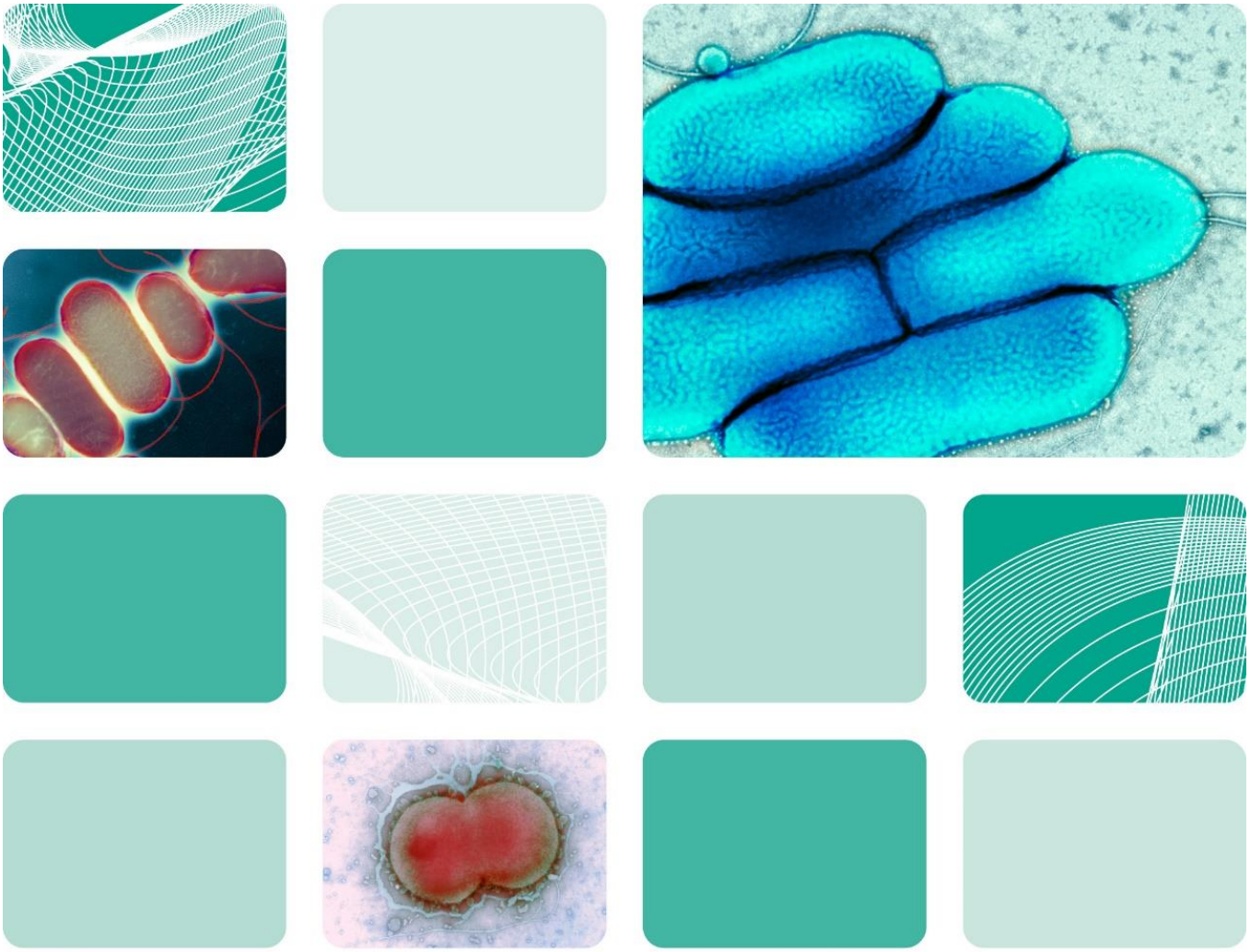
UK Health Security Agency

# UK Standards for Microbiology Investigations

## Identification of Shiga toxin-producing *Escherichia coli* (STEC)



Issued by the Standards Unit, UK Standards for Microbiology Investigations, UKHSA

# Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee.

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:

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# Contents

# 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 | 8/06.08.25 |
| Issue number discarded | 5 |
| Insert issue number | 5.1 |
| **Section(s) involved** | **Amendment** |
| **Whole document.** | Hyperlinks throughout document updated to Royal College of Pathologists website.<br>Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms<br>Partner organisation logos updated.<br>Broken links to devolved administrations replaced.<br>References to NICE accreditation removed.<br>Scope and Purpose replaced with General and Scientific information to align with current UK SMI template. |
| | Addition of Chromogenic, PCR and non-O157 STEC serogroups O26, O145 and O103 throughout the document. |
| | STEC O157 replaced with STEC, to include O157 and non O157. |
| **Title** | Title changed from ‘Identification of Shiga toxin-producing *Escherichia coli* (STEC) using conventional methods to ‘Identification of Shiga toxin-producing *Escherichia coli* (STEC). |
| **Introduction** | Updated the most common non-O157 STEC serogroups O26, O145 and O103 are the most common. |

| | |
|---|---|
| **8.3 Colonial appearance** | Colour and size of colonies on Chromogenic agar added to table |
| **8.6 Storage and referral** | Included addition information on faecal specimens. |
| **11 Public Health responsibilities of diagnostic laboratories** | New section added |
| **Algorithm** | Updated to include chromogenic agar and PCR |

| | |
|---|---|
| **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 *Escherichia coli* including *Escherichia coli* O157' to 'Identification of Shiga toxin-producing *Escherichia coli* (STEC) using conventional methods. |
| **Introduction** | Additional information added about STEC |
| **Technical limitations** | *Escherichia hermannii* has been reclassified to *Atlantibacter hermannii* |
| **Algorithm** | Updated to include *E. coli* 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 (4)
- 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 (4)

- 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 (5) 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 (6).

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), targeting the Shiga toxin gene (stx), and therefore theoretically capable of detecting all STEC types, there has been a seven fold increase in the detection of non-O157 STEC in the UK.

In England, the most common non-O157 STEC serogroups  associated with severe clinical outcomes and outbreaks are: O26, O145 and O103 (2). 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 maybe 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,*(9) 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 semi-selective agar, such as sorbitol MacConkey agar (SMAC), cefixime-tellurite sorbitol (CTSMAC) agar or Chromogenic agar should prove to be useful for rapid detection of *E. coli* serotypes O157, O26, O145 and O103 (9,10).Culture methods are restricted to the detection of a limited range of STEC serogroups and should be used in conjunction with molecular methods, such as PCR, that have a high sensitivity and specificity for all STEC serotypes (11).

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

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 (12,13).

# 6 Safety considerations

The section covers specific safety considerations (14-35) related to this UK SMI, and should be read in conjunction with the general safety considerations.

STEC are Hazard Group 3 organisms. All work with suspected isolates of STEC must be performed under Containment Level 3 conditions. STEC are highly virulent and the infectious dose is low, less than 100 organisms.

STEC may cause severe illness, including HUS, that can be fatal. Laboratory acquired infections have been reported. There is no vaccine available for use for *E. coli* O157 and therapeutic options are contraindicated, as antibiotics are associated with triggering progress to HUS.

The above guidance should be supplemented with local COSHH.

Compliance with postal and transport regulations is essential.

# 7 Target organisms

*E. coli* O157:H7 and other non-O157 serogroups O26, O103, and O145.

*E. coli* serotypes that acquire the ability to produce Shiga toxin exhibit similar characteristics to STEC O157, causing outbreaks of haemorrhagic colitis (bloody diarrhoea) that can progress to HUS.

Not all *E. coli* that grow as non-sorbitol fermenting or sorbitol fermenting colonies on SMAC, CTSMAC, or as purple colonies on Chromagar, and agglutinate with O157, O26, O145 or O103 antisera are STEC. PCR or sequencing by the reference laboratory is required to confirm whether the colonies have stx and are therefore designated as STEC.

# 8 Identification

## 8.1 Microscopic appearance

Gram stain (UK SMI TP 39 - Staining procedures) if required.

STEC are Gram negative rods.

## 8.2 Primary isolation media

Strain of STEC O157:H7 are unable to 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 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 resistant to tellurite

compared with other *E. coli.* There is a risk of failure to detect STEC O157 on SMAC agar lacking cefixime and tellurite due to commensal *E. coli* out competing STEC O157 colonies making it difficult to identify.

- Chromogenic selective agar, can be used for non-O157 serogroups O26, O45, O103, O111, O121 and O145 (36).

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 that detects all non-O157 STEC and differentiates them from non-pathogenic *E. coli* in frontline laboratories (2).

Chromogenic agar can be used alongside antisera for identification plates are commercially available and have been evaluated for the detection of non-O157 serogroups O26, O45, O103, O111, O121 and O145 in faecal specimens. Refer to UK SMI S 7 gastroenteritis.

## 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** |
|---|---|
| Shigella flexneri | Pink colonies. 0.5 to 1mm in diameter |
| Salmonella Typhimurium | Pale pink pinpoint colonies |
| *E. coli* (non-O157) | Generally sorbitol fermenters. Pink colonies. Pinpoint to 0.25mm diameter |
| *E. coli* O157 | Smooth, colourless colonies or slightly greyish, appear with an orange-coloured halo. 2 to 3mm in diameter |
| **Organism** | **Colour and size of colonies on Chromogenic agar** |
| STEC O157 | purple colonies and can be identified by agglutination with O157 antisera |
| Tellurite resistant *E. coli* | purple colonies, and only those colonies agglutinating with available antisera, specifically O26, O103, and O145, which are the most common non-O157 STEC serogroups in the UK. |

## 8.4 Test procedures

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

STEC are 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 mass spectrometry (MALDI-TOF MS)

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 MS 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 (37). Refer to section 5 for technical limitations of MALDI-TOF MS.

### 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 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 (38). Real time PCR has excellent sensitivity and specificity. Multiplex assays are able to detect and differentiate between *stx1* and *stx2* and other pathogens (11).

Commercial real-time PCR systems have been developed which select for Shiga toxin genes, accelerate more sensitive detection compared with traditional culture-based methods (36) and are validated for simultaneous detection of bacterial enteric pathogens either directly from faeces without any pre-enrichment or following overnight pre-enrichment (39).

## 8.5 Further identification

### 8.5.1 Rapid Molecular Methods

Analysis of gene sequences has increased understanding of the phylogenetic relationships of Escherichia and related organisms. 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 (40).

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 (41).

## 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 and STEC non-O157 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.

- If STEC O157 has not been isolated, then faecal samples from cases with appropriate clinical symptoms 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.
- Faecal specimens testing positive for STEC by PCR but culture negative, should be referred for culture if the patient is hospitalised and/or has suspected HUS.
- It is recommended to refer faecal specimens testing positive for STEC by PCR but culture negative if the patient is 5 years old or under, and/or has bloody diarrhoea.
- However if labs lack resources or have the capacity, they may want to send all faecal specimens testing positive for STEC by PCR, but culture negative to the reference laboratory depending on local policies.

# 9 Reporting

## 9.1 Infection Specialist

Presumptive identification of *E. coli* O157 and other STEC serogroups is based on appropriate growth characteristics, biochemical tests, colonial appearance and agglutination with O157, O26, O103 or O145 antiserum or commercial antigen kits. Inform the infection specialist or medical microbiologist of presumptive or confirmed *E. coli* O157 O26, O103 or O145 strains. According to local protocol, the infection specialist should also be informed if the request bears relevant information which suggests infection with STEC such as

- enterocolitis (especially if complicated by severe dehydration, anaemia, haemolytic-uraemic syndrome, neurological dysfunction and or sudden confusion)
- is 5 years old or under
- has bloody diarrhoea
- reports recent travel, farming (or visits to farms)
- reports veterinary or laboratory work
- reports food poisoning
- is a food handler
- is part of an outbreak investigation

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 (30).

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 referral/ 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 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.

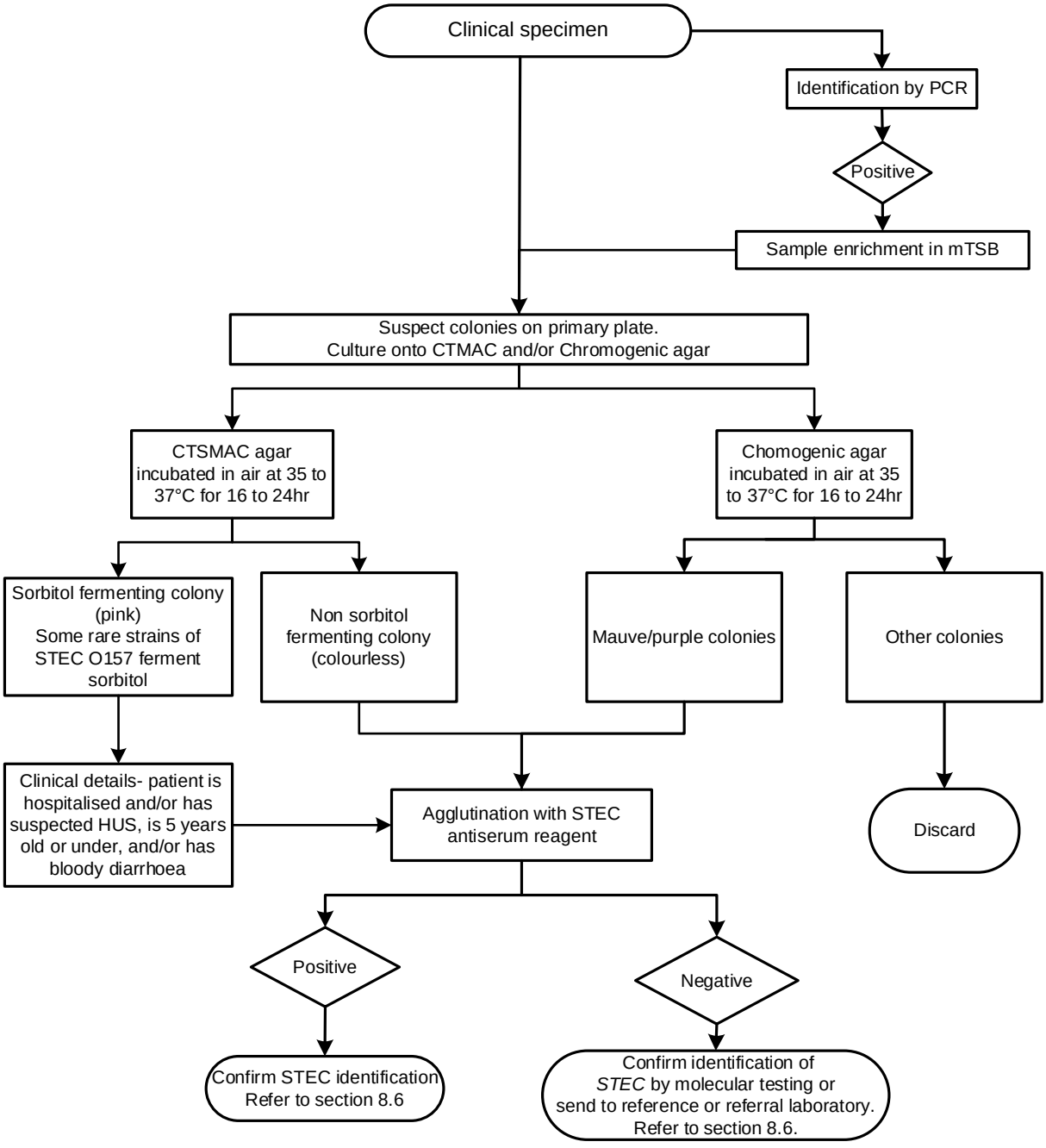
# 11 Public Health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral, to further characterise the organism, as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance, forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

# Algorithm: Identification of STEC




**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
- 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

# References

An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.


1. Mulchandani R, Brehmer C, Butt S, Vishram B, Harrison M, Marchant E et al. Outbreak of Shiga toxin-producing Escherichia coli O157 linked with consumption of a fast-food product containing imported cucumbers, United Kingdom, August 2020. Int J Infect Dis 2021;110 Suppl 1:S62-s8.**2++** 10.1016/j.ijid.2021.04.001

2. Escherichia coli O157 annual totals: Shiga Toxin–Producing Ecoli data 2022 Government website: GOV.UK; 2025. **++**

3. Centers for Disease Control and Prevention. Escherichia coli 2022 ed.vol 07.03.2022. 2022. **++**

4. Buchan B.W FML, Humphries R.M, Dekker J, Ledeboer N.A Escherichia, Shigella, and Salmonella Vol 12th edition Vol 12th edition Washington DC: ASM press; 2019. p. 688-723. **++**

5. Public health operational guidance for Shiga toxin-producing Escherichia coli (STEC); 2023. **++**

6. STEC-O157 information leaflet for casesvol 16.01.2023. PHE publications; 2023. **++**

7. Joseph A, Cointe A, Mariani Kurkdjian P, Rafat C, Hertig A. Shiga Toxin-Associated Hemolytic Uremic Syndrome: A Narrative Review. Toxins (Basel) 2020;12.**++** 10.3390/toxins12020067

8. Jenkins C, Byrne L, Vishram B, Sawyer C, Balasegaram S, Ahyow L et al. Shiga toxin-producing Escherichia coli haemolytic uraemic syndrome (STEC-HUS): diagnosis, surveillance and public-health management in England. J Med Microbiol 2020;69:1034-6.**+** 10.1099/jmm.0.001215

9. Hata H, Natori T, Mizuno T, Kanazawa I, Eldesouky I, Hayashi M et al. Phylogenetics of family Enterobacteriaceae and proposal to reclassify Escherichia hermannii and Salmonella subterranea as Atlantibacter hermannii and Atlantibacter subterranea gen. nov., comb. nov. Microbiol Immunol 2016;60:303-11.**++** 10.1111/1348-0421.12374

10. March SB, Ratnam S. Latex agglutination test for detection of Escherichia coli serotype O157. JClinMicrobiol 1989;27:1675-7.**3-**

11. Parsons BD, Zelyas N, Berenger BM, Chui L. Detection, Characterization, and Typing of Shiga Toxin-Producing Escherichia coli. Front Microbiol 2016;7:478.**3+** 10.3389/fmicb.2016.00478

12. Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. ClinMicrobiolRev 2013;26:547-603.**+** 26/3/547 [pii];10.1128/CMR.00072-12 [doi]

13. Jade Pizzato WT, Sandrine Bernabeu, Rémy A. Bonnin, Emmanuelle Bille, Eric Farfour, Thomas Guillard, Olivier Barraud, Vincent Cattoir, Chloe Plouzeau, Stéphane Corvec, Vahid Shahrezaei, Laurent Dortet, Gerald Larrouy-Maumus. Discrimination of Escherichia coli, Shigella flexneri, and Shigella sonnei using lipid profiling by MALDI-TOF mass spectrometry paired with machine learning. Microbiology Open 2022;Volume 11, issue 4.**2+** https://doi.org/10.1002/mbo3.1313

14. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive 2021. 1-39. **++**

15. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets 2000. **++**

16. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 2005. 1-14. **++**

17. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.**+**

18. Department for Transport, Maritime and Coastguard Agency, HSENI, Civil Aviation Authority. Transport of infectious substances UN2814, UN2900 and UN3373 Guidance note number 17/2012 (revision 7). 2013. **++**

19. Department of Health. Health Protection Legislation (England) Guidance. 1-112. 2010. **++**

20. Gizzie N, Adukwu E. Evaluation of Liquid-Based Swab Transport Systems against the New Approved CLSI M40-A2 Standard. J Clin Microbiol 2016;54:1152-6.**2+** 10.1128/JCM.03337-15

21. Health and Safety Executive. Managing risks and risk assessment at work (accessed 28/07/2021). https://www.hse.gov.uk/simple-health-safety/risk/index.htm. **++**

22. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 2009. **++**

23. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002 (as amended). Approved Code of Practice and guidance L5 (sixth edition). HSE Books. 2013. **++**

24. Health and Safety Executive. Risk assessment: A brief guide to controlling risks in the workplace. HSE. 2014. **++**

25. Health and Safety Executive, Advisory Committee on Dangerous Pathogens. Management and operation of microbiological containment laboratories. HSE. 2019. **++**

26. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books 2003. **++**

27. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967. **++**

28. Home Office. Anti-terrorism, Crime and Security Act. 2001. **++**

29. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices 1998. 1-37. **++**

30. Public Health England. Laboratory reporting to Public Health England: a guide for diagnostic laboratories. PHE. 2020. 1-31. **++**

31. Scottish Government. Public Health (Scotland) Act. 2008. **++**

32. The Royal College of Pathologists. The retention and storage of pathological records and specimens (5th edition). 1-59. 2015. **++**

33. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010. **++**

34. Tyrrell KL, Citron DM, Leoncio ES, Goldstein EJ. Comparison of the Copan eSwab System with an Agar Swab Transport System for Maintenance of Fastidious Anaerobic Bacterium Viability. J Clin Microbiol 2016;54:1364-7.**2+** 10.1128/JCM.03246-15

35. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2019-2020. WHO. 2019. **++**

36. Rice T, Quinn N, Sleator RD, Lucey B. Changing Diagnostic Methods and Increased Detection of Verotoxigenic Escherichia coli, Ireland. Emerg Infect Dis 2016;22:1656-7.**3++** 10.3201/eid2209.160477

37. Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E et al. Rapid identification and typing of listeria species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 2008;74:5402-7.**2-** 10.1128/aem.02689-07

38. Gentle A, Day MR, Hopkins KL, Godbole G, Jenkins C. Antimicrobial resistance in Shiga toxin-producing Escherichia coli other than serotype

O157:H7 in England, 2014-2016. J Med Microbiol 2020;69:379-86.**2+** 10.1099/jmm.0.001146

39. Koziel M, Kiely R, Blake L, O'Callaghan I, Corcoran GD, Lucey B et al. Improved detection of bacterial pathogens in patients presenting with gastroenteritis by use of the EntericBio real-time Gastro Panel I assay. JClinMicrobiol 2013;51:2679-85.**2+** JCM.00809-13 [pii];10.1128/JCM.00809-13 [doi]

40. Butcher H, Elson R, Chattaway MA, Featherstone CA, Willis C, Jorgensen F et al. Whole genome sequencing improved case ascertainment in an outbreak of Shiga toxin-producing Escherichia coli O157 associated with raw drinking milk. Epidemiol Infect 2016;144:2812-23.**2+** 10.1017/s0950268816000509

41. Dallman TJ, Byrne L, Ashton PM, Cowley LA, Perry NT, Adak G et al. Whole-genome sequencing for national surveillance of Shiga toxin-producing Escherichia coli O157. Clin Infect Dis 2015;61:305-12.**2+** 10.1093/cid/civ318