

IEH *E. coli* Test System for detection of non-O157 Shiga-toxin producing *E. coli* and *E. coli* O157 in raw ground beef – AOAC 0100701

SCOPE

This method is an expansion of the IEH *E. coli* O157, *Stx*-Producing *E. coli* (STEC) with Intimin and *Salmonella* Test System (AOAC 100701). This method is applicable for detection of O157 and pathogenic STEC serogroups (O26, O45, O103, O111, O121 and O145) in raw beef.

PRINCIPLES

The IEH *E. coli* Test System rapidly amplifies specific DNA fragments unique to *E. coli* O157 and the top 6 STEC serogroups using polymerase chain reaction (PCR). Initially the organisms are allowed to grow in IEH enrichment medium followed by PCR assays for the presence of the *eae* and *stx* genes common to pathogenic STECs. Genes unique to specific STEC serotypes (ie wzx) can be run at this stage or later in the assay. Samples that produce an initial reactive result (potential positive) undergo a further three PCR assays for confirmation of O157 and STEC serogroups. All three PCR assays are run with and without immuno-magnetic separation. Samples identified as positive using this test must be confirmed using the USDA-FSIS method.

Pre-enrichment

Raw beef samples (375 g) are diluted in 750 mL of pre-warmed IEH enrichment medium and incubated at 42± 1°C for 9-48 h (broth and sample must be at 42 °C for a minimum of 9 h). Each sample will contain one internal amplification positive control per reaction. A positive control culture must be run through the enrichment and initial screening procedure daily or when testing is carried out.

Initial Screening

Enriched samples are screened for the presence of STEC using the IEH *E. coli* O157, *Stx*-Producing *E. coli* (STEC) with Intimin and *Salmonella* Test System using PCR buffers ES6 and STP (STP may be run later during confirmation).

Samples that are negative after the initial screen are reported as negative.

Immuno-magnetic Separation (IMS)

Samples that are potential positive's after screening are run through the IMS procedure. Big 6 STEC serogroups and 0157 are separated and concentrated from the enrichment broth using magnetic beads (manufactured by IEH) coated with antibodies specific to 0157, 026, 045, 0103, 0111, 0121 and 0145.

Molecular Confirmation

Positive samples are re-analysed using the original screening PCR buffer (ES6) and two supplemental PCR buffers (EC7 and STP) with and without IMS. PCR positive samples will be considered as potential positives.

Confirmation

Confirmation must be carried out as per USDA-FSIS or equivalent methods for sample preenrichments that test IEH potential positive, indeterminate, or have an invalid result. Or, the laboratory may review the cause of the indeterminate or invalid result and based on the findings re-analyse the sample by:

- Repeating the IEH Test System analysis using the primary enrichment broth.
- Screening using a department approved method

Confirmation must be carried out at a department approved laboratory.

| CHECKLIST | | |
|--------------------|---|--|
| Pre- enrichment | Is IEH enrichment medium pre-warmed at 42°C before use? | |
| | Is enrichment carried out at 42°C for 9-48 h (broth and sample at 42 °C for a minimum of 9 h)? | |
| | Is the correct amount of enrichment medium used (ie 750 ml)? | |
| | Is a positive control culture run with each batch of samples? | |
| | Is the control culture inoculated into the primary enrichment broth at a level of 10 to 100 cells? | |
| | Is initial screening carred out for stx and eae (note if STP is also run at initial screening to identify specific serogroups)? | |
| | Are <i>E. coli</i> serogroups separated and concentrated from the enrichment broth using IMS? | |
| Screening | Are internal amplification controls run with each batch of samples? | |
| | Are PCR target controls available and how often are they run? | |
| | Are technicians familiar with and trained in the operation of the IEH Automated System? | |
| | Is the shelf-life of media and kits controlled? | |
| Confirmation | Is confirmation carried out from the enrichment culture? | |
| | Is isolation carried out at a department approved laboratory using a department approved method? | |