STUDY REPORT

Thermo Scientific SureTect Salmonella Species PCR Assay Method Extension For Use With the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument AOAC-RI PTM Validation: Method Comparison Study

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Summary

Thermo Scientific™ SureTect™ Salmonella species PCR Assay (candidate method) has been validated in accordance with the AOAC Research Institute *Performance Tested Methods*℠ (PTM) Program for the detection of *Salmonella* from a variety of food and environmental surfaces. The candidate method has been validated for use with the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument to perform PCR, and the Thermo Scientific™ RapidFinder™ Analysis Software v1.0 or greater for data analysis. In addition, plastic surface sponge (100 mL enrichment) and swab (10 mL enrichment) matrices have been added. This report details the method comparison study between the candidate method and the International Organization for Standardization (ISO) 6579-1:2017 for a representative range of matrices.

Methodology

The performance of the candidate method was assessed as an unpaired study in comparison to the ISO 6579-1:2017 reference method. Method developer studies were conducted by Thermo Fisher Scientific on raw chicken breast, bagged lettuce, skimmed milk powder, 375 g raw ground beef, plastic surface swabs (1x1”) and sponges (4x4”).

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Sample Preparation

All samples were added to a homogenizer bag with the appropriate ratio of Buffered Peptone Water (BPW) ISO formulation (as detailed in Table 1) at room temperature. The samples were homogenized thoroughly before incubation.

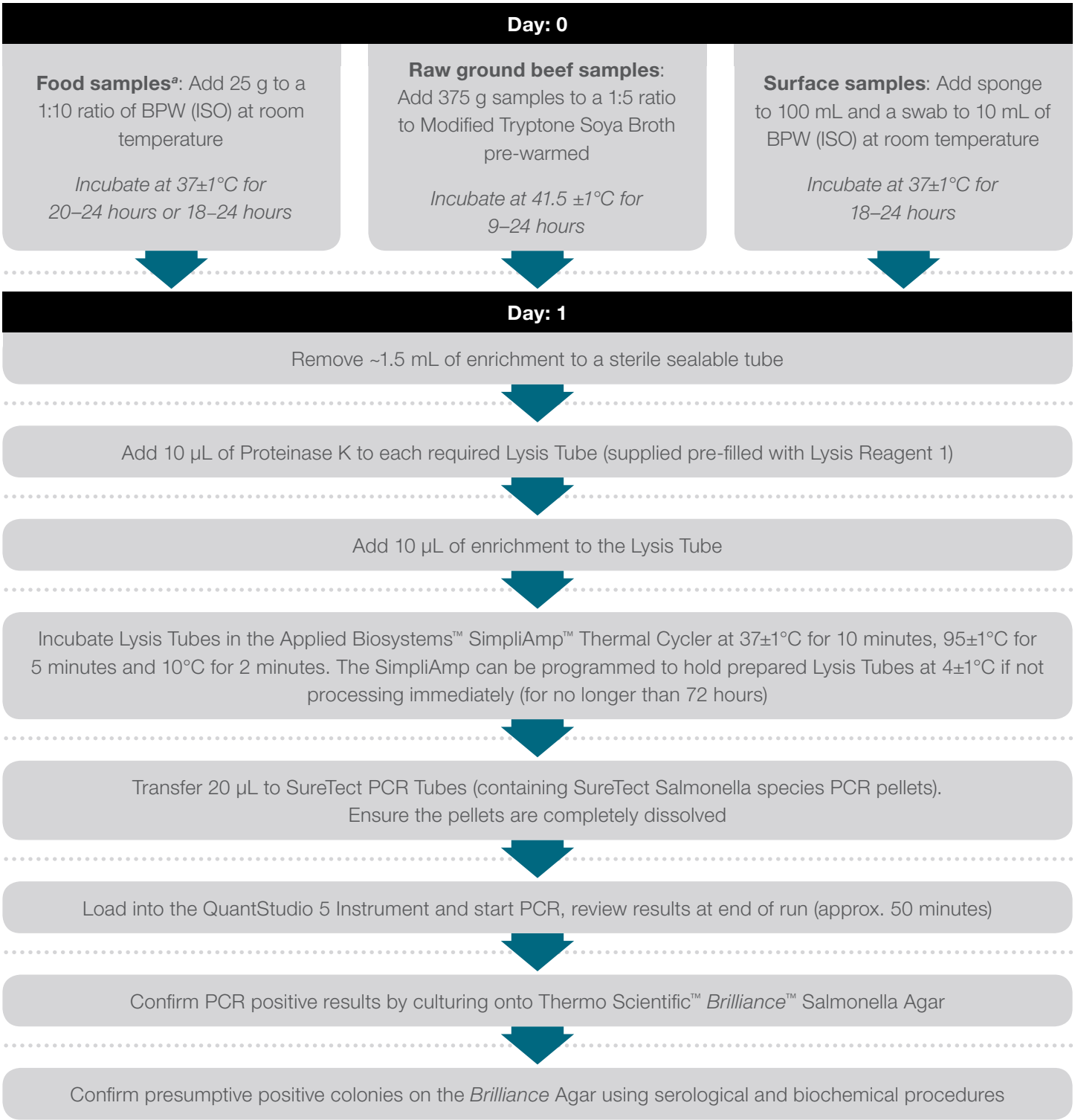
For the plastic surface samples, sterile sampling sponges/swabs were pre-moistened in a suitable diluent. For sampling of areas that had been cleaned or treated with disinfectants, the sponge/swab was pre-moistened with a neutralizing broth, such as Dey-Engley Broth, prior to sampling. The surface was sampled by rubbing the sponge/swab in horizontal and vertical directions across the entire sampling area (4x4" area for sponges and 1x1" area for swabs).

Table 1. Sample enrichment and incubation conditions.

Matrix	Enrichment media	Incubation
Bagged lettuce	1:10 ratio of BPW (ISO)	37±1°C for 20–24 hours
Skimmed milk powder	1:10 ratio of BPW (ISO)	37±1°C for 18–24 hours
Raw ground beef (375 g)	1:5 ratio of BPW (ISO)	41.5±1°C for 9–24 hours
Plastic surface swabs (1x1")	10 mL of BPW (ISO)	37±1°C for 18–24 hours
Plastic surface sponges (4x4")	100 mL of BPW (ISO)	37±1°C for 18–24 hours

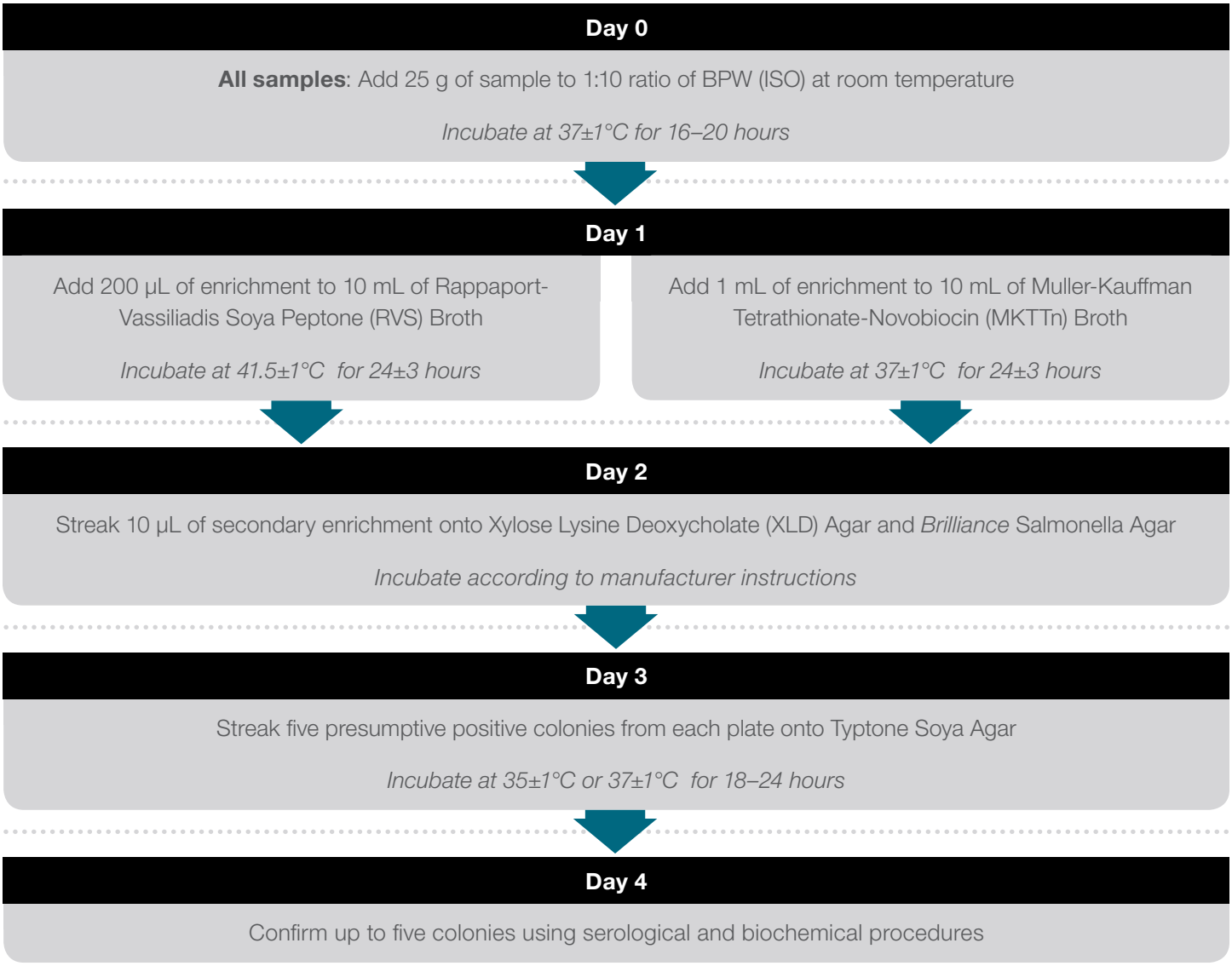
Approximately 1.5 mL of all enrichments were dispensed into a new tube ready to process the sample to the next stage. Enriched samples were stored at 2–8°C for a maximum of 72 hours.

Figure 1. Thermo Scientific SureTect Salmonella species PCR Assay protocol for the detection of *Salmonella* species from food and environmental surfaces



[a] Protocol for food samples included in this validation sudy (raw ground beef, bagged lettuce, skimmed milk powder)

Figure 2. ISO 6579-1:2017 Reference method for the detection of *Salmonella* species from food and surfaces



Results

The culture-confirmed results of the candidate methods in comparison to the ISO 6579-1:2017 reference method are detailed in appendix 1.

There were no statistically significant differences by POD analysis between the confirmed results of the candidate methods and the reference method, or between the presumptive results of the candidate methods and the confirmed results of the candidate methods.

Conclusion

The data presented in this report show that the SureTect Salmonella species PCR Assay is suitable for the detection of *Salmonella* species from a variety of food and environmental surface samples when using the QuantStudio 5 Real-Time PCR Instrument and associated RapidFinder Analysis Software. POD analysis conducted during the validation study demonstrated no statistically significant differences. The AOAC-RI PTM validation certificate (License number: 051303) is available from either www.thermofisher.com or the AOAC Research Institute at www.aoac.org.

Appendix 1

Table 2. SureTect Salmonella species PCR Assay Results: Candidate Method Confirmed (via the Candidate Method) vs Reference Method POD Summary.

Matrix[a]	Inoculating strain(s)	MPN[b]/ test portion	N[c]	SureTect candidate method confirmed via the candidate method result			ISO reference method result			$dPOD_{CC}$[g]	95% CI[h]
				x[d]	POD_{CC}[e]	95% CI	x	POD_R[f]	95% CI		
Raw ground beef (375 g) - 9 hours[l]	RDCC 2315 *Salmonella* Ohio	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1	20	8	0.40	0.22, 0.61	12	0.60	0.39, 0.78	-0.20	-0.46, 0.10
		1.1	5	3	0.60	0.23, 0.88	4	0.80	0.38, 1.00	-0.20	-0.62, 0.31
Raw ground beef (375 g) - 24 hours	RDCC 2315 *Salmonella* Ohio	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.00	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.37, 0.19
		1.10	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Lettuce	TCC 1626 *Salmonella* Anatum	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.30	20	5	0.25	0.11, 0.47	6	0.30	0.15, 0.52	-0.05	-0.31, 0.22
		1.8	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

Matrix[a]	Inoculating strain(s)	MPN[b]/ test portion	N[c]	SureTect candidate method confirmed via the candidate method result			ISO reference method result			$dPOD_{CC}$[g]	95% CI[h]
				x[d]	POD_{CC}[e]	95% CI	x	POD_R[f]	95% CI		
Skimmed milk powder	TCC 0813 *L. monocytogenes*/ 10X *E. faecalis*	N/A[j]	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	16	0.80	0.58, 0.92	-0.05	-0.30, 0.21
		N/A	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46
Plastic surface swabs (1x1")	OCC 722 *Salmonella* Typhimurium	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
		N/A	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46
Plastic surface sponges (4x4")	TCC 1653 *Salmonella* Kentucky	N/A[j]	5	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	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

[a] Matrix = for the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments
[b] 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
[c] N = Number of test portions
[d] x = Number of positive test portions
[e] POD_{CC} = Candidate method confirmed via the candidate method positive outcomes divided by the total number of trials
[f] POD_R = Reference method divided by the total number of trials
[g] $dPOD_{CC}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values
[h] 95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[j] N/A = Not applicable
[l] 9 hour presumptive results compared to the reference method results

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