



## SureTect™ *E. coli* O157:H7 PCR Assay - AOAC 021501

### SCOPE

This method is applicable to testing of raw beef for screening of *E. coli* O157:H7. All samples identified as potentially positive for *E. coli* O157:H7 using this method must be confirmed using a DAFF approved confirmatory method.

### PRINCIPLES

The Thermo Scientific SureTect *E. coli* O157:H7 is a real-time PCR assay designed for rapid detection of *E. coli* O157:H7. This method is used in conjunction with the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument and RapidFinder Analysis Software; the Applied Biosystems 7500 Fast Real-Time PCR Instrument and RapidFinder Express Software or the Thermo Scientific PikoReal Real-Time PCR Instrument and Software. The assay utilizes dye-labelled probes that target genes unique to *E. coli* O157:H7 and an internal positive control. Target DNA, if present, is detected by real-time PCR. Analysis software provides interpretation of results. The IPC template, primers, and probe provide an internal control with each reaction to show that the PCR process has occurred.

The detection of *E. coli* O157:H7 involves the following steps:

- **Sample enrichment**  
Meat samples (375 g) are diluted 1-in-5 (1500 mL) in pre-warmed ( $41.5 \pm 1^\circ\text{C}$ ) mTSB. The sample is homogenised by stomaching for 30 s to 1 min and is incubated at  $41.5 \pm 1^\circ\text{C}$  for 9-24 hours. The sample and enrichment broth must be at  $41.5 \pm 1^\circ$  for a minimum of 9 hours (i.e. sample and broth must reach  $41.5 \pm 1^\circ\text{C}$  and incubated from this point for a minimum of 9 hours. Frozen or chilled samples may take up to 7 hours to reach  $41.5^\circ\text{C}$ . A positive control culture must be run through all procedures daily or when testing is carried out.
- **PCR screening**  
Genes unique to *E. coli* O157:H7 are screened in the sample following the manufacturer's recommended protocol. PCR positive samples will be considered as potential positive.
- **Interpretation**  
Upon completion of the assay the program will provide a test result. Each test sample will be identified as positive (indicated by a red "+" symbol) or negative (a green "-" symbol). A yellow "!" symbol indicates that the test has failed in which case the test must be repeated using the same enrichment cultures. If the repeat test has failed, instructions outlined in the Troubleshooting section of the User Guide must be followed, and the enrichment broth must be analysed using an alternate method or the sample is considered as a deemed positive.
- **Confirmation**  
For all positive samples and samples with a failed result, enriched broth must be confirmed for the presence of *E. coli* O157:H7 at a DAFF approved confirmatory laboratory using a DAFF approved confirmatory method.

CHECKLIST

<b>Enrichment</b>	Is the mTSB enrichment broth warmed to 41.5 ± 1°C before use?	_____
	Is the correct amount of broth used for the weight of sample analysed i.e. diluted 1-in-5; 375 g and 1500 mL?	_____
	Is a positive control culture run with each batch of samples analysed?	_____
	Are control cultures inoculated into the enrichment broth at a level of 10 to 100 cells?	_____
	Is enrichment carried out at 41.5 ± 1°C and is the enrichment broth and sample at 41.5 ± 1°C for a minimum of 9 hours?	_____
<b>PCR Screening</b>	Are the manufacturer’s instructions reproduced in the laboratory manual and followed without modification?	_____
	Are technicians familiar with and trained in the operation of PCR instruments and Software used in this method?	_____
	Is the shelf-life of media, reagents and kits controlled?	_____
<b>Confirmation</b>	Are screen positive samples confirmed at a DAFF approved laboratory using a DAFF approved confirmatory method?	_____