Public Health England

NHS

Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Identification of Vero cytotoxin-producing *Escherichia coli* including *Escherichia coli* O157

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology – Identification | ID 22 | Issue no: 4 | Issue date: 16.06.15 | Page: 1 of 22

© Crown copyright 2015

Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-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.

For further information please contact us at:

Standards Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

PHE Publications gateway number: 2015013

UK Standards for Microbiology Investigations are produced in association with:

Logos correct at time of publishing.

Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK STANDARDS FOR MICROBIOLOGY INVESTIGATIONS: SCOPE AND PURPOSE 6

SCOPE OF DOCUMENT 9

INTRODUCTION 9

TECHNICAL INFORMATION/LIMITATIONS 10

1 SAFETY CONSIDERATIONS 11

2 TARGET ORGANISMS 11

3 IDENTIFICATION 11

4 IDENTIFICATION OF VERO CYTOTOXIN-PRODUCING *ESCHERICHIA COLI* INCLUDING *E. COLI* O157 16

5 REPORTING 17

6 REFERRALS 18

7 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 19

REFERENCES 20

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

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

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	6/16.06.15
Issue no. discarded.	3.2
Insert Issue no.	4
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	The taxonomy section of VTEC O157 has been updated. More information has been added to the Characteristics section. The medically important species are mentioned. Section on Principles of Identification has been updated to include the MALDI-TOF MS.
Technical information/limitations.	Addition of information regarding commercial identification systems, agglutination test and MALDI-TOF MS.
Safety considerations.	The handling of VTEC O157 as well as laboratory acquired infections has been updated.
Target organisms.	The section on the Target organisms has been updated and presented clearly.
Identification.	Updates have been done on 3.2, 3.3 and 3.4 to reflect standards in practice. Section 3.4.4 and 3.4.5 has been updated to include MALDI-TOF MS and NAATs with references. Subsection 3.5 has been updated to include the Rapid Molecular Methods.
Identification flowchart.	Modification of flowchart for identification of Vero cytotoxin-producing *Escherichia coli* including *E. coli* O157 has been done for easy guidance.

Reporting.	Subsections 5.3, 5.4 and 5.5 have been updated to reflect the information required on reporting practice.
Referral.	The addresses of the reference laboratories have been updated.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: scope and purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

Quality assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

Public Health England. (2015). Identification of Vero cytotoxin-producing *Escherichia coli* including *Escherichia coli* O157. UK Standards for Microbiology Investigations. ID 22 Issue 4. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

This SMI describes the identification of presumptive Vero cytotoxin-producing *Escherichia coli* O157 (VTEC O157) isolated from faeces. These strains are associated with a wide spectrum of disease including haemolytic uraemic syndrome (HUS).

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

Vero cytotoxin-producing *E. coli* O157 (VTEC O157) is a member of the genus *Escherichia* and the family Enterobacteriaceae. The emergence of *E. coli* O157 is linked to its genetic evolution and the fact that it has acquired a variety of virulence factors likely through transmission of plasmids between bacteria[1].

Characteristics

VTEC O157 is a Gram negative rod. Most VTEC O157 strains are motile and possess the flagellar antigen H7, but at least 20% in England and Wales are phenotypically non-motile. They are facultatively anaerobic. On sorbitol MacConkey agar (SMAC) or SMAC containing cefixime and tellurite (CT-SMAC), the colonies are colourless and 2-3mm in diameter. VTEC O157 differs from other members of the genus *Escherichia* in that it does not usually ferment sorbitol (a characteristic that is exploited by the selective medium) and is β-glucuronidase negative[2,3]. However, some VTEC O157 strains have been found to ferment sorbitol and to be β-glucuronidase positive[4].

Strains are oxidase negative and usually produce gas from glucose. Some strains show atypical biochemistry eg they are anaerogenic, non-lactose fermenting, indole negative or urease positive.

It has been isolated from urine, stool (human or animals), blood, food and environmental samples[5,6].

Principles of identification

B 30 – Investigation of faecal specimens for enteric pathogens recommends that all diarrhoeal stools are screened for the presence of *E. coli* O157. Presumptive VTEC O157 isolates from primary culture are identified by colonial appearance on CT-SMAC, serology (agglutination with O157-specific antisera) and biochemical tests. 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 care must be taken with the interpretation of the profile[4].

All isolates of presumptive (locally confirmed) *E. coli* O157 should be referred to the Reference Laboratory for confirmation of identification, testing for the presence of Vero cytotoxin genes, serotyping, and phage typing. All identification tests should ideally be performed from non-selective agar to take into account the variations that may occur with biochemical tests such as sorbitol fermentation.

Where the clinical evidence is suggestive of VTEC infection (particularly in children under 15 years and adults over 65 years) and no presumptive sorbitol non-fermenting *E. coli* O157 colonies are observed on CT-SMAC 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 Reference Laboratory for confirmation, detection of VT genes and phage typing

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

In England, Wales and Northern Ireland, confirmation and sub-typing is carried out by the Gastrointestinal Bacteria Reference Unit (GBRU) of the Bacteriology reference Department (BRD) at the Public Health England, Colindale, London.

In Scotland, isolates are referred to the Scottish *E. coli* Reference Laboratory (SERL) at Edinburgh Royal Infirmary.

Technical information/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 they are fit for purpose. It is also essential that appropriate on-going Quality Assurance procedures should be 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 care must be taken with the interpretation of the profile[4]. However, all presumptive *E. coli* O157 from human and non-human sources should be referred to the appropriate specialist laboratories for confirmation.

Agglutination test

Escherichia hermannii is sorbitol negative, cross-reacts and agglutinates in *E. coli* serotype O157 antiserum, and thus it could be mistaken for *E. coli* O157. If this is the case, the use of the commercial latex screen in conjunction with a combination of CT-SMAC/SMAC cultures should prove to be useful for rapid detection of *E. coli* serotype O157[7].

MALDI-TOF MS

One of the limitations is the current inability of MALDI-TOF MS to reliably distinguish pathogenic from non-pathogenic *E. coli* isolates, in addition, numerous reports have also described the difficulty encountered when trying to discriminate *E. coli* from *Shigella* species and this is challenging because of the close genetic relatedness of the organisms. In addition, further research is needed to adapt MALDI-TOF technology to be suitable for strain-specific identifications of *E. coli* isolates[8].

1 Safety considerations[9-25]

VTEC O157 is a Hazard Group 3 organism.

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

VTEC O157 is highly infective, and as few as 10 viable organisms are required for an infective dose[16]. VTEC O157 may cause severe illness that is sometimes fatal. Laboratory acquired infections have been reported[26-29]. However, there is no knowledge of any *E. coli* O157 vaccine currently commercially available in the UK or indeed licensed for use.

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.

2 Target organisms

Commonest serotypes of Vero cytotoxin-producing E. coli O157 isolated and known to cause infections in humans[6,30]

E. coli O157:H7 and *E. coli* O157: NM (non-motile) also known as *E. coli* O157:H negative.

Other *E. coli* serotypes like O145 can act like O157:H7 if they acquire the ability to produce Shiga (Vero) toxin. These other *E. coli* serotypes can also cause outbreaks of bloody diarrhoea with haemorrhagic colitis that can become complicated by haemolytic uraemia.

This procedure may result in the identification of isolates of presumptive *E. coli* O157 that do not produce Vero cytotoxin and some organisms that give equivocal results in section 3.4.

3 Identification

3.1 Microscopic appearance

Gram stain (TP 39 – Staining procedures)

Gram negative rods

3.2 Primary isolation media

Cefixime tellurite-sorbitol MacConkey (CT-SMAC) agar incubated in air at 35-37°C for 16-24hr. CT-SMAC agar is used since classical sorbitol non-fermenting VTEC 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.

Enrichment culture in modified tryptone soya broth (MTSB) may be required in cases of outbreaks (B 30 - Investigation of faecal specimens for enteric pathogens).

3.3 Colonial appearance

On CT-SMAC agar, typical VTEC O157 colonies are smooth, colourless or slightly greyish in appearance which may appear with an orange-coloured halo and are 2-3mm in diameter. Some rare variant strains of VTEC O157 ferment sorbitol and may grow poorly on CT-SMAC/SMAC.

Note: Although CT-SMAC offers a degree of selection for presumptive VTEC 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 CT-SMAC
Shigella flexneri	Pink colonies. 0.5-1mm diameter
Salmonella Typhimurium	Pale pink pinpoint colonies
E. coli (non-O157)	Generally sorbitol fermenters. Pink colonies. Pinpoint to 0.25mm diameter

3.4 Test procedures

3.4.1 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.

3.4.2 Agglutination test

Use VTEC O157 antiserum (latex or other commercial reagent). It is important to perform the appropriate control for autoagglutination.

3.4.3 Biochemical tests

- **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.*

VTEC O157 is almost always lactose positive but rare isolates have been found to be lactose non-fermenters.

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

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS), which can be used to analyse the protein composition of a bacterial cell, 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[31].

One of the limitations is the current inability of MALDI-TOF MS to distinguish pathogenic from non-pathogenic *E. coli* isolates reliably, in addition, numerous reports have also described the difficulty encountered when trying to discriminate *E. coli* from *Shigella* species and this is challenging because of the close genetic relatedness of the organisms. In addition, further research is needed to adapt MALDI-TOF technology to be suitable for strain-specific identifications of *E. coli* isolates[8].

3.4.5 Nucleic acid amplification tests (NAATs)

PCR 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 discrimination of non-pathogenic from pathogenic strains by virtue of specific genes. However, it does have limitations. Although the 16S rRNA gene is generally targeted for the design of species-specific PCR primers for identification, designing primers is difficult when the sequences of the homologous genes have high similarity.

A PCR method (O157 *rfb* PCR) was developed for the rapid detection and confirmation of the O157 serotype. This has been used as a screening test for evidence of the presence of *E. coli* O157 in faecal, food, and environmental samples. It also has an additional advantage in terms of the detection of isolates that have a masked O antigen or when isolates are rough[5].

Commercial real-time PCR systems have been developed and validated for simultaneous detection of bacterial enteric pathogens either directly from faeces without any pre-enrichment or following overnight pre-enrichment[32-34].

3.5 Further identification

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.

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable Number Tandem Repeat Analysis (MVLA) and Whole Genome Sequencing (WGS). All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

Multilocus sequence typing (MLST)

MLST measures the DNA sequence variations in a set of housekeeping genes directly and characterizes strains by their unique allelic profiles. The principle of MLST is simple: the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired. The technique is highly discriminatory, as it detects all the nucleotide polymorphisms within a gene rather than just those non-synonymous changes that alter the electrophoretic mobility of the

protein product. One of the advantages of MLST over other molecular typing methods is that sequence data are portable between laboratories and have led to the creation of global databases that allow for exchange of molecular typing data via the Internet[35].

MLST has been successful for the differentiation of other organisms but has been unable to discriminate among *E. coli* O157:H7 isolates. In a study by Noller et al, no variation was detected in seven housekeeping genes and little variation was noted in two surface protein genes[36].

Multiple-locus variable number tandem repeat analysis (MLVA/VNTR)

Multiple-Locus Variable number tandem repeat Analysis (MLVA) is a method used to perform molecular typing of particular microorganisms. It utilizes the naturally occurring variation in the number of tandem repeated DNA sequences found in many different loci in the genome of a variety of organisms. The molecular typing profiles are used to study transmission routes, to assess sources of infection and also to assess the impact of human intervention such as vaccination and use of antibiotics on the composition of bacterial populations.

This is used successfully to detect *E. coli* O157:H7 in outbreaks and to discriminate among sporadic isolates accurately in outbreaks that would otherwise not have detected by other methods. MLVA appears to have sensitivity equal to that of PFGE and specificity superior to that of PFGE[37].

Pulsed field gel electrophoresis (PFGE)

PFGE detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel. PFGE is known to be highly discriminatory and a frequently used technique for outbreak investigations and has gained broad application in characterizing epidemiologically related isolates. However, the stability of PFGE may be insufficient for reliable application in long-term epidemiological studies. However, due to its time-consuming nature (30hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories[38,39]. The other limitations are that PFGE is labour-intensive, and the results are difficult to analyse and not easily transferable between laboratories[36].

This method is currently the most widely utilized molecular subtyping method for detecting outbreaks of *E. coli* O157:H7. In fact, PFGE has been found to identify outbreaks of *E. coli* O157:H7 that were not detected by traditional epidemiologic methods[36]. In addition to traditional epidemiological investigations, PFGE is used to discriminate between outbreak and sporadic strains of *E. coli* O157:H7[40].

Whole genome sequencing (WGS)

This is also known as “full genome sequencing, complete genome sequencing, or entire genome sequencing”. It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. There are several high-throughput techniques that are available and used to sequence an entire genome such as pyrosequencing, nanopore technology, IIlumina sequencing, Ion Torrent sequencing, etc. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

This has been used successfully to explore the genome of *E. coli* O157:H7 to identify candidate genes responsible for pathogenesis, and to develop better methods of

strain detection and to advance the understanding of the evolution of *E. coli*. With this technique, lateral gene transfer of *E. coli* that was discovered was very extensive[1,41].

Other specialized tests

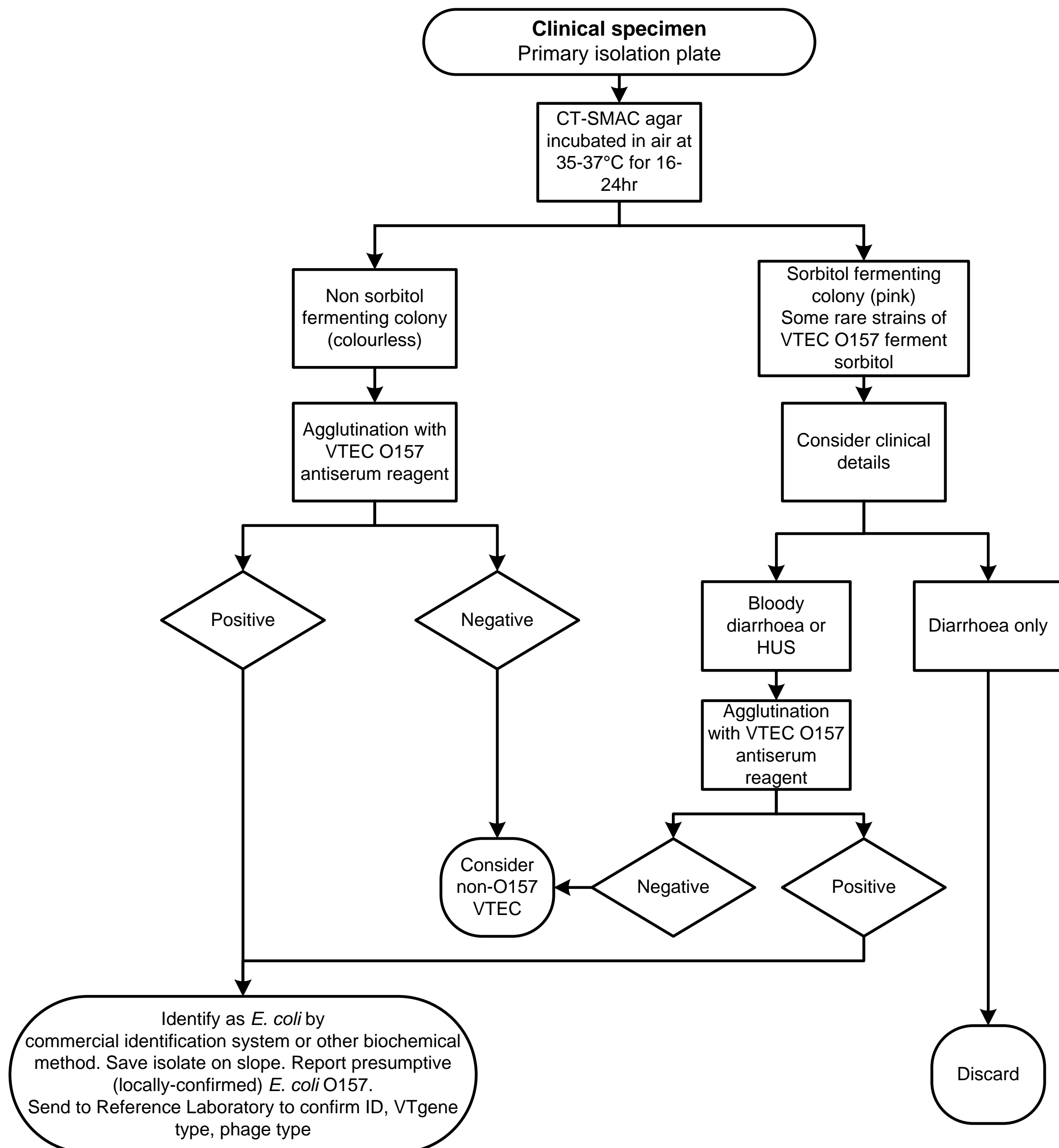
Phage typing

Phage typing has proven to be an important tool for strain characterisation and the results obtained have been used in surveillance, source attribution and outbreak investigations. This has been used successfully in characterisation of *E. coli* O157:H7 isolates but it has also been important tool in understanding the mode of transmission to aid in the control of *E. coli* O157:H7 outbreaks[42].

3.6 Storage and referral

All purified 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 Reference Laboratory for biochemical confirmation, detection of VT genes, serotyping and phage typing.

4 Identification of Vero cytotoxin-producing *Escherichia coli* including *E. coli* O157



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 VTEC infection, the following actions are recommended:

- send a faecal sample to the Reference Laboratory
- send a serum sample to the Reference Laboratory for the testing for the presence of antibodies to *E. coli* O157 lipopolysaccharide

5 Reporting

5.1 Presumptive identification

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.

5.2 Confirmation of identification

Further biochemical tests and/or molecular methods and/or reference laboratory report.

5.3 Medical microbiologist

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

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

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

Follow local protocols for reporting to clinician.

5.4 Public Health England[43]

Refer to current guidelines on CIDSC and COSURV reporting.

5.5 Infection prevention and control team

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

6 Referrals

6.1 Reference laboratory

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

Gastrointestinal Bacterial Reference Unit
Bacteriology Reference Department
Public Health England
61 Colindale Avenue
London
NW9 5EQ

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

Scottish *E. coli* O157/VTEC Reference Laboratory
Department of Laboratory Medicine
Royal Infirmary of Edinburgh
51 Little France Crescent
Old Dalkeith Road
Edinburgh
EH16 4SA
Contact SERL switchboard: Tel. 0131 537 1000 or 0131 536 1000

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm

7 Notification to PHE[43,44] or equivalent in the devolved administrations[45-48]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[45,46], Wales[47] and Northern Ireland[48].

References

1. Peng J, Yang J, Jin Q. The molecular evolutionary history of Shigella spp. and enteroinvasive Escherichia coli. Infection, Genetics and Evolution 2009;9:147-52.

2. Wells JG, Davis BR, Wachsmuth IK, Riley LW, Remis RS, Sokolow R, et al. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare Escherichia coli serotype. J Clin Microbiol 1983;18:512-20.

3. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of Escherichia coli O157:H7 associated with hemorrhagic colitis. J Clin Microbiol 1986;23:869-72.

4. Easton L. Escherichia coli O157: occurrence, transmission and laboratory detection. Br J Biomed Sci 1997;54:57-64.

5. Desmarchelier PM, Bilge SS, Fegan N, Mills L, Vary JC, Jr., Tarr PI. A PCR specific for Escherichia coli O157 based on the rfb locus encoding O157 lipopolysaccharide. J Clin Microbiol 1998;36:1801-4.

6. Heffernan E, Chatur N, Zwirewich C. Escherichia coli 0157 enterohaemorrhagic colitis associated with pyelonephritis: CT findings. Br J Radiol 2009;82:e63-e66.

7. March SB, Ratnam S. Latex agglutination test for detection of Escherichia coli serotype O157. J Clin Microbiol 1989;27:1675-7.

8. 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. Clin Microbiol Rev 2013;26:547-603.

9. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

10. 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. 7-12-1998. p. 1-37.

11. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.

12. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.

13. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.

14. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).

15. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32

16. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.

17. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.

18. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive. 2008.

19. 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.

20. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.

21. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.

22. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.

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

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

25. 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. 24-3-2005. p. 1-14

26. Singh K. Laboratory-acquired infections. Clin Infect Dis 2009;49:142-7.

27. Baron EJ, Miller JM. Bacterial and fungal infections among diagnostic laboratory workers: evaluating the risks. 3 2008;60:241-6.

28. Spina N, Zansky S, Dumas N, Kondracki S. Four laboratory-associated cases of infection with Escherichia coli O157:H7. J Clin Microbiol 2005;43:2938-9.

29. Burnens AP, Zbinden R, Kaempf L, Heinzer I, Nicolet J. A case of laboratory acquired infection with Escherichia coli O157:H7. Zentralbl Bakteriol 1993;279:512-7.

30. De BE, Heuvelink AE. Methods for the detection and isolation of Shiga toxin-producing Escherichia coli. Symp Ser Soc Appl Microbiol 2000;133S-43S.

31. 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.

32. 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. J Clin Microbiol 2013;51:2679-85.

33. Koziel M, Corcoran D, O'Callaghan I, Sleator RD, Lucey B. Validation of the EntericBio Panel II(R) multiplex polymerase chain reaction system for detection of Campylobacter spp., Salmonella spp., Shigella spp., and verotoxigenic E. coli for use in a clinical diagnostic setting. Diagn Microbiol Infect Dis 2013;75:46-9.

34. O'Leary J, Corcoran D, Lucey B. Comparison of the EntericBio multiplex PCR system with routine culture for detection of bacterial enteric pathogens. J Clin Microbiol 2009;47:3449-53.

35. Feil EJ, Spratt BG. Recombination and the population structures of bacterial pathogens. Annu Rev Microbiol 2001;55:561-90.

36. Noller AC, McEllistrem MC, Stine OC, Morris JG, Jr., Boxrud DJ, Dixon B, et al. Multilocus sequence typing reveals a lack of diversity among Escherichia coli O157:H7 isolates that are distinct by pulsed-field gel electrophoresis. J Clin Microbiol 2003;41:675-9.

37. Noller AC, McEllistrem MC, Pacheco AG, Boxrud DJ, Harrison LH. Multilocus variable-number tandem repeat analysis distinguishes outbreak and sporadic Escherichia coli O157:H7 isolates. J Clin Microbiol 2003;41:5389-97.

38. Liu D. Identification, subtyping and virulence determination of Listeria monocytogenes, an important foodborne pathogen. J Med Microbiol 2006;55:645-59.

39. Brosch R, Brett M, Catimel B, Luchansky JB, Ojeniyi B, Rocourt J. Genomic fingerprinting of 80 strains from the WHO multicenter international typing study of listeria monocytogenes via pulsed-field gel electrophoresis (PFGE). Int J Food Microbiol 1996;32:343-55.

40. Bender JB, Hedberg CW, Besser JM, Boxrud DJ, MacDonald KL, Osterholm MT. Surveillance by molecular subtype for Escherichia coli O157:H7 infections in Minnesota by molecular subtyping. N Engl J Med 1997;337:388-94.

41. Perna NT, Plunkett G, III, Burland V, Mau B, Glasner JD, Rose DJ, et al. Genome sequence of enterohaemorrhagic Escherichia coli O157:H7. Nature 2001;409:529-33.

42. Ahmed R, Bopp C, Borczyk A, Kasatiya S. Phage-typing scheme for Escherichia coli O157:H7. J Infect Dis 1987;155:806-9.

43. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. 2013. p. 1-37.

44. Department of Health. Health Protection Legislation (England) Guidance. 2010. p. 1-112.

45. Scottish Government. Public Health (Scotland) Act. 2008 (as amended).

46. Scottish Government. Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States. 2009.

47. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.

48. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967 (as amended).