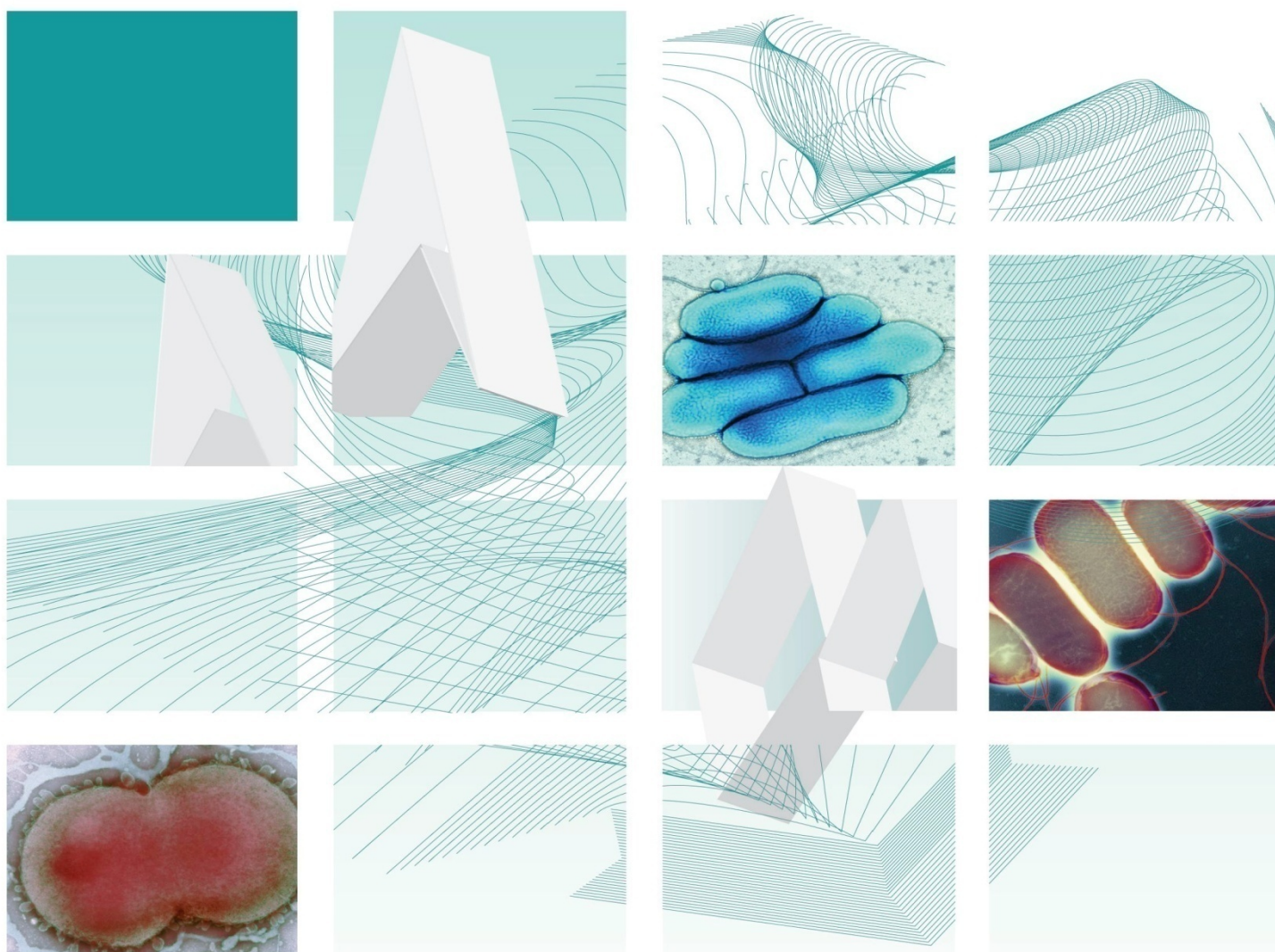




UK Standards for Microbiology Investigations

Investigation of Faecal Specimens for Enteric Pathogens



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

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Contents

ACKNOWLEDGMENTS	2
AMENDMENT TABLE	4
UK SMI: SCOPE AND PURPOSE	6
SCOPE OF DOCUMENT	8
SCOPE	8
INTRODUCTION	8
TECHNICAL INFORMATION/LIMITATIONS	22
1 SAFETY CONSIDERATIONS	25
2 SPECIMEN COLLECTION	25
3 SPECIMEN TRANSPORT AND STORAGE	26
4 SPECIMEN PROCESSING/PROCEDURE	26
5 REPORTING PROCEDURE	31
6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS	32
APPENDIX 1: INVESTIGATION OF FAECAL SPECIMENS FOR ROUTINE BACTERIAL PATHOGENS	34
APPENDIX 2: INVESTIGATION OF FAECAL SPECIMENS FOR ADDITIONAL BACTERIAL PATHOGENS	35
REFERENCES	36



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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.	11/24.04.14
Issue no. discarded.	8
Insert Issue no.	8.1
Section(s) involved	Amendment
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety and notification references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	10/25.03.13
Issue no. discarded.	7
Insert Issue no.	8
Section(s) involved	Amendment
Title.	Name changed from 'bacterial' to 'enteric' pathogens.
Scope.	Recommendation for routine screening of faeces for <i>Campylobacter</i> species, <i>Salmonella</i> species, <i>Shigella</i> species and <i>Escherichia coli</i> VTEC (including O157) on all diagnostic samples, and screening of faeces for <i>Cryptosporidium</i> species on all diarrhoeal samples.
Introduction.	Reorganisation of some text and headings in line with S 7.

	<p>Updated definition of diarrhoea.</p> <p>Discussion of outbreaks with microbiologist and outbreak team added.</p> <p><i>E. coli</i> VTEC section updated to include non-O157 <i>E. coli</i>.</p> <p><i>Clostridium difficile</i> information updated.</p> <p><i>Plesiomonas shigelloides</i> information updated.</p> <p>Addition of <i>Laribacter hongkongensis</i>.</p> <p><i>Yersinia enterocolitica</i> information updated.</p>
Technical Information/Limitations.	<p>Addition of 'Rapid Diagnostic Tests'.</p> <p>Dilution factor for direct plating changed from 1:10 to 1:4.</p> <p>Addition of information regarding <i>Plesiomonas shigelloides</i> identification.</p> <p>Addition of 'Chromogenic Media'.</p>
Section 2: 2.5.3. 2.8.	<p>Table updated for clarity.</p> <p>Reference Laboratory links updated.</p>
Section 4.	<p>Link to causative agents notifiable to the HPA added.</p>
References.	<p>Some references updated.</p>

UK SMI[#]: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also 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. The documents also 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 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.

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

[#] 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.

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.

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Suggested Citation for this Document

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Scope of Document

Type of Specimen

Faeces

Scope

This SMI describes the bacteria responsible for enteric infection and culture methods for their isolation. Rapid diagnostic tests are also available (refer to Technical Information/Limitations), and should be considered for use following validation. The document takes account of UK data from the Infectious Intestinal Disease 2 (IID2) study 2011¹. It recommends routine screening of faeces for *Campylobacter* species, *Salmonella* species, *Shigella* species and *Escherichia coli* VTEC (including O157) on all diagnostic samples, and screening of faeces for *Cryptosporidium* species on all diarrhoeal samples². Consideration should be given to the 'three-day rule' for collection of faecal samples from hospitalised patients (see section, "Collection of faeces samples"). In addition faeces may be screened for individual organisms as indicated by clinical details.

A short section on viruses implicated in enteric disease is included in the introduction.

The microscopy section includes standard methods for the preparation of faecal parasite concentrations and cryptosporidium smears. Investigation of faecal parasites is fully described in [B 31 - Investigation of Specimens other than Blood for Parasites](#).

For details of *Clostridium difficile* refer to [B 10 - Investigation of Faecal Specimens for *Clostridium difficile*](#).

This SMI should be used in conjunction with other SMIs.

Introduction

Diarrhoea

This may be defined as unusual frequency of bowel action (usually at least three times in a 24hr period), passing loose, watery, unformed faeces. The consistency of the stools is more important than the frequency: frequently passed formed stools are not considered to be diarrhoea³. It may be associated with symptoms such as abdominal cramps, nausea and malaise, and with vomiting, fever and consequent dehydration. Patients with visible blood and mucus in the faeces, suggesting inflammation of the bowel, accompanied by symptoms such as abdominal cramps and constitutional disturbance, may be said to be suffering from dysentery.

A wide range of bacterial pathogens, viruses and parasites are capable of causing diarrhoea by a number of mechanisms. For example, diarrhoeal illness may result from multiplication of bacteria in the gut, eg *Salmonella* species or *Shigella* species infection, or ingestion of pre-formed toxins produced by bacteria in food prior to ingestion, eg *Bacillus cereus* toxins. Consumption of food containing irritant chemicals such as heavy metals may also cause diarrhoea.

Outbreaks

Outbreaks may occur as a result of inter- personal infection, through ingestion of infected food and water, and from direct contact with animals. Food borne outbreaks are defined as two or more cases of a similar illness resulting from the ingestion of a common food, and currently are estimated to cause three million deaths worldwide per year. A water borne outbreak is defined as two or more cases of a similar illness resulting from the drinking of water, or contact with water used for recreational purposes, from a common source. Water borne outbreaks are estimated to cause the death of two million children annually as a consequence of diarrhoeal disease.

All outbreak samples should be discussed with a microbiologist and the outbreak response lead (infection control team (hospital) or public health team (community)) to agree appropriate tests based on the clinical and epidemiological information available.

Collection of Faecal Samples

Some clinicians advocate the use of a 'three-day rule'. This rule is derived from the low numbers of faecal pathogens isolated from patients who have been hospitalised for longer than three days. It suggests that faecal samples from these patients should not be cultured except under the following circumstances^{4,5}:

- those inpatients suffering diarrhoea within three days of admission
- adults with nosocomial diarrhoea only if one of the following is applicable:
 - aged 65 or more with pre-existing disease causing permanently altered organ function
 - patients who are HIV positive
 - patients with neutropenia
- suspected nosocomial outbreak
- those with suspected non-diarrhoeal manifestations of enteric infections

The 'three-day rule' does not apply to *C. difficile*; testing for *C. difficile* is required for inpatients as soon as infective diarrhoea is suspected⁶. Conformity to this 'three-day rule' relies on appropriate clinical information accompanying the specimen. The rule may be unreliable if request forms are not fully completed, and problems with late recognition of hospital-associated outbreaks have been reported with the use of such rules⁷. Clinicians and laboratories should consult local policy on the 'three-day rule' for the culture of faeces samples in their departments, and their use should be considered carefully in the light of recent changes in the prevalence and severity of *Clostridium difficile* associated diarrhoea.

Bacteria Commonly Associated with Gastrointestinal Infections

All diagnostic faecal samples, except single organism screens, should be tested for the following organisms:

***Campylobacter* species**

Campylobacter species were first recognised as a cause of abortion in cattle and sheep, and a cause of diarrhoea in cattle and pigs. They were first isolated from the faeces of humans in the early 1970s. *Campylobacter* species are now known to be a major cause of enteritis in the developed world, and are the commonest identifiable

bacterial cause of diarrhoea in the UK as identified by the second Infectious Intestinal Disease study (IID2)¹. *Campylobacter* enteritis in the UK has marked seasonal peaks which occur in May and September. *Campylobacter jejuni* accounts for about 90% of reported infections and most of the remainder are caused by *Campylobacter coli* and *Campylobacter lari*; other *Campylobacter* species have also been isolated from cases of diarrhoea⁸. The species most commonly associated with disease in humans are thermophilic, ie, they will grow at 42°C - 43°C and 37°C, but not at 25°C. *Campylobacter jejuni* subspecies *doylei* and *Campylobacter fetus* (and *C. fetus* subspecies *venerealis*) do not grow at 42°C.

In human hosts, diarrhoea is usually brief, and sequelae are uncommon. Initial symptoms may be severe, with fever and abdominal pain suggesting appendicitis. Faeces frequently contain mucus with blood and leucocytes. *Campylobacter* species infection may occasionally become invasive, with consequences ranging from transient self-limiting bacteraemia, to fulminant Gram negative sepsis. Occasionally infection may produce sequelae such as reactive arthritis, bursitis, endocarditis and neonatal sepsis.

Acute post-infective demyelination may develop, affecting the peripheral nervous system (Guillain-Barré Syndrome), and/or the central nervous system and cranial nerves (eg the Miller-Fisher Syndrome (areflexia, ataxia, and cranial nerve pareses; polyneuritis cranialis)). Specific serotypes are implicated in these conditions⁹.

The clinical presentation of these latter conditions (affecting the brainstem and the cranial nerves) must be distinguished from that of botulism. Deep tendon reflexes are initially preserved in cases of botulism, but are lost early in cases of post-infective demyelinating disease. Nerve conduction is slowed in demyelinating diseases, and the cerebrospinal fluid commonly shows an increase in protein concentration, usually without any accompanying pleocytosis^{9,10}.

Both groups of disorders may culminate in respiratory failure, requiring mechanical ventilation.

Campylobacter fetus is an opportunist organism which may be isolated from blood and other body fluids of immunodeficient patients and is responsible for 8-10% of campylobacter bacteraemia cases.

***Salmonella* species**

Non-typhoid *Salmonella* (*Salmonella* species other than *Salmonella* Typhi, *Salmonella* Paratyphi A, B and C) and *Salmonella* Dublin.

These *Salmonella* species are ubiquitous in animal populations. Human infection is generally associated with consumption of food of animal origin, the drinking of water contaminated by animals, or inter- personal contact. Inter-personal transmission of non-typhoid salmonellae occurs where levels of hygiene may be particularly poor, eg mental healthcare units and schools.

There is marked seasonal variation in occurrence of infection with peaks of incidence during summer and autumn. Infection with *Salmonella* species may be associated with foreign travel, and consumption of imported foodstuffs may be associated with a higher risk of infection¹¹.

Gastroenteritis is the most common condition caused by *Salmonella* species. Symptoms include abdominal pain, diarrhoea, nausea and vomiting, often accompanied by fever. Other clinical manifestations of salmonellosis include

bacteraemia and focal metastatic (haematogenous) infections and the organism may be isolated from other specimens such as blood and urine. A small number of patients may develop an illness that resembles enteric fever (see below). Low numbers of *Salmonella* species may also be present in the faeces of healthy asymptomatic carriers. Certain underlying conditions such as malnutrition, immunosuppression, sickle-cell disease, achlorhydria and inflammatory bowel disease may be associated with more severe infections.

Enteric fever

Although many *Salmonella* species are recorded to have caused invasive infections, those most regularly doing so are *Salmonella* Typhi and *Salmonella* Paratyphi (groups A, B, and C) - the causative organisms of enteric (typhoid) fever. Many *Salmonella* serotypes may be transmitted from animals to man, but *S. Typhi* and *S. Paratyphi* A are usually carried by humans only, and transmitted via human faecal contamination of food or water. Individuals recovering from enteric fever may carry the organism for long periods. Relapsing, non-enteric *Salmonella* species infections may be seen in patients with HIV/AIDS.

Cultures of *S. Typhi* and of *S. Paratyphi* A, B or C, known or suspected, must be handled at Containment Level 3.

Enteric fever is a multi-system disease characterised by:

- prolonged fever
- hypertrophy and activation of the reticulo-endothelial system, particularly the intestinal and mesenteric lymphoid tissue, liver and spleen
- sustained bloodstream infection without endothelial or endocardial colonisation
- metastatic infection and immunologic complications such as immune complex deposition leading to multi-organ dysfunction
- rose spots
- association with constipation (diarrhoea seldom being present until late in the disease course)
- reactive arthritis
- low white blood cell count

The causative organism of an enteric fever may not always be present in faeces. Faecal culture alone is not adequate for the laboratory investigation of enteric fever. Blood cultures should always be collected and enteric fever may be confirmed by isolating *S. Typhi/Paratyphi* from the blood ([B 37 - Investigation of Blood Cultures \(for Organisms other than *Mycobacterium* species\)](#)), bile ([B 15 - Investigation of Bile](#)), bone marrow ([B 38 - Investigation of Bone Marrow](#)) or urine ([B 41 - Investigation of Urine](#)).

Chronic carrier states occur when patients recover from the acute disease (either gastroenteritis or enteric fever) but continue to shed the organism. Therefore, *Salmonella* species may be present in the faeces or urine of patients for one year or longer. The principal site where organisms are harboured is the biliary tract. Obstruction with gallstones or biliary scarring makes eradication of organisms difficult. Similarly, carriage in the urinary tract may be associated with urolithiasis, and with damage caused by urinary schistosomiasis.

***Shigella* species¹²**

Infection with *Shigella* species manifests as a range of symptoms. At its mildest, watery diarrhoea is produced, but this may progress to dysentery with frequent small volume faeces containing blood, mucus and pus. The diarrhoea may be accompanied by fever and abdominal cramps. There is often marked constitutional disturbance (in contrast to cases of dysentery caused by *Entamoeba histolytica*, where the patient may remain relatively well apart from gastrointestinal disturbance).

There are four *Shigella* species:

- *S. dysenteriae*
- *S. flexneri*
- *S. boydii*
- *S. sonnei*

Diagnosis of bacillary dysentery is made by isolation of the infecting organism. Cultures of *S. dysenteriae*, known or suspected, must be handled at Containment Level 3.

All four species are capable of causing dysentery, but *Shigella dysenteriae* serotype 1 causes a particularly severe form of the disease with marked constitutional disturbance. This is due to the production of Shiga toxin, which is closely related to the toxin produced by strains of verocytotoxic *E. coli* O157 (VTEC). As in infection with VTEC, infection with toxigenic *S. dysenteriae* may result in the haemolytic-uraemic syndrome (HUS).

Organisms are primarily transmitted directly from person to person, and multiplication in the environment rarely occurs. Organisms are easily transferred on fingers (faecal-oral spread), via food or water, or by contaminated fomites. *Shigella* species are highly infective, particularly *S. dysenteriae*, which may require as few as 10-100 organisms for an infective dose¹³. Asymptomatic infection has been reported, particularly with strains of *Shigella sonnei*.

Outbreaks may be associated with overcrowding in schools, prisons, mental institutions, and where there are low standards of hygiene. Deaths are more commonly seen during famine and in countries with poor socio-economic circumstances¹³. *S. sonnei* is the commonest species isolated in the UK. *S. dysenteriae* and *S. boydii* are rarely seen in the UK, except as a consequence of foreign travel.

***E. coli* VTEC (including O157)^{14,15}**

E. coli VTEC is an enterohaemorrhagic *E. coli* (EHEC) which produces verocytotoxins. *E. coli* VTEC are also known as Shiga-like toxin producing *E. coli* (STEC).

Verocytotoxin is similar to the 'Shiga' toxin of *Shigella dysenteriae*, and is associated with haemorrhagic colitis and haemolytic uraemic syndrome^{16,17}. There are over 300 known serotypes of VTEC, most of which are not pathogenic: the most common pathogenic serotype in the UK is *E. coli* VTEC O157, and this is the only VTEC for which diagnostic laboratories routinely test¹⁵.

Low numbers of verocytotoxin producing *E. coli* O157 are required to cause infection. Infections vary in severity from mild to bloody diarrhoea (presenting as maroon coloured stools) and may occur in any age group, although it is more common in children. Blood is not always present in faeces in verocytotoxin producing *E. coli*

infections, and the presence of blood must not be used as the sole criterion for selecting specimens for examination for this organism.

The highest incidence of verocytotoxin producing *E. coli* O157 infection is in children <5 years of age. There is a marked seasonal variation, with a peak incidence in the summer and early autumn. Outbreaks have been directly associated with contaminated cooked meats, milk and water, ground beef, beef burgers and indirectly with vegetables, apple cider and mayonnaise¹⁸. Outbreaks may occur in establishments such as nursing homes¹⁸. There have also been outbreaks of *E. coli* O157 infection involving visitors to open farms¹⁹.

This SMI recommends direct plating for *E. coli* VTEC (including O157) of all diagnostic specimens, including those for screening asymptomatic contacts in risk groups. Culture is recommended for all diarrhoeal faecal samples. Suspicious isolates that have been locally confirmed by serological and biochemical tests can be identified as 'presumptive' *E. coli* (VTEC). Cultures of *E. coli* O157 and other VTEC, known or suspected, must be handled at Containment Level 3. There have recently been cases of infection with sorbitol fermenting VTEC O157 (more details are available in the Technical Information section of this document)²⁰.

'Presumptive' (locally confirmed) isolates should be referred to the reference laboratory for detection of verocytotoxin genes, confirmation of identity and phage typing. Most VTEC O157 strains are motile and have the flagella antigen H7, but about 20% of strains are phenotypically non-motile.

As available methods and selective agars are primarily aimed at detecting VTEC O157:H7, faeces from patients with a dysenteric syndrome for which no cause can be found by use of standard microbiological techniques should be sent to a reference laboratory for molecular testing and enhanced culture in order to search for both *E. coli* O157 and other non-O157 verotoxigenic strains of *E. coli*.

Bacteria Commonly Associated with Travel Associated Gastrointestinal Infections and Shellfish Consumption

In addition to the bacteria listed above, several pathogens are associated with cases of travellers' diarrhoea. The destination and season of travel have been shown to affect the predominant causative organisms, and typically symptoms develop within the first week of travel²¹. *Vibrio* species and *Plesiomonas* species have been associated both with travel to endemic areas and with shellfish consumption¹¹. *Vibrio* species and *Plesiomonas* species should be tested for on samples from symptomatic patients who have recently travelled (within two to three weeks previously) to endemic areas (Asia, Africa or Latin America)^{22,23}. *E. coli* (other than *E. coli* O157 VTEC) including enteroaggregative (EAEC), enteroinvasive (EIEC), enteropathogenic (EPEC) and enterotoxigenic (ETEC) may also cause travel associated diarrhoea.

***Vibrio* species**

Vibrio species are natural inhabitants of brackish and salt water worldwide. Several species are pathogenic to man and are usually associated with ingestion of contaminated water or seafood, and travel to endemic areas. The diarrhoea-causing species most frequently isolated are *Vibrio cholerae* (the causative agent of cholera), *Vibrio parahaemolyticus*, *Vibrio fluvialis* and *Vibrio mimicus*²⁴. *Vibrio vulnificus* does not cause diarrhoea, but has been isolated from the blood and tissues of septic patients (especially those with liver disease)²⁵.

Symptoms range from mild (often accompanied by abdominal cramps and vomiting) to explosive diarrhoea.

Strains of *V. cholerae* O1 are the aetiological agents of epidemic cholera. *V. cholerae* O1 has two biotypes: classical and El Tor. *V. cholerae* O1 can also be subdivided into three serotypes: Ogawa, Inaba and Hikojima. Ogawa and Inaba strains agglutinate with specific antisera. Hikojima strains, although rare, agglutinate with both anti-Ogawa and anti-Inaba antisera. In 1993, a new cholera-causing serogroup, *V. cholerae* O139 Bengal, emerged in southern India and spread to several countries in the Asian continent and the Americas²⁶.

The main symptoms of cholera are passage of a profuse watery diarrhoea with mucus, but no blood, giving a 'rice water' appearance. Fluid loss and dehydration is the main cause of death. The incubation period varies from a few hours to a few days. Diarrhoea may last up to six days; long term carriers are rare. Cholera outbreaks are most often associated with contaminated water and contaminated food, particularly undercooked or raw seafood.

V. cholerae O1 and O139 are producers of the cholera toxin (CT), unlike *V. cholerae* non-O1/non-O139 strains which only occasionally produce enterotoxin. CT is very similar to the heat-labile enterotoxin produced by some strains of *E. coli* (ETEC).

Vibrio parahaemolyticus and *V. fluvialis* are responsible worldwide for gastroenteritis (characteristically acute diarrhoea, which may be bloody and lasts about three days) associated with eating contaminated seafood, particularly shellfish²⁴.

***Plesiomonas shigelloides*^{23,27}**

Plesiomonas shigelloides has now been recognised as an important cause of travellers' diarrhoea, particularly in the Indian subcontinent and South East Asia where it may account for around 5% of cases. The organism does not survive well below 8°C so is a pathogen of warm climates, with a peak summer incidence. It is frequently associated with seafood consumption. Incubation is usually less than 48hr and, although the illness is usually self-limiting, symptoms can be severe and patients may remain symptomatic for up to four weeks. It is recommended that investigations for *P. shigelloides* should be undertaken on travellers who would normally be investigated for *Vibrio* species infection.

Enteroaggregative *E. coli* (EAEC)^{28,29}

These form a group of entero-adherent *E. coli* that are associated with chronic diarrhoea, particularly in children, in many parts of the world. EAEC infection symptoms in children include: watery diarrhoea, vomiting, dehydration, and occasionally abdominal pains, fever and passage of bloody faeces. These organisms have been detected in travel acquired infections and may also cause chronic diarrhoea in patients infected with HIV.

EAEC belong to a diverse group of *E. coli* characterised by adherence to HEp-2 and HeLa cells with the formation of characteristic patterns and demonstration of this is considered to be the 'gold diagnostic standard'. Strains belong to a wide range of serotypes; toxin production is likely to be important for pathogenicity in a sub-set these serotypes³⁰. PCR assays targeting EAEC-specific genes are available for the detection of EAEC in faecal specimens; if required, stools should be sent to the appropriate reference laboratory for testing by PCR and for enhanced culture.

A recent outbreak of *E. coli* O104:H4 in Germany, due to an *E. coli* clone combining two virulence genes from two distinct pathogens, has been well documented³¹⁻³³. The enteroaggregative-haemorrhagic *E. coli* (EAHEC) strain evolved from an enteroaggregative *E. coli* by uptake of a Shiga-toxin encoding bacteriophage³². The outbreak strain, a sorbitol fermentor, would most likely have been missed by standard diagnostic techniques which focused on *E. coli* O157³¹.

Enteroinvasive *E. coli* (EIEC)

EIEC cause a dysentery-like illness characterised by fever, abdominal cramps, and watery diarrhoea with blood and mucus which generally contains leukocytes. The condition may closely resemble shigellosis¹⁶.

EIEC invade the colonic mucosa disrupting the epithelial cells. These organisms are rarely encountered in the UK, and routine investigation is not recommended in this SMI. However, faeces from a patient with a dysenteric syndrome, for which no cause can be found by use of standard microbiological techniques, may be sent to a reference laboratory in order to search for these less common bacteria.

Enteropathogenic *E. coli* (EPEC)

These organisms cause infant diarrhoea. They do not appear to produce toxins, but are entero-adherent and damage the villi in the gut. Symptoms include severe, prolonged non-bloody diarrhoea usually with passage of mucus. Vomiting and fever are also common.

Polyvalent antisera for detecting EPEC 'O' antigens are commercially available. These tend not to be used routinely in developed countries because outbreaks with EPEC are now rare. EPEC may be associated with travellers' diarrhoea.

Enterotoxigenic *E. coli* (ETEC)

ETEC cause travellers' diarrhoea, as well as infant diarrhoea in developing countries. They cause mild, watery diarrhoea with abdominal cramps, nausea and low grade fever. Recent outbreaks have been characterised by patients exhibiting prolonged symptoms.

Detection of ETEC requires both DNA-based and culture methods, this is performed mainly in reference laboratories if clinical symptoms and patient history indicate that this would be useful.

Organisms such as *Helicobacter* species, *Arcobacter* species, *Aeromonas* species, *Edwardsiella tarda* and *Laribacter hongkongensis*, may be implicated in travellers' diarrhoea, however investigations for them are rarely indicated and are described later in this document^{23,34,35}.

Bacteria Commonly Associated with Food/Water Poisoning (Toxin Producers)³⁶

Enumeration of the following organisms should be performed in food poisoning and outbreak situations. Vegetative cell or spore counts are essential for food poisoning and outbreak investigations, as organisms may be present in low numbers in healthy adults and children; high counts may indicate infection.

***Staphylococcus aureus*³⁷**

Staphylococcus aureus forms part of the gut flora and may normally be found in small numbers in faeces. In cases of infection, large numbers of *S. aureus* are present in faeces.

Symptoms of *S. aureus* food poisoning include vomiting, nausea and abdominal cramps, often followed by diarrhoea. The incubation period is 1-6hr.

Strains of *S. aureus* which cause food-poisoning produce heat-resistant enterotoxins. *S. aureus* food poisoning occurs after the ingestion of foods in which the organism has grown and produced the enterotoxin(s). The toxins are relatively stable and may be present in the absence of viable organisms after cooking, pasteurisation or prolonged storage of foodstuffs.

Diagnosis is confirmed by culturing the faeces from infected persons as well as from incriminated foods. The organism may also be isolated from vomit, but culture of these specimens is not recommended in this SMI. Culture may not be successful because of the death of the organism following enterotoxin production. However, sufficient enterotoxin may be present in food for detection by immunoassay. Detection of enterotoxin in faeces or vomit is of limited diagnostic value.

Isolated staphylococci should be phage typed and tested for enterotoxin production in a reference laboratory. Toxin-producing strains may be identified as the cause of the infection if identical phage types are isolated from both food and faeces.

***Bacillus cereus* and *Bacillus species*³⁷**

Bacillus cereus is a common cause of food poisoning by ingestion of a toxin rather than infection with living organism. Food poisoning occurs after ingestion of foods in which the organism has multiplied to large numbers and formed the toxins. Two clinical syndromes may ensue - the diarrhoeal syndrome which resembles *Clostridium perfringens* food poisoning and which is due to an enterotoxin, and the emetic syndrome caused by a thermostable peptide. The emetic syndrome is associated with the ingestion of rice and pasta-based foods and is characterised by nausea and vomiting 1-5hr after consumption of the implicated foodstuff. The diarrhoeal type causes diarrhoea and abdominal pain 8-16hr after ingestion of the contaminated food³⁸.

B. cereus may be found in small numbers in the faeces of healthy people. In cases of suspected food poisoning, quantitative culture from faeces and, where available, vomit and food should be attempted. Viable counts from food are generally greater than 10⁴ per gram. Immunoassay can be used to detect any toxin present in the food, but the detection of toxin produced by isolates or present in faeces or vomit is of limited diagnostic value.

Bacillus subtilis and *Bacillus licheniformis* may also be involved in food poisoning episodes.

***Clostridium perfringens*³⁷**

Clostridium perfringens counts in normal human faeces are generally less than 10⁴-10⁵ organisms per gram.

Some strains of *C. perfringens* (typically type A2) are associated with a mild form of food-poisoning occurring in all age groups. The predominant symptoms of this toxic

food poisoning are watery diarrhoea with severe abdominal pain and an incubation period of 8-24hr.

Enteritis necroticans may result from infection with *C. perfringens* type C³⁹. Mortality approaches 40%. The condition is known as 'pig-bel' in Papua, New Guinea (associated with eating undercooked pork) and as 'darmbrand' in Germany. The condition has been reported from various areas of the world.

Laboratory confirmation of *C. perfringens* food poisoning requires at least one of the following criteria to be fulfilled: isolation of the same serotype from the faeces of affected individuals and from food; detection of enterotoxin in the faeces of affected individuals; or faecal spore counts of $>10^5$ organisms per gram.

This SMI recommends that investigation of faeces for *C. perfringens* should only be performed in food poisoning incidents, and that investigations for *C. perfringens* in non-food poisoning cases should not be undertaken routinely. Investigations should only be undertaken with the support of a clinical microbiologist.

C. perfringens spore counts can be determined following alcohol shock treatment. Quantification studies may be helpful because spore counts of $>10^5$ /g are usually only found in food poisoning cases, and confirmation may then be obtained by testing extracts of fresh faecal samples for *C. perfringens* enterotoxin. *C. perfringens* isolates should be retained for serotyping, and faecal specimens retained for toxin testing.

***Clostridium botulinum*^{10,37}**

Clostridium botulinum is the cause of a rare but often fatal form of food poisoning. Botulism is a neuroparalytic disease produced by the neurotoxins of *C. botulinum*, and is classified in four categories:

- Classical food borne botulism which is typically seen in adults, resulting from the ingestion of preformed toxin in contaminated food
- Wound botulism which is the rarest form of the disease. It results from the production of toxin *in vivo* after *C. botulinum* has multiplied in an infected wound
- Infant botulism (although still rare) is the most common infection. It results from multiplication of *C. botulinum* in the infant gut with the production of neurotoxin. Symptoms can range from mild illness to sudden death
- "Classification undetermined" describes cases of botulism in individuals who are over 12 months old in whom no food or wound source of *C. botulinum* can be implicated

Toxins are produced by *C. botulinum* when the organism survives and multiplies in inadequately processed stored foods. The pre-formed toxins may later be ingested with the food and then may be absorbed to the bloodstream and transfer to the peripheral nerve synapses where they block neurotransmission. This causes a descending flaccid paralysis, typically with cranial nerve involvement, sometimes culminating in respiratory arrest¹⁰. The condition must be distinguished from demyelinating polyneuropathy and the Miller Fisher syndrome.

Botulinum toxins are amongst the most toxic substances known, and might conceivably be used in biological warfare or bioterrorism⁴⁰.

The usual severity of the symptoms and the length of time taken for laboratory tests necessitate that a provisional diagnosis must be made by the clinician based on the patient's symptoms. Laboratory findings which confirm the clinical diagnosis include demonstration of toxin in serum or faeces, and detection of the organism in foods (or faeces in the case of infant botulism)¹⁰. These tests should be performed by a reference laboratory and culture is not recommended in this SMI.

Antibiotic Associated Diarrhoea (AAD)

Almost all drugs with an antibacterial spectrum of activity have been implicated causally in AAD. The most frequently implicated drugs are those which have a marked effect on the microflora of the colon. These include broad spectrum beta lactams, cephalosporins, clindamycin and fluoroquinolones⁴¹. The incidence of infection has been shown to decrease once antibiotic usage is controlled. *C. difficile* infection (CDI) is the most common identifiable cause of AAD, however infection with *C. perfringens*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Candida* species and *Salmonella* species have also been implicated^{42,43}.

***Clostridium difficile* Infection**

C. difficile infection is defined as one episode of diarrhoea (loose stool taking the shape of the sample container, or Bristol Stool Chart type 5-7) that is not attributable to any other cause (underlying illness, medication or therapy) and which occurs at the same time as a toxin positive assay (with or without a positive *C. difficile* culture) and/or endoscopic evidence of pseudomembranous colitis^{6,44}.

C. difficile has been associated with outbreaks in hospitals and in extended care facilities for the elderly, and represents an important cause of hospital-acquired infection⁶. The organism may be isolated from the hospital environment and may be found on floors, toilets and bedding⁴⁵.

A two-stage testing approach is recommended by the Department of Health. Refer to current guidelines (or Devolved Nation equivalent)^{44,46}.

For details regarding *C. difficile* testing refer to [B 10 – Investigation of Faecal Specimens for *Clostridium difficile*](#).

Other Microorganisms to Consider

The following organisms should only be looked for in unresolved cases of gastroenteritis and diarrhoea or under special circumstances following discussion with a medical microbiologist.

***Yersinia enterocolitica*⁴⁷**

Yersiniosis, caused by *Yersinia enterocolitica*, is a zoonotic infection. *Y. enterocolitica* can be isolated from wild and domestic animals, environmental samples and food samples. The majority of isolates are non-pathogenic. *Y. enterocolitica* is classified using a combination of biochemical characteristics and serology. Of the six biotypes, five are recognised to be pathogenic (1B, 2-5). *Y. enterocolitica* can also be separated serologically; the majority of pathogenic strains belong to a limited number of O serogroups namely 0:3, 0:9 and 0:5, 27 in the UK, and 0:8 in North America. Infection by other types is rare. A number of foodborne outbreaks caused by *Y. enterocolitica* have occurred in Europe, North America and Japan.

Infection is usually acquired orally from contaminated food, milk or water, and pigs are a frequently identified source of infection. Infection occurs more often in the young (<6y) and the elderly, but in food associated outbreaks all ages may be affected⁴⁸.

After ingestion, the organism proliferates in the lymphoid tissue of the small intestine where it may cause hyperaemia, neutrophil infiltration and ulceration. The incubation period is between four and seven days.

Occasionally, haematogenous spread occurs, leading to sepsis with the formation of abscesses in organs such as the liver and spleen.

Yersiniosis may therefore present with a variety of clinical conditions^{49,50}:

- acute diarrhoea
- mesenteric adenitis/lymphadenitis
- terminal ileitis
- 'pseudo-appendicitis'
- sepsis
- metastatic infections
- immunological sequelae (eg reactive arthritis)

The clinical presentation of mesenteric lymphadenitis is often difficult to differentiate from acute appendicitis.

Culture for this organism is only recommended when clinical suspicion has been aroused, and may be considered in cases of gastroenteritis where *Salmonella* species and *Campylobacter* species have been ruled out, particularly when associated with travel outside the UK.

Isolates should be referred to a reference laboratory, as biotyping and/or serotyping are necessary to establish pathogenicity.

Yersinia pseudotuberculosis

Yersinia pseudotuberculosis colonises many wild and domestic animals as well as birds. Outbreaks are commonly associated with captive rodent colonies, but the organism rarely causes infection in humans. Strains have been isolated from blood, tissues such as lymph nodes, and occasionally from faeces⁴⁷.

***Helicobacter* and *Arcobacter* species**

Helicobacter species including *Helicobacter fennelliae* (formerly *Campylobacter fennelliae*) and *Helicobacter cinaedi* (formerly *Campylobacter cinaedi*) are implicated in the causation of human gastrointestinal disease. *H. fennelliae* and *H. cinaedi* first came to attention as the cause of disease in men who had sex with men. Relevant clinical history may guide further investigations in individual patients.

Several species formerly classified in the genus *Campylobacter* are now assigned to the genus *Arcobacter* (eg *Arcobacter butzleri*) and have been reported as to cause human gastroenteritis^{51,52}. The illness is normally self-limiting; however illness is sometime severe enough to require antibiotics³⁴.

Clostridium septicum

Clostridium septicum is rarely isolated from the faeces of healthy individuals. A clinically important association has been described between *C. septicum* bacteraemia, neutropenia and enterocolitis. Although rare, neutropenic enterocolitis runs a fulminating course which is usually fatal in the absence of surgical and antibiotic intervention. Patients often present with abdominal pain and fever resembling appendicitis. *C. septicum* may be isolated in these cases from the blood or gut contents.

***Aeromonas* species**

Aeromonas species have been implicated as causative organisms of watery, non-bloody diarrhoea²³. Young children and elderly patients may be more susceptible to infection. Although the organisms have been linked to food and water-borne outbreaks, their significance is still uncertain and routine investigation for these species is not recommended.

Edwardsiella tarda

This organism has been associated with sporadic cases of gastroenteritis; it is found worldwide, but is most common in tropical and sub-tropical areas³⁴. There is some evidence that it may cause diseases similar to those caused by *Salmonella* species, including enteric fever⁵³. Most laboratories do not routinely differentiate this organism from other coliforms and its routine isolation is not recommended.

Laribacter hongkongensis

Laribacter hongkongensis is an emerging pathogen associated with travel to South East Asia and shellfish consumption. *L. hongkongensis* should only be tested for when clinically appropriate. Symptoms are similar to those of *Salmonella* species and *Campylobacter* species, and diarrhoea may be bloody and severe^{34,54,55}. Most laboratories do not routinely identify this organism and routine investigation is not recommended.

Non-bacterial Organisms Commonly Associated with Gastrointestinal Infections

Parasites

Many parasites cause enteric diseases and are described in [B 31 - Investigation of Specimens other than Blood for Parasites](#).

Viruses

Viruses are a common cause of diarrhoea and vomiting in children and a major cause of epidemic non-bacterial gastroenteritis in adults. Virus gastroenteritis in children is a significant cause of morbidity and mortality in developing countries. In the UK viruses are normally tested for using PCR and commercially available enzyme immunoassays (EIA).

Viruses implicated include:

Rotavirus

Rotaviruses cause diarrhoea and vomiting most frequently in young children. Epidemics are sometimes seen in nurseries. Occasionally rotaviruses cause

gastroenteritis in the elderly. Peak incidence occurs in the cooler months. Infection is by the faecal-oral route. Rotaviruses also occur in vomit which is an important vehicle for transmission; the virus is disseminated in aerosol droplets resulting in environmental contamination. This SMI does not recommend the examination of vomit for rotavirus.

Rotaviruses are the most common cause of diarrhoea in pre-school aged children, therefore faecal specimens from this group should always be screened for viruses.

Adenovirus

Adenovirus is second in importance to rotaviruses as causes of acute diarrhoea in young children. Outbreaks have been recognised in nurseries and paediatric units. Prolonged diarrhoea and low grade fever are commonly seen.

Adenovirus types 40 and 41 (Group F adenoviruses) are the common causes of adenovirus-related diarrhoeal illness.

Norovirus or small round structured viruses (SRSV)

Norovirus comprise one group of caliciviruses associated with gastrointestinal illness. They are the major cause of non-bacterial epidemic gastroenteritis in the UK. Outbreaks are common within the community at large and within institutions such as hospitals and elderly care homes. All age groups are affected, but outbreaks involving adults predominate. Infection usually spreads by inter-personal contact, but outbreaks caused by faecal contamination of food or water are documented. Norovirus may also be found in vomit and the virus may be transmitted by ingestion of aerosol droplets. Food-borne outbreaks are well recognised, either due to the consumption of sewage-contaminated molluscan shellfish, or more often as a result of contamination of food by infected food handlers. Symptoms include vomiting (often projectile), diarrhoea, headaches, fever, myalgia and abdominal cramps. Incubation time is short, 15-50hr and recovery usually uneventful within about 24hr.

Detection of norovirus by electron microscopy requires faecal samples taken within 48hr of the onset of symptoms. Vomit is not a good specimen for diagnostic purposes and is not recommended in this SMI.

Sapoviruses

Sapoviruses are non-norovirus caliciviruses associated with gastrointestinal symptoms. They cause diarrhoea and vomiting, generally without accompanying fever. Infections occur mainly in infants, young children and the elderly, with an increased incidence in the winter months.

Foodborne outbreaks of sapovirus have been documented^{56,57}.

Astroviruses

Astrovirus infections mostly occur in childhood and symptoms are mild. They include vomiting, abdominal pain, diarrhoea and fever.

Patients who are Immunosuppressed or Immunocompromised

These patients have increased susceptibility to infection, and therefore may be severely affected by organisms that would only cause relatively low grade or asymptomatic infection in immunocompetent persons. Factors which affect host resistance include age (the elderly and the very young are more susceptible), the presence of chronic or debilitating diseases eg AIDS and the use of pharmacological

agents (such as antacid therapy), alcohol, or drugs. Malnutrition severely affects the host's defence systems. An example of increased host susceptibility is the increased incidence of salmonellosis in patients who have AIDS; this group are 20-100 times more likely to be infected than the general population. Furthermore, patients with HIV/AIDS are at risk of developing chronic salmonella carriage with recurrent infections⁵⁸.

Faecal parasites are a common cause of diarrhoea in immunosuppressed or immunocompromised individuals ([B 31 - Investigation of Specimens other than Blood for Parasites](#)).

Infection with the common gastroenteritis viruses may be prolonged in patients who are immunocompromised; this can have important infection control implications. The possibility of herpesvirus infections should also be considered in certain groups. Cytomegalovirus (CMV) colitis may be a cause of diarrhoea in some transplant patients, and CMV may cause exacerbations of symptoms in chronic inflammatory bowel conditions such as Crohn's disease and ulcerative colitis.

Mycobacterium tuberculosis and *Mycobacterium avium-intracellulare* (MAI) have been isolated from faeces in immunocompromised patients. If *M. tuberculosis* is isolated, this may well be due to the ingestion of infected respiratory secretions rather than intestinal disease⁵⁹. The isolation procedure is unreliable and has a low success rate due to the heavy contamination with other bacteria. Culturing faecal samples for mycobacteria is not recommended in this SMI. Both these organisms may be isolated from blood cultures in disseminated infection ([B 40 – Investigation of Specimens for *Mycobacterium* species](#)). There is no requirement for samples containing *Mycobacterium tuberculosis* or MAI to be sent to the reference laboratory for testing.

Yeast may also be isolated from faeces, however isolation of yeast from non-sterile sites may indicate colonisation or contamination in patients, rather than true infection^{60,61}.

Technical Information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective Media in Screening Procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen Containers^{62,63}

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.”

Rapid Diagnostic Tests

Rapid diagnostic tests for identification of pathogens directly from faeces are available, and include enzyme immunoassays (EIA) which detect pathogen antigens or antibodies, and molecular techniques including polymerase chain reaction (PCR). EIA and PCR may perform better than conventional methods, and should therefore be considered for use where available. Evidence suggests that PCR tests are highly accurate for *Salmonella*, *Campylobacter* and *E. coli* O157, however less data is available regarding the effectiveness of testing for toxin producing pathogens (*C. perfringens*, *Bacillus* species, *S. aureus*)⁶⁴. All commercial kits and PCR platforms should be validated prior to use to ensure appropriate clinical interpretation.

Sample Dilution

Sample dilution prior to inoculation may be useful; a study showed no significant differences in the isolation rates of *Salmonella* species or *Campylobacter* species when faecal samples were plated directly or when diluted prior to inoculation to culture media. A 1:4 dilution was shown to reduce significantly the amount of competing flora without compromising isolation of low numbers of pathogens; fewer subcultures for *Campylobacter* species may therefore be required when using a dilute inoculum, reducing labour costs⁶⁵.

Sample dilution (dilution factor and medium used) should be validated locally prior to implementation.

Campylobacter species

The rate of isolation of *Campylobacter* species is higher, and the growth of competing flora is less when an incubation temperature of 42°C is used in preference to 37°C⁶⁶. Recovery of organisms such as *Arcobacter* species and *Helicobacter cinaedi* may, however, be compromised.

There are various technical problems associated with recovery of this diverse group of bacteria from samples of faeces:

- Organisms may be sensitive to selective agents incorporated to campylobacter selective agars (eg *Campylobacter upsaliensis*, *Campylobacter hyointestinalis* and *Helicobacter fennelliae* are sensitive to cephalothin).
- *Arcobacter* species and *H. cinaedi* may not grow at 42°C.
- *C. hyointestinalis* may require a hydrogen tension greater than that regularly supplied by commercially-available microaerobic atmosphere generating kits.

Overall, the contribution to human disease in the UK by this group of bacteria is believed to be small. For this reason the incubation temperature, choice of selective agars, etc recommended in standard methods are primarily aimed at detecting *C. jejuni*, *C. coli* and *C. lari*.

Salmonella species

A study in 2002, comparing xylose lysine desoxycholate (XLD), desoxycholate (DCA), α - β chromogenic medium (ABC) and mannitol lysine crystal violet brilliant green agar (MLCB), found that XLD plus MLCB is the optimal combination when employing direct plating⁶⁷. MLCB was shown to be the best, single direct plating medium for non-typhi salmonellae, whereas XLD remains the most effective for routine diagnostic work.

The results of a study of the performance of lactose and mannitol selenite broths as enrichment media when plated on XLD and DCA for the isolation of *Salmonella* species has led to the proposal that routine diagnostic laboratories subculture mannitol selenite broths to XLD⁶⁸.

Chromogenic agar has also been evaluated and has been shown to be comparable to traditional plated media⁶⁹.

E. coli VTEC (including O157)

Where the clinical evidence is suggestive of VTEC infection (particularly in children under 15 years and adults over 65 years) and no presumed sorbitol non-fermenting *E. coli* O157 colonies are observed on CTSMAC agar, it is recommended that clinical laboratories should²⁰:

- test sorbitol fermenting colonies for agglutination with *E. coli* O157 antiserum
- confirm the identification of agglutination positive O157 colonies as *E. coli*
- all purified isolates of presumed (locally confirmed) *E. coli* O157 (sorbitol non-fermenters or sorbitol fermenting) should be saved on nutrient agar slopes. Cultures should be referred promptly for confirmation, detection of vero cytotoxin genes and phage typing to the reference laboratory
- faecal samples from appropriate cases from whom VTEC O157 has not been isolated should be submitted to a reference laboratory for detection of vero cytotoxin-producing *E. coli* of serogroups other than O157 (non-O157 VTEC)

Plesiomonas shigelloides

There is currently no specific SMI for the identification of *Plesiomonas shigelloides*; the processing method of [ID 20 - Identification of Shigella species](#) or [ID 19 – Identification of Vibrio species](#) may be used. Select pink colonies from XLD or yellow colonies from TCBS which are oxidase positive and confirm using an appropriately validated identification system. *Plesiomonas shigelloides* is a slow growing organism; 48hr incubation may be required.

Chromogenic Media

Chromogenic identification plates are commercially available and have been evaluated for certain clinical samples⁶⁹⁻⁷¹. The use of chromogenic agar may be of value in the isolation and confirmation of pathogens (such as *Salmonella* species, *Shigella* species, *E. coli* (EPEC, EHEC, VTEC/STEC), *Vibrio cholerae* and *Vibrio parahaemolyticus*) from faeces by reducing false positive growth⁶⁹⁻⁷².

Chromogenic media are affected by light and plates should be stored in the dark and not left in the light before or after inoculation. Incubation times for chromogenic media should be as recommended by the manufacturers and media should be validated prior to use.

1 Safety Considerations^{62,63,73-87}

1.1 Specimen Collection, Transport and Storage^{62,63,73-76}

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing^{62,63,73-87}

Containment Level 2.

Diagnostic work with clinical material that could contain Hazard Group 3 organisms (*Salmonella* Typhi, *Salmonella* Paratyphi A, B and C, Vero cytotoxin producing *E. coli* O157 or *Shigella dysenteriae*) does not normally require full Containment Level 3 containment⁷⁹.

If the work to be carried out requires the growth or manipulation of a Hazard Group 3 enteric biological agent then this has to be carried out under full Containment Level 3 conditions⁷⁹.

Note: *S. Typhi*, *S. Paratyphi* A, B and C cause severe and sometimes fatal disease, and laboratory acquired infections have been reported. *S. Typhi* immunisation is available. Guidance is given in the Public Health England immunisation policy.

E. coli O157 VTEC and *S. dysenteriae* type 1 cause severe and sometimes fatal disease and laboratory acquired infections have been reported. Low numbers are required for an infective dose.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet⁷⁹.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

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

2 Specimen Collection

2.1 Type of Specimens

Faeces

2.2 Optimal Time and Method of Collection⁸⁸

For safety considerations refer to Section 1.1.

Collect specimens soon as possible after onset of symptoms.

Collect specimens before antimicrobial therapy where possible⁸⁸.

Specimen may be passed into a clean, dry, disposable bedpan or similar container and transferred into an appropriate CE marked leak proof containers and place in sealed plastic bags. The specimen is unsatisfactory if any residual soap, detergent or disinfectant remains in the pan.

Faecal samples should be liquid or semi formed (ie take the shape of the container).
Faeces may be submitted in suitable liquid transport media.

2.3 Adequate Quantity and Appropriate Number of Specimens⁸⁸

1-2g is sufficient for routine culture. Tests for quantifying food poisoning organisms may require up to 10g.

If more than one specimen is taken on the same day the specimens may be pooled.
Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen Transport and Storage^{62,63}

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible⁸⁸.

Important pathogens such as *Shigella* species may not survive the pH changes that occur in faeces specimens which are not promptly delivered to the laboratory, even if refrigerated.

If processing is delayed, refrigeration is preferable to storage at ambient temperature⁸⁸.

4 Specimen Processing/Procedure^{62,63}

4.1 Test Selection

Select a representative portion of specimen for appropriate procedures such as detection of *C. difficile* ([B 10 – Investigation of Faecal Specimens for *Clostridium difficile*](#)), parasites ([B 31 - Investigation of Specimens other than Blood for Parasites](#)) or viruses depending on clinical details.

4.2 Appearance

Specimens may be described as formed, semi-formed or liquid.

The presence of blood, mucus or parasites should be noted.

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.3.1 Pre-treatment

Routine quantitation by pre-treatment and dilution of the specimen is not recommended in this SMI for the investigation of *Bacillus* species or *C. perfringens*. However, this procedure may be employed in outbreaks when clinically indicated.

4.3.2 Specimen processing

- Spread a portion/drop of faecal material on a culture plate, covering an area equivalent to a quarter to a third of the total area to be used (wooden applicator sticks are often used for this).

- Faeces may be diluted 1:4 in appropriate diluent prior to inoculation of culture medium (see local protocols). It has been shown that dilution significantly reduces the amount of competing flora without compromising isolation of low numbers of pathogens
- For the isolation of individual colonies, spread inoculum with a sterile loop. Alternatively a validated automated plate streaker may be used⁸⁹
- Place a pea-sized portion (or several drops) of faecal material into enrichment broth. After incubation, sub-culture using a sterile loop and inoculate appropriate media ([Q 5 – Inoculation of Culture Media for Bacteriology](#))

Automated and semi-automated specimen processor systems are available from several manufacturers. The current third generation instruments carry out a range of tasks including specimen processing, agar plate streaking, preparation of Gram stained slide films and inoculation of enrichment broth⁸⁹. All automated systems must be validated prior to use and should be used in accordance with the manufacturers' instructions.

Supplementary

Spore count for *C. perfringens*:

- prepare a 1:5 dilution of faeces in PBS (minimum 0.1g of faeces in 0.5mL of PBS) to give a 1:5 suspension
- add an equal volume of 95% v/v ethanol in distilled water, and shake
- leave for 30min at room temperature
- from this 1:10 dilution prepare a further two, tenfold dilution in PBS (1:100, 1:1000). Inoculate 0.1mL aliquots of both these dilutions to neomycin blood agar and incubate anaerobically overnight
- perform a colony count which will permit the calculation of the spore count

Vegetative cell count for *Bacillus* species, *C. perfringens* and *S. aureus*:

- prepare 1:10 and 1:100 dilutions of faeces in phosphate-buffered saline (PBS)
- inoculate 0.1mL aliquots of each dilution to appropriate media for *B. cereus* [polymyxin, egg yolk, mannitol, bromothymol blue agar (PEMBA)], *C. perfringens* or *S. aureus* and incubate overnight
- count colonies and calculate the total viable count

Investigation of faeces for parasites ([B 31- Investigation of Specimens other than Blood for Parasites](#)) should also be performed if clinically indicated.

4.4 Microscopy

4.4.1 Standard

Prepare a medium to thick smear of faeces on a clean microscope slide to stain for *Cryptosporidium* species on diarrhoeal specimens from all symptomatic individuals (except specific screens, eg *Salmonella* species screens on known positives) ([B 31 - Investigation of Specimens other than Blood for Parasites](#))^{90,91}.

4.4.2 Supplementary

Ova, cysts and parasites (OCP) are not routinely included in the primary testing set as yields are extremely low.

If requested, prepare a wet preparation for microscopy for ova, cysts and parasites on a clean microscope slide in 10% (v/v) formalin in water, physiological saline or Ringer's solution (the use of formalin solution is unsuitable for the detection of trophozoites) ([B 31 - Investigation of Specimens other than Blood for Parasites](#)).

Perform faecal concentrations on all specimens where examination of parasites is specifically requested or where there is a definite clinical indication ([B 31 - Investigation of Specimens other than Blood for Parasites](#)).

Microscopy for microsporidia should be considered in patients who have diarrhoea and are HIV positive ([B 31 - Investigation of Specimens other than Blood for Parasites](#)).

Microscopy for *Mycobacterium* species ([B 40 - Investigation of Specimens for Mycobacterium species](#)).

4.5 Culture and Investigation

Using a sterile pipette inoculate each agar plate with specimen (refer to [Q 5 – Inoculation of Culture Media for Bacteriology](#)).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
For all diagnostic specimens (except specific organism screens)	Faeces	Campylobacter selective agar ^{92,93}	39-42	micro-aerobic	≥ 48hr	≥40hr	<i>Campylobacter</i> species
		XLD agar	35-37	air	16-24hr	≥16hr	<i>Salmonella</i> species <i>Shigella</i> species
		Mannitol selenite broth then subculture to: XLD	35-37 35-37	air air	16-24hr 16-24hr	N/A ≥16hr	<i>Salmonella</i> species
		CT-SMAC agar ⁹⁴⁻⁹⁶	35-37	air	16-24hr	≥16hr	<i>E. coli</i> O157
All diarrhoeal specimens and any with obvious blood (semi-formed or liquid faeces) ² Children under 5 years For outbreaks or when	Faeces	MTSB ⁹⁶ then subculture to: CT-SMAC agar	35-37 35-37	air air	16-24hr 16-24hr	N/A ≥16hr	<i>E. coli</i> O157

Investigation of Faecal Specimens for Enteric Pathogens

advised by a senior microbiologist							
For these situations, add the following:							
Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
If a more rapid result is required (eg for non-enteric fever <i>Salmonella</i> outbreaks)	Faeces	MLCB	35-37	air	16-24hr	≥16hr	<i>Salmonella</i> species (except <i>S. Typhi</i> and <i>S. Paratyphi A</i> and <i>B</i>)
Food poisoning (according to clinical details and advice from senior microbiologist)	Faeces	<i>B. cereus</i> selective agar (PEMBA)	35-37 then RT	air air	16-24hr then 16-24hr	16-24hr then 40-48hr	<i>B. cereus</i> <i>B. subtilis</i> <i>B. licheniformis</i>
	Faeces	Neomycin fastidious anaerobe agar	35-37	anaero bic	16-24hr	≥16hr	<i>C. perfringens</i>
Food poisoning (according to clinical details and advice from senior microbiologist)	Faeces	Mannitol salt agar or Baird Parker agar	35-37	air	40-48hr	16-24hr	<i>S. aureus</i>
Culture for <i>C. difficile</i> refer to: B 10 - Investigation of Faecal Specimens for Clostridium difficile	Faeces	CCEY	35-37	anaero bic	40-48hr	≥40hr	<i>C. difficile</i>
Suspected Cholera or suspected infection with <i>V. parahaemolyticus</i> , seafood consumption, and/or recent travel (2-3 weeks) to known cholera area	Faeces	TCBS agar	35-37	air	16-24hr	≥16hr	<i>V. cholerae</i> <i>V. parahaemolyticus</i>
For suspected <i>Vibrio</i> outbreaks or when advised by a senior microbiologist.	Faeces	Alkaline peptone water then subculture	35-37	air	5-8hr	N/A	<i>V. cholerae</i> <i>V. parahaemolyticus</i>

Investigation of Faecal Specimens for Enteric Pathogens

		to: TCBS agar	35-37	air	16-24hr	≥16hr	
Appendicitis Mesenteric lymphadenitis Terminal ileitis Reactive arthritis ⁹⁷ When advised by a senior microbiologist	Faeces	CIN agar	28-30	air	24-48hr	≥24hr	<i>Y. enterocolitica</i> <i>Y. pseudo-tuberculosis</i> <i>Yersinia</i> species
		Tris-buffered 1% peptone (pH 8.0)	8-10 or 28-30	air	7d or 24-48hr		
		then subculture to: CIN agar	28-30	air	24-48hr	≥24hr	
Other organisms for consideration - <i>Mycobacterium</i> species (B 40 - Investigation of Specimens for <i>Mycobacterium</i> species) and parasites (B 31 - Investigation of Specimens other than Blood for Parasites); toxin of <i>C. botulinum</i> .							

Media Key

CCEY	Cycloserine cefoxitin egg yolk agar
CIN	Cefsulodin irgasan (triclosan) novobiocin agar
MLCB	Mannitol lysine crystal violet brilliant green agar
MTSB	Modified tryptone soya broth
CT-SMAC	Cefixime tellurite sorbitol MacConkey agar
TCBS	Thiosulphate citrate bile salts sucrose agar
XLD	Xylose lysine deoxycholate agar

4.6 Identification

Refer to individual SMLs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Bacillus species	genus level
Campylobacter species	genus level
C. difficile	species level
C. perfringens	species level
E. coli O157	species level + serogroup
Salmonella species	genus level
S. Typhi/Paratyphi	species level
Shigella species	species level
S. aureus	species level
Vibrio species	species level
V. cholerae	species level; O1, O139 or not

Yersinia species	species level
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All work on *S. Typhi*, *S. Paratyphi* A, B & C, presumed (locally confirmed) vero cytotoxin-producing *E. coli* O157 and *Shigella dysenteriae* type 1 must be performed in a microbiological safety cabinet under Containment Level 3 conditions.

Organisms may be further identified if this is clinically or epidemiologically indicated. Refer to individual SMLs for organism identification.

4.7 Antimicrobial Susceptibility Testing

Refer to [British Society for Antimicrobial Chemotherapy \(BSAC\)](#) and/or [EUCAST](#) guidelines.

4.8 Referral for Outbreak Investigations

For information regarding outbreak investigation referral, contact specific reference laboratory.

4.9 Referral to Reference Laboratories

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

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should, be sent to the appropriate 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:

England and Wales

<http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370>

Scotland

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

Northern Ireland

<http://www.publichealth.hscni.net/directorate-public-health/health-protection>

5 Reporting Procedure

5.1 Microscopy

5.1.1 Standard

Report presence or absence of *Cryptosporidium* oocysts.

5.1.2 Supplementary

Report presence or absence of ova, cysts and parasites from direct microscopy or faecal concentrate examination.

Parasites ([B 31 - Investigation of Specimens other than Blood for Parasites](#)).

5.1.3 Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report: 16-72hr.

5.2 Culture

Report presence or absence of specific pathogens and results of supplementary investigations.

5.2.1 Culture reporting time

Clinically urgent results to be telephoned or sent electronically.

Written report: 16-72hr stating, if appropriate, that a further report will be issued.

Supplementary investigations: parasites ([B 31 - Investigation of Specimens other than Blood for Parasites](#)).

5.3 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE^{98,99} or Equivalent in the Devolved Administrations¹⁰⁰⁻¹⁰³

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 (HCIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

<http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HealthProtectionRegulations/>

Other arrangements exist in [Scotland](#)^{100,101}, [Wales](#)¹⁰² and [Northern Ireland](#)¹⁰³.

Notify PHE on isolation of:

B. cereus (if associated with food poisoning)

Campylobacter species

C. perfringens (if associated with food poisoning)

E. coli (presumptive [locally-confirmed] VTEC O157 and other possible VTEC strains)

Salmonella species

Shigella species

V. cholerae

Urgent oral notification to the Public Health England Centre within 24 hours of identification is likely to be necessary to protect human health when presumptive identification is made of the following:

Clusters of cases of any of the above list

S. Typhi or *S. Paratyphi*

Salmonella species if a suspected outbreak or a case in a food handler or closed community such as a care home

Shigella species other than *S. sonnei*

S. sonnei if a suspected outbreak or a case in a food handler or closed community such as a care home

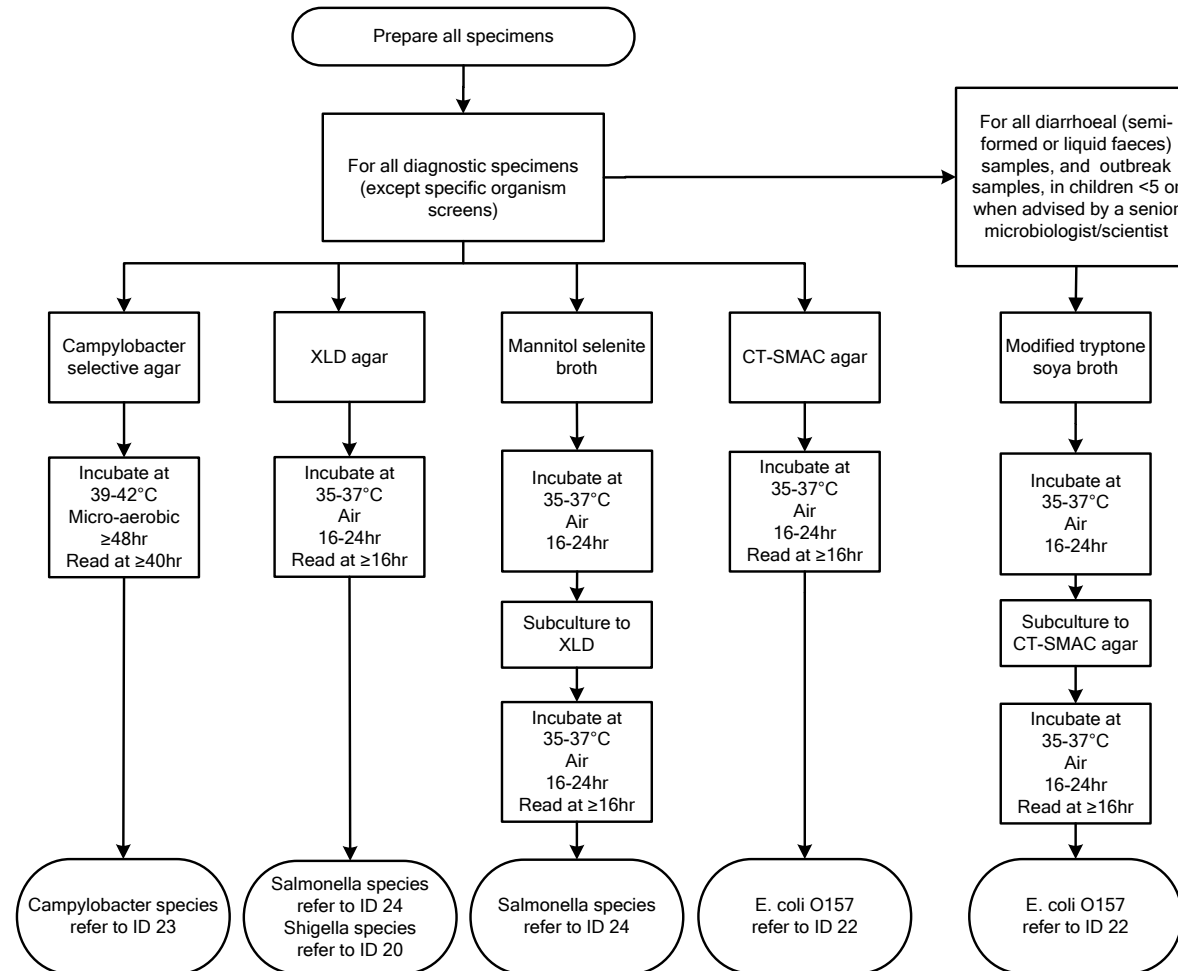
E coli O157 when presumptive (locally confirmed) at the diagnostic laboratory

Other verocytotoxigenic organisms

Confirmatory and typing results should be forwarded to the Public Health England Centre as soon as they are available to expedite appropriate health protection interventions.

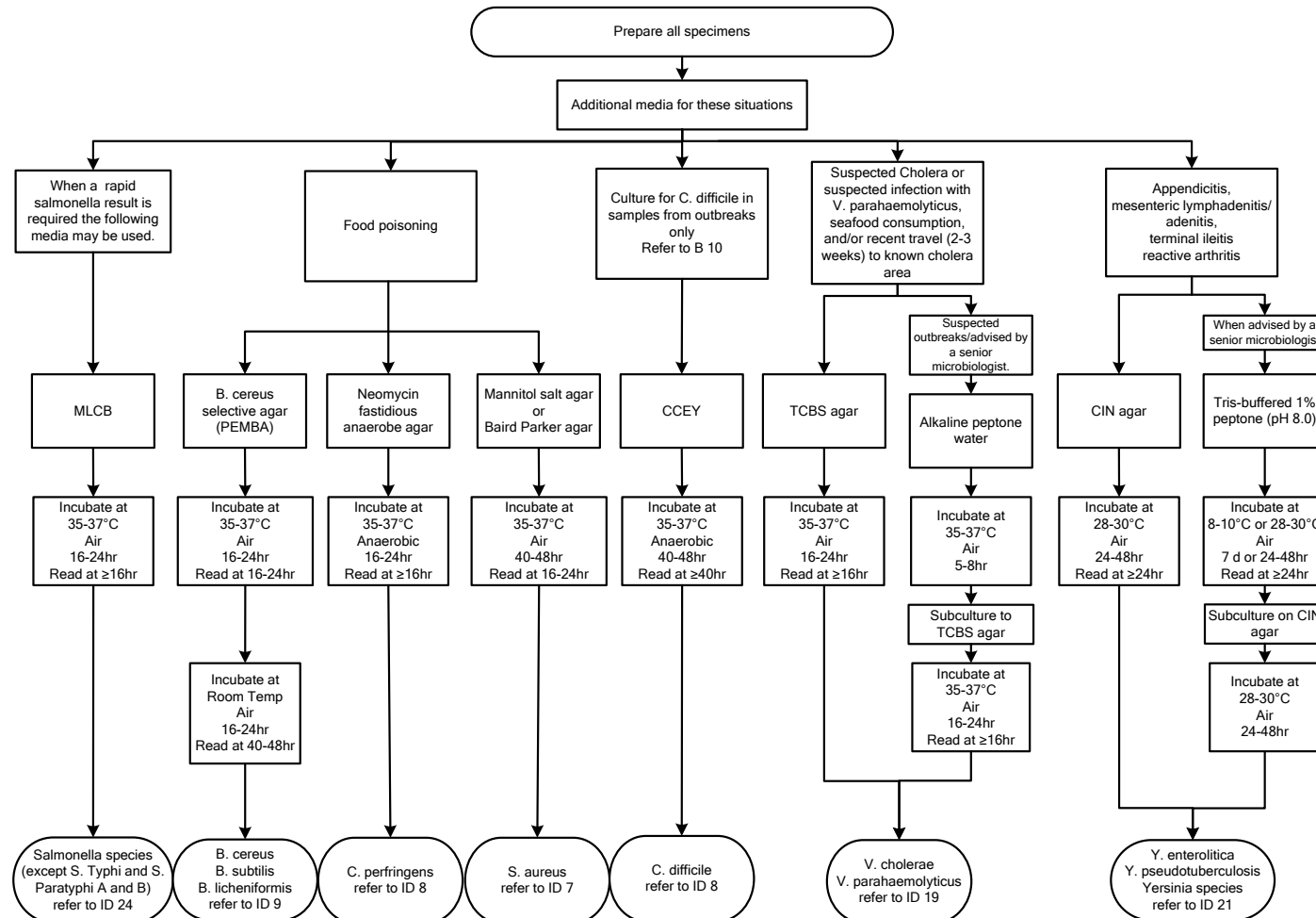
If a diagnosis of botulism is suspected, this should be reported to the CCDC and also to the Public Health England, Colindale, London.

Appendix 1: Investigation of Faecal Specimens for Routine Bacterial Pathogens



To view associated SMI documents please access from: <http://www.hpa.org.uk/SMI>

Appendix 2: Investigation of Faecal Specimens for Additional Bacterial Pathogens



To view associated SMI documents please access from: <http://www.hpa.org.uk/SMI>

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