# 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 ±37.5 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 a DAFF approved confirmatory method. Prior to confirmation, 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 deemed positive for pathogenic STEC for disposition purposes

### Confirmation

Positive samples must be confirmed by a DAFF approved confirmatory method at a department approved confirmatory laboratory or the product deemed positive for one of the indentified STEC for the purposes of disposition.

## CHECKLIST

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| **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 10 h? |   |
|  | Is the correct amount of enrichment broth used? |   |
|  | 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 a DAFF approved confirmatory method? |   |
|  | Is confirmation carried out from the enrichment culture using IMS? |   |