

NATIONAL STANDARD METHOD

# INVESTIGATION OF FAECAL SPECIMENS FOR BACTERIAL PATHOGENS

BSOP 30

Issued by Standards Unit, Department for Evaluations, Standards and Training  
**Centre for Infections**



*Association of Medical Microbiologists*  
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## INVESTIGATION OF FAECAL SPECIMENS FOR BACTERIAL PATHOGENS

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National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

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The reader is informed that all taxonomy in this document was correct at time of issue.

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# AMENDMENT PROCEDURE

Controlled document reference	BSOP 30
Controlled document title	Investigation of faecal specimens for bacterial pathogens

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
8/ 13/10/08	6	6.1	All	<b>All</b>	ESL replaced with DEST
			29 and 30	<b>Appendix</b>	Flowchart inserted

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# INVESTIGATION OF FAECAL SPECIMENS FOR BACTERIAL PATHOGENS

Specimen: Faeces

## SCOPE OF DOCUMENT

This National Standard Method briefly outlines the bacteria responsible for enteric infection and methods for their isolation. It recommends routine screening of faeces for *Campylobacter*, *Salmonella*, *Shigella* and *Escherichia coli* O157<sup>2</sup> on all diarrhoeal (semi-formed or liquid) faeces. However, consideration should be given to the “three day rule” for collection of faeces samples from hospitalised patients (see section below “Collection of faeces samples” page 6). 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 this introduction. Algorithms for the isolation and identification of these viruses are available ([VSOP 2 - Gastroenteritis: sporadic cases](#) and [VSOP 3 - Gastroenteritis: Outbreaks](#) ).

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

For details of *Clostridium difficile* refer to [BSOP 10 - Processing of faeces for Clostridium difficile](#)

## INTRODUCTION

### Diarrhoea

This may be defined as unusual frequency of bowel action, with the passage of loose, unformed faeces. 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 scombrototoxin. Consumption of food containing irritant chemicals such as heavy metals may also cause diarrhoea .

### Outbreaks

Outbreaks may occur as a result of person to person spread of infection, through ingestion of infected food and water and from direct contact with animals. Food borne outbreaks are defined as 2 or more cases of a similar illness resulting from the ingestion of a common food<sup>3</sup> and currently are estimated to cause 3 million deaths worldwide per year<sup>4</sup>. A water borne outbreak is defined as 2 or more cases of a similar illness resulting from the drinking of water from a common source or contact with water used for recreational purposes from a collective source. Water borne outbreaks are estimated to cause the death of 2 million children annually as a consequence of diarrhoeal disease<sup>4</sup>.

### 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

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systems. Examples of increased host susceptibility include the incidence of salmonellosis in patients who have AIDS which is 20-100 times that in the general population<sup>5</sup>; furthermore, patients with HIV/AIDS are at risk of developing chronic *Salmonella* carriage with recurrent infections. Faecal parasites are a common cause of diarrhoea in immunosuppressed or immunocompromised individuals (see [BSOP 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. The isolation procedure is unreliable, has a low success rate and is not recommended in this NSM (See Section 4). Both these organisms may be isolated from blood cultures in disseminated infection. Cultures for MAI may need to be referred to a reference laboratory.

## Collection of faeces samples

Some clinicians advocate the use of a "3 day rule". This rule is derived from the low numbers of faecal pathogens isolated from patients who have been hospitalised for longer than 3 days. It suggests that faecal samples from these patients should not be cultured except under the following circumstances<sup>6,7</sup>:

- those in-patients 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

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 rules<sup>8</sup>. 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

### Campylobacter

*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 1970's. *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. *Campylobacter* enteritis in Great Britain 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 diarrhoea<sup>9</sup>. 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* will 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. Rarely, *Campylobacter* species infection may 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.

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Acute post-infective demyelination may develop, affecting the peripheral nervous system (Guillain-Barré Syndrome), and/or the central nervous system and cranial nerves<sup>10</sup> (eg the Miller-Fisher Syndrome (areflexia, ataxia, and cranial nerve pareses]; polyneuritis cranialis). Specific serotypes are implicated in these conditions.

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 spinal fluid commonly shows an increase in protein concentration, usually without any accompanying pleocytosis<sup>10</sup>.

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

### **Other Campylobacter, Helicobacter and Arcobacter species**

A number of other named *Campylobacter* species and *Helicobacter* species are also implicated in the causation of human gastrointestinal disease (eg *Campylobacter upsaliensis*, *Campylobacter hyointestinalis*, *Helicobacter fennelliae* (formerly *Campylobacter fennelliae*) and *Helicobacter cinaedi* (formerly *Campylobacter cinaed* ).

Several species formerly classified in the genus *Campylobacter* are now assigned to the genus *Arcobacter*<sup>11</sup> (eg *Arcobacter butzleri*). *A. butzleri* has also been reported as a cause of human gastroenteritis<sup>12</sup>.

Some of the organisms above have also been recovered from blood cultures.

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 into campylobacter agars (eg *C. upsaliensis*, *C. hyointestinalis* and *H. 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 engendered by this grouping 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*.

*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. Generally there are not mixed infections in blood cultures, therefore a blood agar plate may be used rather than a selective campylobacter agar plate. *C. fetus* and *C. fetus* subspecies *veneralis* may not grow at 42°C. Other species, such as *Campylobacter concisus*, *Campylobacter rectus* and *Campylobacter curvus* have been associated with dental abscesses and have been isolated from root canals, blood and other sites<sup>13</sup>.

It should be noted that *C. upsaliensis* is associated with dogs, *C. hyointestinalis* (and *C. coli*) are associated with pigs, *H. cinaedi* with hamsters, and *Arcobacter* species with a variety of animals including cattle and pigs<sup>14</sup>. *H. fennelliae* and *H. cinaedi* first came to attention as the cause of disease in men who have sex with men. Relevant clinical history may guide further investigations in individual patients.

Faeces from a patient with a gastroenteritic syndrome, for which no cause can be found by use of standard microbiological techniques, may with prior agreement be sent to a Reference Laboratory in order to search for these less common bacteria.

### **Salmonella<sup>15</sup>**

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

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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 person to person contact. Person to person 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 infection<sup>16</sup>.

Although the majority of isolations are from cases of diarrhoea and/or vomiting, the organisms may be isolated from other specimens such as blood and urine. 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 metastatic (haematogenous) infections. A small number of patients may develop an illness that resembles enteric fever (see below). Low numbers of *Salmonella* species may also be isolated from 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<sup>17</sup>. 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 blood-stream 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 WBC 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 (see [BSOP 37 - Investigation of blood cultures \(for organisms other than Mycobacterium species\)](#) ), bile (see [BSOP 15 - Investigation of bile](#) ), bone marrow (see [BSOP 38 - Investigation of bone marrow](#) ) or urine (see [BSOP 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

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organisms difficult. Similarly, carriage in the urinary tract may be associated with urolithiasis, and with damage caused by urinary schistosomiasis.

### ***Shigella***<sup>18</sup>

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:

- *Shigella sonnei*
- *Shigella flexneri*
- *Shigella dysenteriae*
- *Shigella boydii*

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) – see below. As in infection with VTEC, infection with toxigenic *S. dysenteriae* may result in the haemolytic-uraemic syndrome.

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 dose<sup>19</sup>. 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 of poor socio-economic circumstances<sup>19</sup>. *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.

### ***Escherichia coli***

#### **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 is complicated and is performed mainly in reference laboratories, although commercial kits are available.

#### **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 travel diarrhoea.

#### **Enteroinvasive *E. coli* (EIEC)**

EIEC cause a dysentery-like illness characterised by fever, abdominal cramps, watery diarrhoea with blood and mucus which generally contains leukocytes. The condition may closely resemble shigellosis<sup>20</sup>.

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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 NSM. 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.

### **Verocytotoxic *E. coli* (VTEC)**

*E. coli* O157 is one of several *E. coli* serotypes which produce Vero cytotoxins. The toxin is similar to the Shiga toxin of *Shigella dysenteriae*, and is associated with haemorrhagic colitis<sup>21</sup> and haemolytic uraemic syndrome<sup>20</sup>. Infections vary in severity from mild to bloody diarrhoea and may occur in any age group, although it is more common in children. Blood is not always present in faeces in Vero cytotoxin producing *E. coli* O157 infections and must not be used as the sole criterion for selecting specimens for examination for this organism.

The highest incidence of Vero cytotoxin 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 milk and water, ground beef, beef burgers and indirectly with vegetables, apple cider and mayonnaise<sup>22</sup>. Outbreaks occur in establishments such as nursing homes<sup>22</sup>. There have also been outbreaks of *E. coli* O157 infection involving visitors to open farms<sup>23</sup>. Low numbers of Vero cytotoxin producing *E. coli* O157 are required to cause infection.

Culture for VTEC is recommended in this NSM for all diarrhoeal faecal samples. Suspicious isolates can be confirmed as *E. coli* O157 by serological and biochemical tests. Cultures of *E. coli* O157, known or suspected, must be handled at Containment Level 3. There have recently been cases of sorbital fermenting VTEC (more details available in the Technical Information section of this document)<sup>24</sup>.

Presumptive isolates should be referred to the Reference Laboratory for confirmation of identity, detection of Vero cytotoxin genes and phage typing. Most VTEC O157 are motile and have the flagellar antigen H7, but about 20% of strains are phenotypically non-motile.

Available methods and selective agars are primarily aimed at detecting *E. coli* O157. Faeces from a patient with a dysenteric syndrome, for which no cause can be found using standard microbiological techniques, may be sent to a Reference Laboratory in order to search for other Vero toxigenic strains of *E. coli*. (See also above regarding *Campylobacter* and *Helicobacter* species, and enteroinvasive *E. coli*).

### **Enteroaggregative *E. coli* (EAEC)<sup>25,26</sup>**

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 are detected by adherence to HEp-2 and HeLa cells, with the formation of characteristic patterns and this is considered to be the gold standard. Toxin production does not appear to be important for pathogenicity and strains belong to a diverse range of serotypes.

### ***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. The diarrhoea causing species most frequently isolated are *Vibrio cholerae* (the causative agent of cholera), *Vibrio parahaemolyticus*, *Vibrio fluvialis* and *Vibrio mimicus*<sup>27</sup>. *Vibrio vulnificus* does not cause diarrhoea but has been isolated from the blood and tissues of septic patients (especially those with liver disease)<sup>28</sup>.

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

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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 Americas<sup>29</sup>.

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. Contaminated food, particularly undercooked or raw seafood, can also be a source of infection.

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

*V. parahaemolyticus* and *Vibrio fluvialis*<sup>27</sup> are responsible worldwide, for gastroenteritis (characteristically acute diarrhoea, which may be bloody lasting about three days) associated with eating contaminated seafood, particularly shellfish.

It should be noted that *V. hollisae* will not grow on TCBS.<sup>30</sup>

## Antibiotic Associated Diarrhoea (AAD)

### *Clostridium difficile*

For details of *C. difficile* refer to [BSOP 10 – Processing of faeces for \*Clostridium difficile\*](#). Changes in the gut flora associated with broad spectrum antibiotics and chemotherapeutic agents can result in colonisation by *Clostridium difficile*<sup>31</sup>. It is the commonest identifiable cause of 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 the new fluoroquinolones<sup>32</sup>. The incidence of *C. difficile* infection has been shown to decrease once antibiotic therapy is controlled.

The production of two toxins A (enterotoxin) and B (cytotoxin) causes the characteristic mucosal damage consisting of plaque-like lesions leading to the formation of a pseudomembrane<sup>33</sup>. Not all strains of *C. difficile* produce toxin and therefore not all can cause illness.

The spectrum of disease ranges from a self-limiting mild diarrhoea to the advanced and severe characteristic pseudomembranous colitis. The most accurate diagnosis of pseudomembranous colitis is effected by endoscopic detection of colonic pseudomembranes or microabscesses in antibiotic-treated patients who are suffering from diarrhoea and who have *C. difficile* in their faeces.

The organism has been associated with outbreaks in hospitals and in extended care facilities for the elderly<sup>34</sup>. It represents an important cause of hospital-acquired infection. The organism may be isolated from the hospital environment and may be found on floors, toilets and bedding<sup>35</sup>.

Demonstration of toxins of *C. difficile* in diarrhoeal stools is generally regarded as suggestive of CDAD, in the absence of any other recognised cause for gastrointestinal disturbance. In outbreak situations it is suggested that primary isolation of the organism is undertaken in tandem with toxin detection<sup>36</sup>. The culture of toxin negative faeces followed by toxin testing of the isolate may increase the number of patients diagnosed<sup>37</sup>.

Although considered by some to be the "gold standard", use of tissue culture for the detection of *C. difficile* toxins by virtue of its cytopathic effect (neutralisable with *C. sordellii* antitoxin) requires technical expertise, involves usually a 24 (up to 48) hour delay for the final result<sup>38</sup>. Tissue culture, especially with Vero cells<sup>36</sup>, may detect other faecal cytotoxins that are associated with diarrhoea e.g. *C. perfringens* enterotoxin.

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Cytopathic effect (CPE) that is not neutralised by *C. sordellii* antitoxin may indicate that another pathogen is present.

There are numerous commercially available EIA tests intended to detect the toxins of *C. difficile*. Some detect Toxin A, others A and B<sup>39</sup> although the sensitivity and specificity of these are variable<sup>40,40,41</sup>. Commercial EIAs that detect both toxins A and B are considered more appropriate than those which detect A alone<sup>42</sup> since outbreaks due to A- B+ strains have been recorded.

Latex agglutination kits are available but are not as accurate as EIA due to poor sensitivity<sup>43</sup>. Detection by counter immuno-electrophoresis (CIE) has been suggested but this method lacks sensitivity and specificity<sup>44,45</sup> and is not recommended.

These and other testing procedures are reviewed in a recent report presented to the Department of Health<sup>46</sup>.

There are also a range of Near Patient Tests that are being developed and these may have an increasing role to play in patient diagnostics in the future and have been shown to have very good sensitivities<sup>47,48</sup>.

Typing of strains of *C. difficile* is sometimes useful in the investigation of multiple cases of infection. Typing methods that have been used include bacteriophage/bacteriocin<sup>49</sup> typing and serotyping<sup>50</sup>. PCR ribotyping is gaining acceptance as an internationally recognised method<sup>51</sup> and with in the UK a PCR ribotyping network has been established for use in outbreak situations. Other methods include cell surface protein profiles<sup>52</sup> and other DNA methods of analysis<sup>53,54</sup>.

### **Other organisms<sup>55-58</sup>**

In addition to *C. difficile*, infection with *C. perfringens*, *S. aureus*, *Klebsiella oxytoca*, *Candida* species and *Salmonella* species have been implicated with AAD.

## **Microorganisms sought in special circumstances**

### ***Yersinia enterocolitica***

Yersiniosis is a zoonosis caused by a limited number of O serogroups of *Yersinia enterocolitica*; notably 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 by the oral route from contaminated food, milk or water. Pigs are a frequently identified source of infection. Infection occurs more often in the young (<6y) and the elderly.

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 4 and 7 days.

Occasionally, haematogenous spread occurs, producing septicaemia with the formation of abscesses in organs such as the liver and spleen.

Yersiniosis may therefore present with a variety of clinical conditions:

- Acute diarrhoea
- Mesenteric lymphadenitis
- Terminal ileitis
- "Pseudo-appendicitis"
- Septicaemia
- Metastatic infections
- Immunological sequelae (eg reactive arthritis)

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Culture for this organism is only recommended when clinical suspicion has been aroused. 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 faeces.

### ***Clostridium septicum*<sup>59</sup>**

*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 or 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*<sup>60</sup>**

*Aeromonas* species have been implicated as causative organisms of a watery, non-bloody diarrhoea. Young children and patients who are elderly may be more susceptible to infection<sup>61</sup>. 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.

### ***Plesiomonas shigelloides*<sup>60</sup>**

*Plesiomonas shigelloides* has been isolated from in patients with diarrhoea and abdominal cramps. It has been linked to food and water-borne outbreaks of gastrointestinal infection<sup>62</sup>. Some case reports and epidemiological data support a role for *P. shigelloides* in diarrhoeal disease yet its role is still debated and unclear. Routine investigation for this organism is not recommended.

### ***Edwardsiella tarda*<sup>60</sup>**

This organism has been associated with sporadic cases of gastroenteritis. There is some evidence that it may cause diseases similar to those caused by *Salmonella* species, including enteric fever<sup>64</sup>. However, most laboratories do not routinely differentiate this organism from other coliforms and its routine isolation is not recommended.

## **Parasites**

Many parasites cause enteric diseases and are described in [BSOP 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.

Viruses implicated include:

**Rotaviruses** - these 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 SOP 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.

Detection methods for rotaviruses on faecal samples taken during the acute phase of the illness include electron microscopy, ELISA and latex agglutination.

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**Adenoviruses** - are 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. Commercial methods such as EIA usually detect only these types, whereas EM will detect enteric and nonenteric adenoviruses. Adenovirus PCR is increasingly used in laboratory diagnosis.

**Noroviruses or small round structured viruses (SRSV)** - Noroviruses 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 persons' homes. All age groups are affected, but outbreaks involving adults predominate. Infection usually spreads by person-to-person contact, but outbreaks caused by faecal contamination of food or water are documented. SRSVs are also found in vomit. In this instance the viruses are 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, between 15 to 50 hours and recovery usually uneventful within about 24 hours.

Detection of SRSV by electron microscopy requires faecal samples taken within 48 hours of the onset of symptoms. RT-PCR testing is available in specialised laboratories. Vomit is not a good specimen for diagnostic purposes and is not recommended in this SOP. A commercial ELISA for the detection of noroviruses in faecal samples are available but the reliability of such assays may be compromised by the significant variation among strains of virus<sup>65</sup>. This may be less of a problem with PCR, which is therefore becoming the preferred method for laboratory diagnosis.

**Sapoviruses** - are the 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 increased incidence in the winter months.

Outbreaks have been documented.

Detection of caliciviruses is by electron microscopy of acute faecal samples or by RT-PCR.

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

Detection methods for astroviruses include electron microscopy of acute faecal samples, ELISA test or RT-PCR.

## **Bacteria associated with toxin induced food poisoning**

### ***Staphylococcus aureus***<sup>66</sup>

*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 are vomiting, nausea and abdominal cramps, often followed by diarrhoea. The incubation period is 1-6 hours.

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 vomitus, but culture of these specimens is not recommended in this SOP. Culture may not be successful because of the death of the organism

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following enterotoxin production. However, sufficient enterotoxin may be present in food for detection by immunoassay. Detection of enterotoxin in faeces or vomitus 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<sup>66</sup>**

*Bacillus cereus* is a common cause of food poisoning by ingestion of a toxin rather than infection. 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<sup>67</sup> and is characterised by nausea and vomiting 1-5 hours after consumption of the implicated foodstuff. The diarrhoeal type causes diarrhoea and abdominal pain 8-16 hours after ingestion of the contaminated food.

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

*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, vomitus and food, should be attempted. Viable counts from food are generally greater than  $10^4$  per gram<sup>68</sup>. 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 vomitus is of limited diagnostic value.

### ***Clostridium perfringens*<sup>3, 66</sup>**

*Clostridium perfringens* counts in normal human faeces are generally less than  $10^4$ - $10^5$  organisms per gram<sup>69</sup>.

Some strains of *C. perfringens* (typically type A2) are associated with a mild form of food-poisoning occurring in all age groups<sup>70</sup>. The predominant symptoms of the toxic food poisoning are a watery diarrhoea with severe abdominal pain with an incubation period of 8-24 hours.

Enteritis necroticans may result from infection with *C. perfringens* type C<sup>71</sup>. Mortality approaches 40%. The condition is known as "pig-bel" in Papua, New Guinea, where it may attend feasting on 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 by faecal spore counts of  $>10^5$  organisms per gram.

This SOP NSM 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 as 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*<sup>66</sup>**

*Clostridium botulinum* is the cause of a rare but often fatal food poisoning. Botulism is a neuroparalytic disease produced by the neurotoxins of *C. botulinum*, and is classified in four categories<sup>72</sup>:

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- **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 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 into 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 arrest<sup>73</sup>. The condition must be distinguished from demyelinating polyneuropathy and the Miller Fisher syndrome (see above).

Botulinum toxins are amongst the most toxic substances known, and might conceivably be used in biological warfare or bioterrorism<sup>74</sup>.

The usual severity of the symptoms and the length of time taken for laboratory tests necessitate that a 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)<sup>73</sup>. These tests should be performed by a reference laboratory and culture is not recommended in this SOP.

## TECHNICAL INFORMATION/LIMITATIONS

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: dilution significantly reduces the amount of competing flora without compromising low numbers of pathogens<sup>75</sup>. It was also shown that there were fewer subcultures for *Campylobacter* species when using a dilute inoculum, thus reducing labour costs.

A study in 2002 comparing xylose lysine desoxycholate (XLD), desoxycholate (DCA),  $\alpha$ - $\beta$  chromogenic medium (ABC) and mannitol lysine crystal violet brilliant green agar (MLCB) found that XLD and MLCB is the optimal combination when employing direct plating<sup>76</sup>. 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 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°C<sup>77</sup>. However, recovery of organisms such as *Arcobacter* species and *H. cinaedi* may be compromised.

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 XLD<sup>78</sup>.

Toxin detection is considered to be adequate for investigation of sporadic cases of *C. difficile*. However, in outbreaks when epidemiological studies are required, toxin detection plus isolation of *C. difficile* is the optimal approach. If the workload is too heavy for the laboratory to perform both tests, then the toxin tests should be performed and a faeces specimen should be stored at 4°C or -20°C for later culturing and typing of isolates. A report by the PHLS *Clostridium difficile* Working Group states that all laboratories should be able to isolate *C. difficile* from faecal samples for typing when an outbreak is suspected and typing of isolates is necessary.

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Where the clinical evidence is suggestive of VTEC infection (particularly in children under 15 years and adults over 65 years) and no presumptive sorbitol non-fermenting *E. coli* O157 colonies are observed on CTSMAC agar, we recommend that clinical laboratories should<sup>24</sup>:

- 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 (Laboratory of Enteric Pathogens, HPA Centre for Infections, Colindale) for confirmation, phage typing and detection of VT genes

Faecal samples from appropriate cases from whom VTEC O157 have not been isolated should be submitted to LEP for detection of non-O157 VTEC.

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# 1 SAFETY CONSIDERATIONS<sup>79-88</sup>

## 1.1 SPECIMEN COLLECTION

N/A

## 1.2 SPECIMEN TRANSPORT AND STORAGE

Leakproof container in a sealed plastic bag

## 1.3 SPECIMEN PROCESSING

Containment Level 2 for routine work, or Containment Level 3 if the following organisms are suspected from clinical information or laboratory findings:

***Salmonella Typhi***

***Salmonella Paratyphi A,B & C***

**Vero cytotoxin producing *E. coli* O157 (VTEC)<sup>89</sup>**

***Shigella dysenteriae***

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

VTEC O157 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.

Materials known or suspected to contain botulinum toxins should also be handled at Containment Level 3 to avoid ingestion or inhalation of toxin.

Other infections (eg with toxigenic *V. cholerae*) may also prove dangerous to laboratory staff.

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 SPECIMEN COLLECTION

## 2.1 OPTIMAL TIME FOR SPECIMEN COLLECTION

As soon as possible after onset of symptoms.

## 2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

Specimen may be passed into a clean, dry, disposable bedpan or similar container and transferred to a leakproof container. The specimen is unsatisfactory if any residual soap, detergent or disinfectant remains in the pan.

## 2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

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

If more than one specimen is taken on the same day the specimens may be pooled.

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## 3 SPECIMEN TRANSPORT AND STORAGE

### 3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens of faeces should be transported to the laboratory and processed as soon as possible, because a number of 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.

### 3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

N/A

## 4 SPECIMEN PROCESSING

### 4.1 TEST SELECTION

Select a representative portion of specimen for appropriate procedures such as detection of *C. difficile* toxins ([BSOP 10 - Toxin detection, isolation & identification of Clostridium difficile from faeces](#)) ([BSOP 31 - Investigation of specimens other than blood for parasites](#)) or virology, 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 MICROSCOPY

#### 4.3.1 STANDARD

On all specimens from symptomatic individuals (except specific screens eg *Salmonella* species screens on known positives), prepare a medium to thick smear of faeces on a clean microscope slide to stain for *Cryptosporidium* species<sup>90</sup> ([BSOP 31 - Investigation of specimens other than blood for parasites](#)).

#### 4.3.2 SUPPLEMENTARY

Prepare a wet preparation for microscopy for white and red blood cells, ova, cysts and parasites on a clean microscope slide in either 10% (v/v) formalin in water, physiological saline or Ringer's solution (the use of formalin solution is unsuitable for the detection of trophozoites).

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

Microscopy for microsporidia should be considered in patients who have diarrhoea and are HIV positive (see [BSOP 31 - Investigation of specimens other than blood for parasites](#)).

Microscopy for *Mycobacterium* species ([BSOP 40 - Investigation of specimens for Mycobacterium species](#)).

NB: Methods for staining procedures for parasites and ova are contained in separate SOPs.

### 4.4 CULTURE AND INVESTIGATION

#### 4.4.1 PRE-TREATMENT

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

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#### 4.4.2 SPECIMEN PROCESSING

- 1 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).
- 2 Faeces may be diluted 1:10 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.
- 3 For the isolation of individual colonies, spread inoculum with a sterile loop.
- 4 Place a pea-sized portion (or several drops) of faecal material in to the enrichment broth. After incubation, sub-culture with minimal disturbance to the broth, using a sterile loop to sample from just below the surface of the broth and inoculate appropriate media (see BSOP 54 – Inoculation of culture media).

#### 4.4.3 SUPPLEMENTARY

##### **Spore count for *C. perfringens***

- 1 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.
- 2 Add an equal volume of 95% v/v ethanol in distilled water, and shake.
- 3 Leave for 30 mins at room temperature.
- 4 From this 1:10 dilution prepare two further tenfold dilutions in PBS (1:100, 1:1000). Inoculate 0.1ml aliquots of both these dilutions to neomycin blood agar and incubate anaerobically overnight (see Section 4.4.3).
- 5 Perform a colony count which will permit the calculation of the spore count.

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

- 1 Prepare 1:10 and 1:100 dilutions of faeces in phosphate-buffered saline (PBS).
- 2 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 (see Section 4.4.3).
- 3 Count colonies and calculate the total viable count.  
Investigation of faeces for parasites ([BSOP 31- Investigation of specimens other than blood for parasites](#)) should also be performed if clinically indicated.

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#### 4.4.5 CULTURE MEDIA, CONDITIONS AND ORGANISMS<sup>66, 91, 92</sup>

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
For all diagnostic specimens (except specific organism screens)	XLD agar	35-37	air	16-24 h	≥ 16 h	<i>Salmonella</i> species <i>Shigella</i> species
	Campylobacter selective agar <sup>93,94</sup>	39-42	micro-aerobic	≥48 h	≥ 40 h	<i>Campylobacter</i> species
	Mannitol selenite broth: subculture next day onto XLD	35-37 35-37	air air	16-24 h 16-24 h	N/A ≥ 16 h	<i>Salmonella</i> species
Diarrhoeal specimens <sup>2</sup> (semi-formed or liquid faeces)	CT-SMAC agar <sup>95-97</sup>	35-37	air	16-24 h	≥ 16 h	<i>E. coli</i> O157
For outbreaks or when advised by a senior microbiologist/scientist	MTSB <sup>97</sup>	35-37	air	16-24 h	N/A	<i>E. coli</i> O157
	then subculture to CT-SMAC agar	35-37	air	16-24 h	≥ 16 h	<i>E. coli</i> O157
For these situations, add the following:						
Clinical details/ conditions	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)	MLCB	35-37	air	16-24 h	≥ 16 h	<i>Salmonella</i> species (except <i>S. Typhi</i> and <i>S. Paratyphi</i> A and B)
Food poisoning (according to clinical details and advice from senior microbiologist/scientist)	<i>B. cereus</i> selective agar (PEMBA)	35-37 then RT	air air	16-24 h then 16-24 h	16-24 h then 40-48 h	<i>B. cereus</i> <i>B. subtilis</i> <i>B. licheniformis</i>
	Neomycin fastidious anaerobe agar	35-37	anaerobic	16-24 h	≥ 16 h	<i>C. perfringens</i>
	Mannitol salt agar or Baird Parker agar	35-37	air	40-48 h	16-24 h	<i>S. aureus</i>

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Culture for <i>C. difficile</i> in samples from outbreaks only. Refer to BSOP 10  Note: Test for <i>C. difficile</i> toxin in antibiotic-associated diarrhoea, pseudomembranous colitis, post-antibiotic treatment, all patients over 65 years old <sup>98</sup>	CCEY	35-37	anaerobic	40-48 h	≥ 40 h	<i>C. difficile</i>
Cholera, seafood consumption, recent travel (2-3 weeks) to known cholera area, suspected <i>V. parahaemolyticus</i>	TCBS agar	35-37	air	16-24 h	≥ 16 h	<i>V. cholerae</i> <i>V. parahaemolyticus</i>
For outbreaks or when advised by a senior microbiologist/scientist <sup>99</sup>	Alkaline peptone water	35-37	air	5-8 h	N/A	
	then subculture to TCBS agar	35-37	air	16-24 h	≥ 16 h	
Appendicitis, mesenteric lymphadenitis, terminal ileitis <sup>100</sup> Reactive arthritis	CIN agar	28-30	air	24-48 h	≥ 24 h	<i>Y. enterocolitica</i> <i>Y. pseudotuberculosis</i> <i>Yersinia</i> species
when advised by a senior microbiologist/scientist	tris-buffered 1% peptone (pH 8.0)	8-10 or 28-30	air	7d or 24-48 h	≥ 24 h	
	then subculture on CIN agar	28-30	air	24-48 h		

**Other organisms for consideration - *Mycobacterium* species ( [BSOP 40 - Investigation of specimens for \*Mycobacterium\* species](#) ) and parasites [BSOP 31 - Investigation of specimens other than blood for parasites](#) ); toxin of *C. botulinum***

### Media Key

CCEY	Cycloserine, cefoxitin, egg yolk agar
CIN agar	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 salt agar
XLD	Xylose, lysine, deoxycholate agar

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## 4.5 IDENTIFICATION

All work on *S. Typhi*, *S. Paratyphi* A, B & C, Vero cytotoxin-producing *E. coli* O157 and *Shigella dysenteriae* type 1 must be performed in a microbiological safety cabinet under Containment Level 3 conditions

### 4.5.1 MINIMUM LEVEL OF IDENTIFICATION IN THE LABORATORY

<a href="#">Bacillus species</a>	genus level
<a href="#">Campylobacter species</a>	genus level
<a href="#">C. difficile</a>	species level
<a href="#">C. perfringens</a>	species level
<a href="#">E. coli O157</a>	species level + serotype
<a href="#">Salmonella species</a>	genus level
<a href="#">S. Typhi/Paratyphi</a>	species level
<a href="#">Shigella species</a>	species level
<a href="#">S. aureus</a>	species level
<a href="#">Vibrio species</a>	species level
<a href="#">V. cholerae</a>	species level; O1, 0139 or not
<a href="#">Yersinia species</a>	species level
<a href="#">Parasites</a>	see <a href="#">BSOP 31 - Investigation of specimens other than blood for parasites</a>

Organisms may be further identified if this is clinically or epidemiologically indicated

### 4.5.2 REFERRAL TO REFERENCE LABORATORIES

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

Isolates associated with outbreaks, where epidemiologically indicated and 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.

## 4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Refer to [BSOP 45 - Susceptibility testing](#) .

# 5 REPORTING PROCEDURE

## 5.1 APPEARANCE

Report the presence of blood and/or mucus.

## 5.2 MICROSCOPY

### 5.2.1 STANDARD

Report presence or absence of *Cryptosporidium* oocysts.

### 5.2.2 SUPPLEMENTARY

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

Parasites ([BSOP 31 - Investigation of specimens other than blood for parasites](#) ).

### 5.2.3 MICROSCOPY REPORTING TIME

Urgent microscopy results to be telephoned or sent electronically.

Written report: 16-72 h.

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### **5.3 CULTURE**

Report presence or absence of specific pathogens also, report results of supplementary investigations.

#### **5.3.1 CULTURE REPORTING TIME**

Clinically urgent results to be telephoned or sent electronically.

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

Supplementary investigations: parasites ([BSOP 31 - Investigation of specimens other than blood for parasites](#) ).

### **5.4 ANTIBIOTIC SUSCEPTIBILITY TESTING**

Report susceptibilities as clinically indicated.

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## 6 REPORTING TO THE HPA<sup>101</sup> (LOCAL AND REGIONAL SERVICES AND CDSC CENTRE FOR INFECTIONS)

Refer to the following:

Individual SOPs on organism identification

Health Protection Agency publications:

"Laboratory reporting to the HPA. A guide for diagnostic laboratories"

"Hospital infection control: Guidance on the control of infection in hospitals"

Refer to current guidance on CDSC and COSURV reporting

Local guidelines

Report all isolates of the following:

*B. cereus*

*Campylobacter* species

*C. perfringens*

*E. coli* (VTEC and EPEC strains)

*Mycobacterium* species

*Salmonella* species

*Shigella* species

*V. cholerae*

*V. parahaemolyticus*

*Y. enterocolitica*

*Y. pseudotuberculosis*

If a diagnosis of botulism is suspected report to the CCDC and also to CDSC Duty Officer

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## 7 ACKNOWLEDGEMENTS AND CONTACTS

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For further information please contact us at:

Standards Unit  
Department for Evaluations, Standards and Training  
Centre for Infections  
Health Protection Agency  
Colindale  
London  
NW9 5EQ

E-mail: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

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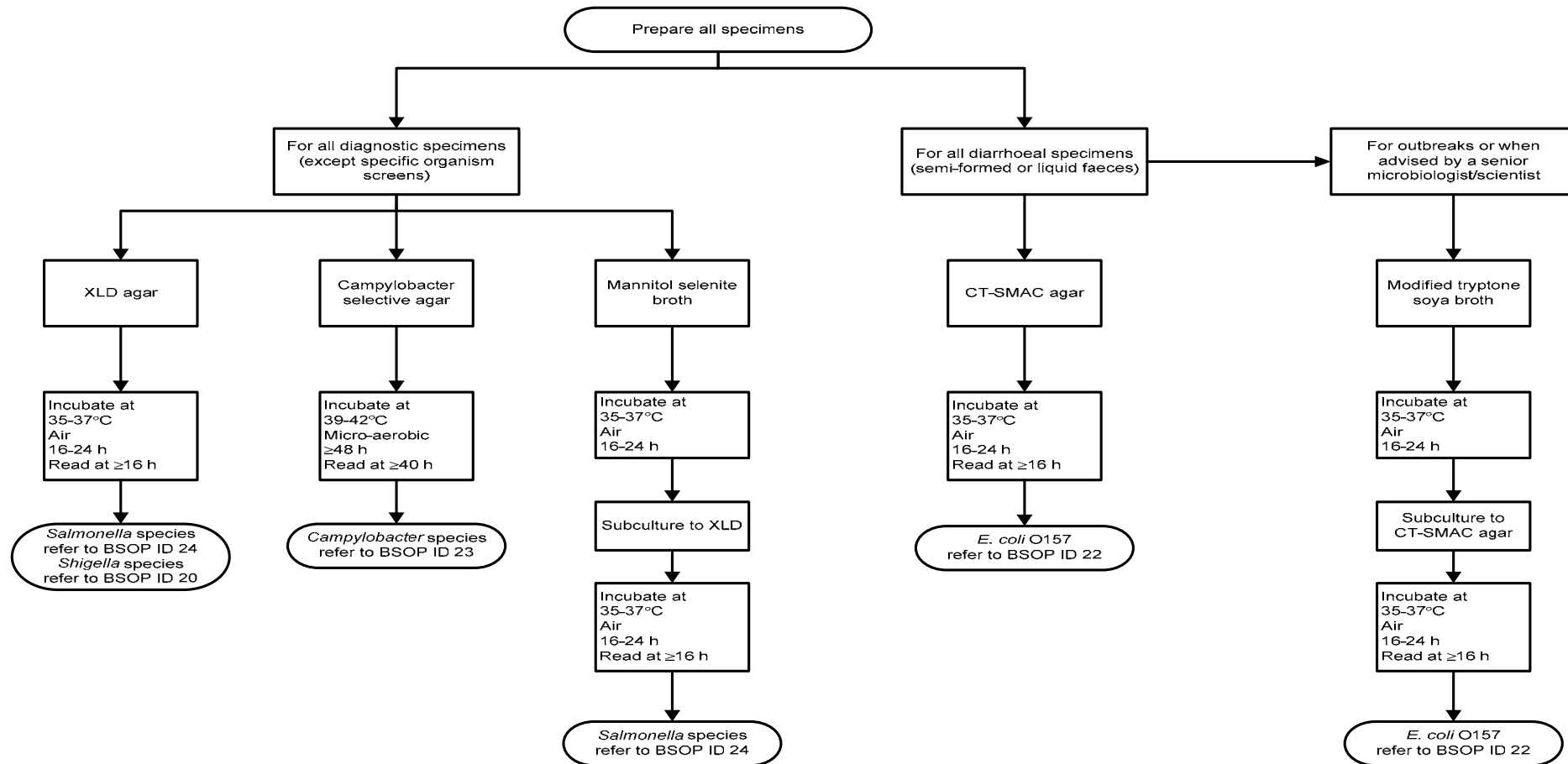
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# APPENDIX 1



## INVESTIGATION OF FAECAL SPECIMENS FOR BACTERIAL PATHOGENS

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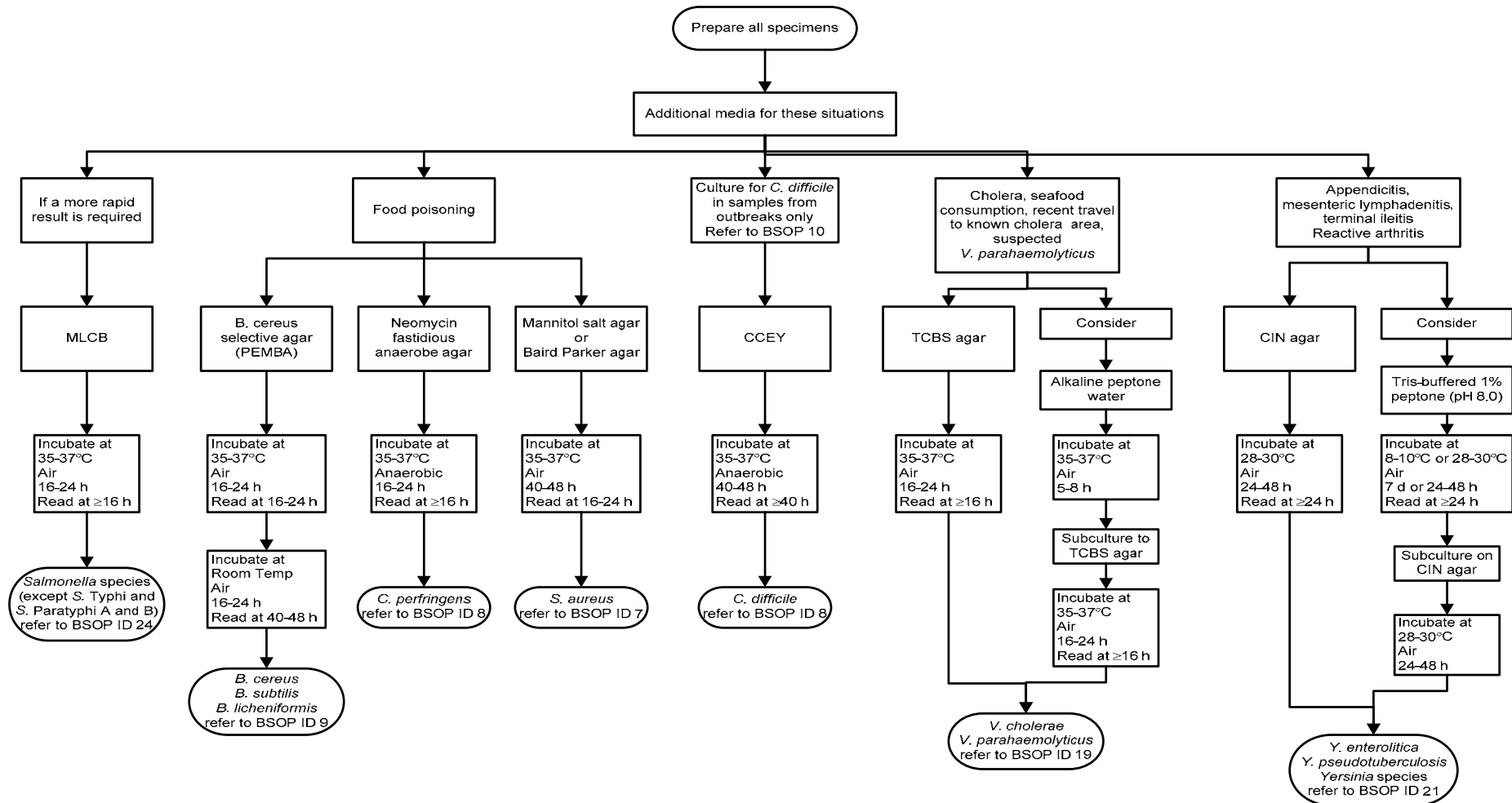
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# APPENDIX 2



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