Australian Government
Department of Agriculture, Water and the Environment

SureTect™ *E. coli* O157:H7 and STEC Screening PCR Assay - AOAC 012102

SCOPE

This method is applicable to testing of raw beef for screening of *E. coli* O157:H7 and top 6 non-O157:H7 STEC. All samples identified as potentially positive for *E. coli* O157:H7 and STEC using this method must be confirmed using a department approved confirmatory method.

PRINCIPLES

The Thermo Scientific SureTect *E. coli* O157:H7 and STEC assays are real-time PCRs designed for rapid detection of *E. coli* O157:H7 and top six STEC (O26, O45, O103, O111, O121 and O145). This method is used in conjunction with the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument and RapidFinder Analysis Software. The Screening PCR Assay includes four different targets (*stx*, *eae*, O157:H7 target 1 and O157:H7 target 2) to identify the presence of *E. coli* O157:H7 and Shiga toxin producing *E. coli.* Both the *stx* and *eae* channels must be positive for a screen positive STEC result. The O157 target 1 and target 2 channels must be positive for a screen positive O157:H7 result, while *stx* and *eae* channels may be positive or negative. The identification PCR Assay differentiates the top 6 STEC serotypes. Both Assays include an internal positive control (IPC) with every reaction. The IPC template, primers, and probe provide an internal control with each reaction to show that the PCR process has occurred. This method is unable to identify O157 serotypes that do not harbour H7 antigen.

The detection of *E. coli* O157:H7 and top 6 STEC involves the following steps:

- **Sample enrichment**
  Meat samples (375 g) are diluted 1-in-5 (1500 mL) in pre-warmed (41.5 ±1°C) mTSB. The sample is homogenized by stomaching for 30 s to 1 min and is incubated at 41.5 ± 1°C for 8-24 hours. The sample and enrichment broth must be at 41.5 ±1° for minimum of 8 hours (i.e. sample and broth must reach 41.5 ±1° and incubated from this point for a minimum of 8 hours. Frozen or chilled samples may take up to 7 hours to reach 41.5°C. A positive control culture must be run through all procedures daily or when testing is carried out.

  E. coli O157:H7 and Shiga toxin genes are detected in the sample using the Screening PCR Assay, following the manufacturer's recommended protocol. PCR positive samples will be considered as potential positives. Identification PCR Assay differentiates the top 6 STEC serotypes (O26, O45, O103, O111, O121 and O145).

- **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 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 and top 6 non-O157 at department approved confirmatory laboratory using a department approved confirmatory method.

CHECKLIST

Enrichment	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 8 hours?	_____
PCR Screening	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 instrument and Software used in this method?	_____
	Is the shelf-life of media, reagents and kits controlled?	_____
Confirmation	Are screen positive samples confirmed at a department approved laboratory using a department approved confirmatory method?	_____