Thermo Scientific SureTect Escherichia coli O157:H7 PCR Method: ISO 16140 Validation According To NF VALIDATION From AFNOR Certification

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Overview

Purpose: To validate the Thermo Scientific™ SureTect™ Escherichia coli O157:H7 PCR (PT0400A) method according to the NF VALIDATION™ process and ISO 16140:2003 requirements to gain validation certification for 25g raw beef samples.

Methods: The SureTect method was compared to the reference method detailed in ISO 16654:2001.

Results: The SureTect Escherichia coli O157:H7 PCR method reliably detected the presence of *E. coli* O157:H7 in raw beef and was granted an NF VALIDATION certificate by AFNOR Certification.

Introduction

The SureTect E. coli O157:H7 PCR Assay is a Real-Time PCR kit for the detection of *E. coli* O157:H7 from foods. This study was conducted using the NF VALIDATION approval process according to ISO 16140:2003 to validate the SureTect E. coli O157:H7 method in comparison to the ISO 16654:2001 reference method for 25g raw beef.

Methods

Expert Laboratory Study

An independent laboratory study was conducted to validate the SureTect method in comparison to the reference method detailed in ISO 16654:2001. In this study the following were assessed:

•Inclusivity and exclusivity, using 50 target and 30 non-target organisms.

•Relative limit of detection (RLOD), at incubation times of 8 and 24 hours.

•Relative accuracy, specificity and sensitivity, using 61 naturally contaminated and artificially spiked samples of raw beef (covering fresh, frozen and seasoned and unseasoned types of minced beef and beef trim).

Collaborative Laboratory Study

Fourteen laboratories from various European countries participated in a collaborative study to analyse replicates of a minced beef matrix. Each laboratory was supplied with blind coded samples: 24 samples for analysis with the ISO method, 24 for analysis with the SureTect method and 1 sample for determination of aerobic total viable count. Each set of 24 samples consisted of 8 unspiked, 8 spiked at ~ 2 CFU/25g and 8 spiked ~ 10 CFU/25g.

Results

Inclusivity And Exclusivity

All 50 *E. coli* O157:H7 isolates were detected by the SureTect method. All 30 exclusivity isolates correctly gave negative results with the SureTect method.

Expert Laboratory Study

The paired data from the accuracy study (fig 1 & 2) demonstrated that the SureTect method gave satisfactory results compared to the ISO reference method. The relative limit of detection of the SureTect method (0.5 CFU/25g) was shown to be very similar to that of the reference method (0.4 CFU/25g) (fig 3).

Statistical analysis according to ISO 16140:2003, gave the relative sensitivity and specificity of the SureTect method as 84.6% and 85.7% respectively for the 8 hour incubation point and 92.3% and 85.7% respectively for the 24 hour incubation point.

FIGURE 1. Accuracy Results At 8 h For The SureTect Method Compared To The ISO Reference Method

SureTect Method	ISO 16654:2001 Reference Method		Total
	Positive	Negative	
Positive	22	5	27
Negative	4	30 (PPNA 1)	34
Total	26	35	61

FIGURE 2. Accuracy Results At 24 h For The SureTect Method Compared To The ISO Reference Method

SureTect Method	ISO 16654:2001 Reference Method		Total
	Positive	Negative	
Positive	24	5	29
Negative	2	30	32
Total	26	35	61

FIGURE 3. Relative Detection Levels For The SureTect And ISO Methods According To ISO 16140:2003

Incubation Time	ISO 16654:2001 Reference Method	SureTect Method
8 h	0.456 [0.3-0.6] CFU/25 g	0.559 [0.4-0.7] CFU/25 g
24 h	0.456 [0.3-0.6] CFU/25 g	0.559 [0.4-0.7] CFU/25 g

Collaborative Laboratory Study

Calculation of the relative accuracy from samples analysed during the collaborative laboratory study demonstrated that the SureTect method has satisfactory performance in comparison to the ISO reference method (fig 4), with similar levels of sensitivity and specificity to the reference method (fig 5).

FIGURE 4. Collaborative Study Paired Results For The SureTect And ISO Reference Methods

SureTect Method	ISO 16654:2001 Reference Method		Total
	Positive	Negative	
24 h Positive	136	8	144
24 h Negative	13	83	96
Total	149	91	240

FIGURE 5. Specificity And Sensitivity Results From The Collaborative Study

	ISO 16654:2001 Reference Method	SureTect Method
Specificity	100%	100%
Sensitivity-Level 1 (2.6 CFU/25 g)	86.3%	80%
Sensitivity-Level 2 (11.1 CFU/25 g)	100%	100%
Sensitivity (combined L1+L2)	93.1%	85.3%

Conclusion

The SureTect Escherichia coli O157:H7 PCR Assay method was shown to be an accurate and rapid method for the detection of *E. coli* O157:H7 from 25 g samples of all types of raw beef and was granted validation certification by AFNOR Certification as NF VALIDATION certificate number UNI 03/10-03/15.

References

1. ADRIA Développement. ISO 16140 Validation Study Of The Thermo Scientific SureTect E. coli O157:H7 Assay For Detection Of Escherichia coli O157:H7 In Raw Beef Meats. [Accessed 25th August 2015] http://nf-validation.afnor.org/en/wp-content/uploads/sites/2/2015/04/Synt-UNI-03-10-03-15_en.pdf

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