

# Thermo Scientific SureTect E. coli O157:H7 PCR Assay: AOAC-RI PTM Validation using the Applied Biosystems QuantStudio 5 PCR Instrument

Amanda Manolis<sup>1</sup>, Jessica Williams<sup>2</sup>, Ben Bastin<sup>3</sup>. <sup>1</sup>Thermo Fisher Scientific, Microbiology Austin, USA, <sup>2</sup>Thermo Fisher Scientific, Microbiology, Basingstoke, UK, <sup>3</sup>Q Laboratories Inc., Ohio, USA.

## INTRODUCTION

The Thermo Scientific™ SureTect™ E.coli PCR Assay (candidate method) has been certified by AOAC-RI *Performance tested method*<sup>SM</sup> (PTM 021501) for the detection of *Escherichia coli* O157:H7 from 375 g ground beef, 375 g raw beef trim, 25 g spinach and 25 mL apple juice.

The aim of the study was to extend the current claims for the candidate method to include the use of the Applied Biosystems™ SimpliAmp Thermal Cycler for sample lysis and the Applied Biosystems™ QuantStudio™ 5 Real-Time Food Safety PCR Instrument with associated Applied Biosystems™ RapidFinder™ Analysis software (QuantStudio 5 Food Safety System) as shown in figure 1.

**Figure 1. Thermo Scientific SureTect Real-Time PCR System**

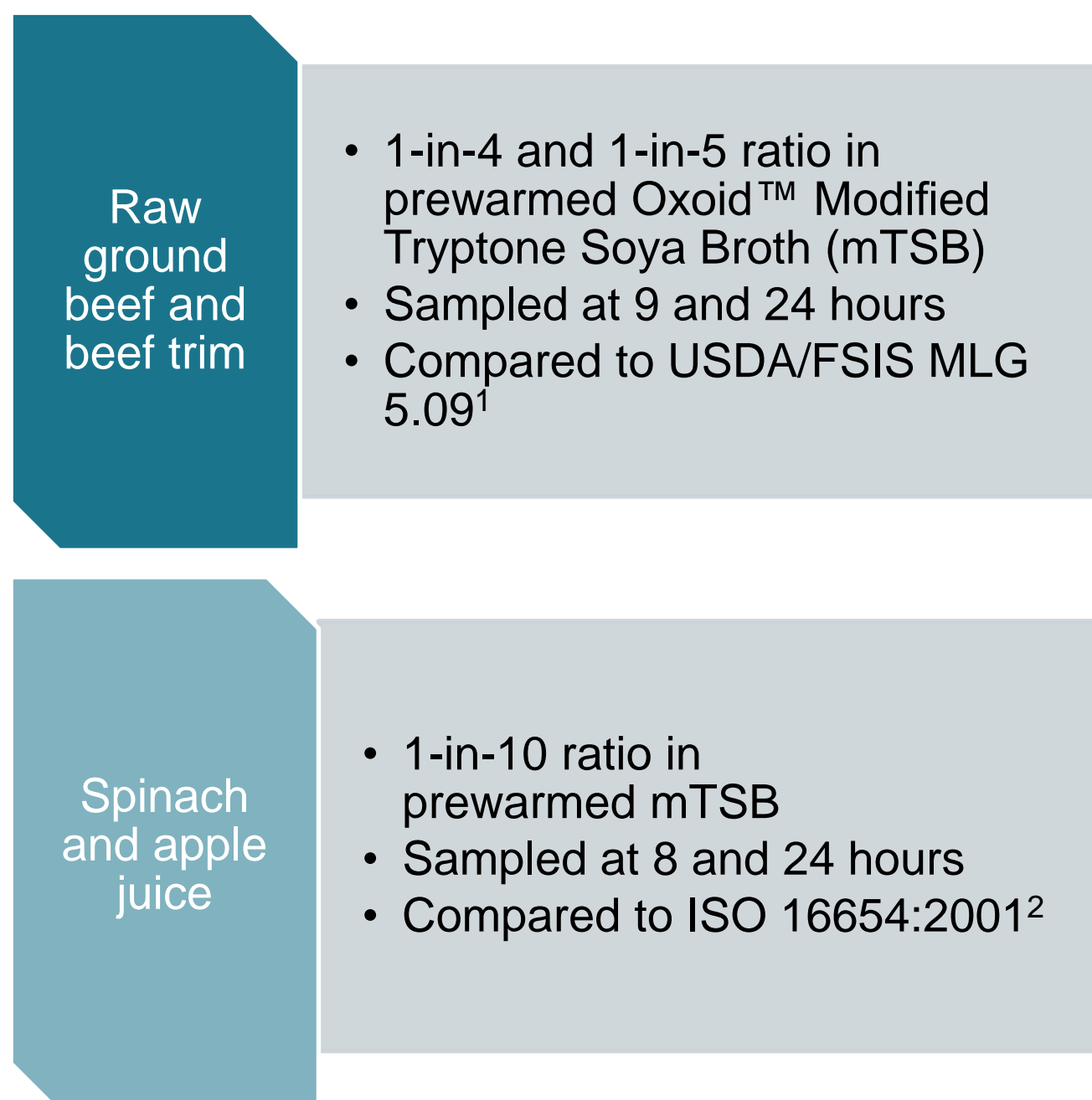


## MATERIALS AND METHODS

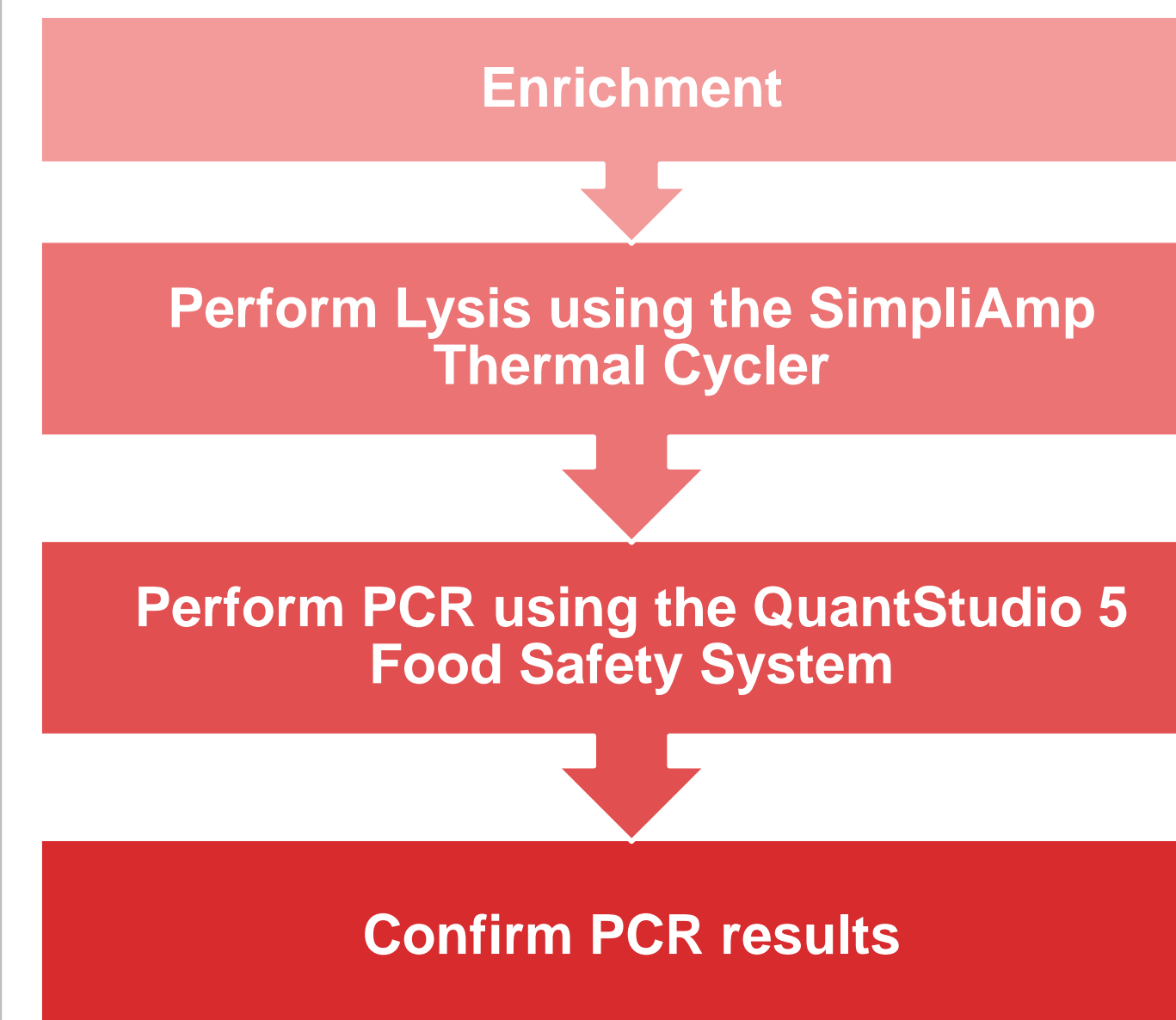
The QuantStudio 5 Food Safety System uses a 6-channel, 96-well cloud-enabled platform suitable for running a wide range of PCR solutions for food pathogen and authenticity testing.

The candidate method was analysed with raw ground beef, raw beef trim and spinach according to the protocols detailed in figures 2 and 3.

**Figure 2. Sample Preparation and Reference Method Summary**



**Figure 3: Summarized Workflow for the SureTect E. coli O157:H7 PCR Assay Extension Study**



## RESULTS

### Inclusivity

All 52 *E. coli* O157:H7 isolates were correctly identified.

### Exclusivity

All 30 exclusivity isolates were correctly excluded.

### Probability of Detection (POD) Analysis

Statistical analysis using Probability of Detection (POD) at 95% confidence levels demonstrated no statistical difference between the candidate and reference method during any of the validation studies (table 1).

**Table 1. Summary of POD Results**

Spike level	N	Candidate method positives	Reference method positives	dPOD	95% CI
n/a	15	0	0	0.00	-0.20, 0.20
Low	60	23	20	0.05	-0.12, 0.22
High	15	15	15	0.00	-0.20, 0.20

There were no statistically significant differences for any matrix individually.  
 N = Number of samples  
 dPOD= difference in POD between methods  
 95% CI= if the 95% confidence interval does not contain a zero the results are statistically significant at the 5% level

## CONCLUSION

The SureTect E. coli O157:H7 PCR method using the QuantStudio 5 PCR Instrument for PCR and RapidFinder Analysis software for data analysis proved to be a suitable substitute to the reference methods for *E. coli* O157:H7 detection in raw ground beef, raw beef trim, spinach and apple juice.

## REFERENCES

1. USDA FSIS MLG5.09: Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges. Effective date 01/15/15.
2. ISO 16654:2001 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Escherichia coli* O157

## TRADEMARKS/ LICENSING

© 2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. PTM is a service mark of AOAC INTERNATIONAL, France. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.

LT2466A  
July 2019

