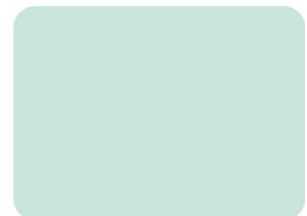
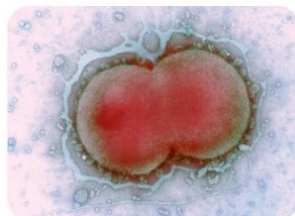
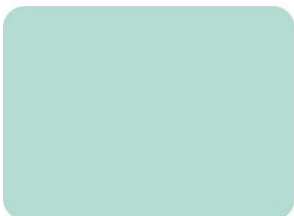
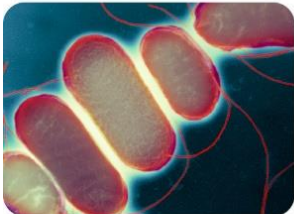
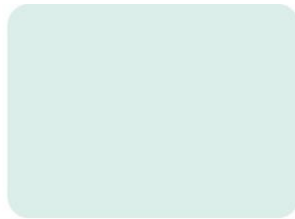
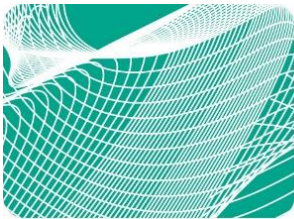




UK Health
Security
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UK Standards for Microbiology Investigations

Identification of Shiga toxin-producing *Escherichia coli* (STEC) using conventional methods



National Institute for Health and Care Excellence (NICE) has renewed accreditation of the process used by the **UK Health Security Agency** to produce **UK Standards for Microbiology Investigations (UK SMIs)**. The renewed accreditation is valid until **30 June 2026** and applies to guidance produced using the processes described in '**UK Standards for Microbiology Investigations Development Process**' (2021). The original accreditation term began on 1 July 2011.

Acknowledgments

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

UK SMIs are produced in association with:



Displayed logos correct as of March 2021

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Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	7/16.05.2023
Issue number discarded	4
Insert issue number	5
Anticipated next review date*	16.05.2026
Section(s) involved	Amendment
All	Whole document has been placed into a new template
Title	Changed from 'Identification of vero toxin-producing <i>Escherichia coli</i> including <i>Escherichia coli</i> O157' to 'Identification of Shiga toxin-producing <i>Escherichia coli</i> (STEC) using conventional methods.
Introduction	Additional information added about STEC
Technical limitations	<i>Escherichia hermannii</i> has been reclassified to <i>Atlantibacter hermannii</i>
Algorithm	Updated to include <i>E. coli</i> O26

*Reviews can be extended up to 5 years where appropriate

1 General information

[View general information](#) related to UK SMIs.

2 Scientific information

[View scientific information](#) related to UK SMIs.

3 Scope of document

This UK Standards for Microbiology Investigations (UK SMI) document describes the presumptive identification of Shiga toxin-producing *Escherichia coli* (STEC) isolated from faeces. These strains are associated with a wide spectrum of disease including haemolytic uraemic syndrome (HUS).

This UK SMI includes culture methods and biochemical tests. Some biochemical tests may not be performed routinely in laboratories except in cases where confirmation by an alternative technique is required or automated methods are not available.

Laboratories are implementing rapid techniques and other molecular methods for the identification of STEC.

UK SMIs should be used in conjunction with other relevant UK SMIs.

4 Introduction

Shiga toxin-producing *E. coli* (STEC) also known as verocytotoxin producing *E. coli* (VTEC) are a group of zoonotic, foodborne pathogenic *E. coli* characterised by the presence of the Shiga toxin gene (*stx*). STEC can produce *stx1* (4 subtypes 1a–1d) and *stx2* (7 subtypes *stx2a*–2g). The presence of *stx2* (specifically *stx2a*) is more likely to cause HUS (1,2).

STEC can cause gastrointestinal symptoms including diarrhoea (often blood-stained), abdominal pain, nausea and vomiting. Illness can start from 1 to 10 days after exposure. Most people start feeling sick 3 to 4 days after exposure to the infecting organism. Most people get better within 5 to 7 days. Some infections are very mild, but others are severe or even life-threatening (3). Following the initial infection, a subset of patients develop HUS, a severe systemic condition that affects the kidneys (2,3).

There are 4 other clinically important pathotypes of *E. coli* that cause gastrointestinal symptoms:

- enterotoxigenic *E. coli* (ETEC) are important causes of diarrhoea in both humans and domestic animals. Infections are seen in individuals returning from regions of endemicity and accounts for 20% to 40% of traveller's diarrhoea (5)
- enteropathogenic *E. coli* (EPEC) are associated with persistent diarrhoea in young children. Close contact such as in day care facilities and poor hygiene, increases the risk of transmission (5)

- enteroaggregative *E. coli* (EAEC) infection is greatest in children living in endemic areas
- enteroinvasive *E. coli* (EIEC) are very similar to *Shigella*. They are capable of invading and multiplying in the intestinal epithelial cells of the distal large bowel in humans

Ruminants are the main animal reservoir for STEC. In the UK, a high proportion of cattle and sheep are colonised with STEC. Small mammals, birds and domestic pets can act as transient carriers. Transmission to humans can occur via direct contact with animals or their environment, and by consumption of contaminated food or water. Outbreaks of STEC infection are common and exposure risks include activities in rural settings, visiting petting farms, consumption of contaminated unpasteurised dairy products, undercooked meat and ready-to-eat produce (2,3).

STEC has a low infectious dose (6) and can spread by person-to-person contact, especially within households and schools. Once symptoms resolve, some people shed STEC in their faeces for weeks following infection. To mitigate the risk of transmission, children aged 5 years old and under, and all those in risk groups (for example food handlers, carers of the elderly, immunocompromised and those with underlying health conditions) are excluded from work, school and childcare settings until they are microbiologically clear (4).

STEC was identified as a cause of HUS in the 1980s and early outbreaks of STEC-HUS in the UK were linked to infection with STEC serotype O157:H7. Consequently, laboratory protocols focused on the detection and identification of this specific serotype in faecal specimens (7,8). Unlike the majority of *E. coli*, STEC O157:H7 do not ferment sorbitol and this characteristic is used to identify STEC on selective media.

As laboratories implement gastrointestinal diagnostics using polymerase chain reaction (PCR), there has been an increase in the detection of non-O157 STEC. In 2019, 768 culture-positive non-O157 STEC cases were reported (655 in England, 113 in Wales). Between 2009 and 2013, only 89 cases reported prior to PCR being implemented (2).

In England, the 5 most common non-O157 serogroups isolated in 2019 were O26 (109/655, 17%) followed by O146 (98/655, 15%), O91 (81/655, 12%), O128ab (37/655, 6%) and O103 (28/655, 4%) (2).

Public health surveillance systems in Scotland identified an increase in the number of reported infections of STEC O26:H11 involving bloody diarrhoea, linked to the consumption of prepacked sandwiches (9).

Accurate and rapid diagnosis of STEC infections is important for the appropriate management of infected patients and for implementation of proper public health intervention.

5 Technical information and limitations

Commercial identification systems

Laboratories should follow manufacturer's instructions when using these kits. It is essential that all commercial kits have evidence of adequate validation, demonstrating that they are fit for purpose. It is also essential that appropriate on-going quality assurance procedures are in place.

Some commercial biochemical tests may give a doubtful or a low percentage profile for *E. coli* O157 because the fermentation of sorbitol is heavily weighted for the identification of *E. coli* strains and therefore care must be taken with the interpretation of the profile. There is also a possibility that organisms may be misidentified because of similar phenotypic and genetic feature. For example, *E. albertii* can be identified as *E. coli*. Hence, all presumptive *E. coli* O157 from human and non-human sources should be referred to the appropriate specialist laboratories for confirmation.

Agglutination test

Atlantibacter hermannii, (10) previously known as *Escherichia hermannii*, is sorbitol negative, cross-reacts and agglutinates in *E. coli* serotype O157 antiserum, and thus it may be mistaken for *E. coli* O157.

Culture methods

The use of the commercial latex screen in conjunction with a combination of cultures on sorbitol MacConkey agar (SMAC) or cefixime-tellurite sorbitol (CTSMAC) agar should prove to be useful for rapid detection of *E. coli* serotype O157 (11).

Culture methods can be time consuming and have limited sensitivity for STEC detection. Other approaches should be considered where possible. Molecular methods have a high sensitivity and specificity for all STEC serotypes (12).

Matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF MS) mass spectrometry

Numerous reports have described the difficulty encountered when trying to differentiate *E. coli* from *Shigella* species. Further research is needed to adapt MALDI-TOF MS technology to be suitable for strain-specific identifications of *E. coli* isolates (13,14).

6 Safety considerations

The section covers specific safety considerations (15-36) related to this UK SMI, and should be read in conjunction with the general [safety considerations on GOV.UK](#).

STEC O157 is a Hazard Group 3 organism.

All work with suspected isolates of STEC O157 must be performed under Containment level 3 conditions.

STEC O157 is highly virulent and the infectious dose is low, possibly less than 100 organisms (6). STEC O157 may cause severe illness that is sometimes fatal. Laboratory acquired infections have been reported (37-39). However, currently there is no vaccine available in the UK or licensed for use for *E. coli* O157.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

7 Target organisms

E. coli O157:H7 and *E. coli* O157: NM (non-motile) also known as *E. coli* O157:H negative and other non-O157 serogroups O26, O45, O103, O111, O121 and O145.

Other *E. coli* serotypes such as O145 can act such as O157:H7 if they acquire the ability to produce Shiga toxin. These other *E. coli* serotypes can cause outbreaks of haemorrhagic colitis resulting in bloody diarrhoea that can become complicated by HUS.

Methods for the detection of STEC may result in the identification of isolates of presumptive *E. coli* O157 that do not produce Shiga toxin and some organisms that give equivocal results.

8 Identification

8.1 Microscopic appearance

Gram stain ([TP 39 – Staining procedures](#)) if required

STEC are identified as Gram negative rods.

8.2 Primary isolation media

STEC O157:H7 do not ferment sorbitol and this characteristic is used to identify presumptive isolates of STEC on selective media CTSMAC. Non-sorbitol fermenting colonies are then agglutinated with antisera to the O157 antigen. Positive colonies are submitted to the appropriate reference laboratory for confirmation and typing.

Some STEC O157 strains have been found to ferment sorbitol and to be β -glucuronidase positive.

Primary isolation media includes:

- CTSMAC agar incubated in air at 35 to 37°C for 16 to 24 hour. CTSMAC agar is used since classical sorbitol non-fermenting STEC O157 are relatively resistant to potassium tellurite compared with other *E. coli*. There is a risk of isolation failure on SMAC agar lacking cefixime and tellurite

- chromogenic selective agar, which can be used for non-O157 serogroups O26, O45, O103, O111, O121 and O145 (40)
- enrichment culture may be required in cases of outbreaks

Where the clinical evidence is suggestive of STEC infection and no presumptive sorbitol non-fermenting *E. coli* O157 colonies are observed on SMAC or CTSMAC agar, clinical laboratories should:

- test sorbitol fermenting colonies for agglutination with *E. coli* O157 antiserum
- confirm the identification of agglutination positive O157 colonies as *E. coli*
- send presumptive isolate(s) to the appropriate reference laboratory for confirmation

Culture methods detect STEC O157 by its inability to ferment sorbitol on selective media (MacConkey agar). However, non-O157 STEC ferment sorbitol and there is no culture method to differentiate non-O157 STEC from non-pathogenic *E. coli* in frontline laboratories (2).

Chromogenic identification plates are commercially available and have been evaluated for certain clinical samples. The use of chromogenic agar may be of value in the isolation and confirmation of *E. coli* (EPEC, EHEC, STEC). Refer to [UK SMI S 7 gastroenteritis](#).

Confirmation for the *stx* gene is required for colonies identified as potential STEC.

8.3 Colonial appearance

- Some rare variant strains of STEC O157 ferment sorbitol and may grow poorly on CTSMAC/SMAC.
- Although CTSMAC offers a degree of selection for presumptive STEC O157, growth of other organisms may be observed. Mixed growth from faecal specimens may contain other sorbitol non fermenters. See table below.

Organism	Colour and size of colonies on CTSMAC
<i>Shigella flexneri</i>	Pink colonies. 0.5 to 1mm in diameter
<i>Salmonella</i> Typhimurium	Pale pink pinpoint colonies
<i>E. coli</i> (non-O157)	Generally sorbitol fermenters. Pink colonies. Pinpoint to 0.25mm diameter
<i>E. coli</i> O157	Smooth, colourless colonies or slightly greyish, appear with an orange-coloured halo. 2 to 3mm in diameter

8.4 Test procedures

8.4.1 Oxidase test ([TP 26 - Oxidase test](#))

E. coli O157:H7 is oxidase negative. Screening with oxidase test may be helpful and should be done on a media containing non-fermentable carbohydrates.

8.4.2 Agglutination test

Suspect colonies can be tested with the appropriate antiserum (latex or other commercial reagent). It is important to perform the appropriate control for autoagglutination.

8.4.3 Biochemical tests

- For commercial identification systems, laboratories should follow manufacturer's instructions and rapid tests and kits should be validated and be shown to be fit for purpose prior to use.
- Subculture to lactose containing media. This may be the purity plate from the commercial identification kit. Chromogenic identification plates are available and may be valuable as an alternative for confirmation of identification of *E. coli*.
- STEC O157 is almost always lactose positive but rare isolates have been found to be lactose non-fermenters.

8.4.4 Matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry

MALDI-TOF MS can be used to analyse the protein composition of a bacterial cell, that has emerged as a new technology for species identification. This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use (41). Refer to section 5 for technical limitations of MALDI-TOF.

8.4.5 Molecular Testing

Nucleic acid amplification tests (NAATs) is usually considered to be a good method for bacterial detection as it is simple, rapid, sensitive and specific. The basis for Polymerase Chain Reaction (PCR) diagnostic applications in microbiology is the detection of infectious agents and the differentiation of non-pathogenic from pathogenic strains by virtue of specific genes.

The implementation of PCR assays targeting common enteric pathogens has detected an increasing number of non STEC serotypes in the UK (42). Real time PCR has excellent sensitivity and specificity. Multiplex assays are able to detect and differentiate between *stx1* and *stx2* and other pathogens (12).

Commercial real-time PCR systems have been developed which select for Shiga toxin genes, accelerate more sensitive detection compared with traditional culture-based methods (40) and are validated for simultaneous detection of bacterial enteric pathogens either directly from faeces without any pre-enrichment or following overnight pre-enrichment (43).

8.5 Further identification

8.5.1 Rapid Molecular Methods

Molecular methods have had an enormous impact on the taxonomy of *Escherichia*. Analysis of gene sequences has increased understanding of the phylogenetic relationships of *Escherichia* and related organisms; and has resulted in the recognition of numerous new species and strains. Molecular techniques have made identification of species more rapid and precise than is possible with phenotypic techniques.

8.5.2 Whole genome sequencing (WGS)

Whole genome sequencing is the principle of sequencing the entire genome of an organism and can be achieved through the use of various available sequencing technologies.

Sequencing can provide valuable information complementing routine microbiological and epidemiological investigations. It has been successfully used for public health surveillance and outbreak detection by performing sequencing of bacterial genomes at a low cost (44).

WGS has the ability to accurately define sporadic cases over time, to enable better characterisation of the population at risk and to assess the relative importance of exposures leading to sporadic infections, which may differ from those leading to outbreaks (45).

8.6 Storage and referral

As STEC is a notifiable disease, for public health management of cases, all isolates of presumptive (locally confirmed) *E. coli* O157 (sorbitol non fermenters or sorbitol fermenting) should be saved on nutrient agar slopes. Cultures should be referred promptly to the appropriate reference laboratory for confirmation of identification, detection of *stx* genes and further typing, including WGS.

All identification tests should ideally be performed from non-selective agar for pure colonies.

Faecal samples from cases with appropriate clinical symptoms from whom STEC O157 has not been isolated should be submitted to the appropriate reference laboratory for detection of STEC strains belonging to serogroups other than O157 by culture and DNA-based methods.

9 Reporting

9.1 Infection Specialist

Presumptive identification of *E. coli* O157 is based on appropriate growth characteristics, biochemical tests, colonial appearance and agglutination with O157 antiserum or commercial antigen kits.

Inform the infection specialist or medical microbiologist of presumptive or confirmed *E. coli* O157 strains.

According to local protocol, the infection specialist should also be informed if the request bears relevant information which suggests infection with *E. coli* O157 such as:

- enterocolitis (especially if complicated by severe dehydration, anaemia, haemolytic-uraemic syndrome, neurological dysfunction and or sudden confusion)
- recent travel, farming (or visits to farms)
- veterinary or laboratory work
- food poisoning
- food handler
- investigation of outbreak situations

Follow local protocols for reporting to clinician

9.2 Confirmation of identification

For confirmation and identification please see section 10 referral to reference laboratories.

9.3 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

Refer to current guidelines on Second Generation Surveillance System (SGSS) reporting (31).

STEC is notifiable under the Public Health (Control of Diseases) Act 1984 and the Health Protection (Notification) Regulations 2010.

In England, local diagnostic laboratories report presumptive cases of STEC to their local Health Protection Teams (HPTs) and then refer samples to the appropriate reference laboratory for confirmation and further testing.

9.4 Infection prevention and control team

Inform the infection prevention and control team of presumptive and confirmed isolates of *E. coli* O157 and other STEC causing a clinical picture characteristic of infection with *E. coli* O157.

10 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the appropriate reference laboratory [see user manuals and request forms](#)

Contact appropriate reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

[England](#)

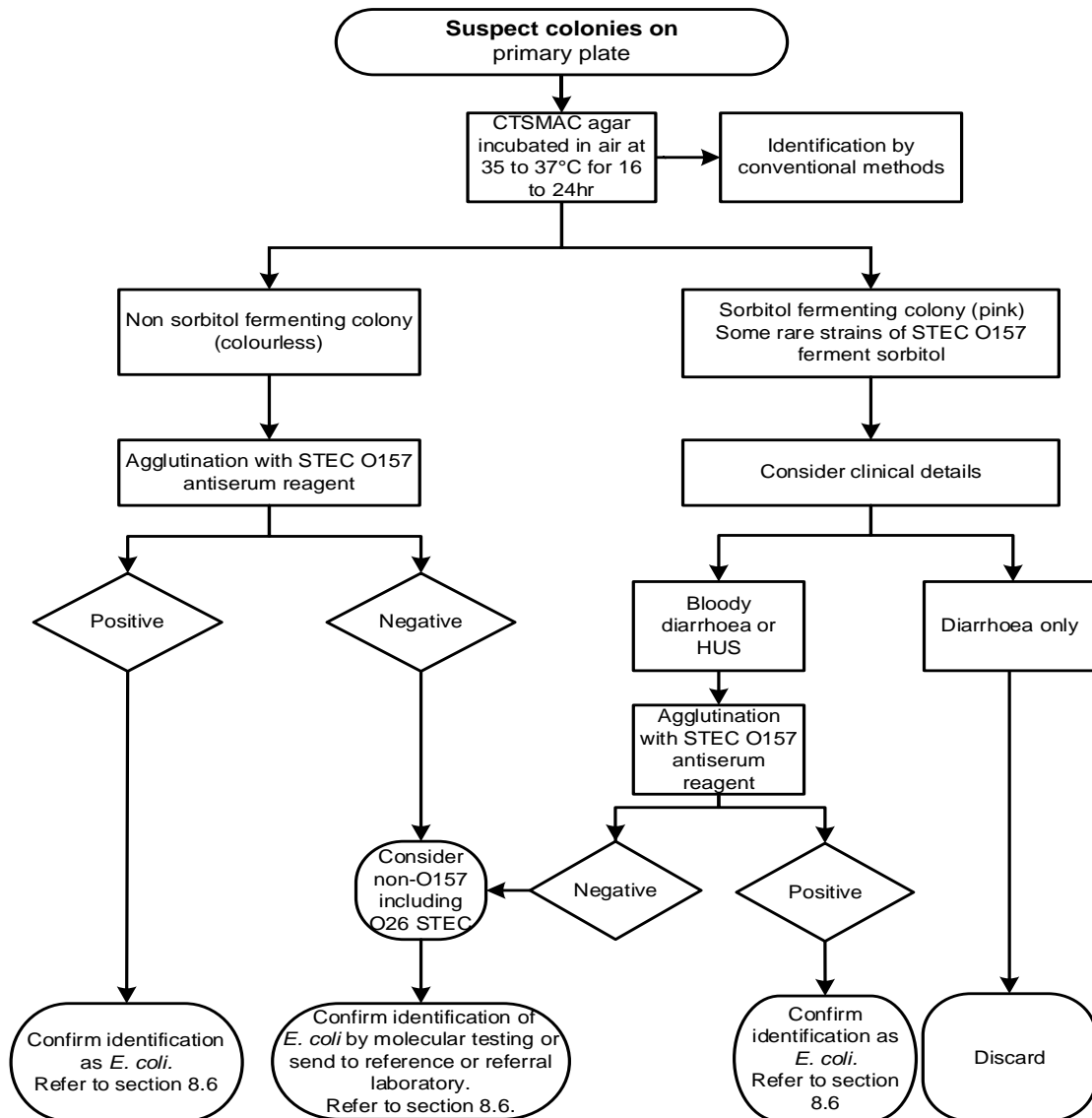
[Wales](#)

[Scotland](#)

[Northern Ireland](#)

Note: In case of sending away to laboratories for processing, ensure that the specimen is placed in the appropriate package and transported accordingly.

Algorithm: Identification of STEC



The flowchart is for guidance only.

Note: Refer to clinical details: in cases and particularly clusters of cases where isolation or identification fails, but the symptoms are consistent with STEC infection, the following actions are recommended:

- send a faecal sample to the appropriate reference laboratory
- send a serum sample to the appropriate reference laboratory for the testing for the presence of antibodies to *E. coli* O157 lipopolysaccharide
- follow local procedure for molecular testing
- if a sample is PCR positive and culture negative, consider repeating the test or send the sample to appropriate reference laboratory

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An explanation of the reference assessment used is available in the [scientific information section on the UK SMI website](#).

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