UK Standards for Microbiology Investigations
Gastroenteritis

“NICE has renewed accreditation of the process used by Public Health England (PHE) to produce UK Standards for Microbiology Investigations. The renewed accreditation is valid until 30 June 2021 and applies to guidance produced using the processes described in UK standards for microbiology investigations (UKSMIs) Development process, S9365, 2016. The original accreditation term began in July 2011.”

Issued by the Standards Unit, National Infection Service, PHE.
Syndromic | S 7 | Issue no: dzp+ | Issue date: xx | Page: 1 of 49
PHE publications gateway number: GW-632

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Acknowledgments

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

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

PHE publications gateway number: GW-632

UK Standards for Microbiology Investigations are produced in association with:
Amendment table

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

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

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<tr>
<td>Whole Document</td>
<td>This new syndromic document supersedes the previous UK SMI S7 document. The content and layout has been changed to widen the scope of the document and include the UK SMI B30.</td>
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*Reviews can be extended up to five years subject to resources available.
1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This document describes infections and relevant investigations that should be considered in accordance with different presentations of diarrhoea or vomiting consistent with gastrointestinal infections in adults and children, in community and healthcare settings including patients who are immunocompromised.

Investigation protocols and target organisms can vary depending on whether the testing is to be performed in response to a sporadic/individual case or as part of an outbreak investigation. Testing of patients with past gastrointestinal infection or contact with a case of gastrointestinal infection for microbiological clearance is included in the scope.

The syndromes included have been selected to reflect the common presenting groups of patients with infective gastroenteritis and diarrhoea. It excludes *Helicobacter pylori* infection, hepatitis viruses, non/protozoal parasitic presentations and any other infections not transmitted through the enteric route.

The investigation of *Clostridium difficile* is not included within the scope of this document due to the varying testing algorithms used in the UK. Please refer to the UK SMI B10: Processing of faeces for *Clostridium difficile* for more detail.

There are an estimated 17 million cases of infectious intestinal disease annually in the UK. Not all community cases of acute diarrhoea and vomiting require laboratory investigation as many are self-limiting.

The document takes account of UK data from the Infectious Intestinal Disease 2 (IID2) study 2011 which emphasised the under diagnosis of enteric viruses in all age groups.

Refer to Public Health England (PHE), National Institute for Healthcare and Clinical Excellence (NICE) and Department of Health (DH) guidelines on gastroenteritis and diarrhoea for more information.

This UK SMI should be used in conjunction with other associated UK SMIs.

4 Background

4.1 Gastroenteritis

Gastroenteritis is the inflammation of the lining of the stomach and the small intestine, characterised by diarrhoea and/or vomiting or it can be described as a transient disorder characterised by the sudden onset of diarrhoea with or without vomiting. This enteric infection can be caused by viruses, bacteria, or parasites. This is also known as "infectious diarrhoea." Depending on the cause of the infection, the
symptoms of gastroenteritis can take from a few hours to a few days after exposure to develop. Most cases resolve without treatment within days, although persistent or severe symptoms may occur which may require hospitalisation and treatment.

4.2 Diarrhoea

This may be defined as stools of a loose or liquid consistency occurring more frequently than is normal for the individual\textsuperscript{10}. Usually this is at least three or more instances in a 24 hour period; however, the consistency of stools is a more important indicator than the frequency. Diarrhoea may be associated with symptoms such as abdominal cramps, nausea, malaise, vomiting, fever and dehydration.

\textbf{Note}: Frequently passed formed stools are not considered to be diarrhoea as advocated by the Bristol Stool Form Scale \textsuperscript{11}.

A wide range of bacterial pathogens, viruses and parasites are capable of causing diarrhoea by a number of mechanisms such as\textsuperscript{8}:

- multiplication of pathogens in the gut, eg \textit{Giardia} species, \textit{Cryptosporidium} species, \textit{Salmonella} species and \textit{Shigella} species
- ingestion of pre-formed toxins produced by bacteria in food prior to ingestion, eg \textit{Bacillus} species, \textit{B. cereus} toxins, \textit{C. perfringens}, \textit{S. aureus} and \textit{C. botulinum} toxins

4.3 Other infections transmitted through the enteric route \textsuperscript{8}

There are several food or water pathogens that do not necessarily give rise to symptoms of gastroenteritis such as botulism, \textit{Helicobacter pylori} infection, listeriosis and poliomyelitis. Diarrhoea may have non-infectious causes, or may be a presentation of sepsis. Microbiological examination is often required to rule out infectious causes of gastroenteritis.

4.4 Organisms implicated

Organisms in the community and presenting to primary care include bacteria, parasites and viruses\textsuperscript{12}.

The table in the appendix outlines the common organisms associated with gastroenteritis, their incubation periods, symptoms, the mode of transmission and the frequency at which these organisms cause infections.

Food poisoning is a notifiable disease under the Health Protection (Notification) Regulations 2010.

5 Clinical presentations of gastrointestinal infections\textsuperscript{10,13}

The clinical presentations can feature in particular epidemiological settings: community or hospital as sporadic cases or outbreaks.

\textbf{Sporadic cases}: also known as ‘non-outbreak’ cases. They are cases that occur at irregular intervals, in scattered or isolated instances.

\textbf{Outbreak cases}: Outbreaks can originate from a single point source but may result in secondary cases as a result of contact with the original cohort. They may occur
through person to person contact, through ingestion of contaminated food and water, contaminated fomites 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 ingredient or cross contamination, and currently are estimated to cause three million deaths worldwide per year.

- Water borne outbreaks are 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.

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.

**Acute watery diarrhoea:** This is defined as diarrhoea not exceeding 14 days of duration and typically with frequency of 3 or more episodes a day. It is characterised by sudden onset, with or without vomiting and abdominal pain, fever, abdominal cramps, lethargy and consequent dehydration may also occur. The incubation period varies from a few hours to a few days. Transmission may occur due to eating improperly prepared foods, drinking contaminated water, sewage contamination or through person to person contact.

Organisms implicated include norovirus, rotavirus, *Salmonella* species, *Campylobacter* species and *C. difficile*. Other organisms that may be considered include the *Vibrio* species including *Vibrio cholerae* (the causative agent of cholera). Consumption of food containing irritant chemicals may also cause diarrhoea.

**Acute bloody diarrhoea:** This is a sudden onset of diarrhoea (passing of liquid or watery stools) where frank blood is present. The presence of visible blood and mucus in the faeces, suggests inflammation of the bowel, with fever, abdominal cramps and constitutional disturbance constitutes dysentery.


**Persistent diarrhoea:** This is diarrhoea of >14 days but fewer than 30 days in duration, and some experts refer to diarrhoea that lasts >30 days as “chronic”. Chronic diarrhoea is a major clinical feature in HIV infection and is a leading cause of morbidity and mortality. It should be noted that viruses (eg norovirus) and bacteria (*Salmonella*, *Shigella* and *Campylobacter* species) can be the cause of persistent diarrhoea in patients who are immunocompromised. Organisms implicated are predominantly parasites - *Giardia*, *Cryptosporidium*, *Cyclospora* and Microsporidia species.

**Vomiting**

Vomiting is an uncontrollable reflex expulsion of gastric contents and can occur either with or without diarrhoea. It can be severe in patients especially in infants and children. Vomiting without diarrhoea can occur with *Bacillus cereus*, norovirus and staphylococcal toxin. Vomiting can present in several scenarios including:
Gastroenteritis

• **Vomiting with diarrhoea** – this type of vomiting is common with toxin producers such as *Vibrio* species, *S. aureus*, *C. perfringens*, etc.

The causative organisms of gastrointestinal infections are described in the appendix and will normally be tested for in specific situations.

### 5.1 Testing pathway for community and hospital settings

The following general information is provided to guide users to navigate the algorithms in sections 5.1.1 and 5.1.2.

**Primary testing**: is the initial set of routine testing/investigation performed to rule out the suspected common pathogens responsible for a clinical presentation. This will normally include *Salmonella*, *Shigella*, *Campylobacter*, STEC (including O157) and norovirus. Testing for *Cryptosporidium* species, *Giardia* species and Rotavirus should be undertaken depending on local policy and clinical presentation. Testing for additional target organism should be dependent on clinical details received with the clinical specimens.

**Secondary testing**: is the further additional set of testing/investigation performed after the initial primary testing undertaken has not provided a confirmed diagnosis or depending on clinical details and thus additional investigations are required.

**Note**: It should be noted that some laboratories offer only traditional testing by microscopy and culture while others offer molecular testing (with varying range of panels) or EIA (antigen) testing; all approaches are currently considered acceptable although the sensitivity of traditional microscopy and culture techniques is limited. Consequently, if performing microscopy, submission of multiple samples is recommended depending on the specific clinical scenario.

STEC can present atypically and may be negative using culture methods, and so such specimens/isolates should be referred following the National Reference Laboratory guidelines 14.

1. **Gastroenteritis in community setting**:

   This algorithm recognises that testing for viral causes of gastrointestinal infections is not normally undertaken unless in children aged <5 years and in patients who are immunocompromised.

   a) **Sporadic cases**

   In this scenario, routine testing of suspected organisms should be performed but additional testing (for instance, if overseas travel has occurred) should be added as described in the algorithm. *C. difficile* is an important cause of community acquired diarrhoea.

   • **Persistent diarrhoea**

   In addition, *Giardia* and *Cryptosporidium* should always be included in primary testing whilst other parasites including amoebae (*Entamoeba histolytica*) and *Cyclospora* should be considered as secondary testing.

   All symptomatic patients should be tested for *Cryptosporidium* species and *Giardia* species irrespective of whether the stool specimens presented take the shape of container or not 15,16.
b) Outbreak cases

- Acute diarrhoea with or without vomiting

This usually occurs at >24 hours incubation. In addition to primary testing, additional tests should be undertaken dependent on clinical presentation. Referral of samples to the local public health laboratories may be necessary if testing is not available in house. In addition to primary testing, *S. aureus*, *Bacillus*, *C. perfringens* and norovirus testing should be performed.

- Acute vomiting with or without diarrhoea

This usually occurs at <24 hours incubation. Testing in this section will include the primary tests as well as *C. perfringens* and norovirus testing.

Consider testing for viruses in certain circumstances following discussion with the local laboratory. Norovirus testing is not recommended as frontline testing in sporadic cases except in patients who are immunocompromised. Testing is dependent on local laboratory policies. However, if a norovirus outbreak is suspected, consider submitting stool samples as early as possible during the acute phase of the illness.\(^{17,18}\) Laboratories may opt to test only during the seasonal increase. Testing for *C. perfringens* should be considered after discussion with the laboratory.

This UK SMI recommends inclusion of *Giardia* species and *Cryptosporidium* species in the primary test set.

2. Gastroenteritis in hospital setting (in-patients):

a) Sporadic cases

- Sporadic cases (< 72 hours)

Diarrhoea occurs within 72 hours of hospitalisation of the patient and so the patient should be investigated as they would in the community setting. *C. difficile* testing should also be indicated if patient has been hospitalised prior to development of symptoms.

For patients who are immunocompromised, in addition to the common causes of gastroenteritis, other viruses that could be considered include cytomegalovirus (CMV), varicella zoster virus and herpes simplex virus as they may also present with gastrointestinal symptoms.

- Sporadic cases (> 72 hours)

Gastrointestinal symptoms presenting >3 days after admission are rarely caused by *Listeria*, *Salmonella*, *Shigella*, *Campylobacter* and STEC (including O157). The UK SMI endorses the ‘3 day’ rule which does not recommend routine testing for these pathogens.\(^ {19}\) All samples should be tested for *C. difficile*.\(^ 6\)

Laboratories considering applying the “three-day rule” should perform analysis of their submission and positivity data and undertake a risk assessment. Clusters of diarrhoea cases must be investigated. The “three-day rule” suggests that faecal samples from patients should undergo microbiological investigation in the following circumstances.\(^ {19,20}\)
• Those inpatients developing diarrhoea within three days of hospital admission
• Adults with nosocomial diarrhoea only if one of the following is applicable:
  o Aged 65 or more with pre-existing disease causing permanently altered organ function
  o Patients who are immunocompromised
  o Patients with neutropenia
  o Suspected nosocomial outbreak (e.g. norovirus, *Salmonella*)
• Those with suspected non-diarrhoeal manifestations of enteric infections.
  Routine testing for norovirus should be considered. Other viruses may be tested for as appropriate. It should be noted that rotavirus positive NAAT results in children who have recently received rotavirus vaccine may be due to detection of vaccine strain.

b) Outbreak cases (< 48 hours)

Testing should include routine examination with *C. difficile*, norovirus and other viruses depending on clinical features.
5.1.1 Gastroenteritis in community setting

Clinical presentation / Patient group

Specific cases

Persistent diarrhoea

Immune competent

Immune compromised

Acute diarrhoea

Additional investigations following clinical details

Routine screen

Salmonella

Shigella

Campylobacter

STEC (including O157)

Giardia

Cryptosporidium

Travel specific information provided eg countries/areas indicated

Mesenteric adenitis

Immunocompromised

OCP

C. difficile

Vibrio

Yersinia

C. difficile

C. perfringens

C. pseudotuberculosis

C. septicum

Norovirus

STEC O157 culture negative but high clinical suspicion

STEC O157 negative but high clinical suspicion

Predominantly vomiting (usually short incubation) (<24hr)

Predominantly diarrhoea (usually >24hr incubation)

OCP - Ova, cysts and parasites including Cyclospora species

MAI - Mycobacterium avium-intracellulare complex

DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 11 NOVEMBER TO 25 NOVEMBER 2019
5.1.2 Gastroenteritis in hospital (in-patients) setting

- **Clinical presentation** / **Patient group**
  - Sporadic cases
    - Acute diarrhoea
      - Routine screen
        - Salmonella, Shigella, Campylobacter, STEC (including O157), Norovirus
      - OCP
      - Vibrio
      - Astrovirus
      - OCP
      - Vibrio
      - Astrovirus
  - Outbreak cases
    - Persistent diarrhoea
      - Routine screen
        - Salmonella, Shigella, Campylobacter, STEC (including O157), Norovirus
      - OCP
      - Vibrio
      - Astrovirus
      - OCP
      - Vibrio
      - Astrovirus

- **Primary testing**
  - Sporadic cases <72hr
    - Acute diarrhoea
      - OCP
      - Vibrio
      - Astrovirus
      - OCP
      - Vibrio
      - Astrovirus
  - Outbreak cases
    - Persistent diarrhoea
      - OCP
      - Vibrio
      - Astrovirus
      - OCP
      - Vibrio
      - Astrovirus

- **Secondary testing**
  - Sporadic cases >72hr
    - Acute diarrhoea
      - OCP
      - Vibrio
      - Astrovirus
      - OCP
      - Vibrio
      - Astrovirus
  - Outbreak cases
    - Persistent diarrhoea
      - OCP
      - Vibrio
      - Astrovirus
      - OCP
      - Vibrio
      - Astrovirus

- **Travel specific information** provided eg countries/areas indicated
  - Shellfish
  - Mesenteric adenitis

- **Immunocompromised**
  - C. difficile
  - Salmonella
  - Shigella
  - Campylobacter
  - STEC (including O157)
  - Norovirus

- **Immunocompetent**
  - C. difficile
  - Norovirus
  - Rotavirus
  - Adenovirus
6 Pre-laboratory processes (pre-analytical phase)

6.1 Specimen Type

Faeces, Blood

Note: vomit swab may be used to test for certain viruses in the absence of faeces but this is not common practice in laboratories\textsuperscript{21}.

Blood cultures are recommended if the patient presents with features of sepsis or is systemically unwell, such as clinical suspicion of enteric fever \textsuperscript{22,23}. Duodenal contents may also be acceptable for such cases.

Colonic biopsy can be considered.

6.2 Specimen collection and handling

Collect specimens as soon as possible after onset of symptoms.

Collect specimens before antimicrobial therapy where possible\textsuperscript{24}.

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

For bacteria testing, faecal samples should be liquid or semi formed (that is, take the shape of the container). Formed stools are unsuitable for investigation for \textit{C. difficile}; these should be rejected and an appropriate comment appended to the report.

For parasite testing, fresh specimens are ideal for microscopy as it allows for the observation of motile trophozoites and cysts\textsuperscript{25}. Preservation of specimens in 10\% formalin is not recommended in this UK SMI.

If faecal specimens for ova, cysts and parasites testing are requested and microscopy is performed (rather than more sensitive molecular or EIA assays), three specimens should be sent at least two days apart as OCP are shed intermittently\textsuperscript{26}.

If faeces are liquid or soft, approximately 5 mL should be collected, and if formed, 1 - 2g is adequate for culture\textsuperscript{24}.

Stool samples are usually collected and referred for investigation in the following situations:

- when the clinician requires a microbiological diagnosis
  - when there is persistent diarrhoea/malabsorption
  - when there is blood, mucous or pus in the stool
  - when there is a history of diarrhoea and/or vomiting, and the patient is systemically unwell
  - when there is a history of recent hospitalisation or for inpatients as soon as infective diarrhoea is suspected.
  - when there is a history of antibiotic therapy
- when public health requires sampling to be carried out. For example:
Gastroenteritis

- when investigating outbreaks of diarrhoea and/or vomiting in contacts of patients infected with organisms such as STEC (including O157) or S. Typhi
- when there is a suspected public health hazard (for example if a patient with diarrhoea is a food handler)
- where a patient requires microbiological clearance for their occupation following past infection or contact with a case of gastrointestinal infection
- when an outbreak is suspected (e.g. petting farm, swimming pool)
  - when the patient is immunocompromised
  - when the patient has travelled within 14 days of symptoms onset. Refer to https://nathnac.net/ for information on endemic areas.

Laboratory test results are dependent on the quality of the specimen submitted. It is important that all specimens are properly labelled with adequate information to assist the laboratory when testing, where possible.

### 6.3 Relevant clinical history details needed on patient request forms when referring samples to the laboratory:

The history of the patient should identify risk factors for unusual causes of acute gastroenteritis and any extra-intestinal causes. In addition to patient identifiable information (such as name, age, etc), patient history (including clinical features and epidemiological information) should be recorded on the request form including:

- Specimen date and time of collection
- Acute/outbreak case
- Immune status
- Healthcare or community-acquired. If patient is hospitalised, date of admission and date of symptom onset should be included
- Recent overseas travel including location
- Water exposure
- Farm animal exposure/animal contact
- Food intake, for example shellfish and chicken
- Recent antibiotic use
- Other relevant information such as suspected food poisoning, contact with cases, food handler and occupation

### 6.4 Specimen transport and storage

Faeces should be submitted in suitable and appropriate transport containers. Specimens should be transported and processed as soon as possible. Important pathogens such as *Shigella* species may not survive the pH changes that occur in faecal specimens when not promptly delivered to the laboratory, even if refrigerated.

If processing is delayed, refrigeration is preferable to storage at ambient temperature.
Refer to current guidance on the safe handling of all organisms documented in the safety considerations section of the UK SMI scientific information.

For more information on specimen transport, refer to UK SMI U 1: National user manual template.

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

6.5 Safety considerations

Containment Level 2

Diagnostic work with clinical material that could contain Hazard Group 3 organisms (S. Typhi, S. Paratyphi A, B and C, STEC including O157 or Shigella dysenteriae) does not normally require full Containment Level 3 conditions. However, all processing work (whether growth or manipulation of a potential/locally confirmed Hazard Group 3 organism) must be performed in a microbiological safety cabinet under Containment Level 3 conditions.

Note: S. Typhi, S. Paratyphi A, B and C as well as STEC (including O157) and S. dysenteriae type 1 cause severe and sometimes fatal disease, and laboratory acquired infections have been reported. Low numbers (as few as 10-100 organisms) are required for an infective dose. Laboratory staff who may handle S. Typhi in the course of their work should be vaccinated. S. Typhi immunisation is available and guidance is given in the Public Health England immunisation policy.

All work with clinical material that could contain enteric viruses may be processed in Containment Level 2 conditions. However, it should be noted that viruses are highly infectious with a low infectious dose such as in noroviruses where approximately 10 virus particles are needed to cause an infection. Extreme care should be taken by laboratory staff when performing any task.

Refer to current guidance on the safe handling of all organisms documented in the safety considerations section of the UK SMI scientific information (see section 2).

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

7 Laboratory processes (analytical phase)

The testing pathway incorporates a range of tests including:

7.1 Microscopy

For parasites, routine testing for Cryptosporidium and Giardia species recommended nationally, subject to local consideration. Microscopy is of low sensitivity when compared to PCR and EIA testing, and evidence suggests a doubling of detection rates using EIA. However, microscopy is still useful in the primary identification of parasites. Refer to the UK SMI TP 39: staining procedures for information on the different stains or wet preparations that may be used.

Ova, cysts and parasites (OCP) are not routinely included in the primary testing set as yields are extremely low. However, if parasitology testing is required other than those already recommended, a request for OCP should be submitted.
7.1.1 Sample preparation
For generic safety considerations, refer to the link in Section 2 of the syndromic document.

Faecal concentrations are carried out on all specimens where examination of parasites is specifically requested, or where there are definite clinical indications and when advised by senior laboratory staff.

All faecal samples from symptomatic individuals should be tested for Cryptosporidium oocysts and Giardia cysts / trophozoites irrespective of specimen consistency. If relevant travel-specific information is provided eg country/area, a full OCP investigation should be performed as relevant using microscopy.

In addition, Microsporidia should be considered in symptomatic, HIV positive, and immunocompromised patients.

**Standard**

Prepare a medium to thick smear of faeces on a clean microscope slide to stain for Cryptosporidium species on all submitted specimens from all symptomatic individuals (except specific screens, for example Salmonella species screens on known positives) (refer to UK SMI TP 39 - Staining techniques).

**Supplementary**

For information on wet preparations for microscopy for ova, cysts and parasites and faecal concentrations of parasites, refer to B 31 - Investigation of specimens other than blood for parasites.

For microscopy of Mycobacterium species, refer to UK SMI B 40 - Investigation of specimens for Mycobacterium species.

7.1.2 Specimen processing for faeces
Sample parts of faeces samples that contain blood, pus, or mucus for direct examination as wet preparations or for staining.

If sampling formed faeces, collect and examine material from various parts of the faecal sample for concentration, wet preparations and for staining.

**Faeces for microscopic examination of protozoa**

If specimen is fresh, examine for motile trophozoites as follows:

1. Place one drop of 0.85% saline on the left-hand side of a clean microscope slide, and one drop of double-strength Lugol's iodine on the right-hand side (the distance between the drops should be sufficient to enable coverslips to be placed over each drop).
2. Using a different swab stick for each preparation, take a small amount of unfixed faeces and thoroughly emulsify in the saline and in the Lugol's iodine.
3. Place cover slips over each preparation on the slide. Examine both entire areas with a low power objective. Use a medium power objective to identify any suspicious morphological features.
4. If required, prepare smears on clean microscope slides for auramine-phenol and/or Giemsa staining (see TP 39 - Staining procedures).
5. Also, concentrate the specimen with the formol-ether* concentration technique described below.

**Modified formol-ether concentration**\(^{33,34}\)

**Note:** Ethyl acetate (not diethyl ether) must be used in a well-ventilated area with no naked flames.

The following method is the recommended technique for faecal concentration. There are many commercial kits for the concentration of faeces available which are based on the Ridley Allen method described below, which is the method of choice used by most clinical laboratories. Commercial concentration kits are often used.

1. Take a sample of faeces about the size of a large pea (approximately 1g) with a swab stick and emulsify it in 7mL of 10% formalin (one volume of 40% formaldehyde diluted with nine volumes of distilled water) in a clean universal container.

2. Sieve by pouring the whole contents of the universal through a sieve (a nylon tea-strainer or a square of wire gauze is suitable) and collect in a suitable container. Sieves are washed in copious amounts of clean water and re-used. Sieving the faeces and formalin mixture prior to centrifugation helps eliminate large pieces of faecal matter from the suspension.

3. Transfer the filtrate into a stoppered glass or polypropylene (ether resistant) container appropriate for centrifugation.

4. Add 3mL of ethyl acetate and a small drop of 0.1% Triton X-100 (helps emulsify the faecal specimen) and vortex for 15 seconds, or shake vigorously for 60 seconds.

5. Centrifuge the specimen at 1200 × g for 3 min.

6. Loosen the fatty layer with a swab stick by passing it around the inside circumference of the tube, removing all residues of the fat from the tube.

7. Tip away the contents of the tube, allowing the last few drops to return to the bottom of the tube to cover the remaining deposit.

8. Resuspend the deposit in the remaining fluid. Place a drop of this on a clean microscope slide and place a coverslip over it.

9. Double strength iodine may be added to a separate preparation to enhance and facilitate comparison of morphological details.

10. Search the entire area using a low power objective; use a medium power objective to examine morphological features.

Commercially available concentrator kits containing sieves of varying pore sizes are available; the size of the pore affects the yield of parasite stages and the amount of debris present\(^{35-38}\). A larger pore size may result in a higher yield of parasite stages, however the increase in debris leads to a denser deposit, making it more difficult to examine the slide; ova and cysts may therefore be obscured. If the pore size is too small, despite having a cleaner slide which is easy to examine, the yield of parasite stages will be reduced. Commercially available faecal concentrator kits should be validated prior to use, and manufacturers’ instructions should be followed.
To maximise the recovery of parasites it is important to sieve the faecal formalin mixture, use a solvent, that is, ethyl acetate with Triton X and centrifuge for the correct time and at the correct centrifugal force\(^3\). Recovery of parasite stages may be greatly diminished if a solvent (for example ethyl acetate) as an extractor of fat and debris is not used\(^3\). A recent study confirms and recommends that 1200 × g for 3 min is optimal for parasite recovery\(^3\).

**Faeces for examination of Cryptosporidium species**

1. Prepare a medium to thick smear of faeces on a clean microscope slide and air dry.
2. Fix in methanol for three minutes.
3. Smears can be stained by either auramine phenol or modified cold Ziehl-Neelsen (see [TP 39 - Staining procedures](#)).

### 7.1.3 Specimen processing for bile, duodenal/jejunal aspirates

**Standard**

1. Centrifuge specimen at 800 × g for 2 min.
2. Decant supernatant to disinfectant.
3. Resuspend the centrifuged deposit in the remaining drops of supernatant.
4. Use the resuspended pellet to prepare smears for staining.
5. Stain using Ziehl-Neelsen and auramine phenol for Cryptosporidium, Giemsa for Cyclospora cayetanesis and C. belli (see [TP 39 - Staining procedures](#)), and iodine or plain wet preparation for S. stercoralis and G. duodenalis.

**Supplementary**

For examination of microsporidia:

1. Centrifuge specimen at 800 × g for 2 min.
2. Decant supernatant to disinfectant.
3. Resuspend the centrifuged deposit in the remaining drops of supernatant.
4. Use the resuspended pellet to prepare smears for staining.
5. Stain with the modified trichrome stain (refer to [TP 39 - Staining procedures](#)).

### 7.1.4 Specific technical limitations (to include any uncertainty of measurement information)

**Concentration methods**

Faecal specimens should be submitted fresh, without formalin, and a concentrate performed routinely prior to OCP investigations.

**Problems with identification**

There are many microscopic artefacts that can be found in faeces which may be confused with trophozoites, oocysts, cysts or ova.
Problems with microscopy

Microscopy cannot be used to discriminate *E. histolytica* (pathogenic) from morphologically identical but non-pathogenic *E. dispar*, *E. moshkovskii*, and other quadrinucleate cysts of *Entamoeba*\(^{40,41}\). Molecular testing is therefore required if cysts resembling *E. histolytica* are identified by microscopy.

Other drawbacks to microscopy generally include its tediousness when large numbers of specimens need to be examined and the lack of microscopic expertise among laboratory staff. Consideration should be given towards implementing more sensitive techniques (e.g. NAAT / PCR testing, EIA testing).

7.1.5 Investigation

Using a sterile loop, spread the inoculum on the slide (refer to Q 5 – *Inoculation of culture media for bacteriology*) and then examine the slides using the appropriate stains or preparations (refer to UK SMI TP 39 - *Staining procedures*).

7.1.6 Identification

**Minimum level**

Identify parasites to species level and their development stage where possible.

7.2 Culture

Culture is important in the primary isolation of bacteria and some parasites from faecal specimens as well as in the primary and secondary testing of bacteria. Laboratories also use the culture test method to provide information on antimicrobial susceptibility to guide clinical management of patients. Refer to section below for the culture media and techniques to use for isolation of bacteria.

7.2.1 Primary culture

Many laboratories utilise molecular techniques for the detection of gastrointestinal pathogens for primary testing however culture techniques described here should be used to detect the pathogens outside the molecular panels in use locally.

For culture and isolation, refer to table in this document.

Culture is important for typing in cases of increased incidence, in outbreak situations and for surveillance of drug resistance.

Culture techniques can be performed as an alternative method to microscopy for some of the parasites such as *Toxoplasma gondii* and *Blastocystis hominis*. Culture techniques are more sensitive than direct smears.

7.2.2 Sample preparation

**Standard**

**Pre-treatment and dilution for bacteria (outbreaks)**

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

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 broth. 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 phosphate-buffered saline (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 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

7.2.3 Specific technical limitations (to include any uncertainty of measurement information)

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 significantly reduce 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 costs\cite{43}.

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°C \cite{44}.
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 in campylobacter
  selective agars (for example 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 recommended in the UK SMIs are primarily aimed at detecting
C. jejuni, C. coli and C. lari.

**Salmonella** species

A study 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
plating \cite{45}. 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 XLD \cite{46}.

Chromogenic agar has also been evaluated and has been shown to be comparable to
traditional plated media \cite{47}.

**STEC (including O157)**

Where the clinical evidence is suggestive of STEC 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 should \cite{48}:

- 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 shiga toxin genes and phage typing to the reference laboratory

• faecal samples from appropriate cases from whom STEC O157 has not been isolated should be submitted to a reference laboratory for detection of shiga toxin producing *E. coli* of serogroups other than O157 (non-O157 STEC)

**Plesiomonas shigelloides**

There is currently no specific UK 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 samples. 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, STEC), *Vibrio cholerae* and *Vibrio parahaemolyticus*) from faeces by reducing false positive growth.

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.

### 7.2.4 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.

<table>
<thead>
<tr>
<th>Clinical details/Conditions</th>
<th>Specimen</th>
<th>Standard media</th>
<th>Incubation</th>
<th>Cultures read</th>
<th>Target organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>For all diagnostic specimens (except specific organism screens)</td>
<td>Faeces</td>
<td>Campylobacter selective agar</td>
<td>37-42</td>
<td>micro-aerobic</td>
<td>≥ 48hr</td>
</tr>
<tr>
<td>Xylose lysine deoxycholate (XLD) agar</td>
<td>35-37</td>
<td>air</td>
<td>16-24hr</td>
<td>≥16hr</td>
<td>Salmonella species</td>
</tr>
<tr>
<td>Mannitol selenite broth then subculture to:</td>
<td>35-37</td>
<td>air</td>
<td>16-24hr</td>
<td>N/A</td>
<td><em>Salmonella</em> species</td>
</tr>
<tr>
<td>XLD</td>
<td>35-37</td>
<td>air</td>
<td>16-24hr</td>
<td>≥16hr</td>
<td></td>
</tr>
<tr>
<td>Clinical details/ conditions</td>
<td>Specimen</td>
<td>Supplementary media</td>
<td>Incubation</td>
<td>Cultures read</td>
<td>Target organism(s)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------</td>
<td>----------------------</td>
<td>------------</td>
<td>---------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>All diarrhoeal specimens and any with obvious blood (semi-formed or liquid faeces)(^{57})</td>
<td>Faeces</td>
<td>Modified tryptone soya broth (MTSB) and then subculture to: CT-SMAC agar</td>
<td>35-37</td>
<td>air</td>
<td>16-24hr</td>
</tr>
<tr>
<td>Children under 5 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For outbreaks or when advised by a consultant microbiologist</td>
<td>Faeces</td>
<td>Cefixime tellurite sorbitol MacConkey (CT-SMAC) agar (^{54-56})</td>
<td>35-37</td>
<td>air</td>
<td>16-24hr</td>
</tr>
</tbody>
</table>

For these situations, add the following:

<table>
<thead>
<tr>
<th>Clinical details/ conditions</th>
<th>Specimen</th>
<th>Supplementary media</th>
<th>Incubation</th>
<th>Cultures read</th>
<th>Target organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>If a more rapid result is required (eg for non-enteric fever Salmonella outbreaks)</td>
<td>Faeces</td>
<td>Mannitol lysine crystal violet brilliant green agar</td>
<td>35-37</td>
<td>air</td>
<td>16-24hr</td>
</tr>
<tr>
<td>Food poisoning (according to clinical details and advice from senior microbiologist)</td>
<td>Faeces</td>
<td>B. cereus selective agar (PEMBA)</td>
<td>35-37</td>
<td>air</td>
<td>16-24hr</td>
</tr>
<tr>
<td></td>
<td>Faeces</td>
<td>Neomycin fastidious anaerobe agar</td>
<td>35-37</td>
<td>anaerobic</td>
<td>16-24hr</td>
</tr>
<tr>
<td>Food poisoning (according to clinical details and advice from senior microbiologist)</td>
<td>Faeces</td>
<td>Mannitol salt agar or Baird Parker agar</td>
<td>35-37</td>
<td>air</td>
<td>40-48hr</td>
</tr>
<tr>
<td>Suspected Cholera or suspected infection with V. parahaemolyticus, seafood consumption, and/or recent travel (2-3 weeks) to known cholera area</td>
<td>Faeces</td>
<td>Thiosulphate citrate bile salts sucrose agar (TCBS) agar</td>
<td>35-37</td>
<td>air</td>
<td>16-24hr</td>
</tr>
<tr>
<td>For suspected Vibrio outbreaks or when advised by a senior microbiologist</td>
<td>Faeces</td>
<td>Alkaline peptone water then subculture to: TCBS agar</td>
<td>35-37</td>
<td>air</td>
<td>5-8hr</td>
</tr>
</tbody>
</table>
### Appendicitis

- Mesenteric lymphadenitis
- Terminal ileitis
- Reactive arthritis

When advised by a senior microbiologist

<table>
<thead>
<tr>
<th>Organism</th>
<th>Incubation</th>
<th>Incubation Media</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Y. enterocolitica</em></td>
<td>≥24hr</td>
<td>CIN agar</td>
</tr>
<tr>
<td><em>Y. pseudotuberculosis</em></td>
<td>≥24hr</td>
<td>CIN agar</td>
</tr>
<tr>
<td><em>Yersinia species</em></td>
<td>≥24hr</td>
<td>CIN agar</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism</th>
<th>Incubation</th>
<th>Incubation Media</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichomonas vaginalis</em></td>
<td>7d</td>
<td>Blood agar</td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td>24-48hr</td>
<td>Blood agar</td>
</tr>
</tbody>
</table>

* Egg-yolk supplement is optional; blood agar can also be used.

** Cultures may be examined after overnight incubation, but should not be removed from the anaerobic cabinet because sporulation is inhibited on selective media, and young cultures may die on exposure to aerobic conditions. Using anaerobic jars, cultures must not be examined before 48 hours incubation.

Other organisms for consideration - *Mycobacterium species* (B 40 - Investigation of specimens for *Mycobacterium species*), toxin of *C. botulinum* and parasites.
Flowchart for Investigation of faecal specimens for routine bacterial pathogens

Prepare all specimens

For all diagnostic specimens (except specific organism screens)

Campylobacter selective agar
- Incubate at 37-42°C Micro-aerobic ≥48hr
- Read at ≥40hr
- Campylobacter species refer to ID 23

XLD agar
- Incubate at 35-37°C Air 16-24hr Read at ≥16hr
- Salmonella species refer to ID 24
Shigella species refer to ID 20

Mannitol selenite broth
- Incubate at 35-37°C Air 16-24hr Read at ≥16hr
- Subculture to XLD
- Salmonella species refer to ID 24

CT-SMAC agar
- Incubate at 35-37°C Air 16-24hr Read at ≥16hr
- Subculture to CT-SMAC agar
- E. coli O157 refer to ID 22

Modified tryptone soya broth
- Incubate at 35-37°C Air 16-24hr
- Subculture to CT-SMAC agar
- Incubate at 35-37°C Air 16-24hr Read at ≥16hr
- E. coli O157 refer to ID 22

For all diarrhoeal specimens and any with obvious blood (semi-formed or liquid faeces); children under 5 years; for outbreaks or when advised by a consultant microbiologist
Flowchart for Investigation of faecal specimens for additional bacterial pathogens

1. Prepare all specimens

2. Additional media for these situations

- When a rapid salmonella result is required the following media may be used:
  - Food poisoning: MLCB agar
  - Neomycin selective agar (PEMBA)
  - Mannitol salt agar or Baird Parker agar
  - CCEY agar

- saline

- Suspected Cholera or suspected infection with V. parahaemolyticus, seafood consumption, and/or recent travel (2-3 weeks) to known cholera area: TCBS agar

- Appendicitis, mesenteric lymphadenitis, adenitis, terminal ileitis, reactive arthritis: CIN agar

3. Culture for C. difficile in samples from outbreaks only: Refer to B 10

4. Suspected outbreaks/advised by a senior microbiologist: Tris-buffered 1% peptone (pH 8.0)

5. Subculture to TCBS agar

6. Salmonella species (except S. Typhi and S. Paratyphi A and B): refer to ID 24

7. B. cereus, B. subtilis, B. licheniformis: refer to ID 9

8. C. perfringens: refer to ID 7

9. S. aureus: refer to ID 7

10. C. difficle refer to ID 8

11. V. cholera: refer to ID 19

12. V. parahaemolyticus: refer to ID 19

13. Y. enterocolitica: refer to ID 21

14. Y. pseudotuberculosis: refer to ID 21

To view associated UK SMI documents please access from: www.gov.uk/phe/uksmi
7.2.5 Identification
Organisms may be further identified if this is clinically or epidemiologically indicated.
Refer to individual UK SMIs for organism identification.

Minimum level of identification in the laboratory

<table>
<thead>
<tr>
<th>Organism</th>
<th>Level of Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus species</td>
<td>genus level</td>
</tr>
<tr>
<td>Campylobacter species</td>
<td>genus level</td>
</tr>
<tr>
<td>C. perfringens</td>
<td>species level</td>
</tr>
<tr>
<td>STEC</td>
<td>species level + serogroup</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>genus level</td>
</tr>
<tr>
<td>S. Typhi/Paratyphi</td>
<td>species level</td>
</tr>
<tr>
<td>Shigella species</td>
<td>species level</td>
</tr>
<tr>
<td>S. aureus</td>
<td>species level</td>
</tr>
<tr>
<td>Vibrio species</td>
<td>species level</td>
</tr>
<tr>
<td>For V. cholerae, to consider whether O1, O139 or not</td>
<td></td>
</tr>
<tr>
<td>Yersinia species</td>
<td>species level</td>
</tr>
<tr>
<td>Parasites</td>
<td>species level and their stages where possible</td>
</tr>
</tbody>
</table>

Note: All work on S. Typhi, S. Paratyphi A, B & C, presumed (locally confirmed) STEC O157 and Shigella dysenteriae type 1 must be performed in a microbiological safety cabinet under Containment Level 3 conditions.

Antimicrobial susceptibility testing
Refer to EUCAST guidelines.

7.3 Enzyme immunoassays (EIA)
These rapid tests detect pathogen antigens or antibodies in faecal samples. They have been found to be useful in the detection of several enteric bacteria, viruses and parasites such as C. perfringens, H. pylori, S. aureus, Adenovirus, Norovirus, Giardia species and Cryptosporidium species. There are several commercially available assays on the market however these may vary in sensitivity and so laboratories should follow manufacturers’ instructions when using these.

However, EIA are still being used by some laboratories for detecting viruses despite their inadequate sensitivity. Where possible, PCR should be used instead. It should also be noted that where EIA is used to detect viruses and the EIA result is negative, a PCR will need to be followed up for confirmation.

7.4 Immunochromatographic lateral flow (ICLF) assays
This point of care test is useful in the rapid identification of pathogens leading to prompt diagnosis of patients. There are many commercially available assays (either as cassette kits or dipsticks) and laboratories should ensure that these are verified prior to use. It has been used successfully in the direct detection of bacteria, viruses and parasites such as Giardia and Cryptosporidium species from clinical specimens (faeces) which is usually confirmed using a quantitative test method.

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UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England
7.5 Nucleic Acid Amplification Tests (NAATs, including PCR) 61

Molecular methods may perform better than phenotypic methods such as culture or microscopy, and should therefore be considered for use where available following validation to ensure appropriate clinical interpretation 62-65. Laboratories are implementing multiplex panels for common pathogens; these have the advantage of being able to detect multiple pathogens in a single run, as well as being both rapid and sensitive with results for gastrointestinal pathogen panels returning in less than a day. The use of multiplex panels by sample type/disease syndrome simplifies routine service through rapidity, reduced cost, reduction of unnecessary antibiotic usage and investigation when compared to single testing66.

Several assays are available for the primary testing of faecal samples replacing traditional methods. Such panels may need to be supplemented by other tests as advised in section 8 of this UK SMI. They have been used successfully in the identification of viruses, bacteria and some parasites67-69. PCR-based assays offer greater sensitivity and specificity than EIAs and culture-based techniques. They are highly accurate for viruses, Salmonella, Campylobacter, STEC (including O157), Giardia species and Cryptosporidium species, however less data is available regarding the effectiveness of testing for toxin producing pathogens (C. perfringens, Bacillus species, S. aureus)30,70.

Due to the high sensitivity of molecular methods the detection of recognised pathogens may not be diagnostic of acute or ongoing infection. Results obtained by molecular testing must be interpreted with caution and clinico-pathological correlation is frequently required.

Note:

1. Laboratories should note that molecular methods do not provide information on antimicrobial susceptibility used to guide clinical management 22.
2. Commercially available panels capture only common organisms known to cause gastrointestinal infection; they will not capture every organism. Consideration must be given for other organisms when clinically indicated.
3. The date of onset is useful when interpreting molecular results as the procedures are unlikely to differentiate between viable and non-viable pathogens and care should be taken to consider results in conjunction with the overall presentation of the patient.
4. If an outbreak is with a known organism such as STEC O157 then consider ordering the specific PCR test for that organism rather than an enteric PCR panel.
5. If there is a strong clinical suspicion but GI multiplex PCR screening is negative consider culture based methods or enrichment for PCR.

7.5.1 Sample preparation

Molecular Tests

For safety considerations, refer to the scientific information.

Follow manufacturers’ instructions when on how to prepare and process the clinical specimens using the manufacturers’ test kit inserts.
7.5.2 Specific technical limitations (to include any uncertainty of measurement information)

Rapid diagnostic test kits/systems

EIA and PCR tests may perform better than conventional methods and should therefore be considered for use where available in diagnostic laboratories. Sensitivity and specificity of these kits/systems vary depending on the manufacturer. Hence, all commercial kits and molecular platforms should be verified prior to use to ensure appropriate clinical interpretation.

Note: It should also be noted that some automated commercial identification systems do not have the ability to differentiate between closely related genera/species and as such may result in clinically significant errors when reporting results, such as the misidentification of *Shigella* species as *E. coli* by MALDI-TOF.

Molecular Assays

Manufacturers produce assays with new gene targets which may not necessarily cover the gene targets now and so laboratory users should ensure that all kits should be validated prior to routine use in the laboratory.

Preservation of specimens

Preservation of faeces in 10% formalin is no longer recommended by the UK SMIs although it may still be used by some laboratories. It should be noted that the drawbacks include its interference with PCR especially after extended fixation time, it is inadequate at preserving morphology of the protozoan trophozoites as well as being unsuitable for some smears stained with trichrome stain.

Concentration of faecal specimens

Faeces to be tested using EIA or other rapid assays should not be concentrated prior to testing because antigens (such as those targeted in diagnosis of *Giardia* species and *Cryptosporidium* species) are lost during the procedure. Most EIAs require the use of fresh or frozen stool specimens. However, there are now some commercially available test kits that use preserved faecal specimens for detection of antigens, and users should check the manufacturers’ instructions.

7.5.3 Investigation

Follow manufacturer instructions on how to prepare the clinical specimens and on the use of the several commercial rapid diagnostic test kits available. Laboratories should ensure that all test kits and platforms are validated prior to use.

If using in-house tests/kits, laboratories should ensure that these have been validated prior to routine use.
7.5.4 Actions for NAAT (including PCR) positive specimens from sporadic cases

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Local confirmation by culture</th>
<th>Referral to reference laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parasites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Giardia species</em></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>Cryptosporidium species</em></td>
<td>No</td>
<td>Refer in outbreak cases. Positive specimens are recommended to be stored for 3 weeks</td>
</tr>
<tr>
<td><em>Entamoeba</em> (<em>Entamoeba histolytica</em>)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>Cyclospora species</em></td>
<td>No</td>
<td>Refer in outbreak cases. Positive specimens are recommended to be stored for 3 weeks</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella species</em></td>
<td>Culture</td>
<td>Send isolate for confirmation and typing</td>
</tr>
<tr>
<td><em>Campylobacter species</em></td>
<td>Culture if treatment is indicated</td>
<td>No</td>
</tr>
<tr>
<td>STEC O157</td>
<td>Culture for O157</td>
<td>Send O157 isolate for confirmation and typing</td>
</tr>
<tr>
<td>Non-O157 STEC</td>
<td>Not applicable</td>
<td>Send faecal specimen to the reference laboratory</td>
</tr>
<tr>
<td><em>Shigella species</em></td>
<td>Culture</td>
<td>Send isolate for speciation (if not performed locally) and typing</td>
</tr>
<tr>
<td><em>Enteroaggregative E. coli</em></td>
<td>Not applicable</td>
<td>No</td>
</tr>
<tr>
<td><em>Vibrio species</em></td>
<td>Culture</td>
<td>Refer for typing</td>
</tr>
<tr>
<td><em>Plesiomonas species</em></td>
<td>Optional</td>
<td>Optional</td>
</tr>
<tr>
<td><em>Aeromonas species</em></td>
<td>Optional</td>
<td>Optional</td>
</tr>
<tr>
<td><em>Yersinia species</em></td>
<td>Culture</td>
<td>Optional</td>
</tr>
</tbody>
</table>

For information on clearance and screening of contacts refer to current guidance74.

7.6 Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS)

This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high throughput use. Refer to UK SMI TP 40 for further information.

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8 Post-laboratory processes (post-analytical phase)

8.1 Microscopy

8.1.1 Interpreting and Reporting Laboratory Results

**Standard**

Report on the presence or absence of bacteria or parasites seen.

**Supplementary**

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

**Note:** For reporting microscopy of parasites, comment on the presence of all development stages of parasites seen, whether they are pathogenic or non-pathogenic.

**Microscopy reporting time**

Urgent microscopy results to be telephoned or sent electronically.

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

8.2 Culture

8.2.1 Interpreting and reporting laboratory results

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

**Culture reporting time**

Clinically urgent results to be telephoned or sent electronically when available.

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

8.2.2 Antimicrobial susceptibility testing and Reporting Table

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Examples of agents to be included within primary test panel (recommended agents to be reported are in bold depending on clinical presentation)</th>
<th>Examples of agents to be considered for supplementary testing (recommended agents to be reported are in bold depending on clinical presentation)</th>
<th>Notes</th>
</tr>
</thead>
</table>
| Shigella spp | Ciprofloxacin
Azithromycin
Ceftriaxone
Amoxicillin
Co-amoxicillin | Chloramphenicol |
Rapid diagnostic tests for identification of pathogens directly from faeces are available. These include enzyme immunoassays (EIA), NAAT (including PCR) testing and lateral flow cassette kits. All commercial kits and molecular platforms should be validated by the laboratory prior to use to ensure appropriate clinical interpretation.

### 8.3 Enzyme immunoassays (EIA)

#### 8.3.1 Interpreting and Reporting Laboratory Results

A positive test result indicates evidence of gastroenteritis infection. However, interpretation should take into consideration, results of all other tests and the clinical picture.

Negative results should be interpreted considering other test results.

Refer to the table within this document for further information regarding clinical presentations of gastrointestinal infections.

**Enzyme Immunoassays assays reporting**

Report results following manufacturers’ interpretation instructions.

#### 8.3.2 Surveillance Reporting

For national surveillance to be effective, laboratories are expected to refer representative proportions of positive samples from gastroenteritis outbreaks to the national reference laboratory for confirmation.

In England, hospital outbreaks of suspected or confirmed norovirus are reported to the Hospital Norovirus Outbreak Reporting System (HNORS) - [http://bioinformatics.phe.org.uk/noroOBK/home.php](http://bioinformatics.phe.org.uk/noroOBK/home.php).

In Scotland, hospital outbreaks of suspected or confirmed norovirus are reported to; [https://www.hps.scot.nhs.uk/a-to-z-of-topics/norovirus/](https://www.hps.scot.nhs.uk/a-to-z-of-topics/norovirus/)

### 8.4 Immunochromatographic lateral flow (ILFA) assays

#### 8.4.1 Interpreting and Reporting Laboratory Results

A positive test result indicates evidence of gastroenteritis infection. However, interpretation should take into consideration, results of all other tests and the clinical picture.

Negative results should be interpreted considering other test results.
Refer to the table within this document for further information regarding clinical presentations of gastrointestinal infections.

Report results following manufacturers’ interpretation instructions.

8.4.2 Interpreting and reporting laboratory results

Surveillance Reporting

For national surveillance to be effective, laboratories are expected to refer representative proportions of positive samples from gastroenteritis outbreaks to the national reference laboratory for confirmation.

In England, hospital outbreaks of suspected or confirmed norovirus are reported to the Hospital Norovirus Outbreak Reporting System (HNORS) - http://bioinformatics.phe.org.uk/noroOBK/home.php.

In Scotland, hospital outbreaks of suspected or confirmed norovirus are reported to; https://www.hps.scot.nhs.uk/a-to-z-of-topics/norovirus/

8.5 Nucleic Acid Amplification Tests (NAATs, including PCR)

8.5.1 NAATs/PCR reporting

Positives

A positive result indicates the presence of the DNA of an organism. Bacterial, parasite or viral DNA detected.

Report result as for example, ‘Salmonella DNA detected”.

Negatives

A negative result does not rule out the presence of organisms that may be present at levels below the detection limits of this assay.

Bacterial, parasite or viral DNA not detected.

Report result as for example, ‘Salmonella DNA not detected”.

If there is a strong clinical suspicion but GI multiplex PCR screening is negative consider culture based methods or enrichment for PCR.

NAATs/PCR reporting time

PCR results should be reported as soon as they are performed and analysed.

8.5.2 Surveillance Reporting

For national norovirus surveillance to be effective, laboratories are expected to refer representative proportions of positive samples from gastroenteritis outbreaks to the national reference laboratory for confirmation.

In England, hospital outbreaks of suspected or confirmed norovirus are reported to the Hospital Norovirus Outbreak Reporting System (HNORS) - http://bioinformatics.phe.org.uk/noroOBK/home.php.

In Scotland, hospital outbreaks of suspected or confirmed norovirus are reported to; https://www.hps.scot.nhs.uk/a-to-z-of-topics/norovirus/
# Appendix 1

## Table 1: Overview of pathogens associated with gastroenteritis

<table>
<thead>
<tr>
<th>Organism</th>
<th>Incubation Period/Infectious dose (ID)</th>
<th>Clinical presentations/features</th>
<th>Mode of transmission/risk factors</th>
<th>Frequency of infections caused by the organism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Common bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> species&lt;sup&gt;23&lt;/sup&gt;</td>
<td>The usual incubation period is 12-72hr. ID is usually $10^3$ organisms Incubation period depends on host factors and the size of the infecting dose.</td>
<td>Abdominal pain, diarrhoea, nausea and vomiting, often accompanied by fever. Other clinical manifestations include bacteraemia and focal metastatic infections. Malnutrition, immunosuppression, sickle-cell disease, achlorhydria and inflammatory bowel disease may be associated with more severe infections.</td>
<td>Foreign travel, and consumption of imported foodstuffs or inadequately cooked or contaminated foods of animal origin, drinking of contaminated water. Important cause of Travellers’ Diarrhoea. Infected food handlers and person-to-person contact&lt;sup&gt;80&lt;/sup&gt; have been implicated in outbreaks of salmonellosis.</td>
<td>There is marked seasonal variation in occurrence of infection with peaks of incidence during summer and autumn.</td>
</tr>
<tr>
<td>Enteric fever caused by <em>Salmonella enterica</em> serovar Typhi&lt;sup&gt;81&lt;/sup&gt;</td>
<td>8-14 days, although can be significantly longer or shorter. ID for S. Typhi is $10^3$ organisms.</td>
<td>Acutely, fever, vomiting and constipation, followed by fever with diarrhoea (may be bloody)</td>
<td>Human faecal contamination of food or water. Travellers to high endemic areas are at risk of contracting typhoid fever due to inadequate sanitation and poor standards of personal and food hygiene.</td>
<td>There is marked seasonal variation in occurrence of infection with peaks of incidence during summer and autumn.</td>
</tr>
<tr>
<td>Organism</td>
<td>Incubation Period/Infectious dose (ID)</td>
<td>Clinical presentations/features</td>
<td>Mode of transmission/risk factors</td>
<td>Frequency of infections caused by the organism</td>
</tr>
<tr>
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<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Enteric fever caused by <em>Salmonella enterica</em> serovar Paratyphi types A, B and C&lt;sup&gt;61&lt;/sup&gt;</td>
<td>Incubation is 1-10 days. ID for S. Paratyphi species is 10&lt;sup&gt;6&lt;/sup&gt; organisms.</td>
<td>Diarrhoea (may be bloody), accompanied by fever and vomiting as well as constipation.</td>
<td>Human faecal contamination of food or water.</td>
<td>There is marked seasonal variation in occurrence of infection with peaks of incidence during summer and autumn.</td>
</tr>
<tr>
<td>Note: Most paratyphoid cases reported in UK travellers are caused by S. Paratyphi A.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| **Shigella** species<sup>52</sup>  
 (*S. flexneri, S. dysenteriae, S. boydii, and S. sonnei*) | 1 – 3 days.  
 As few as 10-100 organisms are required for an ID.  
 For highly virulent strains, <10 organisms are needed to cause infection. | Illness may last up to 2 - 4 weeks.  
 Diarrhoea accompanied by fever, malaise and abdominal pain. This self-limiting, watery diarrhoea may progress to dysentery.  
 *S. dysentriae* infection can be complicated by haemolytic uraemic syndrome which is seen more commonly in children <5 years of age.  
 Asymptomatic infection can occur with all *Shigella* species. | Person to person contact, faecal-oral spread, through contaminated food or water or by contaminated fomites.  
 Note:  
 Outbreaks may be associated with overcrowding in schools, prisons, mental institutions, and where there are low standards of hygiene. | Occurs less commonly. |
| **Campylobacter** species  
 (*C. jejuni* and *C. coli*) | 2 to 10 days.  
 The ID is as low as 500 - 800 organisms. | Self-limited diarrhoea. Initial symptoms may be severe, with fever and abdominal pain mimicking appendicitis.  
 Occasionally, infection may produce sequelae such as reactive arthritis, bursitis, endocarditis and neonatal sepsis. | Consumption of inadequately cooked or contaminated foods and animal products. Person to person transmission can occur if hygiene is poor. | There are marked seasonal peaks which occur in May and September. |
<table>
<thead>
<tr>
<th>Organism</th>
<th>Incubation Period/Infectious dose (ID)</th>
<th>Clinical presentations/features</th>
<th>Mode of transmission/risk factors</th>
<th>Frequency of infections caused by the organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterohaemorrhagic <em>E. coli</em> (EHEC) Also known as STEC (including O157)</td>
<td>3 to 4 days. The incubation period may depend on the number of organisms ingested. ID is &lt;100 organisms or even &lt;10 organisms for <em>E. coli</em> STEC O157.</td>
<td>Mild to bloody diarrhoea, pain in abdomen, nausea, and vomiting. Note: Blood is not always present in faeces in shigatoxin producing <em>E. coli</em> infections. <em>E. coli</em> STEC is associated with haemorrhagic colitis and haemolytic uraemic syndrome (HUS).</td>
<td>Outbreaks have been directly associated with contaminated cooked meats, milk and water, ground beef, beef burgers and indirectly with vegetables, apple cider and mayonnaise. Outbreaks may occur in establishments such as nursing homes and following visits to open farms. There is a marked seasonal variation, with a peak incidence in the summer and early autumn.</td>
<td></td>
</tr>
<tr>
<td>Enteroaggregative <em>E. coli</em> (EAEC)</td>
<td>20 – 48hrs. ID is $10^6 – 10^8$ organisms.</td>
<td>Chronic diarrhoea that may be watery with or without mucus and blood, vomiting, dehydration, and occasionally abdominal pains and fever. Diarrhoea is self-limiting but may develop into a persisting diarrhoea lasting &gt;14 days.</td>
<td>This organism has been detected in travel-associated infections.</td>
<td></td>
</tr>
<tr>
<td>Enteroinvasive <em>E. coli</em> (EIEC)</td>
<td>12 – 72hrs. ID is &lt;100 organisms.</td>
<td>Fever, abdominal cramps, and watery diarrhoea with blood and mucus which generally contains leucocytes.</td>
<td>Foodborne and waterborne outbreaks.</td>
<td></td>
</tr>
<tr>
<td>Organism</td>
<td>Incubation Period/Infectious dose (ID)</td>
<td>Clinical presentations/features</td>
<td>Mode of transmission/risk factors</td>
<td>Frequency of infections caused by the organism</td>
</tr>
<tr>
<td>--------------------------------</td>
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<td>-------------------------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Enteropathogenic <em>E. coli</em> (EPEC)</td>
<td>12-72 hrs. ID is $10^6 - 10^8$ organisms.</td>
<td>Severe, prolonged non-bloody diarrhoea usually with passage of mucus. Vomiting and fever are also common.</td>
<td>EPEC may be associated with travellers’ diarrhoea. Transmitted from person-to-person by the faecal-oral route or via contaminated food.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EPEC is known to cause sporadic cases and outbreaks of diarrhoea among children &lt;2 years of age.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterotoxigenic <em>E. coli</em> (ETEC)</td>
<td>12 to 72 hrs. ID is $10^6 - 10^8$ organisms.</td>
<td>Travellers’ Diarrhoea (mild, watery diarrhoea with abdominal cramps, nausea and low-grade fever). Dehydration and vomiting may occur in some cases.</td>
<td>Travel – associated: visitors to developing tropical/semi-tropical countries. Peak incidence is especially during the warm, wet season.</td>
<td></td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>&lt; 1 week but can be up to 4 weeks.</td>
<td>Symptoms range from a self-limiting mild diarrhoea, to the advanced and severe illness characteristic of pseudomembranous colitis which may be associated with fever, abdominal cramps, and leucocytosis. Complications include intestinal perforation and toxic megacolon.</td>
<td>Outbreaks occur in hospitals and in extended care facilities for the elderly, associated with antibiotic therapy. Transmission is via person to person by the faecal-oral route and by environmental contamination.</td>
<td></td>
</tr>
</tbody>
</table>
## Common parasites

| **Cryptosporidium species** | 1-14 days. ID is as low as 1 oocyst. | Severe, chronic diarrhoeal disease with signs of malabsorption, abdominal pain, low grade fever, nausea and vomiting but other presentations include atypical gastrointestinal disease such as cholangitis, cholecystitis, pancreatitis and hepatitis. Asymptomatic carriage possible. | Primarily spread from person to person via the faecal-oral route, or zoonotically. Causes waterborne and foodborne outbreaks e.g. swimming pools, petting farms. Risk groups include MSM, children attending nurseries, the elderly in care homes. | Infection shows seasonal variation with peak incidence in the spring and especially the autumn. |

**Note:**

Infection is a particular problem for patients who are severely immunocompromised.  

| **Giardia species** | 3-25 days. ID is between 25 and 100 cysts. | Sometimes severe, chronic diarrhoea or a syndrome of chronic diarrhoea, steatorrhoea, malabsorption and weight loss. Other symptoms include: abdominal cramps, bloating and flatulence. Vomiting, fever and tenesmus can also occur, but infection may also be asymptomatic. | Waterborne and foodborne outbreaks have been reported. Outbreaks have also been reported from MSM community. The parasite is primarily spread from person to person via the faecal-oral route, or zoonotically. | Occurs sporadically throughout the year |

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UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England
<table>
<thead>
<tr>
<th>Common viruses</th>
<th>norovirus</th>
<th>rotavirus</th>
<th>sapovirus</th>
<th>adenovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>(norovirus GI/GII)</td>
<td>12-48hr.</td>
<td>24-72hr.</td>
<td>12-48hr and has an ID of as few as 10 virus particles.</td>
<td>5–9 days</td>
</tr>
<tr>
<td>Note: Norovirus Genogroup II genotype 4 is the commonest cause of gastroenteritis in the UK and worldwide.</td>
<td>Approximately 10 virus particles are needed to cause an infection.</td>
<td>Diarrhoea and vomiting occasionally, gastroenteritis in the elderly. Other symptoms may include loss of appetite and dehydration in children.</td>
<td>Diarrhoea and vomiting, generally without accompanying fever. Illness duration is 1 – 4 days.</td>
<td>Acute diarrhoea - prolonged diarrhoea and low-grade fever are commonly seen.</td>
</tr>
<tr>
<td></td>
<td>Vomiting (often projectile), diarrhoea, headaches, fever, myalgia and abdominal cramps. Increased severity may be observed in the elderly and young children.</td>
<td>Outbreaks are common within the community and institutions such as hospitals and elderly care homes. Infection usually spreads by inter-personal contact, faecal contamination of food/water by infected food handlers or even by ingestion of aerosol droplets from vomit.</td>
<td>Foodborne outbreaks via consumption of raw or undercooked shellfish, such as oysters and clams have been documented.</td>
<td>Person to person contact via faecal-oral route. Outbreaks have been recognised in nurseries and paediatric units.</td>
</tr>
<tr>
<td></td>
<td>Recovery is usually within 24hr.</td>
<td>Person to person contact via faecal-oral route, fomites or vomit where the aerosol droplets are disseminated in the environment.</td>
<td>Increased incidence in the winter months.</td>
<td>Person to person contact via faecal-oral route. Outbreaks have been recognised in nurseries and paediatric units.</td>
</tr>
</tbody>
</table>
| **Astrovirus**  
| (Astrovirus serotype 1) | 3 to 4 days | Symptoms are mild which includes vomiting, abdominal pain, diarrhoea and fever. | Faecal-oral route. Transmission in children occurs usually from person to person. | Increased incidence in the winter months. |

| **Other Bacteria** |
| **Vibrio species excluding**  
| V. cholerae and V. parahaemolyticus | 2 - 3 days. | Cholera. Symptoms vary from mild and accompanied by abdominal cramps and vomiting to explosive diarrhoea - passage of a profuse watery diarrhoea with mucus, but no blood, giving a *rice water* appearance. Fluid loss and dehydration are severe complications that can lead to shock and death if untreated. | Ingestion of contaminated water or undercooked seafood, particularly shellfish and travel to endemic areas. Other risk factors include poor sanitation and poverty. | particularly prevalent in warmer months |

| **Vibrio cholerae** | 2 - 3 days.  
| Toxicigenic V. cholerae (O1 and O139 serotypes) are at a ID of $10^3$ and $10^4$ organisms respectively; a non-O1 strain is infective at a much higher dose ($10^6$ organisms). | Cholera. Symptoms vary from mild and accompanied by abdominal cramps and vomiting to explosive diarrhoea - passage of a profuse watery diarrhoea with mucus, but no blood, giving a *rice water* appearance. Fluid loss and dehydration are severe complications that can lead to shock and death if untreated. | Ingestion of contaminated water or undercooked seafood, particularly shellfish and travel to endemic areas. Other risk factors include poor sanitation and poverty. | particularly prevalent in warmer months (during the summer and early autumn). |

<p>| <strong>Vibrio parahaemolyticus</strong> | 2 - 3 days. | Symptoms include abdominal pain, explosive watery or bloody diarrhoea, nausea, vomiting and sometimes fever. Infection is self-limiting. In severe cases, fluid loss and dehydration are common. | Ingestion of contaminated water or undercooked seafood, particularly shellfish and travel to endemic areas. Other risk factors include poor sanitation and poverty. | particularly prevalent in warmer months |</p>
<table>
<thead>
<tr>
<th><strong>Yersinia species</strong> <em>(Y. enterocolitica and Y. pseudotuberculosis)</em></th>
<th>3-7 days.</th>
<th>Yersinosis - symptoms may present as acute diarrhoea, mesenteric adenitis/lymphadenitis, terminal ileitis, ‘pseudo-appendicitis’, sepsis, metastatic infections and immunological sequelae (for example reactive arthritis and erythema nodosum) (^{86,87}).</th>
<th>Contaminated food, milk or water, and pigs are a frequently identified source of infection.</th>
<th>Particularly prevalent in winter months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus species</strong> <em>(Bacillus cereus)</em></td>
<td>Emetic syndrome is 1-6 hr. Food poisoning results from the ingestion of large numbers (&gt;10⁵ cfu/g of food) of toxigenic bacteria or preformed emetic toxin.</td>
<td>Two enteric diseases are seen: Emetic syndrome caused by a thermostable peptide known as cereulide. Diarrhoeal syndrome due to enterotoxins which are haemolysin BL (HBL), non-haemolytic enterotoxin (NHE), and cytotoxin K. Symptoms are mild and usually subside within 24 hr. It includes abdominal pain, watery diarrhoea and occasionally nausea.</td>
<td>Ingestion of foods like rice, pasta, desserts, meat, and dairy products.</td>
<td>Particularly prevalent in warmer months</td>
</tr>
<tr>
<td><strong>B. subtilis and B. licheniformis</strong> may also be involved in food poisoning episodes.</td>
<td>Diarrhoeal syndrome is 8-16 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>1-6 hr and usually resolves in 12 hr</td>
<td>General malaise, vomiting, nausea and abdominal cramps, often followed by diarrhoea. Severe dehydration can occur in children and the elderly.</td>
<td>Ingestion of foods - milk products such as cream-filled pastries, cream pies, butter, cheese, and sandwich fillings.</td>
<td>Particularly prevalent in warmer months</td>
</tr>
<tr>
<td><strong>Clostridium perfringens</strong> <em>(Type A)</em></td>
<td>4-24 hr and symptoms usually subside within 10–24 hrs. ID is &gt;10⁵ of organisms.</td>
<td>Watery diarrhoea with severe abdominal pains and vomiting. Fatalities although rare, can occur, especially in the elderly and other compromised persons.</td>
<td>Ingestion of improperly cooked, stored and reheated foods</td>
<td>Particularly prevalent in warmer months</td>
</tr>
</tbody>
</table>
### Clostridium botulinum

2hrs – 8 days (usually 12 – 36hrs)

Botulism - this includes slurred speech, double vision, difficulty in swallowing, descending flaccid paralysis, typically with cranial nerve involvement, sometimes culminating in respiratory arrest.

- Consumption of inadequately processed stored foods. Person to person spread does not occur.

### Listeria monocytogenes

usually 24hr but can vary from 6hr to even 10 days.

Listeriosis.

- In otherwise healthy individuals, symptoms last for 1 to 3 days, nausea, fever and myalgia. Complications include abdominal pain, nausea, vomiting, dizziness, lymphadenopathy, and sometimes a rash.

- In high risk groups (e.g., patients undergoing treatment for cancer, pregnant women or even have serious underlying health conditions), symptoms are more severe and include fever, myalgia, septicemia, meningitis or even death.

- Consumption of contaminated refrigerated foods like milk, soft cheeses, prepacked sandwiches, pates, etc.

### Cyclospora species

(*Cyclospora cayetanensis*)

2-14 days

ID is 10-100 oocysts

Watery diarrhoea which may be prolonged, with weight loss, anorexia, severe fatigue, nausea, vomiting and abdominal pain.

- Consumption of contaminated soft fruit and vegetables and drinking recreational water. Direct person-to-person transmission is unlikely.

- Travel to endemic countries eg Mexico
### Entamoeba

*(Entamoeba histolytica)*

- 2-4 weeks.
- ID is as low as 1 cyst.

*E. histolytica* may cause ulcerative and inflammatory lesions in the colon producing symptoms of dysentery which include lower abdominal pain, increased frequency of bowel movements and liquid stools. Infection can lead to perforation of the colon, toxic megacolon, ameboma, and perianal ulceration. Invasive spread to major organs is also possible.

This organism can cause diarrhoea in travellers returning from endemic areas and is primarily spread from person to person contact, or via contaminated water consumption. Outbreaks have been reported within the MSM community.

### Other organisms

<table>
<thead>
<tr>
<th>Microsporidia</th>
<th>May be dependent on the number of organisms ingested.2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>(Enterocytozoon bieneusi, Encephalitozoon cuniculi and Encephalitozoon intestinalis)</em></td>
<td>Chronic diarrhoea and wasting syndrome observed in immunocompromised patients. Fever is not observed.</td>
</tr>
<tr>
<td>Contaminated water consumption.</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** It should be noted that in addition to routine testing, samples may be screened for other organisms as indicated by clinical details eg *Vibrio* species, *Plesiomonas shigelloides*, *Aeromonas* species, *Yersinia* species, toxin producers (*Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens* and *Clostridium botulinum*).
References

For the information for the evidence grade ratings given, refer to the scientific information.


3. Primary Care Unit. Management of Infection Guidance for Primary Care for Consultation & Local Adaptation 2010.


