BAC*Gene* STEC Top 7 Workflow – AOAC 022003

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* STEC Top 7 workflow is a qualitative real-time PCR assay that utilizes a BAC*Gene* Mplex STEC Screen, followed by BAC*Gene E. coli* O157:H7 and BAC*Gene* Mplex SERO*type1* and BACGene Mplex SERO*type2* test kits for detection of *E. coli* O157, O26, O45, O103, O111, O121 and O145.

Detection of Top 7 STEC 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 PCR assays are carried out following the manufacturer's recommended protocol.

  Upon completion of the assay the program will provide a test result. Each test sample will be identified as screen positive, indicating that the test sample is positive for *stx*1/*stx*2 and *eae* or negative indicating that the test sample is negative for *stx*1/*stx*2 or *eae*. Samples that are positive for *stx*1/*stx*2 and eae are to be analysed by serogroup specific PCR assays or must undergo confirmation for all top 7 STEC

- **Interpretation**
  Screen positive samples are to be further analysed by BAC*Gene E. coli* O157:H7 and BAC*Gene* Mplex STEC SERO*type* PCR assays. Upon completion of the BAC*Gene E. coli* O157:H7 assay the program will provide a test result. Samples that are O157 positive (negative or positive for H7[1]) are regarded as potential positive for *E. coli* O157 and must undergo confirmation. Samples that are positive for one or more of the top 6 serogroups by BAC*Gene* STEC SERO*type* assays are regarded as potential positive for top 6 non-O157 STEC 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 invalid, the lysate should be diluted in nuclease-free water 1:5 and 1:10 and tested in PCR. If inhibition remains also 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 STEC.

- **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 methods. 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 regard to 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 screen positive samples further analysed by serogroup specific PCR assays?	
	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 methods at a department approved laboratory?	