



GENE-UP® EHEC Detection Method – AOAC 2020.06

SCOPE

This method is applicable for testing of raw ground beef and raw beef trim and some selected foods for *E. coli* (O157, O26, O45, O103, O111, O121 and O145). Standard method (AOAC 2020.06) must be followed without any modification.

PRINCIPLES

The GENE-UP® EHEC Detection Method is a qualitative real-time PCR assay. DNA is extracted using an automated process on the VIDAS or through bead beating using the GENE-UP Lysis Test Kit. DNA is analysed for EHEC virulence factors using the GENE-UP® STEC-*stx* & *eae* 2 assay, followed by GENE-UP® STEC Top 6 and *E. coli* O157:H7 2 PCR assays. The GENE-UP® Thermocycler is utilised to detect fluorescence at several wavelengths to allow for multi-target detections.

Detection of STEC involves the follow steps:

- **Enrichment**
Sample (375 g) is enriched in 1,125 mL of pre-warmed (to 41.5±1°C) buffered peptone water (BPW). Sample and enrichment media are placed in a stomacher bag and homogenised 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.
- **Immuno-concentration¹**
Immuno-concentration is to be carried out using the VIDAS® ESPT or using the bead beating with the GENE-UP® Lysis Kit as per the manufacturer's recommended protocol.
- **PCR Assays²**
Sample preparation for bacterial DNA extraction and PCR assays is carried out following the manufacturer's recommended protocol. PCRs are to be carried out separately: GENE-UP® STEC – *stx* & *eae* (EH1 2) for detection of *stx/eae* genes; if target genes are detected then GENE-UP® *E. coli* O157:H7 (ECO 2) and GENE-UP® STEC Top 6 (EH2) are to be performed for the detection of O157:H7 and top 6 non-O157 serogroups, respectively. Samples negative for these seven serogroups are considered negative for *E. coli* O157:H7 and non-O157 STEC.

Samples with a positive result are regarded as potential positives and must be confirmed. Inhibited samples must be retested as per the standard method. In the case of an inhibited result, the test must be repeated using the same enrichment cultures. If the re-test sample returns a further inhibited result, the equipment supplier must be contacted for advice, and the enrichment broth must be analysed using an alternate method or the sample deemed positive.

¹ Kit # 30229 for VIDAS UP *E. coli* Serogroups (ESPT) or Kit # 414057 for GENE-UP Lysis Kit must be used

² Kit # 423109 for GENE-UP STEC – *stx* & *eae* (EH1 2); Kit # 423108 for GENE-UP *E. coli* O157:H7 (ECO 2) and Kit # 414154 for GENE-UP STEC Top 6 (EH2) must be used.

- **Confirmation with VIDAS ESPT Kit**

a) Concentration is carried out by VIDAS® ESPT2 test and a concentrated sample (30 µL) is to be transferred on to:

- CHROMID® EHEC agar - streak and incubate for 20-24 h at 37 ±1°C.
- SMAC CT agar if *E. coli* O157:H7 is suspected - streak and incubate for 18-24 h at 37 ±1°C
- CHROMID® Coli agar if non-O157 is suspected - streak and incubate 22-26 h at 37 ±1°C

- **Confirmation by Direct Streaking**

b) Enrichment broth is to be directly plated (10µL) on to:

- Supplemented CHROMID® EHEC agar (supplemented with cefixime-tellurite³) - streak and incubate for 20-24 h at 37 ±1°C.
- SMAC CT agar (if *E. coli* O157:H7 is suspected) - streak and incubate for 18-24 h at 37 ±1°C
- CHROMID® Coli agar (if non-O157 is suspected) - streak and incubate 22-26 h at 37 ±1°C

- **Confirmation of isolated colonies**

Between one and five typical colonies are to be selected from each plate and tested for target serogroups either by latex test (SLIDEX *E. coli*, targeted serogroups) or by serogroup specific PCR (GENE-UP® STEC Top 6 and/or GENE-UP® *E. coli* O157:H7 2), followed by performing EHEC gene specific PCR (GENE-UP® STEC-*stx* & *eae* 2).

A positive result is to be reported as confirmed positive.

Discordant results must be tested by diluting 0.1 mL enrichment broth into 9 mL BPW, incubated at 37 ±1°C for 4 – 24 h, followed by repeating the confirmation procedure from the beginning.

(Optional) Positive enrichment broth can also be confirmed by MLG 5/MLG 5B or MLG 5C. In such case confirmation must be carried out at a DAWE approved laboratory.

³ Cefixime-tellurite mixture - Ref. 42606

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?	_____
Immuno-concentration	Which method is utilized for concentration/DNA extraction? VIDAS® ESPT or GENE-Up Lysis Kit?	_____
	Are manufacturers' instructions followed?	_____
PCR Assay	Are correct kits used for different STEC serogroups?	_____
	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 PCR automated instruments and the associated software?	_____
	Is the shelf-life of media and kits controlled?	_____
Confirmation	Is confirmation carried out from the enrichment culture (BPW)?	_____
	Is VIDAS® ESPT2 Kit or direct plating methods carried out in the confirmation step?	_____
	Are 1-5 typical colonies run through GENE UP® STEC top 6 and GENE-UP® E. coli O157:H7 2 assay or latex agglutination test?	_____
	If positive, are STEC genes verified by GENE-UP® STEC- <i>stx</i> & <i>eae</i> PCR assay?	_____
	Is a discordant result (if any) retested and reconfirmed?	_____
	Is confirmation carried out using MLG 5/MLG 5B or MLG 5C at a DAWE approved laboratory?	_____