# 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 STECinvolves the follow steps:

### Enrichment

Sample (375 ±37.5 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-concentration[[1]](#footnote-1)

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 Assays[[2]](#footnote-2)

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.

### Confirmation with VIDAS ESPT Kit

1. 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

1. Enrichment broth is to be directly plated (10µL) on to:
* Supplemented CHROMID® EHEC agar (supplemented with cefixime-tellurite[[3]](#footnote-3)) - 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.

**Note:** Where the GENE-UP method is used for screening only, confirmation testing must occur at a DAFF approved confirmatory laboratory using a DAFF approved confirmatory method.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-*stx* & *eae* PCR assay? |   |
|  | Is a discordant result (if any) retested and reconfirmed? |   |
|  | If confirmation testing is not conducted using GENE-UP methodology, is confirmation conducted at a DAFF approved confirmatory laboratory using a DAFF approved confirmatory method? |   |

1. Kit # 30229 for VIDAS UP *E. coli* Serogroups (ESPT) or Kit # 414057 for GENE-UP Lysis Kit must be used [↑](#footnote-ref-1)
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. [↑](#footnote-ref-2)
3. Cefixime-tellurite mixture - Ref. 42606 [↑](#footnote-ref-3)