BAC*Gene E. coli* O157:H7 Workflow – AOAC 022002

SCOPE

This method is applicable to raw ground beef, raw beef trim, and other selected foods. This method has been validated in 375 g of raw ground beef and beef trim samples.

PRINCIPLES

The BAC*Gene E. coli* O157:H7 workflow is a qualitative real-time PCR assay that utilizes BAC*Gene E. coli* O157:H7 and BAC*Gene* Mplex STEC Screen test kits for detection of *E. coli* O157:H7. Target DNA fragments when detected are amplified and then identified with a fluorescence-labelled hybridization probe.

Detection of *E. coli* O157:H7 involves the follow steps:

- **Enrichment**
  Sample (375 g) is enriched in 750 mL (1:2) of pre-warmed (to 41.5±1°C) mTSB. Sample and enrichment media are placed in a stomacher bag fitted with a side filter and homogenized by hand massage or using a stomacher. Incubation is carried out at 41.5 ± 1°C for 10 - 24 h. It is essential that the temperature of the broth and sample is at 41.5±1°C for a minimum of 10 h. A positive and a negative control culture must be run through all procedures daily or when testing is carried out.

- **PCR Assay**
  Sample preparation for bacterial DNA extraction and BAC*Gene E. coli* O157:H7 PCR assay is carried out following the manufacturer's recommended protocol. Samples that return an O157 positive (H7[1] negative or positive) must be tested using the BAC*Gene* Mplex STEC Screen PCR assay for confirmation of the Shiga toxin encoding genes *stx1*/*stx2* and *eae* as per the manufacturer's recommended protocol.

- **Interpretation**
  Samples that are positive for O157 by BAC*Gene E. coli* O157:H7 assay and positive for stx1/stx2 and eae genes by BAC*Gene* Mplex STEC Screen PCR assay are regarded as potential positive and must undergo confirmation.

  If the internal positive control is invalid in negative samples, the test must be repeated using the same enrichment cultures. If the internal positive control for the re-test sample is still invalid, the enrichment sample should be diluted 1:5 and 1:10 in nuclease-free water and re-tested in PCR. If inhibition remains in the diluted samples the equipment supplier must be contacted for advice, and the enrichment broth must be analysed using an alternate method or the sample deemed positive for *E. coli* O157:H7.

- **Confirmation of positive results**
  For all positive samples and negative samples with an invalid positive control result, enriched media must be analysed using a department approved confirmatory method. Confirmation must be carried out at a Department approved laboratory.

[1] Testing for the H7 gene/antigen is not required

CHECKLIST

Enrichment	Is the enrichment media pre-warmed to 41.5 ± 1°C before use?	
	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 10 h?	
	Is the correct amount of enrichment broth used?	
	Is a positive and a negative control run with each batch of samples/daily?	
	Are reference cultures inoculated into enrichment media at a level of 10-100 cells per sample?	
PCR Assay	Are manufacturer´s instructions followed in regards of cross-contamination prevention?	
	Are internal controls run with each batch of samples?	
	Are technicians familiar with and trained in the operation of PCR automated instruments and the associated software?	
	Are O157 positive samples further analysed by BAC*Gene* Mplex STEC Screen PCR assay?	
	Is the shelf-life of media and kits controlled?	
Confirmation	Is confirmation carried out from the enrichment culture (mTSB)?	
	Is confirmation carried out using a department approved confirmatory method at a department approved laboratory?	