard confirmation protocol. Additional 1 positive confirmed using extended confirmation protocol.

m 11 of the 12 positives confirmed using standard protocol. An additional 1 positive confirmed by testing more than the recommended 20 colonies.

n NR = Not reported. *Salmonella* strains that are not a specific PCR target cannot have presumptive PCR results for the specific strain, therefore POD and dPOD cannot be calculated.

**Table 8. RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit Results with QuantStudio 5 PCR Instrument: Candidate method confirmed result (using candidate method confirmation protocol) vs. Candidate method confirmed result (using reference method confirmation protocol)**

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw chicken thighs with skin	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A <sup>k</sup>	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		1.84	20	19	0.95	0.76, 1.00	19	0.95	0.76, 1.00	0.00	-0.19, 0.19
		1.77	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.34	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		0.20	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		1.01	20	11	0.55	0.34, 0.74	12	0.60	0.39, 0.78	-0.05	-0.33, 0.24
		1.51	5	2	0.40	0.12, 0.77	2	0.40	0.12, 0.77	0.00	-0.46, 0.46
Raw chicken thighs with skin (independent lab)	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.87	20	16	0.80	0.58, 0.92	16	0.80	0.80, 0.92	0.00	-0.25, 0.25
		3.63	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.89	20	12	0.60	0.39, 0.78	12	0.60	0.60, 0.78	0.00	-0.28, 0.28
		2.28	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.81	20	10	0.50	0.39, 0.78	10	0.50	0.50, 0.70	0.00	-0.28, 0.28
		2.37	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43
Raw chicken wings with skin	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		3.14	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Chicken nuggets	<i>Salmonella</i> Montevideo C <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.52	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		1.32	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.50	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		3.01	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46

Table continued on the next page ►

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw pork sausage	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Ohio C <sub>1</sub> , <i>Salmonella</i> Typhimurium B and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		3.80	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
		>4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.63	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		0.8	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.16, 0.75
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.36	20	14	0.70	0.48, 0.85	12 <sup>m</sup>	0.60	0.39, 0.78	0.10	-0.18, 0.36
		0.27	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46
	<i>Salmonella</i> Ohio C <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.25	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
		4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel environmental surface sponges	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel environmental surface sponges (independent lab) <sup>l</sup>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	15	0.75	0.53, 0.89	15	0.75	0.75, 0.89	0.00	-0.26, 0.26
		N/A	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	10	0.50	0.45, 0.66	0.00	-0.28, 0.28
		N/A	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	9	0.45	0.26, 0.66	9	0.45	0.50, 0.70	0.00	-0.28, 0.28
		N/A	5	5	1.00	0.57, 1.00	5	1.00	1.00, 1.00	0.00	-0.43, 0.43

a MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

b N = Number of test portions.

c x = Number of positive test portions.

d POD<sub>CC</sub> = Candidate method confirmed (using *Brilliance* Salmonella Agar method) positive outcomes divided by the total number of trials.

e Candidate method confirmed using *Brilliance* Salmonella Agar method.

f Candidate method confirmed using reference method. All strains were confirmed by serotyping.

g POD<sub>CC2</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.

h dPOD<sub>CC</sub> = Difference between the candidate method confirmed result (using *Brilliance* Salmonella Agar method) and candidate method confirmed result (using reference method) POD values.

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

j RapidFinder presumptive PCR result for *Salmonella* species reflects all species present and therefore does not discriminate between the species.

k N/A = Not applicable.

l Matrix tested in the independent laboratory.

m 11 of the 12 positives confirmed using standard protocol. An additional 1 positive confirmed by testing more than the recommended 20 colonies.

**Table 9. RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit Results with QuantStudio 5 PCR Instrument: POD summary of Candidate method confirmed result (using candidate method confirmation protocol) vs. USDA FSIS MLG 4.09/FDA BAM Ch.5 reference method result**

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw chicken thighs with skin	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A/	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.84	20	19	0.95	0.76, 1.00	18	0.90	0.70, 0.97	0.05	-0.15, 0.26
		1.77	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A/	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		0.34	20	15	0.75	0.53, 0.89	8	0.40	0.22, 0.61	0.35	0.04, 0.58
		0.20	5	4	0.80	0.38, 1.00	1	0.20	0.0, 0.62	0.60	0.00, 0.88
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A/	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		1.01	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		1.51	5	2	0.40	0.12, 0.77	3	0.60	0.23, 0.88	-0.20	-0.60, 0.32
Raw chicken thighs with skin (independent lab)	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A/	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.87	20	16	0.80	0.58, 0.92	17	0.85	0.64, 0.95	-0.05	-0.29, 0.19
		3.63	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A/	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.89	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.32, 0.23
		2.28	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Kentucky C <sub>3</sub>	N/A/	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.81	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.37, 0.19
		2.37	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw chicken wings with skin	<i>Salmonella</i> Typhimurium B	N/A/	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
		3.14	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Chicken nuggets	<i>Salmonella</i> Montevideo C <sub>1</sub>	N/A/	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.52	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	-0.15, 0.41
		1.32	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	<i>Salmonella</i> Typhimurium B	N/A/	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.50	20	15	0.75	0.53, 0.89	16	0.80	0.58, 0.92	-0.05	-0.30, 0.21
		3.01	5	3	0.60	0.23, 0.88	5	1.00	0.57, 1.00	-0.40	-0.77, 0.12

Table continued on the next page ►

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw pork sausage	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Ohio C <sub>1</sub> , <i>Salmonella</i> Typhimurium B and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		3.80	20	20	1.00	0.84, 1.00	19	0.95	0.76, 1.00	0.05	-0.12, 0.24
		>4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.63	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		0.8	5	4	0.80	0.38, 1.00	2	0.40	0.12, 0.77	0.40	-0.16, 0.75
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.36	20	14	0.70	0.48, 0.85	5	0.25	0.11, 0.47	0.45	0.14, 0.66
		0.27	5	3	0.60	0.23, 0.88	1	0.20	0.00, 0.62	0.40	-0.16, 0.75
	<i>Salmonella</i> Ohio C <sub>1</sub>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.25	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.32, 0.23
		4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel environmental surface sponges	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	13	0.65	0.43, 0.82	15	0.75	0.53, 0.89	-0.10	-0.36, 0.18
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
		N/A	5	4	0.80	0.38, 1.00	2	0.40	0.12, 0.77	0.40	-0.16, 0.75
Stainless Steel environmental surface sponges (independent lab) <sup>j</sup>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.28, 0.28
		N/A	5	5	1.00	0.57 -1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.19, 0.37
		N/A	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 based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

b N = Number of test portions.

c x = Number of positive test portions.

d POD<sub>C</sub> = Candidate method presumptive positive outcomes confirmed positive using Brilliance Salmonella Agar method.

e Reference methods = MLG 4.09 for poultry and meat products; BAM Ch. 5 for environmental surface sponges.

f POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials.

g dPOD<sub>C</sub> = Difference between the candidate method confirmed (using Brilliance Salmonella Agar) 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.

i RapidFinder presumptive PCR result for *Salmonella* species reflects all species present and therefore does not discriminate between the species. All strains were confirmed by serotyping.

j N/A = Not applicable.

k Matrix tested in the independent laboratory.



**Table 10. RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit Results with QuantStudio 5: POD summary of Candidate method confirmed result (using reference method confirmation protocol) vs. USDA FSIS MLG 4.09/FDA BAM Ch.5 reference method result**

Matrix	Inoculating strain(s)	MPN <sup>a</sup> /test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw chicken thighs with skin	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.84	20	19	0.95	0.76, 1.00	18	0.90	0.70, 0.97	0.05	-0.15, 0.26
		1.77	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
	<i>Salmonella</i> Enteritidis D1	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		0.34	20	15	0.75	0.53, 0.89	8	0.40	0.22, 0.61	0.35	0.04, 0.58
		0.20	5	4	0.80	0.38, 1.00	1	0.20	0.00, 0.62	0.60	0.00, 0.88
	<i>Salmonella</i> Kentucky C3	N/A	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.43, 0.43
		1.01	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.32, 0.23
		1.51	5	2	0.40	0.12, 0.77	3	0.60	0.23, 0.88	-0.20	-0.60, 0.32
Raw chicken thighs with skin (independent lab)	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Kentucky C <sub>3</sub> and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.87	20	16	0.80	0.58, 0.92	17	0.85	0.64, 0.95	-0.05	-0.29, 0.19
		3.63	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Enteritidis D1	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.89	20	12	0.60	0.30, 0.70	13	0.65	0.39, 0.78	-0.05	-0.32, 0.23
		2.28	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Kentucky C3	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.81	20	10	0.50	0.39, 0.78	12	0.60	0.43, 0.82	-0.10	-0.37, 0.19
		2.37	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw chicken wings with skin	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.99	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
		3.14	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Chicken nuggets	<i>Salmonella</i> Montevideo C1	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.52	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	-0.15, 0.41
		1.32	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.50	20	15	0.75	0.53, 0.89	16	0.80	0.58, 0.92	-0.05	-0.30, 0.21
		3.01	5	3	0.60	0.23, 0.88	5	1.00	0.57, 1.00	-0.40	-0.77, 0.12

Table continued on the next page ►

Matrix	Inoculating strain(s)	MPN <sup>a</sup> / test portion	N <sup>b</sup>	RapidFinder Salmonella Multiplex PCR Kit presumptive PCR result			RapidFinder Salmonella Multiplex PCR Kit result confirmed (using candidate confirmation method) <sup>e</sup>			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>f</sup>	95% CI		
Raw pork sausage	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Ohio C <sub>1</sub> , <i>Salmonella</i> Typhimurium B and <i>Salmonella</i> Enteritidis D <sub>1</sub> )	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		3.80	20	20	1.00	0.84, 1.00	19	0.95	0.76, 1.00	0.05	-0.12, 0.24
		>4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.63	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		0.8	5	3	0.60	0.23, 0.88	2	0.40	0.12, 0.77	0.20	-0.32, 0.60
	<i>Salmonella</i> Enteritidis D <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.36	20	12 <sup>i</sup>	0.60	0.39, 0.78	5	0.25	0.11, 0.47	0.35	0.04, 0.58
		0.27	5	3	0.60	0.23, 0.88	1	0.20	0.00, 0.62	0.40	-0.16, 0.75
	<i>Salmonella</i> Ohio C <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.25	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.32, 0.23
		4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel environmental surface sponges	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	13	0.65	0.43, 0.82	15	0.75	0.53, 0.89	-0.10	-0.36, 0.18
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
		N/A	5	4	0.80	0.38, 1.00	2	0.40	0.12, 0.77	0.40	-0.16, 0.75
Stainless Steel environmental surface sponges (independent lab) <sup>j</sup>	<i>Salmonella</i> spp. totali ( <i>Salmonella</i> Poona G <sub>1</sub> and <i>Salmonella</i> Typhimurium B)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Typhimurium B	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Poona G <sub>1</sub>	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		N/A	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 based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

b N = Number of test portions.

c x = Number of positive test portions.

d POD<sub>CP</sub> = Candidate method presumptive positive outcomes confirmed positive using Brilliance Salmonella Agar method.

e Reference methods = MLG 4.09 for poultry and meat products; BAM Ch. 5 for environmental surface sponges.

f POD<sub>CC</sub> = Reference method confirmed positive outcomes divided by the total number of trials.

g dPOD<sub>CP</sub> = Difference between the candidate method confirmed (using Brilliance Salmonella Agar) 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.

i RapidFinder presumptive PCR result for *Salmonella* species reflects all species present and therefore does not discriminate between the species. All strains were confirmed by serotyping.

j N/A = Not applicable.

k Matrix tested in the independent laboratory.

l 11 of the 12 positives confirmed using standard protocol. An additional 1 positive confirmed by testing more than the recommended 20 colonies.