Study Report

SureTect Escherichia coli O157:H7 PCR Assay Workflow NF VALIDATION ISO 16140: Relative Level of Detection

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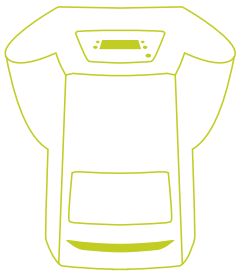

SureTect™ PikoReal™ Real-Time PCR Instrument

Summary

As part of the NF VALIDATION™ ISO 16140 certification of the Thermo Scientific™ SureTect™ Escherichia coli O157:H7 Assay workflow (alternative method) a relative level of detection (RLOD) study was conducted by ADRIA Développement, Quimper, France. The following is a summary of that study.

Methodology

Choice of Strains

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.

Protocol

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.

Alternative Method

Twenty-five gram samples of raw beef were homogenized with 225 ml of pre-warmed (41.5±1 °C) Buffered Peptone Water (BPW) (ISO). Samples were then enriched by incubating for 8 to 24 hours at 41.5±1 °C.

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 each of the required number of enriched samples were added to the 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 E. coli O157:H7 PCR tablets. The PCR Tubes were then immediately sealed and transferred to the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument for processing.

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ISO Reference Method

Twenty-five grams of all samples were analyzed according to ISO 16654:2001. Each sample was enriched by incubating at 41.5±1 °C for 6 and 18 to 24 hours in 225 ml of Modified Tryptone Soya Broth (mTSB) supplemented with 20 mg/l novobiocin. Following enrichment, immunomagnetic separation (IMS) was performed on 1 ml of the mTSB enrichment. Fifty microlitres of the resulting suspension were then streaked onto Cefixime Tellurite Sorbitol MacConkey (CT-SMAC) Agar and CHROMagar™ O157 Agar. Both plates were incubated for 18 to 24 hours at 37±1 °C before confirming presumptive positive colonies by biochemical and serological identification tests as detailed in the reference method.

Results

The levels of detection for the alternative method and the ISO reference method were determined according to the ISO 16140:2003 standard (Table 1) and according to the ISO 16140-2:2016 standard (Table 2). The aim was to determine the relative level of detection for both incubation times of the alternative method workflow analyzed during the AFNOR Certification validation study.

Table 1:Relative detection level results for the ISO reference method and alternative method according to the Spearman-Kärber test, according to ISO 16140:2003

Matrix/Strain pairs	Incubation time	Relative level of detection	
		ISO reference method (CFU/25 g)	Alternative method (CFU/25 g)
Beef trim/*E. coli* O157:H7 Ad933	8 hours	0.456 [0.327-0.634]	0.559 [0.402-0.777]
Beef trim/*E. coli* O157:H7 Ad933	24 hours	0.456 [0.327-0.634]	0.559 [0.402-0.777]

Table 2: Relative detection level results for the alternative method 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	1.542 [0.714-3.331]
Beef trim/*E. coli* O157:H7 Ad933	24 hours	1.362 [0.641-2.891]

Conclusions

The relative level of detection study conducted as part of the NF VALIDATION demonstrated that the level of detection of the SureTect Escherichia coli O157:H7 PCR Assay workflow was similar to that of the ISO reference method, detailed in ISO 16654:2001. When the results were determined following the ISO 16140:2003 standard, the level of detection range for the raw beef trim matrix was 0.327-0.634 CFU/25 g compared to a range of 0.402 – 0.777 CFU/25 g for the alternative method, demonstrating that the SureTect Escherichia coli O157:H7 PCR Assay is an accurate alternative method for the detection of *E. coli* O157:H7 from raw beef. When following the ISO 16140-2:2016 standard, it was determined that the relative level of the alternative method met the acceptability limits for an unpaired study. The NF VALIDATION certificate and the validation report summary for this study are available from **http://nf-validation.afnor.org/en/**.

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