

AOAC-RI Performance Tested Method Validation Of The Thermo Scientific SureTect Escherichia coli O157:H7 PCR Assay

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Overview

Purpose: Validation of the Thermo Scientific™ SureTect™ Escherichia coli O157:H7 PCR Assay (PT0400A) according to AOAC Research Institute (RI) *Performance Tested Methods*SM validation criteria.

Methods: The SureTect method was compared to the reference method detailed in either the USDA FSIS MLG 5.08 or ISO 16654:2001 depending on the food matrix.

Results: The SureTect Escherichia coli O157:H7 PCR Assay reliably detected the presence of *E. coli* O157:H7 in both raw beef and produce matrices.

Introduction

The SureTect Escherichia coli O157:H7 PCR Assay (PTM#021501) is a Real-Time PCR kit for the detection of *E. coli* O157:H7 from foods using dedicated software to interpret the PCR result. This study¹ was conducted using the AOAC RI *Performance Tested Methods*SM program² to validate the SureTect Escherichia coli O157:H7 Assay against the ISO 16654:2001 reference method for spinach and apple juice and USDA FSIS MLG 5.08 reference method for raw beef, through both the method developer and independent laboratory studies required to gain AOAC-RI PTM validation certification.

Methods

SureTect Analysis

The SureTect method was followed as detailed in the product instructions for use to analyse 25g samples of spinach and apple juice and 375g samples of raw ground beef and beef trim. All matrices were enriched in pre-warmed modified Tryptone Soya Broth (mTSB) before processing 10µl of the enrichment through the SureTect lysis protocol. All results were confirmed using both the SureTect confirmation protocol and the confirmation protocols in the relevant reference method.

Reference Methods

Spinach and apple juice were validated in comparison to the ISO 16654:2001 reference method and beef matrices were validated in comparison to the USDA FSIS MLG 5.08 reference method.

Inclusivity And Exclusivity

Fifty-eight different isolates of *E. coli* O157:H7 were analysed at a level of ~10⁴ CFU/ml with the SureTect method according to AOAC-RI PTM requirements. For exclusivity testing, 54 difference organisms were cultured as per AOAC-RI PTM study requirements and analysed with the SureTect method at a level of approximately 10⁸ CFU/ml.

Results

Inclusivity And Exclusivity

All 58 *E. coli* O157:H7 isolates were detected and all but one of the exclusivity isolates remained undetected by the SureTect method. The exception was an *E. coli* O157:NM exclusivity isolate which gave a positive result.

Previous studies³ have shown that O157:NM isolates can possess the H7 gene but not express it. Three other O157:NM isolates all gave PCR negative results.

Food Matrix Analysis

There was no statistically significant difference, between the two methods (figure 1).

FIGURE 1. Method Developer & Independent Laboratory Study Results For The Reference And SureTect Escherichia coli O157:H7 PCR Methods

Matrix/Inoculating Organism	Level	MPN/25g	No. Test portions	ISO	SureTect	
					Presum*	Con*
375g raw ground beef 1:4 ratio 9 h	Low	0.60	20	8	11	10
	High	4.38	5	5	5	5
375g raw ground beef 1:4 ratio 24 h	Low	0.60	20	8	10	10
	High	4.38	5	5	5	5
375g raw ground beef 1:5 ratio 9 h	Low	0.60	20	8	11	11
	High	4.38	5	5	5	5
375g raw ground beef 1:5 ratio 24 h	Low	0.60	20	8	11	11
	High	4.38	5	5	5	5
375g raw beef trim 1:4 ratio 9 h	Low	0.49	20	7	6	7
	High	4.38	5	5	5	5
375g raw beef trim 1:4 ratio 24 h	Low	0.49	20	7	7	7
	High	4.38	5	5	5	5
375g raw beef trim 1:5 ratio 9 h	Low	0.49	20	7	9	9
	High	4.38	5	5	5	5
375g raw beef trim 1:5 ratio 24 h	Low	0.49	20	7	10	9
	High	4.38	5	5	5	5
25g spinach 8 h	Low	0.88	20	11	10	10
	High	0.73	5	5	5	5
25g spinach 24 h	Low	0.88	20	11	10	10
	High	0.73	5	5	5	5
25g apple juice 8 h	Low	2.56	20	18	14	14
	High	4.37	5	5	5	5
25g apple juice 24 h	Low	2.56	20	18	14	14
	High	4.37	5	5	5	5
25g Spinach 8 h Independent lab study	Low	0.28	20	5	3	3
	High	0.58	5	3	3	3
25 g spinach 24 h Independent lab study	Low	0.28	20	5	3	3
	High	0.58	5	3	3	3
375g raw ground beef 1:4 9 h Independent lab study	Low	1.1	20	8	14	14
	High	3.0	5	4	5	5
375g raw ground beef 1:4 24 Independent lab study	Low	1.1	20	8	6	6
	High	3.0	5	4	5	5

* Presumptive (Presum) and Confirmed (Con) SureTect results

Conclusion

The SureTect Escherichia coli O157:H7 PCR Assay was shown to be an accurate method for the detection of *E. coli* O157:H7 from a representative range of raw beef and produce matrices. The assay is designed to be user friendly through the use of pre-dispensed lysis reagent, tableted PCR reagents and automatic interpretation of results.

References

1. Cloke, J., *et al.* Validation Of The Thermo Scientific SureTect Escherichia coli O157:H7 Real-Time PCR Assay for Raw beef and Produce Matrices. Journal of AOAC International. Pending Publication.
2. AOAC International, Method Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces 2012.
3. Wang, G., Clark, C. G and Rodgers, F. G. 2002. Detection in *Escherichia coli* of the Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR. J. Clin. Micro. 40:3613-3619

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