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

Study Report

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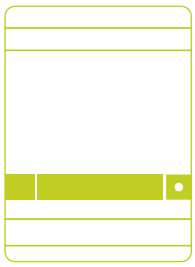
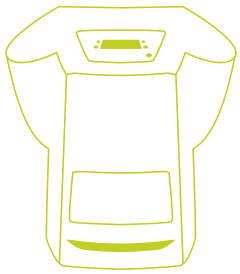


Applied Biosystems™ 7500 Fast Real-Time PCR Instrument



SureTect™ PikoReal™ Real-Time PCR Instrument

Summary

As part of the NF VALIDATION™ ISO 16140 extension study for the Thermo Scientific™ SureTect™ Escherichia coli O157:H7 PCR Assay workflow (alternative method) a relative level of detection (RLOD) study was conducted by ADRIA Développement, Quimper, France. The extension study aims to validate the use of the SureTect Escherichia coli O157:H7 PCR Assay with Applied Biosystems™ 7500 Fast Real-Time PCR System and with the Applied Biosystems™ Rapid Finder™ Express version 2.0 Software against the reference method stated in ISO 16654:2001. 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 raw beef trim matrix.

Protocol

Samples were prepared to give three batches of the matrix which consisted of 5 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.

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Alternative Method:

Twenty-five gram samples of raw beef trim were homogenized with 225 ml of pre-warmed (41.5±1 °C) Buffered Peptone Water (ISO) (BPW (ISO)). Samples were incubated for 8 and 18 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 the 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.

For each run, a negative control sample was prepared by adding 10 µl sterile nuclease free water to a SureTect Lysis Tube and ran through the Applied Biosystems 7500 Fast System.

The PCR Tubes containing the samples and the negative control were then immediately sealed and transferred to the Applied Biosystems 7500 Fast system for processing with the RapidFinder Express v2.0 Software.

ISO Method:

Twenty-five gram samples were analyzed according to ISO 16654:2001. Each sample was enriched by incubating at 41.5±1 °C for 18 to 24 hours in 225 ml of prewarmed (at 41.5±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 additional 12 to 18 hours incubation. 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 level of detection for the alternative method and the ISO reference method were determined according to the ISO 16140-2:2016 standard (Table 1). The aim was to determine the relative level of detection for both incubation times of the alternative method workflow analyzed during the AFNOR Certification extension study.

Table 1: 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	1.151 [0.519-2.553]
Beef trim / *E. coli* O157:H7 Ad933	18-24 hours	1.0 [0.478-2.092]

Conclusions

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. The NF VALIDATION certificate and validation report summary for this study are available from **nf-validation.afnor.org/en/.**

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