UK Standards for Microbiology Investigations

Identification of *Escherichia coli* O157

Issued by the Standards Unit, Microbiology Services, PHE

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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 http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

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.

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UK Standards for Microbiology Investigations are produced in association with:
Identification of *Escherichia coli* O157

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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.

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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 http://www.hpa.org.uk/SMI/Partnerships. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and processes 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.

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#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.

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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 http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313. 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.
Identification of Escherichia coli O157

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.

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. However, some VTEC O157 strains have been found to ferment sorbitol and to be β-glucuronidase positive.

Strains are oxidase negative and usually produce gas from glucose. Some strains show atypical biochemistry e.g. 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.

Principles of Identification

B 30 – Investigation of faecal specimens for bacterial 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 other E. coli strains, and care must be taken with the interpretation of the profile.

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
Identification of Escherichia coli O157

Escherichia coli O157 colonies are observed on CT-SMAC agar, we recommend that 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 Infections Reference Unit (GIRU) of the Laboratory for Gastrointestinal Pathogens (LGP) 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 must 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 other E. coli strains4, and care must be taken with the interpretation of the profile. 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 0157 antiserum, and thus it could be mistaken for E. coli 0157. 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 01577.

1. Safety Considerations8-24

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 dose15. VTEC O157 may cause severe illness that is sometimes fatal. Laboratory acquired infections have been reported25-28. 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**\(^6,29\)

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

**NB:** Other *E. coli* serotypes like 0145 can act like 0157: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 16-24hrs. 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 \((B 30 - Investigation of faeces specimens for bacterial pathogens)\).

### 3.3 Colonial Appearance

On CT-SMAC agar, typical VTEC O157 colonies are colourless or slightly greyish in appearance and 2-3mm in diameter. Some rare variant strains of VTEC O157 ferment sorbitol and may grow poorly on CTSMAC/SMAC.

**NB:** 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.

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DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 15 NOVEMBER - 13 DECEMBER 2013
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

**Agglutination test**

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

**Biochemical tests**

Commercial identification systems

Laboratories must follow manufacturer’s instructions and rapid tests and kits must be validated and be shown to be fit for purpose prior to use.

**Subculture to lactose containing media**

Chromogenic identification plates are available and may be valuable as an alternative for confirmation of identification of E. coli. This may be the purity plate from the commercial identification kit.

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

### 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 identification and sensitivity methods have been developed for isolates from clinical samples; these include molecular techniques such as Real-time Polymerase Chain reaction (PCR), Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable-Number Tandem-Repeat Analysis (MLVA), Whole Genome Sequencing (WGS) and Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF) Mass Spectrometry. All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

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

**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 simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use.

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.

Real-time Polymerase Chain reaction (RT-PCR)

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.

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, and 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.

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.

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 accurately discriminate among sporadic isolates 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 $^{34}$.

**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 $^{35,36}$. The other limitations are that PFGE is labour-intensive, and the results are difficult to analyse and not easily transferable between laboratories $^{33}$.

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 $^{33}$. In addition to traditional epidemiological investigations, PFGE is used to discriminate between outbreak and sporadic strains of *E. coli* O157:H7 $^{37}$.

**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, Illumina 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 $^{1,38}$.

**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 $^{39}$.
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 *E. coli* O157

The flowchart is for guidance only

**NB:** Refer to clinical details: in cases and particularly clusters of cases where isolation or identification fails, but the symptoms are consistent with VTEC O157 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

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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 card 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
Refer to current guidelines on CDSC and COSURV reporting.

5.5 Infection Control Team
Inform the infection 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 nation reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

Gastrointestinal Infections Reference Unit
Microbiology Services
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

Scotland

Northern Ireland
http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm
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. Immediate cases should be notified orally 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.

NB: The Health Protection Legislation Guidance (2010) includes reporting of HIV & STIs, HCAIs and CJD under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

Other arrangements exist in Scotland, Wales and Northern Ireland.
References


8. 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".


Identification of *Escherichia coli* O157


