Thermo Scientific SureTect Escherichia coli O157:H7 PCR Assay: NF Validation Using the Applied Biosystems 7500 Fast PCR Instrument

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ABSTRACT

Introduction
The Thermo Scientific™ SureTect™ Escherichia coli O157:H7 PCR Assay is a real-time PCR assay intended for the detection of *Escherichia coli* O157:H7 from raw beef meats (seasoned and unseasoned), which has previously gained NF VALIDATION™ by AFNOR Certification using the Thermo Scientific™ SureTect™ PikoReal™ PCR instrument and Thermo Scientific™ SureTect™ Software version 1.2.

Purpose
The purpose of this study was to conduct an NF VALIDATION by AFNOR Certification extension study to validate use of the SureTect Escherichia coli O157:H7 PCR Assay on the Applied Biosystems™ 7500 Fast PCR Instrument with Applied Biosystems™ RapidFinder™ Express version 2.0 Software (the alternative method) for raw beef meats (seasoned and unseasoned).

Methods
A method comparison study and relative limit of detection (RLOD) study was conducted. For the alternative method, all samples underwent an enrichment step followed by direct lysis. Following direct lysis, PCR was run and results were automatically interpreted by the software. The reference method was conducted according to ISO 16654:2001.

Results
Food samples were tested using the alternative and reference methods. For the RLOD study, *an E. coli* strain from the culture collection at ADRIA Développement was spiked into a raw beef trim matrix and analyzed as per the alternative method protocol and the reference method. The alternative method demonstrated equivalent performance to the reference method. The alternative method RLOD met the acceptability limits for an unpaired study, according to the ISO 16140-2:2016 (LOD for the alternative method shall not be higher than 2.5 time the LOD of the reference method).

Significance
The alternative method proved to be a suitable substitute to reference method for *E. coli* O157:H7 detection.

INTRODUCTION

The SureTect Escherichia coli O157:H7 PCR Assay is a real-time PCR kit for the detection of *E. coli* O157:H7 from raw beef meat samples. The kit combines pre-dispensed lysis reagents and lyophilised and tableted PCR reagents to simplify and improve assay handling, along with software to automatically interpret and display results. This NF VALIDATION by AFNOR Certification ISO 16140 extension study was conducted to extend the use of the SureTect Escherichia coli O157:H7 PCR Assay to the Applied Biosystems 7500 Fast 96-well PCR Instrument with RapidFinder Express v2.0 Software for raw beef meat samples.

MATERIALS AND METHODS

Method comparison study
A total of 60 samples were analyzed as part of the expert laboratory study, which was designed to validate the performance of the alternative method for raw beef meats (seasoned and unseasoned) on the Applied Biosystems 7500 Fast system.

Thirty-three samples were artificially contaminated (by seeding with 11 different isolates of *E. coli* O157:H7). Thirty samples returned a positive result. The remaining three samples failed to return a positive result with either the ISO reference method or the alternative method. The highest inoculum level was 3.2 CFU/25 g.

Relative limit of detection (RLOD) study
For this study, *Escherichia coli* O157:H7 Ad399 was selected from the culture collection at ADRIA Développement and was spiked into a beef trim matrix.

Samples were prepared to give three batches of the matrix which consisted of five samples at 0 CFU/25 g, 20 samples spiked at 0.5-1 CFU/25 g to achieve fractional positive results and 5 samples at 2 CFU/25 g. The samples were analyzed using the reference method detailed in ISO 16654:2001, prior to inoculation in order to verify the absence of *E. coli* O157:H7. After inoculation, samples were analysed using the ISO reference method and the alternative method.

SureTect Escherichia coli O157:H7 PCR Assay method
Twenty-five gram samples of raw beef were homogenized with 225 ml of pre-warmed (41.5±1 °C) Buffered Peptone Water (ISO) (BPW (ISO)). Samples were incubated for up to 24 hours at 41.5±1 °C with samples taken for testing at 8 hours and 24 hours.
Ten microlitres of SureTect Proteinase K Reagent were added to each of the required SureTect Lysis Tubes (supplied prefilled with Lysis Reagent 1). Ten microlitres of enriched samples were added to each of the required number of Lysis Tubes, which were then heated at 37±1 °C for 10 minutes, followed by 95 ±1 °C for 5 minutes. The tubes were cooled at room temperature prior to transferring 20 µl aliquots of the lysates to SureTect PCR Tubes containing SureTect Escherichia coli O157:H7 PCR tablets .
A negative control sample was prepared by adding 10 µl sterile nuclease free water to a SureTect Lysis Tube instead of enriched sample. The negative control then followed the same steps as the enriched samples. Positive PCR results were confirmed by streaking 50 µl of the enrichment onto Cefixime Tellurite Sorbitol MacConkey (CT-SMAC) Agar and then incubated at 37±1 °C for 18 - 24 hours. Typical (presumptive positive) colonies were then confirmed using the Thermo Scientific™ Oxoid™ Escherichia coli O157 Latex Test and the Thermo Scientific™ Remel™ Wellcolex™ Escherichia coli O157:H7 Kit.

ISO reference method
Twenty-five gram samples were analyzed according to ISO 16654:2001. Each sample was enriched by incubating at 41.5±1 °C for a total of 18 - 24 hours in 225 ml of pre-warmed (45±1 °C) Modified Tryptone Soya Broth (mTSB) supplemented with 20 mg/l novobiocin. Immunomagnetic separation (IMS) was performed on 1 ml of the mTSB enrichment after 6 hours of incubation and if no positive result was obtained, after an additional 12 to 18 hours incubation. Fifty microlitres of the resulting suspension were then streaked onto CT-SMAC Agar and CHROMagar™ O157 Agar. Both streaked agar plates were incubated for 18 - 24 hours at 37±1 °C before confirming any typical colonies by biochemical and serological identification tests as detailed in the reference method.

RESULTS

Method comparison study
The SureTect Escherichia coli O157:H7 PCR Assay workflow was shown to be a reliable alternative to the ISO reference method for the detection of *E. coli* O157:H7 from both seasoned and unseasoned raw beef products.

Three negative deviation results were recorded during the method comparison study. The presence of *E. coli* O157:H7 was not confirmed in these samples and the expert lab concluded the negative deviations were probably due to the fact that this study was an unpaired study and that the portion of sample used for the alternative method contained no target cells.
This may also have been the case for the reference ISO method for the one positive deviation that was observed.

The relative sensitivity, specificity and accuracy of the SureTect Escherichia coli O157:H7 PCR Assay method are listed in Table 2.

Relative limit of detection (RLOD) study
The level of detection for the alternative method and the ISO reference method were determined according to the ISO 16140-2:2016 standard (Table 3). The aim was to determine the relative level of detection for both matrices analyzed during the AFNOR Certification validation study.

Figure 1. SureTect Escherichia coli O157:H7 PCR Assay protocol used for all raw beef meat samples using the Applied Biosystems 7500 Fast Instrument

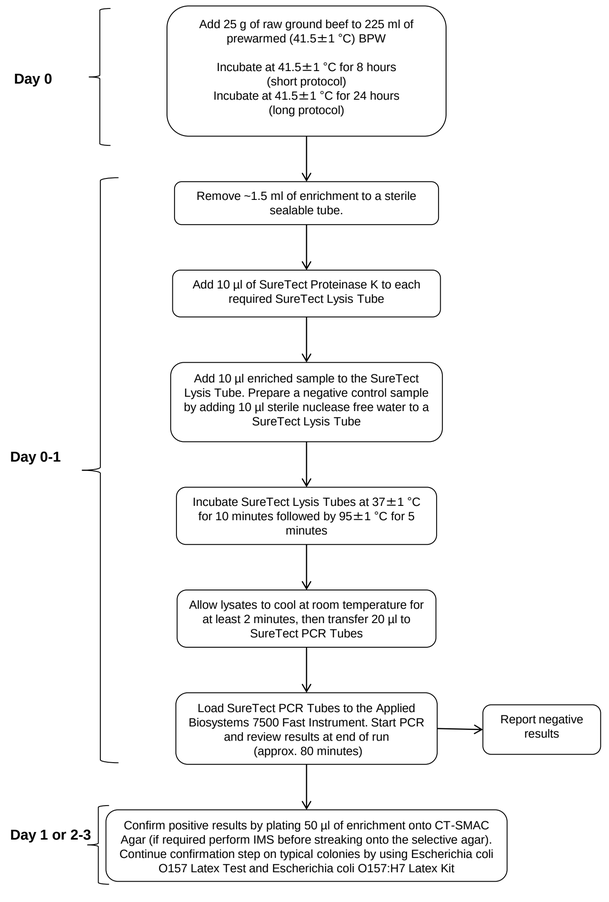

Table 1. SureTect Escherichia coli O157:H7 PCR Assay versus the ISO reference method

SureTect Escherichia coli O157:H7 PCR Assay Method	ISO 16654:2001 reference method		Total
	positive	negative	
8 h positive	26	1	27
8 h negative	3	30	33
18-24 h positive	26	1	27
18-24 h negative	3	30	33

Table 2. Relative sensitivity, specificity and accuracy of the SureTect Escherichia coli O157:H7 PCR Assay

	SureTect Assay methods comparative study for both incubation times (8 h and 18-24 h)
Relative Accuracy	93.3%
Relative Sensitivity	90.0%
False positive ratio	0.0%

Table 3: Relative detection level results for the SureTect Escherichia coli O157:H7 PCR Assay according to ISO 16140-2:2016

Matrix/Strain pairs	Incubation time	Relative level of detection (CFU/25 g)
Beef trim / *E. coli* O157:H7 Ad933	8 hours	1.151 [0.519-2.553]
Beef trim / *E. coli* O157:H7 Ad933	18 -24 hours	1.0 [0.478-2.092]

CONCLUSIONS

The method comparison study conducted as part of this NF VALIDATION extension study demonstrated that the alternative method is equivalent in performance for the food samples analyzed to the ISO reference method detailed in ISO 16654:2001, when using the Applied Biosystems 7500 Fast System and RapidFinder Express v2.0 Software.

The relative level of detection study conducted as part of the NF VALIDATION extension study demonstrated that the level of detection of the SureTect Escherichia coli O157:H7 PCR Assay workflow met the acceptability limits for an unpaired study, as detailed in the ISO 16140-2:2016, using the Applied Biosystems 7500 Fast System with the RapidFinder Express v2.0 Software

REFERENCES

1. ISO 16654:.2001 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of Escherichia coli O157
2. ISO 16140-2:2016. Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

TRADEMARKS/LICENSING

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