Australian Government
Department of Agriculture and Water Resources

Assurance GDS® MPX ID for Top 6 STEC – AOAC 101502

SCOPE

This method is applicable for detection of Top 6 Shiga toxin-producing *E. coli* (O26, O45, O103, O111, O121 and O145) in beef trim as a secondary screening method following a positive result using the Assurance GDS® MPX Top 7 STEC assay (AOAC 071301).

PRINCIPLES

GDS® MPX ID is designed to detect Top 6 Shiga toxin-producing non-O157 STEC and is used in conjunction with the GDS MPX Top 7 STEC assay. Initially samples are analysed by the GDS® MPX Top 7 STEC assay (AOAC 071301). Any positive sample is analysed by GDS MPX ID, which utilizes a proprietary immunomagnetic separation (IMS)-based procedure to capture Top 6 STEC followed by detection of specific genes using multiplex PCR assays.

Detection of target STEC involves the follow steps:

- **Enrichment**
  Samples (375 g) are diluted in 1.5 L of pre-warmed (42°C) mEHEC medium. Incubation is carried out for 10 h at 42°C. It is essential that the temperature of the broth and sample is at 42 ± 1 °C for a minimum of 10h. A positive control culture (i.e. *E. coli* O157 *stx* negative control) must be run through all procedures daily or when testing is carried out.

- **Top 7 STEC assay (AOAC 071301)**
  Samples are analysed by the Assurance GDS® MPX Top 7 STEC assay (AOAC 071301) as per manufacturer's instructions. Samples undergo proprietary IMS followed by GDS® MPX Top 7 STEC PCR. All screen positive samples must be confirmed for *E. coli* O157:H7 by the MLG 5 method. Prior to confirmation by the relevant FSIS MLG method, Top 6 non-O157 STEC screen positive samples can be individually identified by GDS® MPX ID for Top 6 non-O157 STEC.

- **Separation and concentration**
  Top 6 STEC are separated and concentrated from the enrichment broth using proprietary IMS-based sample preparation procedure to capture O26, O45, O103, O111, O121 and O145 prior to GDS® MPX ID PCR assay.

- **PCR Confirmation**
  Screened samples, identified as positive, are analysed for Top 6 serogroups using GDS Group 1 and Group 2 STEC PCR following the manufacturer's recommended protocol. Upon completion of the assay the GDS® Rotor-Gene program will provide results for the presence of individual Top 6 STEC. Each test sample will be identified as positive, indicating that the test sample is positive for pathogenic STEC, negative indicating that the test sample is negative for pathogenic STEC, or "No Amp" indicating that amplification did not occur.

  A "No Amp" reading may be due to reagent or test failure or operator error. In this event the test must be repeated using the same enrichment cultures. If the result continues to show "No Amp" the equipment supplier must be contacted for technical services. In this case, the enrichment broth must be analysed using an alternate method or the product deem positive for pathogenic STEC for disposition purposes

- **Confirmation**
  "Positive" or "No Amp" samples must be confirmed by the relevant FSIS MLG method at a department approved confirmatory laboratory or the product deemed positive for one of the indentified STEC for the purposes of disposition.

CHECKLIST

Enrichment	Is the sample enriched in mEHEC medium pre-warmed at 42°C before use?	_____
	Is enrichment carried out 42°C and is the enrichment broth and sample at 42 °C for a minimum of for 10 h?	_____
	Is the correct amount of enrichment broth used (i.e. 1.5L mEHEC broth)?	_____
	Is a positive control run with each batch of samples/daily?	_____
	Are reference cultures inoculated into enrichment media at a level of 10-100 cells/mL?	_____
	Are the enriched samples also analysed for *E. coli* O157:H7 using Assurance GDS O157 (AOAC 2005.04)?	_____
Assay	Do screen positive samples undergo IMS prior to GDS MPX ID Group 1 and Group 2 STEC PCR assays?	_____
	Are manufacturer's instructions available for reference?	_____
	Are internal controls run with each batch of samples?	_____
	Are technicians familiar with and trained in the operation of the GDS® MPX Automated System and the Rotor-Gene Program?	_____
	Is the shelf-life of media and kits controlled?	_____
Confirmation	Is confirmation carried out using an approved method at a department approved laboratory?	_____
	Is screen positive sample (AOAC 071301) confirmed for *E. coli* O157 by MLG 5?	_____
	Is confirmation carried out from the enrichment culture using IMS?	_____