

# Thermo Scientific SureTect E. coli O157:H7 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™ E. coli O157:H7 PCR Assay (candidate method) has been validated in accordance with the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> (PTM) Program for the detection of *Escherichia coli* from a variety of food and environmental surfaces. The candidate method has been validated for use with Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument to perform PCR and Thermo Scientific™ RapidFinder™ Analysis Software v1.0 or greater for data analysis. This report details the method comparison study between the candidate method and the appropriate reference method (detailed on next page) for a representative range of matrices.

## Methodology

The performance of the candidate method was assessed as an unpaired study in comparison to the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) Microbiology Laboratory Guidebook (MLG) 5.09 'Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges' reference method for raw beef (1:4 and 1:5 enrichments).

The performance of the candidate method was assessed as an unpaired study in comparison to the ISO 16654:2001 'Microbiology of food and animal feeding stuffs—Horizontal method for the detection of *Escherichia coli* O157' reference method for fresh spinach.

Method developer studies were conducted by Q Laboratories Inc, OH, USA.

## Sample preparation

For raw ground beef samples, 375 g of sample was added to a homogenizer bag along with a 1:4 or 1:5 ratio of prewarmed Oxoid™ Modified Tryptone Soya Broth (mTSB). The samples were homogenized thoroughly and incubated at  $41.5\pm 1^{\circ}\text{C}$  and sampled after 9 and 24 hours of incubation.

For fresh spinach samples, 25 g of sample was added to a 1:10 ratio of prewarmed mTSB. The samples were homogenized thoroughly and incubated at  $41.5\pm 1^{\circ}\text{C}$  and sampled after 8 and 24 hours of incubation.

**Figure 1. Thermo Scientific SureTect *E. coli* O157:H7 PCR Assay protocol for the detection of *E. coli* O157:H7 from raw ground beef and fresh spinach.**

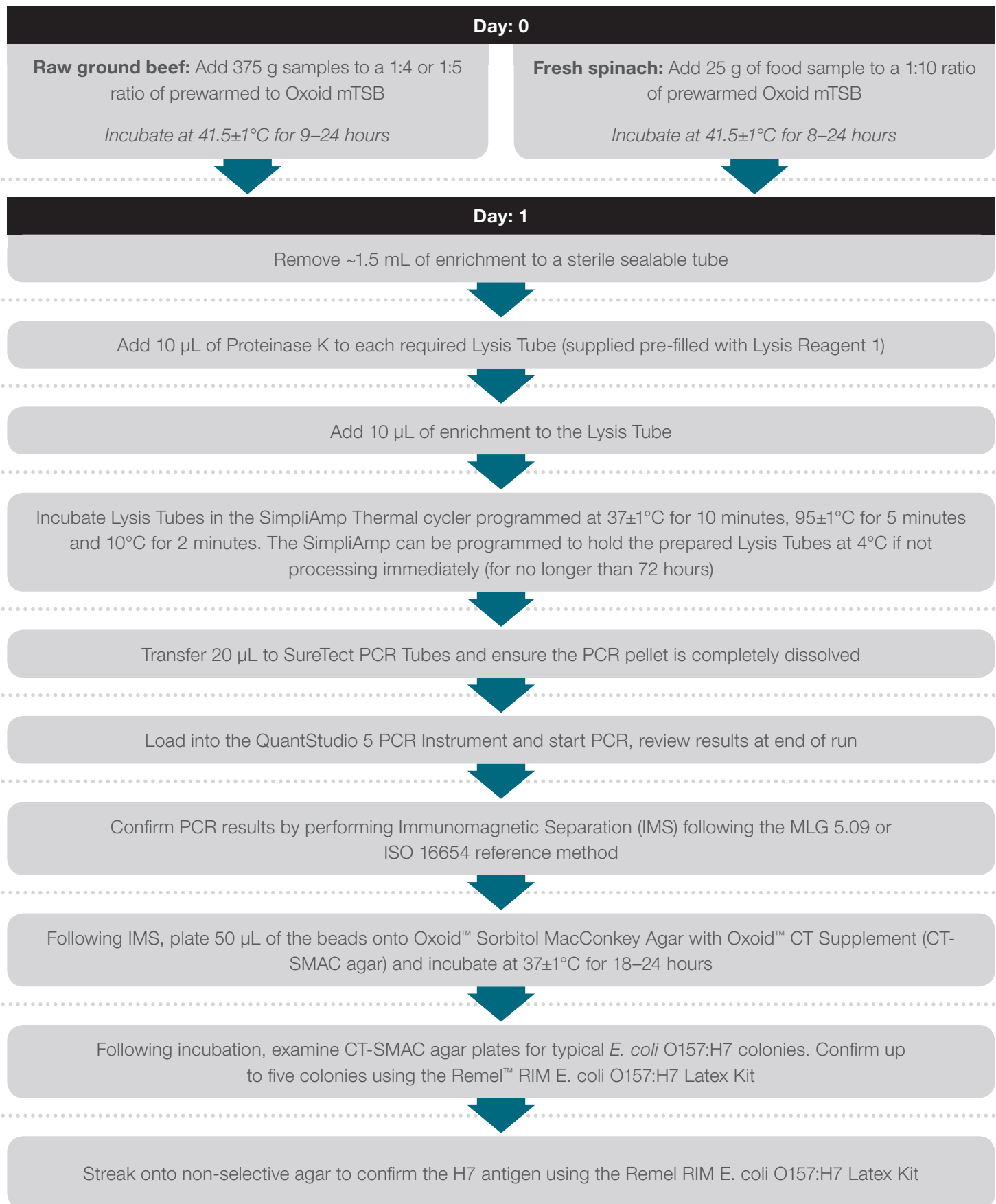


Figure 2. USDA FSIS MLG 5.09 Reference method for the detection of *E. coli* O157:H7 from raw beef.

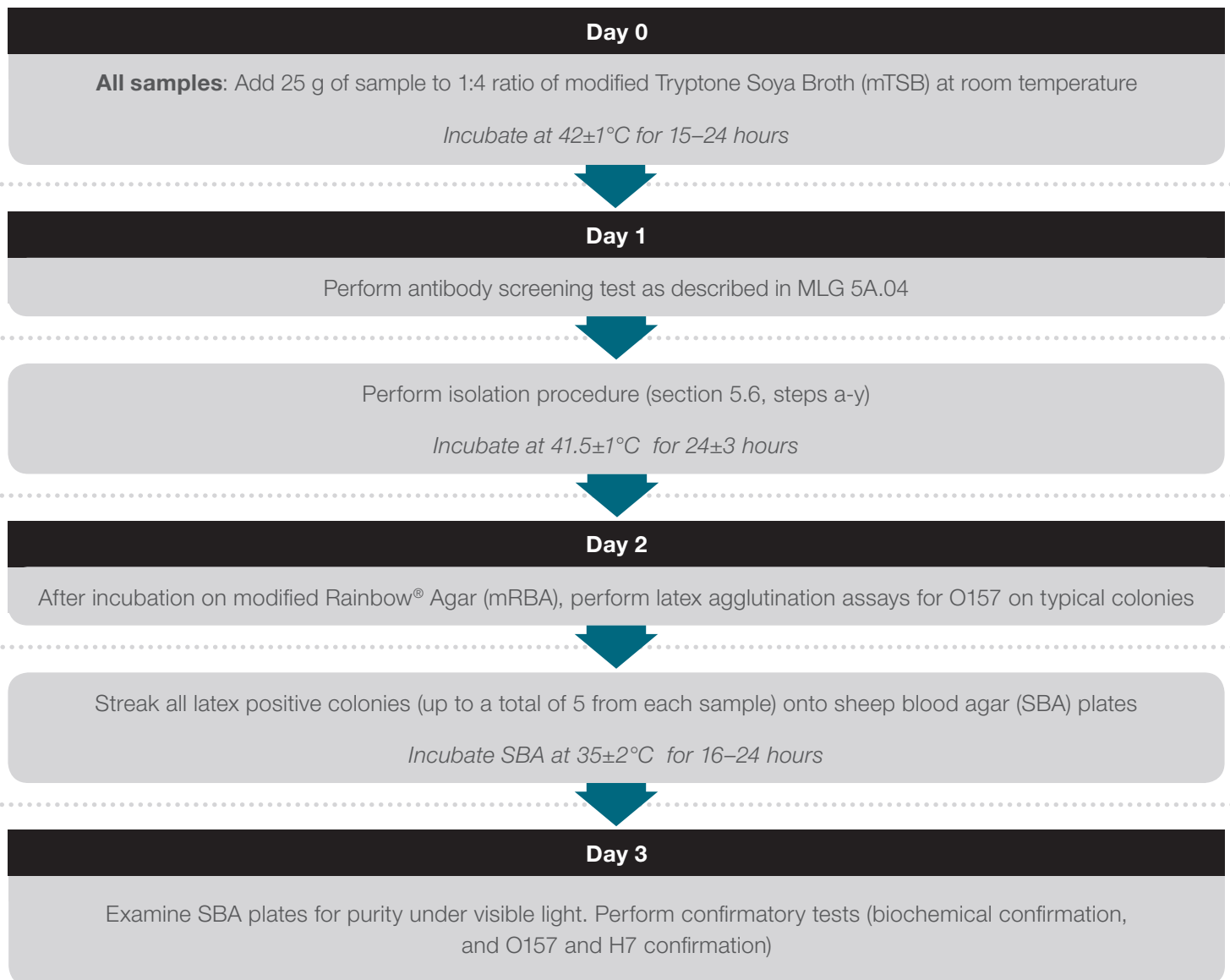


Figure 3. ISO 16654:2001 Reference method for the detection of *E. coli* O157:H7 from fresh spinach.



## Results

The results of the candidate methods confirmed (via the candidate confirmation method) in comparison to the USDA/FSIS MLG 5.09 and ISO 16654:2001 reference methods is detailed in appendix 1.

The results from the method comparison study showed no statistically significant differences by POD analysis between the candidate methods (including presumptive results, and

confirmed results via candidate and reference methods) and the reference method, or between the candidate presumptive result and the candidate method confirmed (via the candidate method and the reference method).

## Conclusion

The data presented in this report show that the SureTect *E. coli* O157:H7 PCR Assay is suitable for the detection of *E. coli* O157:H7 from a variety of food 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 between the candidate

method and the corresponding reference method.

Inclusivity and exclusivity testing demonstrated that both candidate methods successfully detected all 52 *E. coli* O157:H7 isolates and correctly excluded all 30 closely related non-target isolates. The AOAC-RI PTM validation certificate (License number: 021501) is available from either [www.thermofisher.com](http://www.thermofisher.com) or the AOAC Research Institute at [www.aoac.org](http://www.aoac.org).

## Appendix 1

**Table 2.** SureTect *E. coli* O157:H7 PCR Assay results: candidate method confirmed (via the candidate method) vs reference method POD summary.

Matrix	Strain	MPN <sup>1</sup> / test portion	N <sup>2</sup>	Candidate			Reference			dPOD <sub>c</sub> <sup>6</sup>	95% CI <sup>7</sup>
				X <sup>3</sup>	POD <sub>cc</sub> <sup>4</sup>	95% CI	X	POD <sub>cc</sub> <sup>5</sup>	95% CI		
Raw ground beef (1:4 enrichment ratio)	<i>E. coli</i> O157:H7 ATCC 35150	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.49 (0.25, 0.85)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw ground beef (1:5 enrichment ratio)	<i>E. coli</i> O157:H7 ATCC 35150	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.49 (0.25, 0.85)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh spinach	<i>E. coli</i> O157:H7 ATCC 51657	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.40 (0.18, 0.69)	20	7	0.35	0.18, 0.57	6	0.30	0.15, 0.52	0.05	-0.23, 0.32
		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

<sup>1</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator version 1.6 provided by AOAC RI, with 95% confidence interval

<sup>2</sup>N = Number of test portions

<sup>3</sup>X = Number of positive test portions

<sup>4</sup>POD<sub>cc</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>5</sup>POD<sub>cc</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>6</sup>dPOD<sub>c</sub> = POD values for the difference between the candidate method results and reference method confirmed results

<sup>7</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

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