Australian Government
Department of Agriculture

FSIS Procedure for the use of *Escherichia coli* O157:H7 Screening Tests for Meat and Meat Products - MLG 5A.04

SCOPE

This method is applicable to the analysis of raw ground beef, beef trim, fermented sausages and cooked meat patties.

PRINCIPLES

This method uses a commercial PCR screening procedure; the BAX System Real-time PCR Assay for *E. coli* O157:H7. All samples identified as potentially positive for *E. coli* O157:H7 using this test must be confirmed using ISO 16654:2001, MLG 5 or FDA BAM Chapter 4A using the IMS option.

- **Pre-enrichment**
  Samples are prepared with a 1:4 ratio of product and enrichment broth (i.e. 325 ± 32.5 g sample with 975 ± 19.5 mL mTSB[1]), stomached and incubated at 42 ± 1°C for 15-22 h.

- **BAX System Real-time PCR Assay for *E. coli* O157:H7**
  Perform screening test using 20 µL of enriched sample. Follow the current BAX System User's Guide for preparing reagents, performing PCR test and reading the results. The BAX *E. coli* O157 test is an automated method that uses polymerase chain reaction (PCR) technology for the detection of *E. coli* O157 in meat. Initial screening is for *eae*/*stx* genes. DNA is combined with DNA polymerase, nucleotides, and primers. The mixture then undergoes a series of timed heating and cooling cycles. Heating denatures the DNA, separating it into single strands. As the mixture cools, the primers recognise and bind to the targeted DNA sequences. The DNA polymerase then uses the nucleotides to extend the primers, thus creating 2 copies of the targeted DNA fragment; repeating the cycle results in an exponential increase in the number of target DNA fragments. A fluorescent dye then binds with double-strand DNA and emits a fluorescent signal in response to light. After amplification, the BAX System begins a detection phase in which the fluorescent signal is measured.

- **Confirmation**
  BAX negative samples are reported as negative. In all cases of BAX-positive, BAX-indeterminate or a BAX-signal-error the mTSB culture must be tested using ISO 16654:2001, FSIS MLG 5 or FDA BAM Chapter 4A (isolation must include an Immunomagnetic Separation) step. Confirmation must be carried out at a department approved laboratory.

[1] Tryptone Soya Broth (Oxoid # CM0989B or current) 33.0 g; Casaminoacids (casein acid hydrolysate) 10.0 g; Sterile water 1.0 L. Rehydrate by stirring, then autoclave 20 min at 121°C.. Final pH 7.4 ±0.2 at 25°C.

CHECKLIST

Pre-enrichment	Is the sample enriched in mTSB?	
	Is enrichment carried out at 42 ± 1°C for 15-22 h?	
	Are positive culture and/or DNA controls run with each batch of samples?	
	Are reference cultures inoculated into primary enrichment broth at a level of 10 to 100 cells?	
BAX screening	Are manufacturer's instructions available for reference?	
Isolation	Is isolation carried out at a department approved laboratory using a department approved method?	
Confirmation	Is confirmation carried out at a department approved laboratory?	
	Are colonies confirmed using appropriate biochemical and serological tests?	
	Are tests for toxin or toxin genes carried out?	